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Influence of pH on Microbial Hydrogen Metabolism in Diverse Sedimentary Ecosystems

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Hydrogen transformation kinetic parameters were measured in sediments from anaerobic systems covering a wide range of environmental pH values to assess the influence of pH on hydrogen metabolism. The concentrations of dissolved hydrogen were measured and hydrogen transformation kinetics of the sediments were monitored in the laboratory by monitoring hydrogen consumption progress curves. The hydrogen turnover rate constants (k_t) decreased directly as a function of decreasing sediment pH, and the maximum hydrogen uptake velocities (V_{max}) varied as a function of pH within each of the trophic states. Conversely, the half-saturation concentrations (K_m) were independent of pH. The steady-state hydrogen concentrations were at least 2 orders of magnitude lower than the half-saturation constants for hydrogen uptake. Dissolved hydrogen concentrations were at least fivefold higher in sediments from eutrophic systems than from oligotrophic and dystrophic systems. The rates of hydrogen production determined from the assumption of steady state decreased with sediment pH. These data indicate that progressively lower pH values inhibit microbial hydrogen-producing and -consuming processes within sedimentary ecosystems.

Hydrogen metabolism plays an important role in the degradation of carbon compounds in anaerobic sediments (6, 16, 19). The production of oxidized fermentation intermediates, such as acetate, is accompanied by hydrogen production. Hydrogen consumption can couple the degradation of complex organic matter to methane production in sulfate-limited sedimentary ecosystems. A balance of hydrogen-producing and hydrogen-consuming processes appears essential to the efficient anaerobic digestion of organic matter in freshwater or marine sediments. The final fate of reducing equivalents transferred as hydrogen determines the extent to which reduced carbon compounds can be oxidized in environments depleted of molecular oxygen. Many of the microorganisms responsible for the anaerobic degradation of polymers, carbohydrates, fatty acids, and alcohols are obligate hydrogen producers. The unfavorable energetics of some of these reactions in the presence of even low concentrations of hydrogen has led to the discovery of syntrophic microbial interactions which depend on interspecies hydrogen transfer (10, 17, 18) for function. Interspecies hydrogen transfer describes a close association of hydrogen-producing acetogens and hydrogen-consuming methanogens capable of maintaining hydrogen concentrations at levels that provide conservable free energy for both sets of reactions.

Two of the major groups of organisms involved in hydrogen consumption and the terminal stages of anaerobic metabolism are sulfate reducers and methanogens. The metabolism of both groups is greatly limited by low pH (10, 11, 13, 15, 20). Inhibition of interspecies hydrogen transfer might be expected to cause a decrease in the rates of hydrogen-producing and -consuming processes and result in accumulation of organic matter in low-pH environments.

Few studies have examined the environmental factors which influence hydrogen metabolism in anaerobic ecosystems. Measurement of dissolved hydrogen concentrations in anaerobic ecosystems has been hindered by the lack of adequate analytical methods. This limitation has recently been overcome by the development of sensitive detection procedures for trace reducing gases (2, 3). The purpose of this report is to present dissolved hydrogen concentrations and hydrogen transformation kinetic parameters from seven anaerobic sediments of various pHs. Three of the sediments were from oligotrophic lake systems, two were from eutrophic lake systems, and two were from dystrophic lakes associated with acid peat bogs. The sediment pH values ranged from 8.0 to 4.9.

The location and ecological features of Knaack Lake and Lake Mendota have been reported previously (3, 6, 16). The other lakes are located in Northern Wisconsin adjacent to the University of Wisconsin Trout Lake Biological Station.

All chemicals used were reagent grade or better and were obtained from Sigma Chemical Co., St. Louis, Mo., or Mallinckrodt, Paris, Inc., Paris, Ky. Gases were obtained from Matheson (Joliet, Ill.). All gases were at least 99.9% pure and were passed over copper-filled Vycor furnaces (Sargent Welch Scientific Co., Skokie, Ill.) to remove oxygen. Traces of hydrogen were removed from the bases by passing them through Hopkalit (Dragerwerke, Lübeck, Federal Republic of Germany). Tritium gas was a gift of Paul DeLuca, Physical Sciences Laboratory, University of Wisconsin, Madison.

Hydrogen was detected with an H_2 analyzer (2, 3) based on the HgO-to-Hg vapor conversion technique.

The concentrations of dissolved gases in sediment were determined by extracting 45 ml of sediment in 45 ml of a nitrogen headspace as previously described (3). A 10-ml sample of the equilibrated headspace gas was transferred by syringe to a flask completely filled with saturated Na_2SO_4 solution and 100 μg of $HgCl_2$ per ml. The sample was

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transported to the laboratory as a gas bubble. All transfers were made with a glass syringe.

An in vivo hydrogenase assay was used to measure the total catalytic potential of hydrogenases in the sediments. This method measures the incorporation of tritium gas into the liquid phase (12) and was shown to assay for the catalytic potentials of both hydrogen-producing and hydrogen-consuming hydrogenases under the experimental conditions used in this study.

The hydrogen uptake kinetics were determined by measuring the hydrogen consumption progress curves in sediments equilibrated with a 2% hydrogen atmosphere (3). The measurements were made in the absence of a headspace to avoid phase transfer limitations. The apparent maximum hydrogen uptake velocity (V_{max}) for whole sediments was determined from the linear decrease in hydrogen at saturating conditions. The hydrogen turnover rate constant (k_t) was determined from the logarithmic portion of the hydrogen consumption progress curve. The apparent half-saturation constant (K_m) was determined by fitting the logarithmic portion of the hydrogen consumption progress curve to Michaelis-Menten kinetics: $K_m = [V_{max}(S)]/[V - (S)]$, where V is the observed rate of hydrogen uptake and (S) is the hydrogen concentration. At very low hydrogen concentrations, K_m can be approximated by $K_m = V_{max}[(S)/V]$, and because $(S)/V = 1/k$, the K_m can be determined from $K_m = V_{max}/k_t$, where k_t is the pseudofirst-order rate constant for hydrogen uptake (1). This is only true at low hydrogen concentrations, when plots of V versus (S) are first order. Kinetic parameters were determined at hydrogen concentrations down to an order of magnitude below the observed K_m values. This overcomes some of the problems of determining uptake kinetics of mixed populations described by Williams (14). At the same time, hydrogen turnover rate constants must be determined at hydrogen concentrations high enough to ensure that simultaneous hydrogen production is negligible compared with hydrogen consumption. In the sediments studied, steady-state hydrogen concentrations were so low compared with the half-saturation constant that the observed hydrogen uptake kinetics were not significantly biased by simultaneous hydrogen production. The turnover rate constant can only be assumed to remain constant if there is no change in the biomass (as can be expected during the short incubation times used in this study). The dissolved hydrogen values presented in this study are the in situ steady-state concentrations. This conclusion was supported by laboratory incubation (at in situ temperature) of sediments to which hydrogen had been added. In each case, hydrogen concentrations returned to the levels measured in the field. The hydrogen production rates were estimated by multiplying the turnover rate constants by the hydrogen concentrations and assuming that hydrogen production and consumption balance.

The tritium incorporation rate was independent of pH (Table 1). Comparison among systems suggested that the total catalytic potential of hydrogenase is more closely related to the overall rates of organic transformation than to the actual rates of hydrogen production or consumption. In this regard, it should be noted that the highest catalytic potentials were found in eutrophic systems, intermediate values were found in oligotrophic systems, and the lowest catalytic potentials were found in dystrophic systems. Because tritium incorporation is a measure of total catalytic potential, these rates are significantly higher than the measured rates of hydrogen uptake.

The apparent K_ms for all of the systems measured fell between 4 and 14 μM (Table 1). No consistent differences in either pH or trophic status were noted. The apparent V_{max} for whole sediments displayed a trend toward lower hydrogen uptake rates at low pH within oligotrophic and dystrophic systems. Although pH did not affect the half-saturation constants of the microbial populations for hydrogen uptake, pH directly influenced the maximal rates at which hydrogen was consumed. At low pH, the maximal rate of hydrogen consumption was slower than at neutral pH values.

The turnover rate constants decreased proportionally with the decrease in sediment pH (Fig. 1). No correlation between the turnover rate constants and temperature was observed. This analysis indicated a pronounced decrease in the rate of hydrogen uptake as a function of decreasing sediment pH. The data suggested that the overall rate of hydrogen metabolism was slowed as a result of decreased sediment pH. This slowing of the hydrogen transformation rates was reflected in both hydrogen-consuming and hydrogen-producing reactions, and hydrogen did not accumulate to greater levels in acid sediments than in neutral sediments.

Because V_{max}, but not K_m, varied as a function of pH, the relationship between k_t and pH may be the result of either a

TABLE 1. Comparison of hydrogen transformation kinetic parameters in anaerobic sediments of various pHs[a]

Ecosystem	pH	Hydrogenase catalytic potential (mmol/liter-h)	Maximum uptake velocity (μmol/liter-h)	Half-saturation constant (μmol/liter)	Hydrogen production rate (μmol/liter-h)
Eutrophic					
Lake Mendota	7.2	171.6	488.4	14	1.08
Knaack Lake	6.2	155.7	88.0	6	0.64
Oligotrophic					
Sparkling Lake	8.0	24.1	233.2	6	0.39
Lake Allequash	6.0	32.3	176.3	11	0.13
Lake Mary	5.6	20.6	16.6	4	0.02
Dystrophic					
Trout Bog	5.8	7.3	70.4	11	0.06
Crystal Bog	4.9	6.5	37.8	12	0.03

[a] Hydrogenase catalytic potential was determined in vivo as the incorporation of $^3H_2$ into ^3H_2O. Sediment (10 ml) was incubated in a 56-ml pressure vial at 37°C in the presence of an H_2-N_2 (10/90% [vol/vol]) headspace. Incorporation of $^3H_2$ was linear for 60 min. The other H_2 transformation parameters were determined from the H_2 consumption progress curve. Sediments (100 ml) were equilibrated with a 2% H_2 headspace in a 200-ml glass syringe. The headspace was removed, and H_2 consumption was monitored during incubation at in situ temperatures. H_2 production rates were determined by multiplying the H_2 turnover rate constants by the steady-state H_2 concentrations.

direct effect of pH on hydrogen metabolism or a result of differences in the total biomass of hydrogen utilizers. The latter effect may indeed represent the indirect influence of pH on those factors which determine microbial biomass, such as availability of substrate (e.g., hydrogen and carbon dioxide) or lower population levels of anaerobes with H_2 metabolism. In the absence of techniques for directly measuring the biomass of hydrogen-utilizing organisms in natural sediments, it is not possible to distinguish between these two explanations. However, the differences in the hydrogenase catalytic potential represented by the tritium incorporation results do suggest that the latter interpretation has some validity. We have reported drastically lower populations of methanogens and H_2-producing anaerobes in Crystal Bog versus Lake Mendota (5).

The sediment concentrations of dissolved hydrogen fell between 0.005 and 0.044 μmol/liter (Table 2). The dissolved hydrogen pools were fivefold higher in the sediments of eutrophic systems than in those of either oligotrophic or dystrophic systems. The latter sediments had high organic matter content (up to 80% by weight) but were very low in dissolved inorganic nutrients. No consistent variation in dissolved hydrogen occurred as a function of pH. Steady-state dissolved hydrogen concentrations appeared to be related to trophic status but not to sediment pH values. In all cases, the steady-state concentrations of hydrogen were at least 2 orders of magnitude lower than the K_m values. A similar result has been observed previously for a single eutrophic lake of near-neutral pH (9). Interestingly, addition of hydrogen to acid Crystal Bog sediments did not enhance methane production (data not shown). This was not the case for Lake Mendota sediment, in which methanogenesis is hydrogen limited (16).

TABLE 2. Comparison of ecosystem physicochemical parameters and steady-state hydrogen concentrations in anaerobic sediments of various pHs[a]

Ecosystem	pH	Temperature (°C)	Dry wt (%)	Hydrogen concn (μmol/liter)
Eutrophic				
Lake Mendota	7.2	10	9.3	0.031
Knaack Lake	6.2	4	2.3	0.044
Oligotrophic				
Sparkling Lake	8.0	5	1.9	0.010
Lake Allenquash	6.0	12	2.0	0.008
Lake Mary	5.6	4	5.0	0.005
Dystrophic				
Trout Bog	5.8	4	3.6	0.009
Crystal Bog	4.9	15	3.2	0.009

[a] Surface sediments were collected by Eckman dredge. Temperature and pH measurements were made immediately by using a thermistor, Sargent Welch pH meter, and a combination glass electrode. Dry weights were determined after drying at 105°C for 36 h. Dissolved gases were collected by anaerobically transferring 45 ml of sediment into 90-ml extraction flasks. The flasks were vigorously agitated for <2 min to equilibrate the dissolved gases with the N_2 headspace. Headspace samples (10 ml each) were transferred by syringe to flasks containing a saturated Na_2SO_2 solution and 200 mg of HgCl per liter. Gas samples were transported to the laboratory as bubbles and analyzed within 6 h.

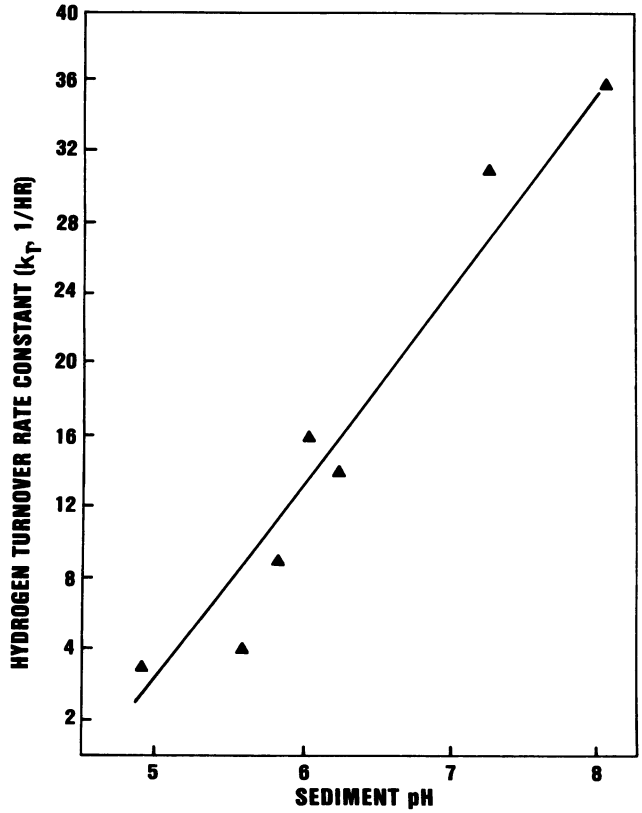

FIG. 1. Hydrogen turnover rate constants as a function of sediment pH in anaerobic environments. Sediment (100 ml) in a 200-ml glass syringe was equilibrated with a 2% H_2 headspace, and then the headspace was removed. H_2 consumption progress curves were determined during incubation at in situ temperatures. Turnover rate constants were determined from the logarithmic decrease of H_2 at concentrations slightly higher than those in situ.

In general, the data showed that overall rates of hydrogen metabolism were slowed as a result of decreased sediment pH. These results provide evidence that the dynamic state of hydrogen-producing and -consuming microbial populations is affected by pH. The decreased steady-state hydrogen turnover rate constants and the specific hydrogen uptake rates of the microbial populations indicate that total hydrogen metabolism is limited by low pH. The decreased rates of hydrogen-producing and hydrogen-consuming processes have a profound impact on the oxidation of reduced carbon compounds in anaerobic environments. The normal flow of carbon and electrons in anaerobic sediments involves channeling of a large proportion of reducing equivalents through the dissolved hydrogen pool to enhance thermodynamic and metabolic efficiencies of anaerobic bacterial food chains (19).

Acidification of anaerobic environments can occur in several ways. Accumulation of humic and fulvic acids can lead to acidification. Physicochemical processes may also result in acidification of anaerobic systems. However, imbalances in the microbial digestion process can also contribute to acidification (19). An imbalance in interspecies electron transfer processes leads to accumulation of fatty acids and alcohols and a lowering of pH that inhibits methanogenesis. The dynamics of hydrogen metabolism inhibition are correlated to pH-dependent accumulation of organic fatty acids and alcohols in acid bog systems (5).

The potential for lake acidification due to acid rain may lead to progressively lower sediment pH levels, and this is under investigation (7, 8). This has led to concern over the possibility of decreased rates of organic decomposition within the sediments. To the extent to which it occurs, acidification of anaerobic sediments can be expected to

decrease overall rates of interspecies electron transfer and slow the overall rate of organic matter decomposition. A second factor which affects organic matter decomposition is changes in the rate of sulfate reduction due to the input of sulfur oxide species (4). It is hypothetically possible that the balance between the rate of organic matter input and the rate of breakdown of this organic matter in natural anaerobic sediments is disturbed by the acidification process, and enhanced organic deposition leads to the starting materials for peats and lignites.

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