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Thermodynamics of H_2-consuming and H_2-producing metabolic reactions in diverse methanogenic environments under in situ conditions

(Sulfate reduction; methanogenesis; acetogenesis; lactate fermentation; ethanol fermentation; fatty acid fermentation)

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1. SUMMARY

In situ concentrations of hydrogen and other metabolites involved in H_2-consuming and H_2-producing reactions were measured in anoxic methanogenic lake sediments, sewage sludge and fetid liquid of cottonwood. The data were used to calculate the Gibbs free energies of the metabolic reactions under the conditions prevailing in situ. The thermodynamics of most of the reactions studied were exergonic with Gibbs free energies being more negative for H_2-dependent sulfate reduction > methanogenesis > acetogenesis and for H_2-producing lactate fermentation > ethanol fermentation. Butyrate and propionate fermentation, on the other hand, were endergonic under in situ conditions. This observation is interpreted by suggesting that butyrate and propionate is degraded within microbial clusters which shield the fermenting bacteria from the outside H_2 (and acetate) pool.

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2. INTRODUCTION

The anaerobic degradation of organic compounds such as carbohydrates to methane and carbon dioxide is an exergonic process. However, there is no single microorganism able to perform this reaction all on its own. Instead, the degradation is brought about by a complex metabolic food chain which ends in the production of methane [1–3]. In this mineralization process, H_2 is next to acetate the most important intermediate utilized by methanogenic bacteria in freshwater environments. In addition, H_2 may be consumed by chemolithotrophic homoacetogenic bacteria and, in the presence of sulfate, by chemolithotrophic sulfate-reducing bacteria. On the other hand, H_2 is an obligatory product of bacteria which degrade reduced fermentation intermediates such as alcohols or fatty acids to acetate. Both bacterial groups, the H_2 consumers and the H_2 producers, are assumed to be limited in their metabolic activities by the partial pressure of H_2 in their natural habitat. This limitation is due to the thermodynamics of the metabolic reactions

which are highly sensitive to the H_2 partial pressure. While the H_2 consumers depend on a minimum H_2 partial pressure necessary to allow an exergonic reaction, many of the H_2 producers can tolerate H_2 only below a certain H_2 partial pressure for the same reason. Hence, the whole anaerobic microbial community has to maintain the environmental H_2 partial pressure within a range that is permissive for H_2-producing as well as H_2-consuming reactions. Otherwise, the mineralization of organic matter to gaseous products could not be completed under anaerobic conditions.

The permissive range of the H_2 partial pressure has been estimated in thermodynamic calculations by assuming standard conditions with respect to the other reaction partners. Based on this assumption, the permissive range for methanogenic degradation of ethanol and propionate would be 0.1–10000 Pa H_2 and 0.1–10 Pa H_2, respectively (McCarty, 1981 in [4,5]). However, these predicted ranges have so far not been confirmed by thermodynamic calculations based on real in situ concentrations of metabolites or by direct measurements of the in situ H_2 partial pressures. The lack of H_2 data is mainly due to analytical difficulties in measuring the low H_2 partial pressures prevailing in methanogenic environments. These problems have only recently been overcome [6,7].

Here we report on concentrations of H_2 and of other metabolites involved in H_2-producing and H_2-consuming reactions which were measured in diverse methanogenic environments under in situ conditions. The data are used to calculate the actual Gibbs free energies of the reactions for these conditions, and to calculate the range of H_2 partial pressures which would be thermodynamically permissive for these reactions.

3. MATERIALS AND METHODS

Lake Mendota is a dimictic eutrophic lake and Knaack Lake is a meromictic dystrophic lake, both in Wisconsin, U.S.A. Sediment samples were taken by means of an Ekman grab during summer stratification [8,9]. Sewage sludge was obtained from the anaerobic digestor of the Nine Springs Municipal Sewage Plant in Madison, WI [7,10]. Fetid liquid of wetwood was sampled from a cottonwood tree (*Populus deltoides*) near Madison, WI, by means of wood corers and syringes [11–13].

Hydrogen and methane concentrations were determined after extraction at the field site followed by gaschromatographic analysis [7]. H_2 was detected by an H_2 analyzer based on the HgO-to-Hg vapour conversion technique [14,15] with a detection limit of 0.5 nM dissolved H_2. CH_4 was measured by a flame ionization detector with a detection limit of 1 μM dissolved CH_4. Partial pressures were calculated from the dissolved gas concentrations by using Bunsen solubility coefficients for the in situ temperatures [16,17].

The concentration of total inorganic carbon ($CO_3^{2-} + HCO_3^- + H_2CO_3$) in sediments and sewage sludge was measured as described [18]. The concentration of bicarbonate (HCO_3^-) was calculated from the dissociation constant at the in situ pH using a pK_a for HCO_3^- of 6.4. The HCO_3^- concentration in fetid liquid of wetwood was calculated from the CO_2 partial pressure in the gas collected from the wetwood tree [11,13] using the Henry constant for CO_2, the pK_a for HCO_3^- and the in situ pH [19].

Dissolved sulfate and total sulfide concentrations were determined as described [20]. The concentration of HS^- was calculated from total sulfide and the in situ pH using a pK_a of 7.0. The concentrations of lactate, acetate, propionate and ethanol were measured by gas chromatography as already described [8,9,12,21].

The standard Gibbs free energies (ΔG_0) of H_2-utilizing and H_2-producing reactions (Table 1) were calculated from tabulated Gibbs free energies of formation (ΔG_f) of the individual reactants and products [22]. H_2 and CH_4 were assumed as gaseous compounds; all other compounds as dissolved. The Gibbs free energy (ΔG) of an individual reaction under non-standard conditions was calculated for the individual methanogenic environments by using the in situ concentrations of the involved reactants and products, the in situ pH (H^+ concentration), temperature, and the standard Gibbs free energy of reaction (ΔG_0);

Table 1

Gibbs free energies of various reactions calculated for standard conditions

ΔG_0, standard Gibbs free energy, i.e., reactants and products at 1 M concentration or 1 atm partial pressure; $\Delta G_0'$, standard Gibbs free energy at pH 7; $\Delta G_0' = \Delta G_0 + m\ 2.303\ RT\ \lg\ 10^{-7}$; with m = net number of protons formed in the reaction

Reaction	ΔG_0 (kJ)	$\Delta G_0'$ (kJ)
H_2 consumption		
(1) $4\ H_2 + SO_4^{2-} + H^+ \rightarrow 4\ H_2O + HS^-$	−192.0	−152.2
(2) $4\ H_2 + HCO_3^- + H^+ \rightarrow 3\ H_2O + CH_4$	−175.4	−135.6
(3) $4\ H_2 + 2\ HCO_3^- + H^+ \rightarrow 4\ H_2O + CH_3COO^-$	−144.4	−104.5
H_2 production		
(4) $CH_3CHOH\ COO^- + 2\ H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2\ H_2$	+35.9	−4.0
(5) $CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	+49.5	+9.6
(6) $CH_3CH_2CH_2COO^- + 2\ H_2O \rightarrow 2\ CH_3COO^- + H^+ + 2\ H_2$	+88.2	+48.3
(7) $CH_3CH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3\ H_2$	+116.4	+76.5

e.g., for reaction (1):

$$\Delta G = -192.0 + RT \ln \frac{[HS^-]}{p(H_2)^4[SO_4^{2-}][H^+]} \quad (1)$$

where $p(H_2)$ = H_2 partial pressure (atm) and value in brackets = concentration (M) of the individual compound.

The permissive H_2 partial pressure ($p(H_2)^*$) which allow for a $\Delta G < 0$ was calculated from the in situ value of ΔG and the in situ H_2 partial pressure ($p(H_2)$) by

$$\log p(H_2)^* = \log p(H_2) - \Delta G/(n\ 2.303\ RT) \quad (2)$$

where n = stoichiometric number of H_2 produced.

4. RESULTS

The temperature, pH and partial pressures or concentrations of metabolites in the diverse anoxic

Table 2

In situ concentrations of metabolites in various anoxic environments

Condition metabolite	Lake Mendota sediment	Knaack Lake sediment	Sewage sludge	Fetid liquid of cottonwood
T (°C)	10	4	32	20
pH	7.2	6.2	7.2	7.0
H_2 (Pa)	3.7	4.8	26.9	14
CH_4 (kPa)	189	167	375	313
HCO_3^- (mM)	6.45	1.54	4.26	123
HS^- (μM)	586	1.4	n.d. [a]	< 5
SO_4^{2-} (μM)	25	< 10	n.d.	< 10
Acetate (μM)	32	151	360	32000
Propionate (μM)	0.88	0.3	2.3	2800
Butyrate (μM)	< 0.2	< 0.2	n.d.	1000
Lactate (μM)	77	138	n.d.	100
Ethanol (μM)	43	174	113	2500

[a] n.d., not determined

methanogenic environments studied are shown in Table 2. The individual values are average values of at least triplicate samples and determinations. The standard deviation was highest in samples of fetid liquid from wetwood, but was generally less than $\pm 80\%$. The in situ Gibbs free energies per mol H_2 converted in a number of H_2-consuming and H_2-producing reactions as well as the H_2 partial pressures that are permissive for the reaction to be exergonic are listed in Table 3.

It should be noted that the in situ Gibbs free energy is very sensitive to the in situ H_2 partial pressure since it is effective in the second to fourth power. Therefore, a good accuracy in determining H_2 partial pressures is most important for thermodynamic calculations. The precision of determination of dissolved H_2 was better than $\pm 10\%$, the total accuracy was slightly less ($\pm 15\%$). However, the in situ concentrations of H_2 in replicate samples from the same environment may vary by $\pm 80\%$. Hence, the in situ ΔG values indicate a generalized thermodynamic condition.

Compared to H_2, the in situ Gibbs free energy is much less sensitive to the concentrations of the other metabolites, e.g., propionate. Propionate degradation in sewage sludge would still be an endergonic reaction with a $\Delta G$of $+2.2$ kJ per mol H_2 formed, even if the in-situ concentration of propionate were 50-fold higher. Such a concentration (0.1 mM) would already be in a range that is typical for digestion of animal rather than municipal waste [23,24]. Moreover, high concentrations of long-chain fatty acids are generally paralleled by high concentrations of the product acetate, which neutralizes the thermodynamically beneficial effect of a high substrate concentration.

Most of the reactions studied in the various methanogenic environments showed negative ΔG-values, indicating that they are exergonic for in situ conditions (Table 3). However, propionate degradation was endergonic in all of the environments studied. Butyrate degradation was only exergonic in Lake Mendota sediment, but was endergonic in Knaack Lake sediment and in wetwood. In sewage sludge, the ΔG-value for butyrate degradation could not be determined, since the pool size of butyrate has not been analyzed. Thermodynamic calculations show, however, that the butyrate pool must be > 0.74 μM in order to make butyrate degradation exergonic under in situ conditions.

5. DISCUSSION

The values of the Gibbs free energies show that most reactions in fact are thermodynamically possible under in situ conditions. The only exceptions are the metabolism of propionate to acetate, CO_2 and H_2, which is endergonic under in situ conditions in all the diverse methanogenic environments studied, and the degradation of butyrate in Knaack Lake sediment and in wetwood. The permissive H_2 partial pressure needs to be 20–90% lower to result in $\Delta G = 0$ and needs even to be lower than that to allow a net yield of free energy. Nevertheless, propionate is apparently degraded in the sediments of Lake Mendota, Knaack Lake and in sewage sludge [10], and the same may be assumed for butyrate [24,25], although it has not been shown explicitely for the methanogenic environments studied in this work. Since sulfate reduction essentially does not occur in sewage sludge and Knaack Lake sediments [8] and is only very low in Lake Mendota sediments [20], the sulfate-dependent propionate degradation by e.g., *Desulfobulbus propionicus* [26], cannot be important. Moreover, studies with radiolabeled propionate demonstrate that it is transformed nearly stoichiometrically to CH_4 and CO_2 in the environments studied [10]. The catabolism probably proceeds via the succinate pathway and intermediate acetate release [10,27,28], the reducing equivalents being transferred to a H_2-oxidizing methanogenic partner organism. A syntrophic co-culture of this kind has been isolated, however, this is very difficult to cultivate, and enzymological studies are lacking so far [29]. A similar syntrophic co-culture based on the degradation of butyrate has recently been characterized enzymologically, indicating that butyrate is catabolized by beta-oxidation [30].

The question remains how butyrate and propionate can be oxidized in the environments studied where H_2 partial pressures were too high to render the reaction exergonic. Mixotrophic utilization of the fatty acids could be one explana-

tion, i.e., endergonic degradation of butyrate or propionate coupled to the exergonic catabolism of a second substrate. However, all syntrophic cultures isolated so far with fatty acids as substrates proved to be highly specialized and unable to use any substrate other than fatty acids [29,31,32]. Oxidation of fatty acids by co-metabolism is thus not a likely explanation for our observations.

The most simple explanation would be the existence of methanogenic or homoacetogenic bacteria which are themselves able to degrade butyrate or propionate. In this case, the obligatory release of H_2 would be avoided by coupling reaction [6] or [7] to reaction [2] or [3]. The ΔG-values of butyrate and propionate-dependent methanogenesis or homoacetogenesis can be calculated for the in situ conditions of the various methanogenic environments from the values listed in Table 3. They are negative, i.e., exergonic, in every case, however, butyrate or propionate-utilizing methanogenic or homoacetogenic bacteria are yet unknown.

The only explanation presently at hand is that fatty acid-oxidizing fermenting bacteria and H_2-oxidizing methanogenic bacteria form clusters inside which the H_2 partial pressure is considerably lower than in the free pool accessible to our measurements. It is easy to conceive that interspecies H_2 transfer can proceed much more effectively within such a cluster, and that this thermodynamically favourable effect could even be enhanced, if acetate-degrading methanogens also participated in metabolite transfer. It has recently been shown that the H_2 flux through the free H_2 pool can account for only a small fraction of the total rate of H_2-dependent CH_4 formation [7,33]. It was concluded from these results that a considerable part of the overall H_2 transfer within the sediments occurs in clusters of different bacteria juxtaposed on one another. Cluster formation and close contact between partner organisms seems to enhance the efficiency of syntrophic propionate degradation significantly, as is shown by the observation that an enrichment culture growing as a biofilm on a suitable surface grows about 10–100 times faster than a similar syntrophic culture growing in suspension [29] (Szewzyk and Schink, unpublished). In Knaack Lake sediments homoacetogenic bacteria may replace methanogens as juxtaposed syntrophic partners since electron and carbon flow in Knaack Lake sediments is dominated by acetogenic metabolism [8]. Propionate could also be degraded within anaerobic protozoa containing syntrophic methanogenic bacteria [34] so that the environmental H_2 partial pressure would not be thermodynamically effective.

In contrast to butyrate and propionate, H_2 production by lactate or ethanol degradation is thermodynamically possible in all of the environments studied. Methanogenic ethanol turnover is significant in the lake sediments and in sewage sludge [21], and methanogenic lactate turnover is significant in lake Mendota sediment during fall turnover [9]. With regard to these metabolites, a considerably higher H_2 partial pressure and consequently a higher turnover would be permissive. A higher H_2 partial pressure would also exert a stimulatory effect on H_2-dependent methanogenesis and homoacetogenesis. Obviously, however, this is not the case in the anoxic methanogenic environments studied. Hence, H_2-producing reactions apparently are not limited in general by the in situ H_2 concentrations and, H_2-consuming reactions have to operate at a relatively low yield of free energy per mol H_2 consumed. In this context it is interesting to note that the energy metabolism of H_2-oxidizing methanogens appears to be most efficient at H_2-limiting conditions and to be uncoupled when H_2 is supplied in excess [35,36].

In all the environments studied, the energy yield is considerably higher for H_2-dependent methanogenesis than for H_2-dependent acetogenesis. This may explain the relatively low abundance of chemolithotrophic homoacetogenic bacteria in most anoxic environments [37] and their small contribution to carbon and electron flow [38]. In Knaack Lake sediments, however, carbon and electron flow is dominated by acetogens and consequently, H_2 turnover and H_2-dependent methanogenesis are very small [8,39].

The methanogenic environments studied do not contain sufficient amounts of sulfate to allow sulfate reduction, except for Lake Mendota sediments. In this lake, sulfate reduction is the thermodynamically most favorable reaction for H_2

Table 3

Gibbs free energies and permissive H_2 partial pressures for H_2-consuming and H_2-producing reactions in various anoxic environments under in situ conditions

Reaction	Lake Mendota		Knaack Lake		Sewage Sludge		Cottonwood	
	ΔG (kJ/mol H_2)	P_{H_2} [a] (Pa)	ΔG (kJ/mol H_2)	P_{H_2} (Pa)	ΔG (kJ/mol H_2)	P_{H_2} (Pa)	ΔG (kJ/mol H_2)	P_{H_2} (Pa)
H_2 consumption								
(1) Sulfate reduction	−12.3	> 0.02	n.d. [b]	n.d.	n.d.	n.d.	n.d.	n.d.
(2) Methanogenesis	−6.7	> 0.21	−8.7	> 0.11	−8.2	> 1.07	−10.4	> 0.19
(3) Homoacetogenesis	−2.5	> 1.30	−2.5	> 1.58	−2.8	> 8.83	−4.2	> 2.51
H_2 production								
(4) Lactate degradation	−32.5	< 3750000.0	−28.8	< 1270000.0	n.d.	n.d.	−18.8	< 32000.0
(5) Ethanol degradation	−19.1	< 12500.0	−14.8	< 2900.0	−14.7	< 13000.0	−14.0	< 3400.0
(6) Butyrate degradation	−5.6	< 12.28	+2.2	< 1.83	n.d.	n.d.	+2.8	< 4.93
(7) Propionate degradation	+0.6	< 2.83	+4.7	< 0.62	+3.6	< 6.60	+4.3	< 2.35
In situ H_2 partial pressure (Pa)		3.7		4.8		26.9		14.0

[a] Permissive H_2 partial pressure, i.e. P_{H_2} at which the Gibbs free energy of reaction is negative under in situ conditions.

[b] n.d. = not determined.

consumption. Despite the favorable thermodynamics, H_2-dependent sulfate reduction is negligible [33,40]. Obviously, H_2-dependent sulfate reduction is limited by other factors than H_2 partial pressure. This observation questions the validity of H_2 competition between H_2-consuming sulfate reducers and methanogens as ecophysiological basis for sulfate-dependent inhibition of methanogenesis [33,40–42].

A similar conclusion can be drawn for the acetate-dependent methanogenesis and sulfate-reduction in Lake Mendota sediments showing ΔG-values of -16.6 kJ and -37.8 kJ per mol acetate consumed, respectively (values calculated from data in Table 2). A similar relation has recently been observed in an anaerobic digestor with ΔG-values of -32 kJ and -49 kJ per mol acetate consumed in methanogenesis and sulfate reduction, respectively [43]. Despite the more favorable thermodynamics of sulfate reduction, acetate degradation was dominated by methanogens in both environments.

The results of our measurements and thermodynamic calculations are by and large in accordance with the concept that the H_2 partial pressures in anoxic environments are in such a range that both H_2-producing and H_2-consuming reactions are thermodynamically possible. Our results also demonstrate, however, that thermodynamic considerations alone are not sufficient to explain the existence or non-existence of metabolic reactions in methanogenic environments, and that the reactions are not necessarily limited by the available H_2.

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