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Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments

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Abstract—Factors controlling the concentration of dissolved hydrogen gas in anaerobic sedimentary environments were investigated. Results, presented here or previously, demonstrated that, in sediments, only microorganisms catalyze the oxidation of H_2 coupled to the reduction of nitrate, Mn(IV), Fe(III), sulfate, or carbon dioxide. Theoretical considerations suggested that, at steady-state conditions, H_2 concentrations are primarily dependent upon the physiological characteristics of the microorganism(s) consuming the H_2 and that organisms catalyzing H_2 oxidation, with the reduction of a more electrochemically positive electron acceptor, can maintain lower H_2 concentrations than organisms using electron acceptors which yield less energy from H_2 oxidation.

The H_2 concentrations associated with the specified predominant terminal electron-accepting reactions in bottom sediments of a variety of surface water environments were: methanogenesis, 7–10 nM; sulfate reduction, 1–1.5 nM; Fe(III) reduction, 0.2 nM; Mn(IV) or nitrate reduction, less than 0.05 nM. Sediments with the same terminal electron acceptor for organic matter oxidation had comparable H_2 concentrations, despite variations in the rate of organic matter decomposition, pH, and salinity. Thus, each terminal electron-accepting reaction had a unique range of steady-state H_2 concentrations associated with it.

Preliminary studies in a coastal plain aquifer indicated that H_2 concentrations also vary in response to changes in the predominant terminal electron-accepting process in deep subsurface environments. These studies suggest that H_2 measurements may aid in determining which terminal electron-accepting reactions are taking place in surface and subsurface sedimentary environments.

INTRODUCTION

IN SEDIMENTARY ENVIRONMENTS, the redox reactions of greatest geochemical significance are often the reduction of terminal electron acceptors (most notably oxygen, nitrate, Mn(IV), Fe(III), sulfate, and carbon dioxide) coupled to the oxidation of organic matter. These microbially catalyzed redox reactions are generally segregated into distinct zones (PONNAMPERUMA, 1972; YOSHIDA, 1975; CHAMP *et al.*, 1979; FROELICH *et al.*, 1979; REEBURGH, 1983).

Measurements of the rate of each potential redox reaction would provide the most direct determination of which redox reaction predominates within a given sediment. However, this is rarely, if ever, feasible. Methods for measuring the *in situ* rates of some redox reactions have not been developed (for example, Fe(III) and Mn(IV) reduction). Furthermore, estimating the rates of even one redox reaction (for example, the rate of sulfate reduction with the [^{35}S]-sulfate technique; JØRGENSEN, 1978) is labor intensive and requires expensive analytical equipment. In the case of aquifers, it can be extremely expensive to sample the solids where the redox reactions take place, and the rates of reactions are so slow that unacceptably long incubation periods may be required to make rate estimates. Therefore, a readily measured indicator to describe or predict the predominant redox activity in sediments would be a useful geochemical tool.

A variety of indicators have been previously proposed. Electrode measurements of redox potential have been the classic geochemical indicator of redox processes (BASS-BECKING *et al.*, 1960; PONNAMPERUMA, 1972). However, in practice, redox measurements have little practical value or theoretical significance (BERNER, 1981; STUMM and MORGAN, 1981; LINBERG and RUNNELS, 1984; THORSTENSON, 1984). This is because redox electrodes do not respond to many of the important redox couples in sediments and because most environments are not at redox equilibrium.

The fact that redox potentials, as measured with electrodes, can not be used to predict the predominant redox reaction in anaerobic sediments is clear from a compilation of redox potentials reported to be associated with various reactions in a series of review articles (Table 1). In general, the measured redox potential associated with a particular redox reaction encompasses a wide range. There is a significant overlap in the redox potentials reported for different reactions. Furthermore, different investigators have found quite different redox potentials to be associated with the same redox reactions. For example, WATANABE and FURUSAKA (1980) report 200 to −200 mV as the range of the redox potential in sediments in which organic matter oxidation with the reduction of Fe(III) is the predominant redox reaction. However, depending on which studies were referenced (Table 1), redox potentials in this range could also be interpreted as indicating that reduction of oxygen, Mn(IV), sulfate, or carbon dioxide was the predominant redox reaction.

Given the limitations of redox measurements, it has been suggested that the presence or absence of redox reactive compounds, such as oxygen, hydrogen sulfide, iron species, or methane, may be a more informative indicator of the pre-

Table 1. Redox potential as measured with electrodes for anaerobic sediments with various terminal electron acceptors.

Redox Reaction	Measured Redox Potential (millivolts)	Literature Reference
Nitrate Reduction	400 - 100	Watanabe and Furusaka (1980)
	600 - 500	Yoshida (1975)
	350 - 250	Jones (1982)
	710	Reeburgh (1983)
Mn(IV) Reduction	400 - -100	Watanabe and Furusaka (1980)
	600 - 400	Yoshida (1975)
	500 - 400	Jones (1982)
	470	Reeburgh (1983)
Fe(III) Reduction	200 - -200	Watanabe and Furusaka (1980)
	600 - 300	Yoshida (1975)
	350 - 50	Jones (1982)
	60	Reeburgh (1983)
Sulfate Reduction	0 - -200	Watanabe and Furusaka (1980)
	0 - -190	Yoshida (1975)
	-100 - -300	Jones (1982)
	-200	Reeburgh (1983)
Carbon Dioxide Reduction	-200 - -300	Watanabe and Furusaka (1980)
	-150 - -190	Yoshida (1975)
	-200 - -300	Jones (1982)
	-250	Reeburgh (1983)

dominant redox reactions in a given sediment (BERNER, 1981; STUMM and MORGAN, 1981; LINBERG and RUNNELS, 1984). None of these indicators would be a universally useful indicator by itself as each indicator applies only to one redox reaction. A further limitation of the use of these indicators is that the presence of a product of a redox reaction (for example, ferrous iron) does not indicate that the redox reaction (in this example, ferric iron reduction) is occurring at the site or time at which the product is detected.

The ideal indicator of the predominant redox reactions in sediments should be applicable to a range of redox reactions and should be dynamic enough to be indicative of the redox reactions that are currently going on at the site being sampled. The indicator should be readily quantified and permit clear distinctions between the various potential redox reactions in sediments.

In theory, hydrogen concentrations could be an indicator of which microbially catalyzed redox reactions are taking place in sedimentary environments. Hydrogen is known to be a fleeting but important intermediate in the decomposition of organic matter in methanogenic environments such as the rumen and anaerobic sewage sludge digestors (WOLIN, 1982; ZEIKUS, 1983). During the initial steps of the anaerobic decomposition of organic matter in these environments, a wide variety of organisms produce hydrogen during their metabolism of carbohydrates, amino acids, aromatics, or fatty acids. As rapidly as the hydrogen is produced, methanogenic bacteria oxidize it with the reduction of carbon dioxide to methane. The rapid turnover of the hydrogen pool between hydrogen producers and consumers has been termed interspecies hydrogen transfer (IANNOTTI *et al.*, 1973).

Hydrogen is also a key intermediate in aquatic sediments. In sediments with methane production as the predominant terminal electron-accepting process, hydrogen is an intermediate for *ca.* 40% of the electron flow (WINFREY and ZEIKUS, 1977; LOVLEY and KLUG, 1982). Interspecies hydrogen transfer results in hydrogen as an intermediate for 10 to 30% of the electron flow in sediments with sulfate as the terminal electron acceptor (SØRENSEN *et al.*, 1981; LOVLEY and KLUG, 1986). Hydrogen is also produced and consumed in sediments with Fe(III) reduction as the terminal electron-accepting process (LOVLEY and PHILLIPS, 1987).

We previously speculated that sediments with Fe(III) reduction, sulfate reduction, or methane production as the terminal electron-accepting reaction had distinct hydrogen concentrations that were characteristic for each particular reaction (LOVLEY and PHILLIPS, 1987). If so, and if there are also hydrogen concentrations characteristic of nitrate and Mn(IV) reduction, then hydrogen concentrations might be a useful indicator of the predominant terminal electron-accepting process in anaerobic sedimentary environments. The purpose of this study was to theoretically and experimentally examine this possibility.

THEORETICAL CONSIDERATIONS

The segregation of anaerobic terminal electron-accepting processes into distinct zones implies that in each zone there will be one predominant hydrogen-consuming reaction. This can be true even if a particular sediment zone temporarily contains more than one type of hydrogen-consuming organism. For example, when sulfate concentrations are not limiting to the metabolism of sulfate reducers, they maintain the concentration of hydrogen in sediments so low that methane production from hydrogen is thermodynamically unfavorable (LOVLEY *et al.*, 1982). Under these conditions, methane can not be produced even when there are large populations of potentially active methanogens in the sediment. In a similar manner, Fe(III)-reducing bacteria metabolize hydrogen at concentrations much lower than those metabolized by sulfate reducers (LOVLEY and PHILLIPS, 1987). Thus, when Fe(III) reduction is not limited by Fe(III) availability, Fe(III) reducers prevent hydrogen from being metabolized by sulfate reducers or methanogens. Studies with pure cultures of hydrogen-consuming organisms have further indicated that for each electron acceptor for anaerobic hydrogen metabolism there is a threshold level below which hydrogen can not be further metabolized (LOVLEY, 1985; CORD-RUWISCH *et al.*, 1988). As previously suggested in sediment studies (LOVLEY *et al.*, 1982), the threshold for hydrogen uptake by pure cultures appears to be controlled by thermodynamic constraints, as the hydrogen uptake threshold can be altered by changing the product-reactant ratio in the cultures (GOODWIN, unpublished data).

Hydrogen uptake kinetics have been studied with a wide variety of pure cultures and natural assemblages of hydrogen-consuming bacteria (STRAYER and TIEDJE, 1978; KRISTJANSSON *et al.*, 1982; LOVLEY *et al.*, 1982; ROBINSON and TIEDJE, 1982; CONRAD *et al.*, 1983a,b; KRISTJANSSON and SCHÖNHEIT, 1983; LOVLEY and KLUG, 1983; ROBINSON and TIEDJE, 1984; CONRAD *et al.*, 1985; LUPTON and ZEIKUS, 1984). These studies have all indicated that, as long as the hydrogen concentration remains above the minimum threshold for hydrogen uptake, hydrogen uptake by bacteria can be modelled with Michaelis-Menten kinetics according to the equation:

$$v = \frac{V_{max}}{K + [H_2]} \times [H_2] \times B \quad (1)$$

where v (units of moles of hydrogen per time) is the rate of hydrogen uptake, V_{max} (units of moles of hydrogen per time

per gram of cells) is the maximum rate of hydrogen uptake when hydrogen uptake is not limited by hydrogen availability, K (units of moles of dissolved hydrogen per liter) is the hydrogen concentration at which v equals $0.5V_{max}$, B is the number of grams of the hydrogen-consuming organism, and $[H_2]$ is the concentration of dissolved hydrogen in moles per liter.

The growth of microorganisms on hydrogen can be defined by:

$$\frac{dB}{dt} = (v \times Y) - (b \times B) \quad (2)$$

where dB/dt is the rate of growth of the organism (units of grams of cells formed per time), Y is the yield coefficient (units of grams of cells formed per mole of hydrogen consumed), and b is the cell decay coefficient (units of time^{-1}).

Modelling of sediment processes is typically simplified by assuming steady-state conditions (BERNER, 1980). Under steady-state conditions, the rate of hydrogen uptake, v, equals the rate of hydrogen production, and the hydrogen-consuming population reaches a steady state in which there is no net growth, i.e., $dB/dt = 0$. Given these assumptions, and by combining Eqns. (1) and (2), it can be demonstrated that at steady-state conditions the hydrogen concentration can be defined as follows:

$$[H_2] = \frac{K}{(V_{max} \times Y/b) - 1}. \quad (3)$$

Equation (3) indicates that under steady-state conditions the hydrogen concentration depends solely upon the physiological characteristics of the organisms consuming the hydrogen. Other factors, such as the rate of hydrogen production, do not influence the predicted steady-state hydrogen concentration. Equation (3) is merely a specific case of the generalization made by MCCARTY (1972) and BILLEN *et al.* (1980) that, at steady-state conditions in environments such as sediments, where there is no significant physical dilution of the microbial population, the steady-state concentration of microbial substrates can be predicted solely from a knowledge of the physiological characteristics of the microorganisms consuming the substrate.

Of the physiological parameters that can affect the steady-state hydrogen concentration, least is known about the cell decay coefficient. This coefficient includes all losses of cellular material including such factors as: endogenous respiration, cell death, cell lysis, and grazing by predators (MCCARTY, 1972) as well as physical dilution from environments such as digestors and the rumen. There appears to be little reason to believe that this parameter differs greatly between the various metabolic types of sediment organisms. In general, V_{max} values also appear to be relatively constant, regardless of the electron acceptor for metabolism (MCCARTY, 1972). This may be because all organisms are limited by constraints on the maximum rate of electron transport (MCCARTY, 1972). The data of ROBINSON and TIEDJE (1984) indicates that this generalization holds for hydrogen metabolism by anaerobic bacteria.

In contrast to V_{max} and b, both the K and Y parameters for hydrogen metabolism are strongly influenced by the energy yield that is available from hydrogen oxidation. K decreases as the potential energy available from hydrogen oxidation increases (Table 2). Possible reasons for this have been discussed previously (KRISTJANSSON *et al.*, 1982; KRISTJANSSON and SCHÖNHEIT, 1983). As the potential energy yield from a microbially catalyzed reaction increases, Y is expected to increase (MCCARTY, 1972). This generalization appears to be true for hydrogen metabolism, as in a comparison of pure cultures of hydrogen-metabolizing sulfate reducers and methanogens, the Y for sulfate reducers was fourfold higher than the Y for methanogens (ROBINSON and TIEDJE, 1984).

Given that K decreases and Y increases as the potential energy yield from hydrogen oxidation increases, Eqn. (3) predicts that the steady-state concentration of hydrogen in sediments will follow the order: methanogenic > sulfate-reducing > Fe(III)-reducing > Mn(IV)-reducing > nitrate-reducing. Furthermore, since the hydrogen concentration is solely dependent upon the physiological characteristics of the organisms consuming hydrogen, each hydrogen-consuming process should have a unique steady-state hydrogen concentration that is characteristic of that reaction. Therefore, sediments with the same predominant terminal electron-accepting process would be expected to have the same steady-state hydrogen concentration. However, this generalization would not apply to sediments that contain factors (such as inhibitory compounds) which could alter the physiological characteristics of the hydrogen-consuming organisms.

Influence of juxtapositioning and interspecies formate transfer

Several studies have speculated that most of the organic matter decomposition in methanogenic environments may take place in microbial aggregates in which all of the microbial populations necessary for the conversion of organic matter to

Table 2. Potential energy yield and half-saturation constants (K) for hydrogen oxidation.

Reaction	Potential Standard-Free Energy[a] (kJ per H_2)	K[b]
$O_2 + 2H_2 \longrightarrow 2H_2O$	237[c]	67 nM[d]
$2NO_3^- + 5H_2 + 2H^+ \longrightarrow N_2 + 6H_2O$	224[c]	< 1 μM[e]
$NO_3^- + 4H_2 + 2H^+ \longrightarrow NH_4^+ + 3H_2O$	150[c]	unknown
$MnO_2 + H_2 \longrightarrow Mn(OH)_2$	163[f]	unknown
$2Fe(OH)_3 + H_2 \longrightarrow 2Fe(OH)_2 + 2H_2O$	50[f]	unknown
$SO_4^{2-} + 4H_2 + H^+ \longrightarrow HS^- + 4H_2O$	38[c]	1.4 μM[g]
$HCO_3^- + 4H_2 + H^+ \longrightarrow CH_4 + 3H_2O$	34[c]	4.7 μM[g]

[a] Energy available from the reaction under standard conditions at pH7.

[b] Half-saturation constants for mixed assemblages of bacteria.

[c] From Thauer *et al.* (1977).

[d] From Conrad *et al.* (1983).

[e] From Thauer *et al.* (1977).

[e] From Kurt *et al.* (1987).

[f] Calculated from standard free energies of formation given in Stumm and Morgan (1981).

[g] From Lovley *et al.* (1982).

methane and carbon dioxide are tightly grouped or "juxtapositioned" in flocs (CONRAD *et al.*, 1985; THIELE *et al.*, 1988). It has been argued that this juxtapositioning creates two hydrogen pools, one within the floc and an external pool. However, there is a question over whether the external hydrogen pool is lower (OZTURK *et al.*, 1988) or higher (CONRAD *et al.*, 1985, 1986) than the hydrogen concentration within the flocs. Recently, it has been further proposed that interspecies formate transfer replaces interspecies hydrogen transfer as an important pathway of decomposition within flocs (THIELE and ZEIKUS, 1988). However, even if juxtapositioning or interspecies formate transfer are substantiated as important aspects of anaerobic sediment metabolism, this does not invalidate the use of hydrogen concentrations as an indicator of the predominant terminal electron-accepting reaction in sediments. Current analytical techniques only measure the external pool (CONRAD *et al.*, 1985). As noted above, the steady-state hydrogen concentration should be independent of the rate of hydrogen production. Therefore, whether hydrogen or formate is the predominant intermediate in metabolism and whether most of the metabolism takes place within juxtapositioned flocs or not, the theoretical analysis outlined above is valid as long as there is some hydrogen production and consumption outside the flocs.

MATERIALS AND METHODS

Potomac River sediments

Freshwater sediments were collected at various times throughout the year with an Eckman dredge from the previously described site near the mouth of Gunston Cove in the Potomac River, Maryland (LOVLEY and PHILLIPS, 1986a,b). Canning jars were filled to the top with the brown surficial sediment. The bottles were sealed with a lid and transported to the laboratory. These sediments were then manipulated in various ways. All transfers and incubations were performed under strict anaerobic conditions under N_2-CO_2 (93:7). Sediments were incubated in serum bottles (Wheaton Scientific[1]) or anaerobic pressure tubes (Bellco Glass, Inc.) which were sealed with thick butyl rubber stoppers (Bellco Glass, Inc.).

The predominant terminal electron-accepting process in the freshwater sediments was determined by measuring the concentrations of nitrate, copper sulfate-extractable Mn(II), HCl-extractable Fe(II), sulfate, and methane over time as outlined below. For example, sediments in which nitrate had been depleted, Mn(II) was no longer accumulating, Fe(II) was accumulating, and there was no sulfate reduction or methane production were considered to be Fe(III)-reducing sediments. Repeated hydrogen measurements were made for each sediment type to ensure that hydrogen concentrations had stabilized and presumably were approaching steady-state for that particular process. For nitrate-reducing sediments, measurements could only be made over the course of 1–2 days before the nitrate was depleted. Mn(IV) reduction was the predominant terminal electron-accepting process for 5 days in the study in which natural Mn(IV) forms were the electron acceptor and hydrogen could be measured in Mn(IV)-reducing sediments for two weeks in sediments with added synthetic MnO_2. For all other hydrogen measurements reported here for the first time, hydrogen could be monitored with the same terminal electron accepting process predominating for at least a two-week period. Methanogenic and sulfate-reducing sediments which could be incubated for months with the same terminal electron-accepting process predominating were found to maintain the same hydrogen concentration over these extended incubations.

[1]Use of trade names is for identification purposes and does not constitute endorsement by the U.S. Geological Survey.

For measurements of the hydrogen concentration in sediments in which Fe(III) was the predominant terminal electron acceptor (Fig. 1), and for the studies on hydrogen concentrations during the transition from Fe(III) reduction to sulfate reduction and methane production (Fig. 4), sediment (100 ml) was transferred into 160-ml serum bottles. For some of the measurements of hydrogen concentrations with Fe(III) as the terminal electron-accepting process, as well as the measurements of hydrogen concentrations during nitrate and Mn(IV) reduction in sediments with no electron donor amendments, sediments were maintained under aerobic conditions overnight by stirring them under air with a magnetic stir bar to prevent accumulation of reduced products until the incubations could be initiated. These sediments were then incubated under anaerobic conditions and monitored as described above.

One set of sediments from Gunston Cove was collected during low-flow, high-tide conditions and had unusually high (>1 mM) sulfate concentrations. During the anaerobic incubation of this sediment, there was a distinct period when Fe(III) reduction was complete, methane production had not started, and sulfate reduction was the predominant terminal electron-accepting process. This permitted the measurement of hydrogen concentrations in freshwater sediments which had not been artifically amended with sulfate but in which sulfate reduction was the predominant terminal electron-accepting process.

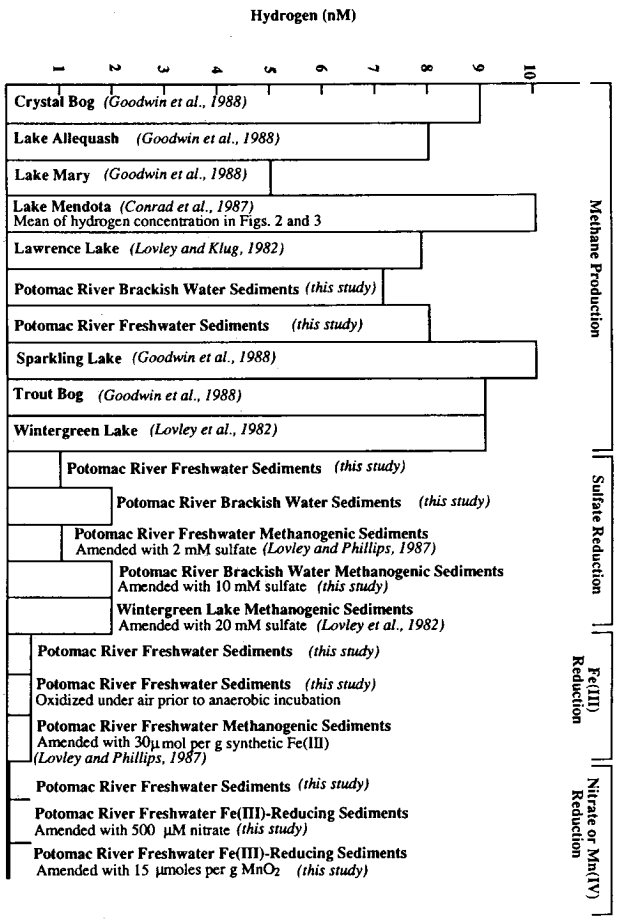

FIG. 1. Hydrogen concentrations in sediments with different predominant terminal electron-accepting processes. Unless otherwise noted, the sediments received no amendments. Hydrogen concentrations for Crystal Bog, Lake Allequash, Lake Mary, Sparkling Lake, and Trout Bog were obtained by extracting hydrogen from freshly collected sediments. All of the other values were estimated by incubating sediments and measuring hydrogen concentrations over time (days to months) to ensure that a steady-state concentration was approximated.

The metabolism in sediments in which Fe(III) reduction was the terminal electron-accepting process could be switched to nitrate reduction or Mn(IV) reduction with the addition of nitrate or Mn(IV). Nitrate (400 μM, final concentration) or MnO_2 (15 μmoles per gram wet sediment, final concentration) were added from an anaerobic solution of sodium nitrate (40 mM) or an anaerobic slurry of a poorly crystalline synthetic MnO_2 (400 μmoles per g of slurry).

Some of the sediments were transferred into 1-liter bottles and incubated for more than a month to deplete electron acceptors for terminal metabolism other than carbon dioxide. Methane production was the terminal electron acceptor in these sediments. These sediments were transferred in 10-ml aliquots into anaerobic pressure tubes and were used for the studies summarized in Fig. 3. Terminal metabolism in these sediments was switched from methane production to nitrate reduction or Mn(IV) reduction with the aseptic addition of nitrate (500 μM final concentration) or MnO_2 (40 μmoles per g wet sediment) from sterile anaerobic stocks. When required, biological activity in the sediments was inhibited by autoclaving (121°C, 15 minutes) the sediments within the tubes.

Brackish water sediments were collected at two sites with a Petite Ponar dredge. Sediments with sulfate reduction as the predominant terminal electron-accepting process were collected from the previously described site in the lower portion of the Potomac River Estuary (LOVLEY and PHILLIPS, 1986a). The brown surficial layer of the sediment was discarded and canning jars were filled with the dark grey sulfidogenic sediments. Brackish water sediments in which methane production was the predominant terminal electron-accepting process were collected at a nearby deep site (24 m). Previous studies (CALLENDER and LOVLEY, unpublished data) had indicated that sulfate was depleted within the surficial layers of these sediments and that there was active methane production (500 μmoles methane per kg of wet sediment per day) at lower depths. The sediments were transported in canning jars. In the laboratory, the sediments were transferred in 100-ml aliquots to 160-ml serum bottles, which were then sealed with butyl rubber stoppers. Sulfate was added to some of the methanogenic sediments from an anaerobic stock solution of 2 M sodium sulfate to give a concentration of added sulfate of 20 mM.

Unless otherwise noted, all of the sediments were incubated in a horizontal position on a wrist-action shaker. All incubations were at 20°C in the dark.

Hydrogen concentrations in the Middendorf aquifer, South Carolina

Dissolved hydrogen concentrations in waters of the Middendorf aquifer, South Carolina, were determined. The wells were oriented along the regional hydrologic gradient from the recharge area near Aiken to the discharge area near Charleston. The hydrologic framework and ground-water flow pattern of the Middendorf aquifer have been described previously (AUCOTT *et al.*, 1987; AUCOTT and SPEIRAN, 1985). Each well was flushed for a minimum of 15 minutes before sampling. This was sufficient to remove at least two well volumes at each site. The sampling procedure followed CONRAD *et al.* (1985). Water samples were collected in calibrated, 90-mL glass flasks fitted with two stopcocks. Half of the N_2 headspace was displaced with a 45-mL water sample. The flask was shaken vigorously for 1 minute to equilibrate hydrogen between the aqueous phase and the headspace. A glass syringe was used to transfer 10 mL of the equilibrated headspace to a flask completely filled with saturated Na_2SO_4 solution containing 100 μg/mL of $HgCl_2$. The sample was transported to the laboratory as a gas bubble. Hydrogen and methane concentrations were determined in the laboratory as described below. Dissolved sulfate and nitrate in water samples which had been transported to the laboratory on ice were determined as outlined below.

Analytical methods

Most of the analytical methods have been described previously, and the appropriate references should be consulted for a detailed description. Fe(II) extractable in 0.5 N HCl and Mn(II) extractable in acidic copper sulfate solution were determined on whole sediments as previously described (LOVLEY and PHILLIPS, 1986; LOVLEY and PHILLIPS, 1988). Dissolved iron in aquifer samples was determined with atomic absorption spectrophotometry with an air-acetylene flame. Pore water for nitrate and sulfate determinations was obtained by centrifugation. The supernatant was filtered through a Versapor filter (0.45 μm pore diameter). The anions were quantified by high performance liquid chromatography (LOVLEY and PHILLIPS, 1987). Methane was determined by gas chromatography with a flame ionization detector (LOVLEY and PHILLIPS, 1986b).

Hydrogen was measured with a reduction gas analyzer (Trace Analytical). Headspace samples (0.1–1.0 ml) which contained hydrogen in equilibrium with the dissolved hydrogen in the sediment were separated on a 0.5 m column of Carbosieve II (Supelco, Inc.) run at room temperature with N_2 as the carrier. The headspace gas that was removed was replaced with N_2 or N_2:CO_2 (93:7). In order to make the hydrogen measurements readily comparable with other studies, the dissolved hydrogen concentration was calculated from the hydrogen partial pressure and hydrogen solubility constants (WIHELM *et al.*, 1977). For brackish water sediments, the influence of salinity on hydrogen solubility (WIESENBURG and GUINASSO, 1979) was considered. It has previously been demonstrated that even when sediments are not shaken, dissolved hydrogen comes into equilibrium with gaseous hydrogen within 20 minutes (NOVELLI *et al.*, 1987); and shaken sediments come into equilibrium within seconds (CONRAD *et al.*, 1985; ROBINSON and TIEDJE, 1982). As noted above, the hydrogen concentrations in this as well as previous studies (LOVLEY *et al.*, 1982; LOVLEY and PHILLIPS, 1987) were determined on sediments that were shown to maintain the same hydrogen concentration with the same predominant terminal electron accepting process for days to months. Furthermore, studies with Fe(III)-reducing, sulfate-reducing, and methanogenic sediments have demonstrated that if the hydrogen concentration is artificially increased or decreased, it rapidly returns to the steady-state concentration (LOVLEY *et al.*, 1982; LOVLEY and PHILLIPS, 1987; unpublished data).

RESULTS AND DISCUSSION

Hydrogen concentrations in sediments

Measurements of hydrogen concentrations in a variety of sediments indicated that hydrogen concentrations were controlled by the terminal electron-accepting process which predominated in the sediment (Fig. 1). Sediments with the same terminal electron-accepting process had remarkably similar hydrogen concentrations. This was true whether an electron acceptor was added to the sediments to establish the particular redox reaction or whether the sediments were unamended. The hydrogen concentrations in sediments with different terminal electron-accepting processes were distinctly different. As predicted from the apparent thermodynamic control on the physiological characteristics of hydrogen-consuming bacteria (see Theoretical Considerations), hydrogen concentrations for the various sediments followed the order: methanogenic > sulfate-reducing > Fe(III)-reducing > Mn(IV)- and nitrate-reducing.

Some hydrogen measurements reported in the literature were not included in the summary in Fig. 1. Several values for methanogenic sediments were excluded for reasons outlined in the section on methanogenic sediments. Most notably, the hydrogen concentrations reported by Novelli and coworkers for various marine sediments (NOVELLI *et al.*, 1987) and freshwater sediments for Lake Washington (KUIVILA *et al.*, submitted) have not been included. Those studies reported hydrogen concentration for sediments with sulfate reduction or methane production as the predominant terminal electron-accepting process that were approximately ten-fold higher than those summarized in Fig. 1 for the same processes. The reasons for such a wide discrepancy are not clear and are currently under investigation (KUIVILA and

LOVLEY, unpublished data). The studies of Novelli and coworkers follow the same trend observed in Fig. 1, with decreasing hydrogen concentrations as the electrochemical potential of the terminal electron acceptor becomes more positive. Thus, despite some analytical differences, all of the hydrogen measurements available to date suggest that sediment hydrogen concentrations respond in the same manner to changes in the terminal electron-accepting process.

Methanogenic sediments

The most data were available for methanogenic sediments (Fig. 1). The estimates of dissolved hydrogen in methanogenic sediments were obtained by two different methods. In one method, dissolved hydrogen was extracted either from freshly collected sediments (GOODWIN *et al.*, 1988) or sediments incubated without a headspace (CONRAD *et al.*, 1987). The other hydrogen estimates were the result of measuring the gaseous hydrogen in equilibrium with dissolved hydrogen in sediment incubations. Both methods indicated that methanogenic sediments typically have dissolved hydrogen concentrations of about 8 nM (Fig. 1). This conclusion was recently supported by measurements made by a third method in which the hydrogen partial pressure in gas bubbles from submerged methanogenic rice paddy soils was determined (SCHÜTZ *et al.*, 1988). With the exception of one anomously high value, the hydrogen partial pressures that were measured corresponded to a mean dissolved hydrogen concentration of 9 nM.

In accordance with Eqn. (3), the hydrogen concentrations in most of the methanogenic sediments were similar, despite wide variations in the rate of organic matter decomposition. For example, hydrogen concentrations in methanogenic sediments of Wintergreen Lake and the freshwater Potomac River were comparable, even though the rates of hydrogen production (as estimated from methane production rates) were sometimes as much as 25 times faster in Wintergreen Lake sediments.

However, hydrogen concentrations higher than those summarized in Fig. 1 may sometimes be observed in methanogenic sediments of eutrophic lakes because high inputs of organic matter or sampling manipulations can temporarily increase the rate of hydrogen production and disturb the steady-state (LOVLEY and KLUG, unpublished data). Hydrogen concentrations as high as 60 nM have been observed in methanogenic sediments of eutrophic Lake Mendota (CONRAD *et al.*, 1987b). However, these high concentrations did not represent steady-state conditions because when sediments from the same time of year (June) were incubated under conditions approximating those *in situ*, the hydrogen concentrations stabilized at approximately 6 nM (CONRAD *et al.*, 1987b). Methanogenic sediments from dystrophic Knaack Lake, were reported to have a hydrogen concentration of 44 nM (GOODWIN *et al.*, 1988). This value is unlikely to represent steady-state conditions as when the sediments were incubated the hydrogen concentration decreased more than 40-fold (CONRAD *et al.*, 1987a). Therefore, the data presented for Lake Mendota is a summary of data from laboratory incubations (CONRAD *et al.*, 1987b) in which it could be demonstrated that hydrogen concentrations approximated steady-state and no data for Knaack Lake were included.

Brackish water methanogenic sediments from the Potomac River Estuary had hydrogen concentrations comparable to the freshwater methanogenic sediments, and methanogenic sediments from acidic bogs had hydrogen concentrations comparable to those in methanogenic sediments that were near neutral pH. These results suggest that, within the range of environments tested, differences in pH and salinity had little effect on the hydrogen uptake characteristics of the methanogens.

Previous studies have definitively demonstrated that the hydrogen concentrations in sediments with methane production as the terminal electron-accepting process are under the biological control of methanogenic bacteria. Chloroform at low concentrations (100 μM) selectively inhibits the metabolism of methanogenic bacteria in sediments (LOVLEY *et al.*, 1982). Addition of 100 μM chloroform to sediments in which methane production was the terminal electron-accepting process completely inhibited hydrogen uptake and resulted in the accumulation of hydrogen over time as fermentative bacteria continued to produce hydrogen from organic matter (LOVLEY and KLUG, 1982; LOVLEY *et al.*, 1982). If the hydrogen concentrations in the sediments were controlled by nonenzymatic redox reactions, the addition of such a small quantity of chloroform would be unlikely to have any effect on the hydrogen concentration.

Sulfate-reducing sediments

Previous studies have suggested that sediments with sulfate reduction as the predominant terminal electron-accepting process have a lower hydrogen concentration than methanogenic sediments (LOVLEY *et al.*, 1982; LOVLEY and PHILLIPS, 1987; CONRAD *et al.*, 1987b). All of the fresh and brackish water sediments with sulfate reduction as the terminal electron-accepting process that were tested had similar hydrogen concentrations (Fig. 1). This was true whether sulfate reduction was naturally the predominant terminal electron-accepting process or whether sulfate was added to convert methanogenic sediments to sulfate-reducing sediments.

There is little question that the hydrogen concentration in sulfate-reducing sediments is a function of the metabolism of hydrogen-consuming, sulfate-reducing bacteria. Molybdate, a specific inhibitor of sulfate-reducing bacteria, inhibits hydrogen oxidation coupled to sulfate reduction in sediments (SØRENSEN *et al.*, 1981; LOVLEY *et al.*, 1982). The addition of molybdate to sulfate-reducing marine sediments resulted in a steady accumulation of hydrogen over time, because sulfate reduction could no longer serve as a sink for hydrogen produced by hydrogen-forming bacteria (SØRENSEN *et al.*, 1981). When molybdate was added to sulfate-reducing freshwater sediments, the hydrogen concentration initially increased and then restabilized at concentrations characteristic of a methanogenic sediment (LOVLEY *et al.*, 1982). This was because the sediments contained a residual population of methanogenic bacteria that became active as soon as the hydrogen concentration increased to levels which they could metabolize.

Fe(III)-reducing sediments

A previous laboratory study with synthetic amorphic Fe(III) oxide suggested that Fe(III)-reducing bacteria can metabolize

hydrogen to concentrations lower than can be metabolized by sulfate-reducing bacteria (LOVLEY and PHILLIPS, 1987). Additions of Fe(III) to a sediment in which sulfate reduction was the predominant terminal electron-accepting process resulted in a switch in terminal electron flow from sulfate reduction to Fe(III) reduction with a concomitant lowering of the sediment hydrogen concentration. Not only were the steady-state concentrations of hydrogen lower in Fe(III)-reducing sediments, but if hydrogen was added to increase the hydrogen concentration in the sediments, the Fe(III)-reducing bacteria rapidly brought the hydrogen concentration down to the steady-state level.

The results presented here demonstrate that Fe(III)-reducing bacteria maintain the same low hydrogen concentrations when natural Fe(III) forms are the electron acceptor (Fig. 1). Although limited in scope, this data is consistent with the hypothesis that sediments with Fe(III) reduction as the predominant terminal electron-accepting reaction have similar steady-state hydrogen concentrations.

There are no known specific inhibitors for dissimilatory Fe(III)-reducing bacteria (LOVLEY, 1987). Therefore, it is not as straightforward as it is with methane production or sulfate reduction to demonstrate that microorganisms catalyze hydrogen oxidation coupled to Fe(III) reduction. However, it was observed that if sediments in which Fe(III) reduction was the terminal electron-accepting process were incubated under a headspace containing one atmosphere of hydrogen (replenished daily), the rate of Fe(III) reduction was much greater than the rate in sediments receiving no hydrogen additions (Fig. 2). Even with the added hydrogen, there was no detectable Fe(III) reduction in sediments which were treated with heat (121°C, 15 minutes) to kill the microorganisms (Fig. 2). When an inoculum (10% of total volume) of sediment containing viable Fe(III)-reducing bacteria was added to the heat-killed sediments incubated under hydrogen, there was active reduction of Fe(III) after a brief lag period. Therefore, the heat treatment had not affected the availability of the sediment Fe(III) for reduction but had killed the microorganisms necessary to catalyze hydrogen oxidation coupled to Fe(III) reduction. These results indicate that Fe(III)-reducing bacteria metabolize hydrogen in the sediment and that there is no hydrogen oxidation coupled to Fe(III) reduction in the absence of viable bacteria.

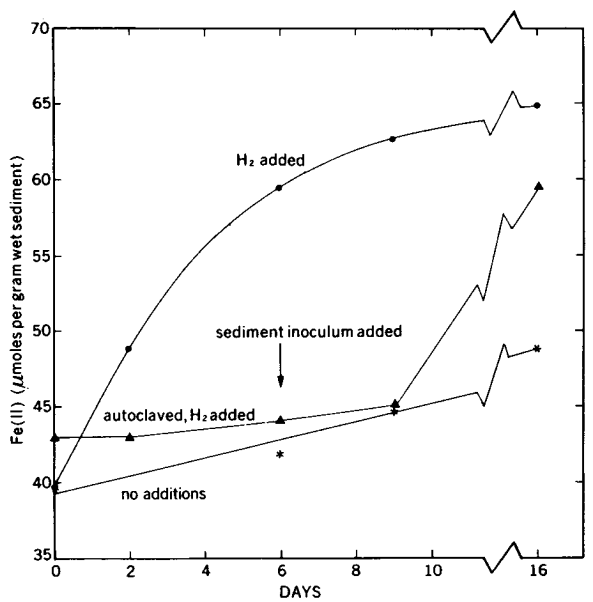

FIG. 2. Fe(III) reduction in various treatments of sediments. HCl-extractable Fe(II) was measured in sediments receiving no additions and in sediments with added hydrogen. Autoclaved sediments with added hydrogen were sampled aseptically for the first six days of incubation. After sampling the sediments for Fe(II) on day six, a 10-percent inoculum of the sediment which was incubated with added hydrogen and was not autoclaved was added to the autoclaved sediments incubated under hydrogen. The results shown are from single bottles and are representative of triplicates for each treatment. The standard error for the Fe(II) determinations was less than 5%.

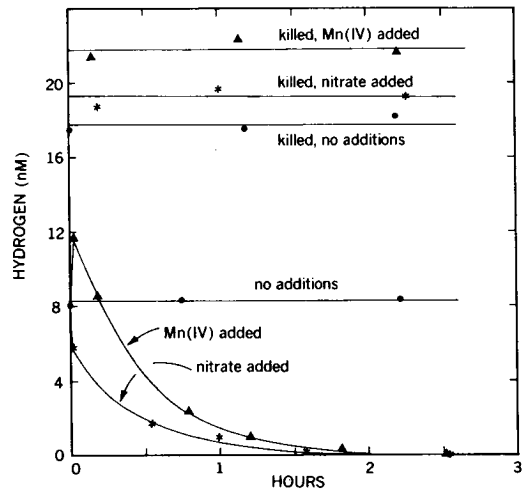

FIG. 3. Hydrogen metabolism coupled to biological Mn(IV) and nitrate reduction. Additions of nitrate or Mn(IV) were made to sediments in which methane production was the predominant terminal electron-accepting process. Similar additions were also made after destroying biological activity with heat. The results shown are from single tubes and are representative of replicate tubes of each treatment.

Mn(IV)- and/or nitrate-reducing sediments

Hydrogen was below the detection limit (0.05 nM) when Mn(IV) or nitrate was the terminal electron acceptor for metabolism. Hydrogen dynamics in sediments with nitrate or Mn(IV) as the predominant terminal electron acceptor have not been previously studied in detail. Organisms capable of coupling hydrogen oxidation to nitrate reduction have been reported (PAYNE, 1981), but only recently has an organism capable of growing by coupling hydrogen oxidation to Mn(IV) reduction been demonstrated (LOVLEY and PHILLIPS, in preparation).

To determine if biological consumption of hydrogen coupled to nitrate or Mn(IV) reduction could be responsible for the maintenance of hydrogen concentrations below 0.05 nM, nitrate or Mn(IV) was added to methanogenic sediments (Fig. 3). Hydrogen rapidly decreased to undetectable levels with the addition of nitrate or Mn(IV) while remaining constant in sediments receiving no additions. Heating the sediments to destroy biological activity resulted in hydrogen concentrations that were 2- to 2.5-fold higher than that in untreated sediments (Fig. 3). This was presumably the result of hydro-

gen-consuming bacteria being killed slightly sooner than the hydrogen-producing bacteria during the heating. In spite of these higher hydrogen concentrations, there was no consumption of hydrogen when nitrate or Mn(IV) was added to the sediments without biological activity. These results suggest that hydrogen oxidation can be coupled to the reduction of nitrate or Mn(IV) in sediments and that this activity is dependent upon the activity of microorganisms.

Comparison of observed hydrogen concentrations with those predicted by microbial physiology

One indication of whether the hydrogen concentrations in the sediments are controlled by the physiological characteristics of the hydrogen-consuming organisms is how well the hydrogen concentrations that were observed in the sediments compare with what would be predicted from the known physiological parameters of the organisms. The K for hydrogen uptake by methanogenic and sulfate-reducing bacteria in pure culture (KRISTJANSSON *et al.*, 1982; KRISTJANSSON and SCHÖNHEIT, 1983; ROBINSON and TIEDJE, 1984) are very similar to the K values for methanogens and sulfate reducers living in sediments (LOVLEY *et al.*, 1982). The Y and the V_{max} parameters for hydrogen uptake by methanogenic bacteria and sulfate reducers in sediments have not been directly measured because of a lack of suitable techniques. It is reasonable to assume that these parameters also approximate the values measured with pure cultures. A summary of pure culture data (ROBINSON and TIEDJE, 1984) reported that the hydrogen metabolism parameters for methanogens and sulfate-reducers were respectively: Y (g protein × mole H_2^{-1}) 20, 85; V_{max} (mole H_2 × g protein^{-1} × min^{-1}) 1.1×10^{-4}, 1.1×10^{-4}; K (μM) 4.1, 1.2. The b for hydrogen-consuming bacteria in sediments was experimentally estimated as 7×10^{-6} min^{-1} (LOVLEY, 1982). From these parameters it can be calculated from Eqn. (3) that the steady-state hydrogen concentrations in sediments with methane production or sulfate reduction as the hydrogen-oxidizing process should be 13 and 1 nM, respectively. These steady-state hydrogen concentrations, predicted solely on the basis of the physiological characteristics of the organisms, are similar to the hydrogen concentrations actually observed. This finding strongly supports the contention that the hydrogen concentrations in sediments are controlled by the physiological characteristics of the hydrogen-consuming organisms. Furthermore, Eqn. (3) accu rately predicts the steady-state hydrogen concentration in methanogenic sludge digestors (unpublished data) and the rumen (W. SMOLENSKI and J. ROBINSON, pers. commun.) when the physiological parameters for pure cultures of methanogens and the appropriate b parameters are entered into the equation. Comparable calculations have not been made for Fe(III) reduction, Mn(IV) reduction, or nitrate reduction because of a lack of data on the organisms catalyzing these reactions. However, the results to date suggest that the model for hydrogen metabolism presented here is applicable to a wide variety of environments and that physiological parameters of pure cultures of hydrogen consuming organisms can aid in the interpretation of hydrogen dynamics in natural environments.

Interpretation of hydrogen concentrations in sediments not at steady state

Except for nitrate and Mn(IV) reduction which may proceed simultaneously under steady-state conditions, one microbially catalyzed redox reaction will predominate in a given sediment interval or microzone (PONNAMPERUMA, 1972; FROELICH *et al.*, 1979; REEBURGH, 1983). The segregation of Fe(III) reduction, sulfate reduction, and methane production appears to be the result of the organisms catalyzing the more thermodynamically favorable reaction maintaining the concentration of electron donors below the concentrations that are required for the metabolism of the organisms catalyzing the less energetically favorable reaction (LOVLEY *et al.*, 1982; LOVLEY and PHILLIPS, 1987).

However, microbially catalyzed reactions can coexist when the more energetically favorable reaction is limited by electron acceptor availability. Under these conditions, the more favorable reaction can no longer maintain the hydrogen concentration below the minimum threshold necessary for hydrogen metabolism by the less favorable reaction. Coexistence of hydrogen-consuming processes due to such electron-acceptor limitations have been noted previously (LOVLEY *et al.*, 1982; LOVLEY and PHILLIPS, 1986a, 1987).

To examine the effect of coexistence of terminal electron-accepting processes on hydrogen concentrations, hydrogen was measured in surficial freshwater sediments from the Potomac River as they underwent a succession from Fe(III) reduction to methane production (Fig. 4). Within 1.5 days of collection, these sediments had no detectable nitrate and were depleted of microbially reducible Mn(IV) as indicated by the lack of Mn(II) accumulation over time (data not shown). Fe(III) reduction was initially the predominant terminal electron-accepting process (Fig. 4). There was no detectable sulfate reduction or methane production (Fig. 4). During this initial stage, hydrogen concentrations were characteristic of Fe(III)-reducing sediment.

At 19 days of incubation, the start of sulfate reduction was detected (Fig. 4). Between 19 and 40 days of incubation, there

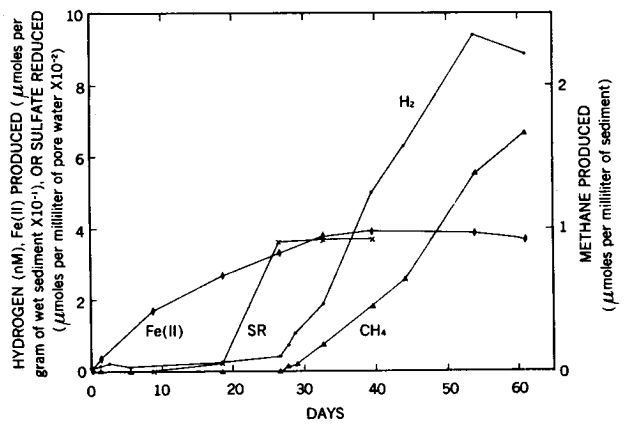

FIG. 4. Hydrogen concentrations, Fe(III) reduction, sulfate reduction, and methane production in surficial freshwater sediments from the Potomac River incubated under anaerobic conditions. The results shown are from a single bottle of sediment and are representative of replicate incubations.

was a period of concurrent Fe(III) reduction and sulfate reduction, followed by concurrent Fe(III) reduction and methane production. Hydrogen concentrations rose steadily during this time. With the completion of Fe(III) reduction, hydrogen concentrations stabilized at *ca.* 9 nM (Fig. 4, and data not shown).

These results suggest that at the start of incubation, when the most readily reducible Fe(III) was still available, Fe(III)-reducing organisms were able to completely outcompete sulfate reducers and methanogens for hydrogen and other electron donors. Fe(III) reduction was the predominant hydrogen-consuming process in the sediments and the hydrogen concentrations were typical of Fe(III)-reducing sediments. However, as the incubation progressed, Fe(III) reduction became limited by Fe(III) availability, and Fe(III) reducers could no longer completely outcompete sulfate reducers for electron donors. As sulfate was depleted, methanogens began to successfully compete with Fe(III) reducers. During the period of concurrent Fe(III) reduction and methane production, the hydrogen concentrations were characteristic of those expected, under steady-state conditions, for sediments with sulfate reduction as the predominant terminal electron-accepting process.

These results demonstrate that in non-steady-state environments which are undergoing rapid transitions with no one terminal electron-accepting process predominating, hydrogen concentrations can not be interpreted unambiguously. Another potential situation where there could be more than one electron acceptor for organic matter oxidation within a readily sampled sediment interval is the formation of microzones in which a reaction different from that in the bulk sediment predominates. The hydrogen concentration determined by measuring the bulk sediment will primarily reflect the processes in the bulk sediment and will not give an indication of processes in microzones.

Hydrogen concentrations in the Middendorf aquifer, South Carolina

An indicator of the predominant microbially catalyzed redox reactions in aquatic sediments would be most useful for environments such as deep aquifers, where direct measurement of the rate of redox reactions is difficult or impossible. As a preliminary indication of the feasibility of using hydrogen as an indicator of redox reactions in aquifers, hydrogen and other pertinent parameters were measured along the flow path of the Middendorf aquifer, South Carolina. Geochemical evidence suggests that there is a transition from organic matter oxidation with oxygen reduction near the recharge area to successive zones of nitrate reduction, Fe(III) reduction, sulfate reduction, and methanogenesis down gradient (Fig. 5, and unpublished data). Dissolved hydrogen concentrations appeared to respond to these transitions in the predominant terminal electron-accepting process (Fig. 5). This suggests that hydrogen concentrations might be a useful tool in analyzing redox reactions in deep subsurface environments.

However, from the geochemical data alone, it is impossible to confidently state what electron acceptors are being reduced

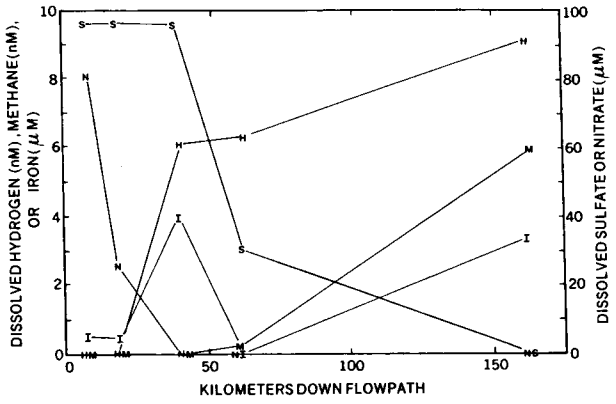

FIG. 5. Hydrogen (H), sulfate (S), nitrate (N), dissolved iron (I), and methane (M) along the flow path of the Middendorf aquifer, South Carolina.

at any of the sites along the flow path. Further studies are required in which hydrogen concentrations and the rates of terminal electron-accepting processes are directly measured in cores of aquifer material. Although much more technically difficult and expensive, such studies are the only appropriate method to adequately test the true utility of hydrogen measurements for characterizing the redox geochemistry of aquifers.

There is considerable controversy over whether microbial activity significantly influences the geochemistry of deep subsurface environments. If the hydrogen measured in the Middendorf aquifer and other aquifers (unpublished data) can definitely be demonstrated to be biogenic, this would strongly support the hypothesis that redox reactions in subsurface environments are microbially catalyzed (D. THORSTENSON and F. CHAPELLE, pers. commun.).

SUMMARY AND CONCLUSIONS

The investigations reported here demonstrate the potential usefulness of dissolved hydrogen concentrations as an indicator of the predominant terminal electron-accepting process in anaerobic sedimentary environments.

Theoretical considerations indicate that the steady-state hydrogen concentration in sediments is controlled by the organisms catalyzing the predominant terminal electron-accepting process. The more positive the electrochemical potential of the electron acceptor for hydrogen oxidation, the greater the potential energy yield to bacteria catalyzing the hydrogen oxidation. The greater the potential energy yield, the higher the affinity of the organisms for hydrogen uptake and the greater the yield of cellular material per hydrogen metabolized. A higher hydrogen affinity and cell yield permit organisms to maintain lower steady-state hydrogen concentrations. Since the hydrogen concentration should be dependent solely upon the physiological characteristics of the organisms consuming hydrogen, sediments with the same predominant terminal electron-accepting process should have the same steady-state hydrogen concentration, regardless of other factors, such as the rate of organic matter decomposition.

The hydrogen measurements we have made to date agree with this theoretical analysis. Methane production, sulfate reduction, Fe(III) reduction, and nitrate and Mn(IV) reduction each appear to have unique hydrogen concentrations associated with them, when any of these processes is the predominant terminal electron acceptor. These findings, coupled with the commercial availability of highly sensitive detectors for the gas chromatographic determination of hydrogen, suggest that hydrogen measurements could have wide applicability to the study of the geochemistry of anaerobic sedimentary environments.

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