

# H<sub>2</sub> Concentrations in a Landfill Leachate Plume (Grindsted, Denmark): In Situ Energetics of Terminal Electron Acceptor Processes

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Empirical H<sub>2</sub> concentration ranges are currently related to specific redox processes, assuming steady-state conditions at which only one microbiologically mediated redox process occurs due to competitive exclusion of others. Here the first H<sub>2</sub> data from a landfill leachate plume are presented, and an alternative partial equilibrium approach is used. The approach implies that TEAPs (terminal electron-accepting processes) occur at negative  $\Delta G_r$  values, close to thermodynamic equilibrium, and that the fermentative H<sub>2</sub> production is overall rate limiting. It eliminates the steady-state prerequisite and may explain the occurrence of concomitant TEAPs. Concentrations of H<sub>2</sub> and redox process reactants and products were measured in 52 sampling points, downgradient of the Grindsted Landfill (Denmark), and used to calculate in situ  $\Delta G_r$  values of TEAPs, assuming partial equilibrium. H<sub>2</sub> generally ranged from 0.004 to 0.88 nM, with most values around 0.2 nM. Fe reduction was, according to the empirically defined ranges, the most prominent TEAP, but concomitant methanogenesis and sulfate reduction occurred as well. This indicated a need for an alternative approach to explaining the H<sub>2</sub> distribution, and the measured H<sub>2</sub> concentrations are viewed as being controlled by a partial equilibrium. A derived theoretical relation between H<sub>2</sub> concentrations and temperature indicates temperature effects to be more important than currently appreciated. Calculated in situ  $\Delta G_r$  values can, combined with a threshold value, predict which TEAPs can occur via H<sub>2</sub> oxidation. For our samples,  $\Delta G_r$  for methanogenesis was always  $> -7$  kJ/mol, and CO<sub>2</sub> reduction should only occur in stagnant porewater at higher H<sub>2</sub> concentrations or by direct interspecies transfer. In contrast, sulfate and Fe reduction occur close to or slightly below a threshold of  $-7$  kJ/mol H<sub>2</sub> and may occur concomitantly at partial equilibrium.

## Introduction

H<sub>2</sub> measurements have been developed into a tool for deducing the redox state of natural anaerobic aquatic systems (1, 2). The concept is based on experimental data as well as microbiological and thermodynamic theory. According to this, a TEAP (terminal electron-accepting process) at steady state is characterized by a specific H<sub>2</sub> level due to competitive exclusion of other microbiologically mediated TEAPs. The competitive edge is related to differences in the thermodynamic energy yield of the redox processes and physiological characteristics of the various bacteria (2). One of the advantages of using H<sub>2</sub> is that the pool of H<sub>2</sub> found in natural systems is so small that it is cycled within minutes, implying that the measured concentration relates to processes that actually occur in the sampled sediment.

Recently, the possibility of using H<sub>2</sub> measurements to map redox zones within petroleum-contaminated aquifers has been addressed (3–5). A precise knowledge of the ongoing redox processes in a plume is important for prediction of the fate of the pollutants in the plume.

In this paper, we present the first H<sub>2</sub> measurements from a mixed landfill leachate plume emanating from the Grindsted Landfill, Denmark. The redox conditions in the Grindsted Landfill leachate plume have previously been studied in terms of the distribution of redox-sensitive species in groundwater samples (6) and TEAP rates in unamended sediment bioassays (7). Both approaches have shown a redox state gradient from methanogenic conditions close to the landfill to aerobic conditions 250 m away. However, the redox processes in the plume do not occur in well-separated zones, but to some extent they occur simultaneously, indicating that competitive exclusion is not always fully efficient. This concomitance of redox processes has also been observed in a petroleum polluted aquifer (8) and in marine sediments (9). These observations call for an alternative approach to using H<sub>2</sub> as an indicator of microbiologically mediated TEAPs. We propose that measured H<sub>2</sub> and TEAP reactant concentrations may be combined, through simple thermodynamic calculations, into an actual potential in situ energy yield, representing the potential for a given hydrogen oxidizing TEAP. With this approach, we can explain the observation of concomitant TEAPs, evaluate where in the leachate plume the different TEAPs can take place, and derive relative reactivities of iron oxides.

The existing approach to using H<sub>2</sub> concentrations is only valid if the system studied is in a steady state, a prerequisite that is very difficult to be certain of. This is especially the case for systems with iron oxide reduction in which a range of iron oxides are present. In such a system, the most available iron oxide will steadily be removed, and the system might never reach a true steady state as long as there are iron oxides present. The approach proposed here is applicable also in systems that are not in a steady state.

## Materials and Methods

**Field Site.** The site is described with regard to geology and sediment geochemistry in ref 10. The profile in Figure 1 summarizes the main geological features of the sandy aquifer along the center line of the plume. The groundwater flow is toward the right side of the figure (NW) with estimated flow velocities of 50 m/yr in the upper Quaternary sediments and 10 m/yr in the lower Miocene (micaceous sands) sediments (6). Annual infiltration is approximately 400 mm/yr. The groundwater temperature is 8–10 °C.

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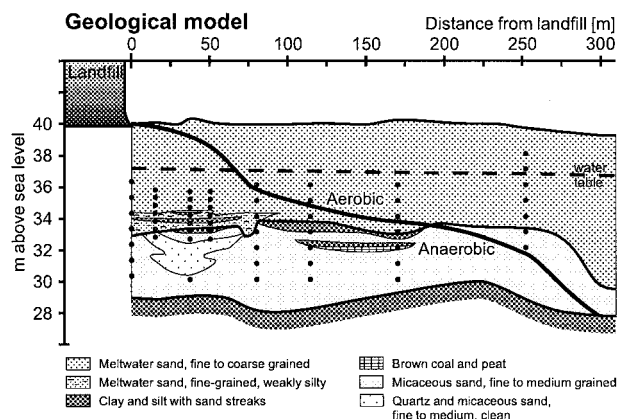


FIGURE 1. Geology along the flowline transect at the Grindstedt Landfill site. Dots indicate sampling points. Note the boundary between the upper Quaternary sediments and the lower Tertiary micaceous sands.

The landfill contains municipal as well as industrial waste (11) and has no measures to prevent leachate from migrating into the aquifer. The age of the plume is estimated to >20 yr.

**Sampling and Analysis.** MLSs (multi-level samplers) were installed at eight distances from the landfill to a depth of 10 m below surface. A 3/4 in. stainless steel pipe was driven into the aquifer, a bundle of 4 mm Teflon tubing was inserted into this, and the steel pipe was retrieved (12). The lower 5 cm of the Teflon tubing was perforated to form a screen. Fifty-five sampling points were installed. The MLSs were left in the ground for more than 3 months in order to avoid elevated  $H_2$  concentrations in the samples (12).

$H_2$  samples were taken using the bubble-stripping method developed by Chapelle (13), adapted to smaller screens and lower flow rates (12). Water was continuously pumped with a peristaltic pump at 100 mL/min through a 4 mL bubble of  $N_2$  gas retained in a vertically held glass tubing. Equilibrium with the gas bubble was obtained in approximately 20 min, and a sample of the gas bubble was taken with the glass tubing held horizontally through a septum over a bulge in the tubing. Samples were taken after 20 and 25 min, and if the difference was less than 5%, it was assumed that equilibrium had been obtained.

The  $H_2$  concentration was determined with a RGD2, reduced gas detector, from Trace Analytical (Menlo Park, CA). The separation of gases was done with a 3 ft long, 1/8 in. wide, 13 × 40/60 column at ambient temperature. Calibration was carried out using dilutions of 20 or 50  $\mu\text{L/L}$

gas standards of  $H_2$  in  $N_2$  with a nominal precision of  $\pm 10\%$ . Gas concentrations in microliters per liter headspace were converted into concentrations in the water phase using Henry's law (1  $\mu\text{L/L}$  headspace = 0.88 nM, at 8 °C). Other measured groundwater parameters in the samples were determined as described in ref 6.

**Free Energy Calculations.** The energy available to the microorganisms from  $H_2$  oxidizing TEAPs has been calculated as Gibbs free energies of reaction ( $\Delta G_r$ ), from Gibbs energies of formation ( $\Delta G_f^\circ$ ), at a groundwater temperature of 8 °C. Reactions, equations, and thermodynamic values used in the calculations are shown in Table 1. Similar calculations of in situ energy yields in a field setting are found in ref 14 for sulfate reduction, methanogenesis, and methane oxidation in a marine sediment.

Corrections for temperature were made using the enthalpy, and the Van't Hoff equation in the form:

$$\Delta G^\circ_{T_2} = \frac{\Delta H^\circ(T_1 - T_2) + T_2 \Delta G^\circ_{T_1}}{T_1}$$

where  $\Delta H^\circ$  is the enthalpy,  $T_1$  is the absolute temperature at standard conditions (298.15 K), and  $T_2$  is the absolute groundwater temperature (281.15 K), see refs 15 and 16.

From the equations of Table 1, it is clear that the energy yield for a given reaction is related to the chemistry of the local environment. For example, pH will be very important to the  $\Delta G_r$  of iron oxide reduction—with decreasing pH (increasing  $H^+$  activity), the energy available to the bacteria will increase as the  $\Delta G_r$  decreases. To make calculations as precise as possible, solute activities rather than concentrations were used. The derivation of solute activities was based on activities obtained from speciations made with PHREEQC (17) of earlier full analysis samples from the same plume transect (6). For the full analysis samples, analytical relations relating activities of the relevant species calculated by PHREEQC to concentrations of the major and most important complexing ions present in both data sets were derived. These relations were then used to estimate the activities for the samples in Table 2. Given that the natural log of the activity enters into the equations (Table 1), these corrections are not critical, and corrections using just activity coefficients lead to very similar values. Changing the  $HS^-$  activity from  $10^{-7}$  to  $5 \times 10^{-7}$  only increases the calculated  $\Delta G_r$  for sulfate reduction by 1 kJ/mol  $H_2$ . Also the error introduced by using interpolated alkalinities where data were missing should be small.

Previous studies have shown that in steady-state systems a threshold  $\Delta G_r$  of  $\sim -15$  to  $-7$  kJ/mol  $H_2$  for a given TEAP

TABLE 1. Equations and Thermodynamic Values Used for Calculating In Situ Gibbs Free Energies for Terminal Electron-Acceptor Processes (TEAPs) with  $H_2$ <sup>a</sup>

TEAP	eq used for calculating in situ $\Delta G_r$	$\Delta G_{281.15}$ (kJ/mol)	$\Delta H_r^\circ$ (kJ/mol)
$H_2 + 2FeOOH + 4H^+ \rightleftharpoons 2Fe^{2+} + 4H_2O$	$\Delta G_r = \Delta G^\circ + RT \ln \frac{[Fe^{2+}]^2}{[H_2][H^+]^4}$	-178.5 -157.5	-198.7
$4H_2 + SO_4^{2-} + H^+ \rightleftharpoons HS^- + 4H_2O$	$\Delta G_r = \Delta G^\circ + RT \ln \frac{[HS^-]}{[H_2]^4[SO_4^{2-}][H^+]}$	-262.4	-235
$HCO_3^- + 4H_2 + H^+ \rightleftharpoons CH_4 + 3H_2O$	$\Delta G_r = \Delta G^\circ + RT \ln \frac{[CH_4]}{[H_2]^4[H^+][HCO_3^-]}$	-229.8	-237.8

<sup>a</sup>  $R$  is the gas constant,  $T$  is the absolute temperature in K,  $[ ]$  indicate species activities. Thermodynamic data on solutes are from ref 15. The  $\Delta G_f^\circ$  values for iron oxide reduction corresponds to the reduction of the least ( $\Delta G_f^\circ = -472.8$  kJ/mol) and most stable ( $\Delta G_f^\circ = -483.3$  kJ/mol) lepidocrocites (derived from ref 25) the  $\Delta H_r^\circ$  values are calculated using  $\Delta H_f^\circ = -559.4$ , which is for goethite since there is no available value for lepidocrocite. The error introduced by this approximation is minor because the effect of  $\Delta H$  is not high for small temperature differences, and the temperature effect on  $\Delta G^\circ$  is small. The major effect comes from the second term of the equation.

TABLE 2. Concentrations of Redox-Sensitive Species Used in the Calculations of Gibbs Free Energies for H<sub>2</sub> Oxidizing TEAPs, at the 52 Sampling Points Downgradient of the Landfill

dist. (m) <sup>a</sup>	masl (m) <sup>a</sup>	O <sub>2</sub> (μM)	Mn (μM)	Fe <sup>2+</sup> (mM)	SO <sub>4</sub> <sup>2-</sup> (μM)	S(-II) <sup>b</sup> (μM)	CH <sub>4</sub> (mM)	alkal. (mequiv/L)	pH	H <sub>2</sub> (nM)
0	36.20	<5	39	2.53	17.4	1.3	0.97	21.0	6.43	0.16
0	35.20	<5	33	1.47	807	1.3	0.53	nm <sup>c</sup>	5.46	0.24
0	33.20	<5	34	1.33	51.9	1.3	1.51	22.2	6.35	0.21
0	32.20	<5	56	1.45	7.6	31	1.46	26.8	6.35	0.59
0	31.20	<5	67	2.39	11.8	1.3	1.15	24.5	6.71	0.71
0	30.20	<5	51	2.51	35.9	1.3	1.62	22.2	6.53	0.71
15	35.68	<5	36	2.80	43.9	9.4	0.91	20.4	6.33	0.10
15	35.18	<5	25	3.42	nm	9.4	1.01	nm <sup>c</sup>	6.49	0.25
15	34.68	<5	34	1.56	144	9.4	1.08	22.7	6.58	0.27
15	34.18	<5	44	2.11	606	3.1	1.10	nm <sup>c</sup>	6.59	0.095
15	33.68	<5	61	1.25	8.7	3.1	1.60	27.5	6.54	0.11
15	33.18	<5	57	1.39	9.0	1.3	1.11	nm <sup>c</sup>	6.54	0.32
15	32.68	<5	57	1.34	19.5	1.3	1.89	29.1	6.51	0.19
37	35.56	<5	nm	2.39	49.2	1.3	1.19	nm <sup>c</sup>	6.54	0.17
37	35.06	<5	55	2.64	35.8	3.1	1.25	25.6	6.74	0.12
37	34.56	<5	43	2.15	155	3.1	1.00	nm <sup>c</sup>	6.69	0.19
37	34.06	<5	39	2.57	257	9.4	1.00	25.1	7.13	0.10
37	33.56	<5	41	2.37	332	9.4	0.81	nm <sup>c</sup>	6.82	0.18
37	33.06	<5	41	2.47	72.9	9.4	0.83	22.2	6.94	0.087
37	32.56	<5	49	2.34	17.8	9.4	1.44	nm <sup>c</sup>	6.95	0.19
37	30.00	<5	51	4.47	157	1.3	1.21	24.9	6.68	0.65
50	35.56	<5	nm	4.12	9.8	6.3	0.92	20.2	6.74	0.069
50	35.06	<5	46	3.90	14.2	6.3	0.88	nm <sup>c</sup>	6.77	0.026
50	34.56	<5	32	3.35	210	6.3	0.58	23.7	6.86	0.21
50	34.06	<5	34	3.52	146	6.3	0.71	nm <sup>c</sup>	6.89	0.064
50	33.56	<5	44	3.52	37.1	6.3	0.98	25.3	6.82	0.075
50	33.06	<5	48	4.37	39.1	6.3	0.94	nm <sup>c</sup>	6.76	0.19
50	32.56	<5	49	3.42	27.2	6.3	1.46	23.7	6.72	0.15
80	36.00	100	35	0.49	191	3.1	0.00	nm <sup>c</sup>	6.23	0.004
80	35.00	<5	212	2.44	250	3.1	0.20	16.1	6.92	0.29
80	34.00	<5	nm	2.42	47.5	3.1	0.68	21.9	7.11	0.10
80	33.00	<5	31	2.38	33.9	3.1	0.94	21.2	7.16	0.16
80	32.00	<5	58	2.98	225	3.1	0.69	23.1	7.21	0.21
80	31.00	<5	54	3.41	207	3.1	0.93	26.6	7.16	0.30
80	30.00	<5	44	4.54	166	3.1	1.23	27.6	7.12	0.24
114	36.00	31	12	0.02	534	<0.3	0.00	0.81	5.21	0.22
114	35.00	28	19	0.01	460	<0.3	0.16	nm <sup>c</sup>	4.98	0.12
114	34.00	<5	77	0.01	362	<0.3	0.25	1.03	5.49	0.004
114	31.00	<5	268	0.05	359	<0.3	0.16	8.11	6.68	0.28
114	30.00	<5	364	0.06	135	<0.3	0.16	17.3	6.80	0.045
170	36.00	125	5.4	0.01	101	<0.3	0.00	0.96	5.21	0.014
170	35.00	65	9.2	0.02	204	<0.3	0.00	nm <sup>c</sup>	5.09	0.030
170	34.00	25	21	0.01	130	<0.3	0.00	nm <sup>c</sup>	5.00	0.43
170	33.00	<5	62	0.04	366	<0.3	0.05	0.99	5.19	0.014
170	32.00	<5	22	0.07	426	<0.3	0.07	nm <sup>c</sup>	5.62	0.11
170	31.00	<5	11	0.09	413	<0.3	0.15	nm <sup>c</sup>	5.72	2.24
170	30.00	<5	11	0.13	446	<0.3	0.12	nm <sup>c</sup>	5.71	0.83
252	36.00	113	6.3	0.02	116	<0.3	0.00	0.37	5.16	0.052
252	35.00	150	4.4	0.02	231	<0.3	0.00	nm <sup>c</sup>	5.51	0.015
252	34.00	178	5.2	0.02	156	<0.3	0.00	0.10	5.14	0.079
252	33.00	163	6.8	0.01	155	<0.3	0.00	nm <sup>c</sup>	4.98	0.88
252	32.00	128	6.0	0.02	290	<0.3	0.00	0.23	5.15	0.024

<sup>a</sup> Distance from landfill and vertical position in meters above sea level. <sup>b</sup> Total dissolved sulfide values from earlier sampling, if <0.3, 0.3 was used in calculation; nm, not measured. <sup>c</sup> Interpolated value used in calculation.

is observed, corresponding to the minimum energy necessary for the bacteria to be able to store the gained energy as ATP (e.g., refs 14, 18, and 19) (negative  $\Delta G_r$  implies a positive energy yield). In the calculations where the threshold enters, we use the value of -7 kJ/mol H<sub>2</sub> found in ref 18 for methanogenesis at 4 °C. This particular value was chosen because ref 18 has a quite complete treatment of the thermodynamics of their systems. The value appears to change with temperature as it was found to be -5.6 kJ/mol at 20 °C (16). It should be kept in mind that the value is not an absolute value, and it need not be the same for all TEAPs. The threshold value does appear to be similar at least for

sulfate reduction (14), and because the threshold value is related to the ATP energy storage system, which is common to all the TEAPs, the threshold value for the different TEAPs should be comparable. This also implies that values from field settings should be comparable to laboratory and theoretical values, as it also appears from ref 14. The exact threshold value used is not critical to the argumentation, the important point is that generally the bacteria tend to and are able to bring the system close to equilibrium, as close as the threshold value allows.

An assumption of a partial equilibrium is implied by applying the thermodynamic calculations (20). Partial in

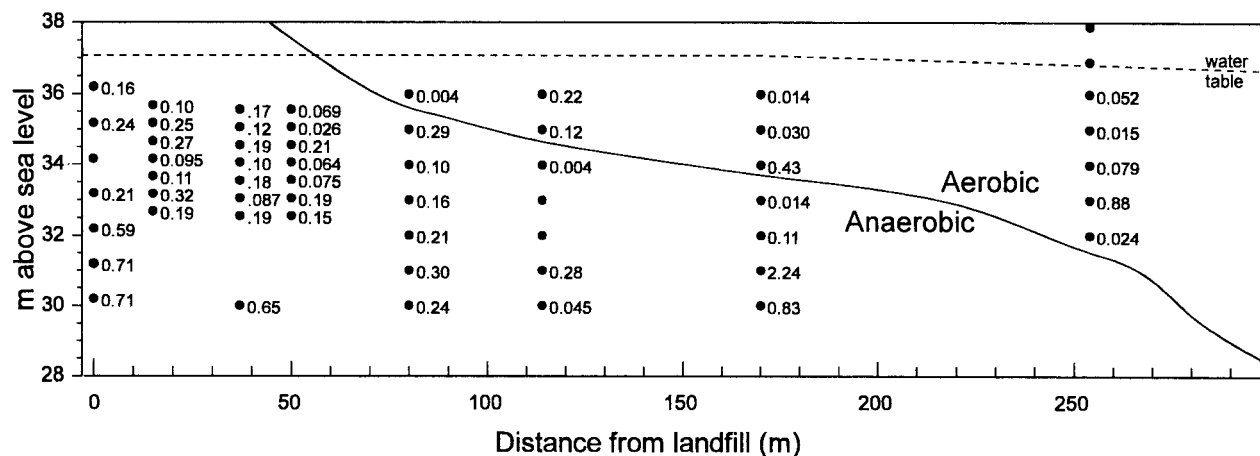


FIGURE 2.  $H_2$  concentrations (in nM) at 52 sampling points in the Grindsted Landfill leachate plume.

the way that the overall rate of the processes that degrade complex organic matter is determined by the rate of the primary fermentative step producing, for example, acetate and  $H_2$ , while comparably fast TEAPs can be viewed as taking place at close to thermodynamic equilibrium. True equilibrium ( $\Delta G_r = 0$ ) is not reached, because  $\Delta G_r$  is not zero but reaches the threshold value described above. This results from, for example, the  $H_2$  concentration being higher than it would be at true equilibrium. At true equilibrium the  $H_2$  concentration would be about an order of magnitude lower than measured values (e.g., 20 times lower for the sample used for making Figure 4). The assumption of partial equilibrium probably works best for  $H_2$  oxidizing processes because the very small pool of  $H_2$  and the derived rapid turnover indicate that the kinetics for oxidation of  $H_2$  are indeed very fast, implying that equilibrium is approached for the oxidation and that the fermentative production of  $H_2$  is overall rate limiting. The presented calculated energies only refer to the energy produced via  $H_2$  oxidation since this is what enters into the expression—other processes such as oxidation of acetate by the same terminal electron acceptor might occur as well. At the very low  $H_2$  concentrations in the plume, fermentation to  $H_2$  should be thermodynamically very favorable (14, 21).

The iron oxides pose a particular problem to the calculation of available energies because the  $\Delta G^\circ_r$  of naturally occurring iron(III) oxides is ill-defined due to a varied mineralogy and crystallinity (23). The actual available energy for iron oxide reduction will depend on these factors. Lepidocrocite and goethite were found to be the most abundant iron oxides in similar Quaternary sediments (24), and of these lepidocrocite is the most unstable and therefore the one most liable to be reduced. Here two different stability constants representing the range for lepidocrocite (25) were chosen to illustrate the effect of iron oxides of different availability as electron acceptor. Incubations (26) have shown that the thermodynamically least stable iron oxides (less negative  $\Delta G^\circ_r$ ) are reduced faster in microbiologically mediated reduction—indicating a thermodynamic control also on microbiological Fe reduction. It should mean that the iron oxide most liable to undergo reduction at a given time and place within the aquifer will be the least stable in the given mixture.

## Results and Discussion

**Observations.** The  $H_2$  distribution from the landfill and downgradient through the leachate plume is shown in Figure 2 and Table 2. The cross-section shows that, apart from one value, all values are below 1 nM. The ranges relating  $H_2$  concentrations to specific TEAPs given in ref 2 would lead

to the conclusion that the plume was entirely dominated by Fe-reducing conditions, as 35 out of 52 measurements were between 0.1 and 0.5 nM—the range that according to ref 2 is characteristic of Fe-reducing conditions. Of the remaining measurements, 10 are below this range implying either Mn or  $NO_3^-$  reduction, six values are within 0.5–1.0 nM—which is not really a diagnostic range according to ref 2, and only one value, 2.24 nM, clearly indicates  $SO_4^{2-}$  reduction.

The redox-related chemistry of the 52 groundwater samples shown in Table 2 is consistent with previous results (6) from the same transect. There is a substantial increase in  $Fe^{2+}$  from 0 m and out to > 50 m from the landfill, clearly indicating iron(III) oxide reduction in this part of the plume. The Quaternary sands contain about 20  $\mu\text{mol/g dw}$  iron oxides, and the Tertiary micaceous sands contain about 0.5  $\mu\text{mol/g dw}$ . There is no obvious change in concentrations downgradient of the landfill. After 80 m, the Fe concentration drops abruptly and the Mn concentration goes up, indicating a reduction of manganese oxide perhaps with some of the  $Fe^{2+}$  (25, 26). Bioassays from ref 7 also indicate Mn reduction at 113 m, 34.5–30 m above sea level. Substantial sulfate reduction seems most prominent in the zone from 0 to 50 m in a narrow layer around 34 m above sea level and in the lower part around 170 m. Both zones show relatively high sulfate concentrations that disappear downgradient. Still sulfide has been detected within a large part of the plume, and sulfate reduction has been observed in bioassays (7) from other places in the plume. Methanogenesis occurs at low rates (7) closest to the landfill and especially in the same layer where sulfate reduction seems to occur. The overall impression is that the redox processes are not well separated in specific redox zones, though iron oxide reduction is prominent especially in the proximal part of the plume. The prominent iron oxide reduction is, as mentioned, what would be predicted by using the empirical  $H_2$  scale given in ref 2, although the presence of concomitant sulfate reduction and methanogenesis in the proximal part of the plume would not be identified nor explained by using this approach. This could, as discussed previously, be a result of non-steady-state conditions or it could, as we propose, be an intrinsic feature of this and many other systems.

**Available Energies.** To gain further understanding of the complicated processes of the strongly anaerobic part of the leachate plume, we calculated the  $\Delta G_r$  for oxidation of  $H_2$  for the 41 available anaerobic sampling points. These calculations combine the information in the data on redox-sensitive species with the information in the data on  $H_2$  concentrations. This was done for the different TEAPs suggested in Table 1. Aerobic samples were not included because oxic processes may occur without an intermediary  $H_2$ -producing fermenting



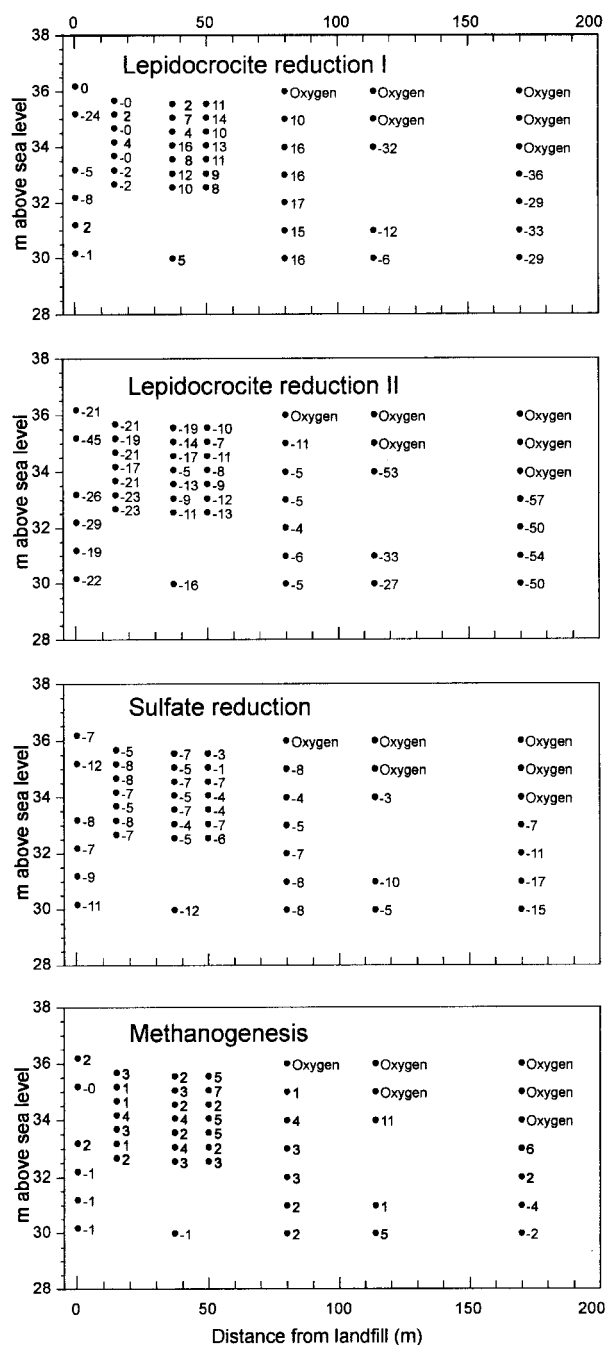


FIGURE 3. Calculated  $\Delta G_r$  (in kJ/mol) of  $H_2$  oxidation by iron oxide (lepidocrocite I,  $\Delta G_r^\circ = -483.3$  kJ/mol; II,  $\Delta G_r^\circ = -472.8$  kJ/mol), sulfate, and  $CO_2$  reduction within the landfill leachate plume.

step. Nitrate reduction is not included because all of the nitrate is reduced close to the anaerobic/aerobic boundary. The calculation of  $\Delta G_r$  for manganese oxide reduction is not reasonable without more data on manganese oxide mineralogy. The results for the discussed anaerobic TEAPs (reduction of two different iron oxides, sulfate reduction, and methanogenesis, all via  $H_2$  oxidation) are shown in Figure 3. The calculations make it possible to delineate zones where a given TEAP involving  $H_2$  oxidation may or may not occur—depending on whether  $\Delta G_r$  is above or below the necessary threshold.  $\Delta G_r$  values below the threshold should be particularly favorable. The calculated values refer to the main moving part of the water since this is the water represented in the groundwater sample.

$\Delta G_r$  values above the threshold do not entirely exclude that a given redox process proceeds via  $H_2$ . The process can

still occur in stagnant subdomains if the  $H_2$  concentration in these is higher due to fermentation of organic matter or if  $H_2$  is directly transferred from fermenting to  $H_2$  consuming bacteria (29). This way part of the produced  $H_2$  may never reach the moving (sampled) water, because once it leaves the stagnant zones it is immediately used up in, for example, Fe reduction and never turns up in the sample water. At locations in the plume where  $\Delta G_r$  for a given TEAP is higher than the threshold value, this TEAP must, if it is occurring in the nonstagnant part of the sediment, proceed with another electron donor (acetate, for example) (or in the case of methanogenesis by fermentation).

Figure 3 shows very negative  $\Delta G_r$  values for  $H_2$  oxidation by Fe reduction using the thermodynamic data for the most unstable of the two iron oxides considered, while no energy seems available if the most stable of the two iron oxides is used in the calculations. The iron content of the sediment is of the order of micromoles per gram (10), but currently we have no tools available to identify the iron minerals actually contributing to the oxidation of  $H_2$ . A range of iron oxide minerals, with a spectrum of stability constants, may potentially be present in the plume-affected sediment, and it is not unlikely that  $H_2$  oxidation by iron reduction can take place in the plume at a threshold value or at a more negative  $\Delta G_r$ . This suggests that, in terms of  $H_2$  oxidation, iron oxide reduction could be dominating in the nonstagnant parts of the groundwater. The  $\Delta G_r$  for sulfate reduction is close to the threshold in several locations in the plume, suggesting that sulfate reduction is indeed controlled by a partial equilibrium. It appears that sulfate reduction may take place locally, but probably does not account for the major part of the  $H_2$  oxidation. Methanogenesis via  $H_2$  oxidation for most parts of the plume must take place in stagnant zones, since the  $\Delta G_r$  values are all positive. For methanogenesis to occur via  $H_2$  in the nonstagnant part of the groundwater, we can calculate that the  $H_2$  concentration with the conditions 31.2 m above sea level at the edge of the landfill should be around 10 nM, to bring the  $\Delta G_r$  value below  $-7$  kJ/mol  $H_2$ .

On a general level, the energy calculations fit reasonably well with bioassay results (7). In these the Fe reduction rates are the highest rates, while low-rate sulfate reduction is seen in many of the bioassays. It also fits the observation of Fe-reducing bacteria in high numbers in most of the plume (28) and the mentioned pronounced downgradient increase in the Fe concentration seen in proximal parts of the plume.

The zone around 80 m is characterized by relatively high  $\Delta G_r$  values for Fe-reduction and relatively low  $\Delta G_r$  values for sulfate reduction, in accordance with bioassays (7) from this part of the plume where comparable rates of both Fe and sulfate reduction were measured.

Methanogenesis occurred at low rates in a few bioassays of sediment sampled very close to the landfill (7). The  $\Delta G_r$  values are positive, so methanogenesis must either be related to fermentation of acetate or  $H_2$  oxidation in stagnant porewater.

In conclusion, the processes indicated by the groundwater chemistry and the bioassays correspond to the calculated  $\Delta G_r$  values, indicating that the partial equilibrium approach is applicable to the interpretation of our  $H_2$  data.

**Temperature Effects.**  $\Delta G_r$  is calculated for the non-standard temperature of 8 °C (the approximate temperature of the plume at the time of sampling), and although one might expect a minor effect of small temperature differences, the effects are important.

Varying the temperature in experimental batches with active methanogenesis may have a pronounced impact on the  $H_2$  level (18, 19). The observed shift in the  $H_2$  level in these studies was highly correlated to the change in  $\Delta G_r$  due to the shift in temperature. This emphasizes that the  $H_2$  level is not just a function of the given redox process, but it

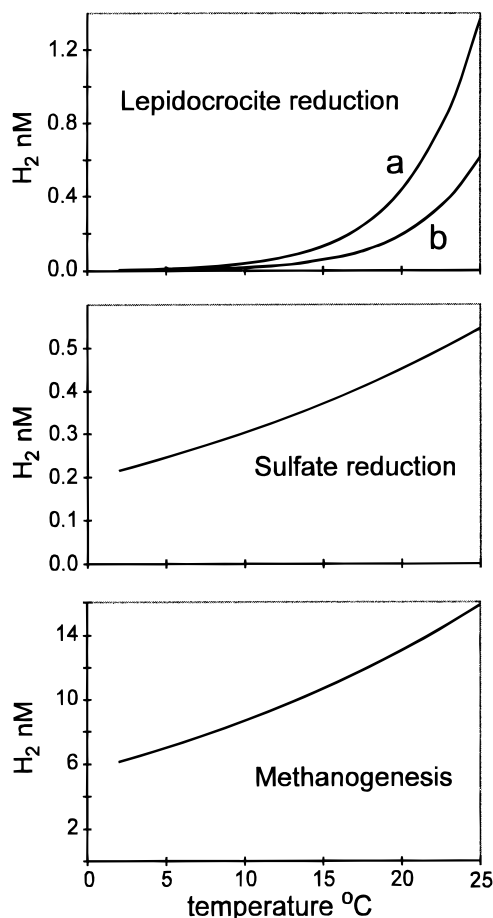


FIGURE 4. Theoretical effect of temperature on steady-state  $H_2$  levels based on the assumption of a threshold  $\Delta G_r$  (see text for details). Lepidocrocite a has  $\Delta G_r^\circ = -474.8$ , b has  $\Delta G_r^\circ = -473.8$ .

is closely tied to the actual in situ  $\Delta G_r$ . It appears as if the bacteria drive the  $H_2$  level down as far as possible—until the  $\Delta G_r$  reaches the threshold. Lower temperatures may, in some environments, shift the  $H_2$  concentration downward (19). In the case of methanogenesis, it can decrease to  $< 3$  nM, which is within the currently accepted range related to sulfate reduction. The theoretical thermodynamic effect of temperature on steady-state  $H_2$  concentrations for the three discussed TEAPs is illustrated in Figure 4 (two lepidocrocites are included). These plots serve to illustrate the effect of temperature and are not exact predictions of  $H_2$  levels at different temperatures. The actual effect of temperature will be a combination of both thermodynamic and physiological effects, and the calculated values are sensitive to the threshold value used. The plots were produced from the equations in Table 1, which were rearranged to give the  $H_2$  concentration. The  $\Delta G^T$  values (temperature-corrected  $\Delta G^\circ$  values) and the  $\Delta G_r$  values (representing the threshold value) were inserted. A linear variation of the threshold from  $-7$  to  $-5.6$  kJ/mol  $H_2$  from  $4.0$  to  $20.0$  °C (18) was assumed. The groundwater composition at 37 m from the landfill 34.56 m above sea level was used (the actual concentration calculated will vary slightly according to the groundwater sample used). The plots indicate that the characteristic  $H_2$  level for the different TEAPs might decrease with decreasing temperature. Therefore, the  $H_2$  levels derived from previous studies made at temperatures of  $20$  °C are not necessarily applicable to settings with lower temperatures even if competitive exclusion is effective.

Figure 4 suggests that, in an aquifer at  $8$  °C, methanogenesis and sulfate reduction could bring the  $H_2$  concentra-

tion down to about half of the value found at  $20$  °C, and Fe reduction could take place down to concentrations that are an order of magnitude lower. The interpretation of the redox zones in the Grindstedt Landfill leachate plume, obtained using the existing approach to interpreting  $H_2$  measurements, would not change dramatically due to temperature effects. In other cases, neglecting temperature effects may lead to a misinterpretation of the redox environments present.

**Effects of Reactants.** The correlation of the  $H_2$  level to temperature demonstrated in ref 19 supports the assumption of partial equilibrium. If changes in  $\Delta G_r$  due to shifts in temperature affect the  $H_2$  level, changes in  $\Delta G_r$  related to other factors should also affect the  $H_2$  level. It could be changes in concentrations of reactants or products as shown in ref 31 (and as it has been assumed in producing Figure 3) or it could be variations in iron oxide stabilities. This implies that, given a system dominated by iron oxide reduction but with variations in the iron oxide mineralogy, the  $H_2$  level would reflect the stability of the iron oxide so that more stable iron oxides would lead to higher  $H_2$  concentrations (this effect is also shown in Figure 4). This idea is supported by our data. Even though the proximal part of the plume seems to have Fe-reduction occurring over the entire depth, there is a pronounced difference in the amount of  $H_2$  present. Low values are seen in the upper Quaternary sediments, while the highest values are in the Miocene sediments below. These lower sands contain much less iron oxide as compared to the Quaternary layers, possibly implying that the more reactive iron oxides are gone. Also the ratio of Fe(III) extracted in  $0.5$  and in  $5.0$  M HCl is higher for the Quaternary sands, indicating a higher proportion of more unstable, more reactive iron oxides.

Generally, the  $H_2$  concentrations seem to reflect the reducing potential of the system. High  $H_2$  concentrations represent very reduced systems regardless of the actual redox process. A system dominated by Fe reduction could be misinterpreted as a system more oxidized than another system dominated by sulfate reduction based on just data of dissolved  $Fe^{2+}$  but supplemented by  $H_2$  measurements and energy calculations—it might turn out to be more reduced. Such an Fe-reducing system may allow for simultaneous sulfate reduction and may be capable of reductive dechlorination of chlorinated aliphatic hydrocarbons.

It appears that, as long as there are electron acceptors present, they will be used if the reduction of these has a  $\Delta G_r$  below or equal to the necessary threshold. The  $H_2$  level will be given by the in situ circumstances so that the final result is a  $\Delta G_r$  equal to this threshold. If the Fe(III) oxides present are relatively stable, then the  $H_2$  level will increase to a level that might result in sulfate reduction and perhaps methanogenesis producing the necessary energy. All processes might then occur concomitantly. This could be promoted by a low temperature, because the differences in  $\Delta G_r$  values of the different TEAPs are smaller.

## General Implications

$H_2$  concentrations are closely linked to the energetics of the TEAPs in the system, and these concentrations enable calculations of actual available energies for various microbiologically mediated processes in natural systems. The calculations lead to a more informative interpretation of  $H_2$  data that does not rely on an assumption of steady state and effective competitive exclusion. The approach appears to be very useful in predicting where specific TEAPs may and may not occur in strongly anaerobic contaminant plumes (in the volume represented by the sample). This implies a potential of predicting where in an anaerobic plume the degradation of a given organic xenobiotic may occur (provided the reaction is known, it involves  $H_2$  and that

thermodynamic values are available). This is the case for many chlorinated compounds (32, 33).

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