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Geochemical and microbiological zonation of the Middendorf aquifer, South Carolina

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Abstract

We consider the nature and origin of the chemical and microbiological zonation of the Cretaceous Middendorf aquifer, in the Coastal Plain of South Carolina, in light of the chemical evolution of groundwater along a flow path there. Some types of microbial activity, most notably aerobic respiration and iron reduction, lead to a net generation of acid as CO_2, but reaction of groundwater with minerals in the aquifer consumes the acid, driving up pH and controlling the evolution of the groundwater's major ion chemistry. The thermodynamic energy available to the various functional groups of microbes, except the autotrophic hydrogen-oxidizing methanogens, is everywhere along the flow path sufficient to drive microbial growth. The concentrations of acetate, formate, and lactate do not vary systematically from zone to zone along the flow path, as might be expected if each zone were dominated by a single functional group of microbes. The overall rate of microbial respiration in the aquifer appears to be limited by the rate of the initial fermentation of organic matter. We found no compelling evidence indicating that one functional group of microbes excludes others from any of the aquifer's zones. Sulfate reduction, for example, may be non-existent in the high-iron zone associated with iron reduction, or may account for up to 90% of the respiration occurring there. Zonation of the groundwater into high-iron and low-iron facies may result from a minor change along the aquifer in the balance between the activities of iron and sulfate reducers.

Keywords: Middendorf aquifer; Zonation; Geomicrobiology; Sulfate reduction; Iron reduction

1. Introduction

The Cretaceous Middendorf aquifer in South Carolina is perhaps the best studied example of a geochemically and microbiologically zoned regional aquifer (Aucott and Speiran, 1985; Chapelle and Lovley, 1990, 1992; McMahon and Chapelle, 1991; Speiran and Aucott, 1994). Groundwater in the clastic aquifer recharges along the Fall Line and flows southeast beneath the Atlantic Coastal Plain and the Atlantic Ocean (Fig. 1). Along the direction of flow, groundwater passes through a series of geochemical zones, and microbial activity in each zone is interpreted to be dominated by a different functional group of microorganism (Chapelle and Lovley, 1992; Lovley and Goodwin, 1988).

Near the recharge area, the water is oxygenated. Dioxygen concentration here decreases along flow, presumably due in large part to its consumption by aerobic microbes. Once dioxygen is depleted, water enters first a zone rich in dissolved ferrous iron likely

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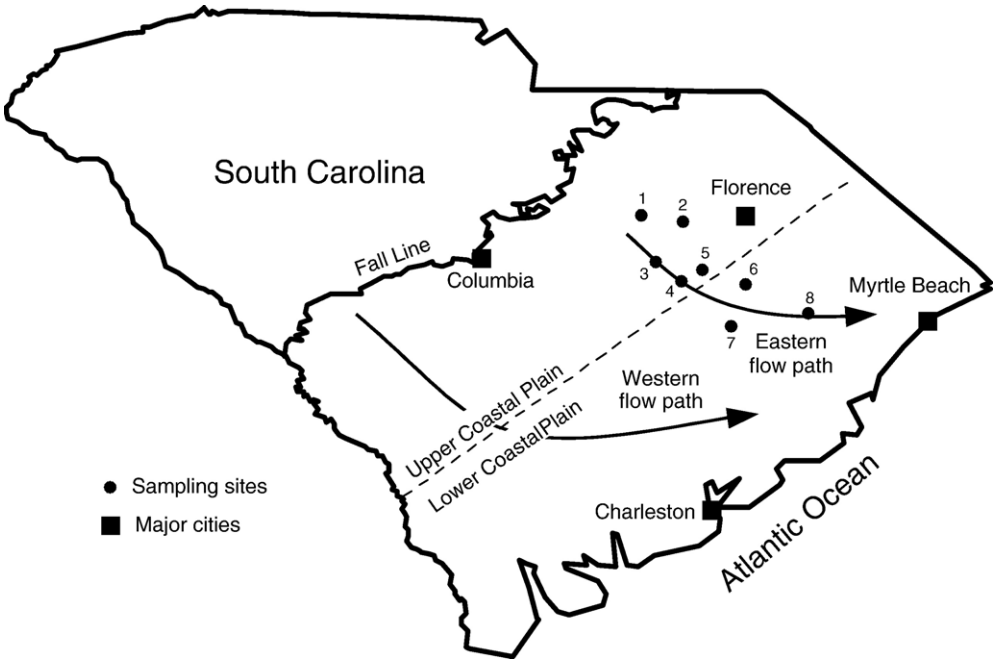

Fig. 1. Groundwater sampling sites (filled dots) and site numbers (Table 1) in the Middendorf aquifer, South Carolina. Arrows show groundwater flow paths in the eastern and western aquifer.

supplied by iron-reducing bacteria, then an iron-poor zone in which sulfate reducing bacteria are believed to predominate. In the latter zone, small amounts of dissolved methane accumulate in the groundwater, due to the action of aceticlastic or autotrophic hydrogen-oxidizing (i.e., hydrogenotrophic) methanogens, or both.

Microbial activity in the aquifer is believed to be supported by the breakdown of complex organic matter in fine-grained sediments through the action of fermenting bacteria. As the bacteria degrade the organic matter, they produce simple organic molecules, such as acetate and formate, and dihydrogen. These species migrate into aquifer sediments, where they serve as electron donors for respiring organisms. The microbiological zones reflect the distribution of electron accepting processes: oxygen respiration, reduction of ferric iron in the sediments, and sulfate reduction and methanogenesis. The net result of the non-methanogenic microbial activity is that each mole of reduced carbon in the initial organic matter becomes a mole of bicarbonate or carbon dioxide dissolved in groundwater as it is oxidized within the aquifer. Aceticlastic methanogens produce half a mole of CO_2 and half a mole of methane for each mole of carbon fermented, and hydrogenotrophic methanogens yield methane in a molar ratio of 1:3 to 1:2 to the CO_2 generated during fermentation, depending on the initial oxidation state of the carbon.

Some of the controls on zonation within the aquifer are clear. The aquifer along the upper Coastal Plain consists of non-marine sediments that contain abundant ferric iron and little calcite. Ferric iron is available here to serve as an electron acceptor and, once reduced, accumulates as ferrous iron in the groundwater. Along the lower Coastal Plain, the aquifer grades into marine sediments that contain little ferric iron but abundant calcite. Iron reduction in these sediments is limited, but the less energetically favorable processes of sulfate reduction and methanogenesis proceed. Sulfate reduction produces sulfide, which reacts with dissolved iron or ferrous iron in minerals to form sulfide minerals, stripping iron from solution. The transition from high-iron to low-iron groundwater, then, likely reflects the distribution of sedimentary facies within the aquifer.

Other aspects of the nature of the aquifer's zonation are less apparent, and a number of questions have not been completely addressed. These questions include:

- What are the roles of microbial activity and water–rock interaction in controlling the pH and major ion chemistry of groundwater in the aquifer?
- How does the chemical energy available in the aquifer to support microbial respiration and methanogenesis vary along the direction of flow? Does the amount of energy available affect the distribution of microbial activity?

- What is the limiting factor that controls the rate of microbial respiration and fermentation in the aquifer? Is it the supply of electron donors, the availability of electron acceptors, the availability of chemical energy, or the kinetic properties of the microbes themselves?
- Are the zones dominated by individual functional groups of microbes to the exclusion of other groups? Or, are more than one group active in the same place (Jakobsen and Postma, 1999; Postma and Jakobsen, 1996)? For example, are sulfate reducing bacteria active–or perhaps even predominant–in the high-iron zone, or do the iron-reducing bacteria effectively exclude their activity?

To better address these questions, we attempt in this paper to interpret the evolution of groundwater composition in the Middendorf aquifer along the eastern flow path of Aucott and Speiran (1985).

2. Hydrogeologic setting

The Middendorf aquifer, composed of late Cretaceous sediments, serves as an important water resource along the Atlantic Coastal Plain of South Carolina (Fig. 1). It overlies crystalline bedrock and the Cape Fear formation, and is covered by a wedge of Cretaceous and Tertiary sediments that increase to a depth of more than 1 km near the Atlantic Ocean (Aucott and Roy, 1986). The aquifer crops out along the Fall Line, where groundwater is recharged, and dips southeast. Groundwater flows to the southeast in the upper Coastal Plain and turns east in the lower Coastal Plain, where it discharges through overlying confining units to the land surface and ocean. As evidenced by the amount of ^{226}Ra in seawater, a considerable amount of groundwater discharges to the coastal ocean in South Carolina. The groundwater flux to these coastal waters is about 40% of the river-water flux (Moore, 1996). Near the recharge area, the groundwater is young and aerobic, and its velocity is relatively high, up to 20 m/year (Sargent and Fliermans, 1989). Downgradient from the recharge area, the groundwater is anaerobic and flows more slowly (Chapelle and Lovley, 1990).

The base of the Middendorf aquifer appears to be separated from the aquifer in the Cape Fear formation by an effective confining unit, because water quality and hydraulic head in the two units differ. The top of the Middendorf aquifer is confined by a sandy clay formation that is the lower part of the Black Creek formation, and the confining unit appears to be leaky (Aucott, 1988; Aucott and Speiran, 1985). In the middle to lower Coastal Plain, Middendorf wells are under flowing artesian conditions that may prevent groundwater influx from the upper aquifer (Aucott, 1988). Groundwater in the deep Middendorf aquifer, therefore, probably originates largely in the aquifer's recharge area, rather than entering from adjacent formations.

The depositional environment of the Middendorf aquifer has a significant effect on the geochemistry of groundwater. In the upper Coastal Plain, the Middendorf aquifer is composed of non-marine sediment containing a large amount of ferric oxyhydroxide. Iron reducing bacteria utilize the ferric iron and form a zone in which the concentration of ferrous iron in the groundwater is high. The sand fraction is primarily quartz and potassium feldspar with a lesser amount of plagioclase. The clay fraction consists of kaolinite, illite, and smectite (Chapelle and Lovley, 1992; Speiran and Aucott, 1994). The groundwater is generally soft, acidic, and low in dissolved solids. Dissolved inorganic carbon (DIC) here is largely biogenic, and the DIC concentration and pH increase along the direction of flow. Since silica makes up a large part of the dissolved constituents, the dissolution of silicate minerals appears to be an important geochemical process (Speiran and Aucott, 1994).

In the lower Coastal Plain, the Middendorf aquifer grades into marine sediments that contain little ferric oxyhydroxide but abundant calcite. Due to the limited availability of ferric iron, iron reducers do not seem to be active here and little ferrous iron is found in groundwater. The Cl concentration is small compared to seawater, indicating that seawater intrusion (Brown and Schoonen, 2004; Chapelle, 1993; Pucci et al., 1992) has had a limited effect on groundwater composition. The most important ions in the groundwater are sodium and bicarbonate (Lee and Strickland, 1988). Biogenic DIC increases more sharply here than in the non-marine sediments. The sediments are composed of fine to medium grained quartz, sodium rich marine clay minerals, shell material, and abundant organic matter (Speiran and Aucott, 1991). According to Speiran and Aucott (1994), the major processes affecting groundwater composition here are the dissolution of calcium carbonate and the exchange of calcium in solution for sodium absorbed on clay minerals. Since the Middendorf aquifer in the middle and lower Coastal Plain is more deeply buried and contains more saline groundwater than in the upper Coastal Plain, it is less commonly utilized as a water resource (Reihm, 2002).

3. Sampling and analysis

We sampled and analyzed groundwater along the eastern flow path of Aucott and Speiran (1985) from eight municipal and public water supply wells completed in the Middendorf aquifer. The wells range in location from the recharge area to the lower Coastal Plain (Fig. 1). The water supply well at Bishopville (Site number 1), within the aquifer's recharge area, was chosen as a reference point. Locations of the other sampling points (Table 1) are expressed in distance downgradient from this well. Wells from Bishopville through Woods Bay State Park (Sites 1–5) are located in the upper Coastal Plain, where sediments are largely non-marine; those from Lake City to Hemingway (Sites 6–8) are completed in the mostly marine sediments of the lower Coastal Plain. Groundwater chemistry depends on position within the Coastal Plain. Samples for the non-marine sediments have low sodium and chloride concentrations, whereas those taken from the marine sediments are relatively rich in these ions (Table 1).

Before sampling a well, we let the water flow until pH, Eh, temperature, and dissolved dioxygen readings became stable. These parameters typically stabilized within 30 min. For major and trace element analyses, we added 0.625 mL purified concentrated nitric acid to 125 mL HDPE sample bottles, which we filled with raw and filtered (0.2 μm) groundwater. After closing the cap without any headspace, we stored the sample at 4 °C for transport to the laboratory, where it was analyzed by inductive coupled plasma mass spectroscopy (ICP-MS). Unless noted, the nominal analytical precision for these and other wet chemical analyses is ±10%. To analyze

Table 1
Chemistry of groundwater samples from the Middendorf aquifer, SC[a]

	Bishopville	Lamar	Mayesville	Turbeville	Woods Bay State Park	Lake City	Kingstree	Hemingway
Site number	1	2	3	4	5	6	7	8
Latitude[b]	34.20725N	34.17354N	33.98555N	33.89227N	33.94509N	33.87266N	33.67254N	33.73144N
Longitude[b]	80.26966W	80.07062W	80.20647W	80.07795W	79.97951W	79.76547W	79.83630W	79.46163W
Distance (km)	0	12.6	23	39.3	40.8	64.8	81.5	94.4
Depth (m)	104		151	143		128	286	274
pH	4.93	5.53	6.35	7.18	6.64	8.4	8.11	8.45
Temperature	19.04	19.02	19.31	18.70	20.39	18.80	23.83	23.60
Conductivity (μS)	12.4	23.6	30.6	89.7	90	170	n/d[c]	761
Oxygen (mg/L)	7.65	0.30	0.47	0.26	0.30	0.14	0.3	0.45
Eh (mV)	440	324	265	149	194	66	63	259
Ammonium (mM)	0.52	0.73	0.98	0.83	0.29	0.18	0.48	2.22
Acetate (μM)	0.76	0.72	1.14	0.39	0.64	0.71	0.67	0.51
Formate (μM)	0.15	0.13	0.35	0.19	0.19	0.14	<0.1	0.15
Lactate (μM)	0.79	0.19	0.08	0.04	0.04	0.03	0.04	<0.03
Ferrous iron (μM)	<0.2	6.2	92.0	25	45.5	<0.2	0.27	<0.2
Methane (μM)	<0.01	0.058	0.11	0.084	0.52	0.21	0.60	2.3
DIC (mM)	0.6	0.68	0.75	1	0.9	1.4	3.45	6.8
DOC (ppm as C)	0.4	0.5	0.6	0.9	0.3	0.4	0.4	0.7
DOC (unfiltered)	0.4	0.4	0.5	0.9	0.4	0.5	0.6	0.7
Sulfate (μM)	10	82	85	86	71	104	400	79
Sulfide (μM)	0.21	0.39	0.17	<0.1	n/d[c]	0.25	n/d[c]	<0.1
Nitrate (μM)	4.1	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Aluminum (μM)	0.11	0.04	0.07	0.04	0.04	0.07	0.07	0.11
Bromide (μM)	0.13	0.24	0.18	0.1	0.16	0.16	2.1	2.3
Calcium (mM)	0.01	0.21	0.093	0.11	0.049	0.05	0.052	0.035
Chloride (mM)	0.14	0.17	0.11	0.056	0.11	0.14	0.99	0.96
Fluoride (mM)	<0.005	<0.005	<0.005	<0.005	<0.005	0.036	n/d[c]	0.095
Magnesium (mM)	0.009	0.025	0.056	0.083	0.048	0.023	0.018	0.015
Potassium (mM)	0.004	0.025	0.083	0.23	0.13	0.09	0.06	0.04
Silicon (mM)	0.16	0.19	0.47	0.59	0.41	0.46	0.39	0.25
Sodium (mM)	0.06	0.19	0.09	0.28	0.43	1.57	5.83	9.21
Total cations (mM)	0.08	0.46	0.42	0.73	0.70	1.73	5.96	9.3

[a] Sampling was performed in November, 2002.
[b] Latitude and longitude given using WGS84.
[c] n/d="not determined".

for dissolved organic carbon (DOC), we collected raw and filtered (0.2 μm) groundwater into 60 mL serum bottles without headspace, added 0.5 mL concentrated sulfuric acid, capped the bottle with a butyl stopper, and stored the bottles at 4 °C for later analysis. The organic carbon content of the samples was combusted at 625 °C using a catalyst and analyzed with a Shimadzu TOC-V CPH total organic carbon analyzer.

Certain potentially unstable parameters were determined or fixed at the field site. We measured temperature, conductivity, dissolved dioxygen, and oxidation–reduction potential using a HYDROLAB Datasonde 4A probe. We titrated the filtered groundwater with either hydrochloric acid or sodium hydroxide to an endpoint pH of 4.4 or 8.3. By reproducing the titration curve with a reaction model (Bethke, 1996), we calculated the amount of dissolved inorganic carbon in each sample. We determined ferrous iron concentration using the Ferrozine method (Gibbs, 1976) and sulfide concentration using methylene blue method (American Public Health Association, 1995). The detection limits for ferrous iron and sulfide were about 0.2 and 0.1 μM, respectively. Since sulfide concentrations fell near the detection limit, the precision of these measurements was not as high as for other analytes. To measure the concentration of nitrate, we reduced nitrate to nitrite using a Cd reduction column (Dorich and Nelson, 1984), formed a reddish purple azo dye with the nitrite, and measured absorbance with a spectrophotometer (American Public Health Association, 1995). We determined sulfate concentration using the turbidimetric method with barium chloride and ammonium concentration using the phenate method (American Public Health Association, 1995). The detection limit for sulfate by this method is about 5 μM.

To analyze for acetate, lactate, and formate, we collected 500 mL of filtered (0.2 μm) groundwater in a glass bottle and raised the pH to above 11 by adding 2 mL of 2 N KOH at the site. We capped the bottle without headspace and stored it at 4 °C for transport to the laboratory. Within 10 days of sampling, we freeze-dried 250 mL of the sample and reconstituted the dried sample with 2.5 mL 30% phosphoric acid (Chapelle and Lovley, 1990). This procedure concentrates the sample 100 times. We analyzed the concentrated samples using a Waters HPLC and a BioRad Aminex HPX-87H ion exclusion column heated to 60 °C. The eluent used for the analysis was 0.005 N H_2SO_4 and the concentration was determined by measuring absorbance at 210 nm using a UV absorbance detector. The detection limits for acetate, formate and lactate were about 0.1, 0.1, and 0.03 μM, respectively. Analytical precision for acetate, formate, and lactate concentrations above 0.5 μM was about ±20%. Formate and lactate were found in small concentrations, so their measurements were likely less precise than suggested by this uncertainty.

We sampled dissolved methane using a gas stripping method similar to that used by Chapelle et al. (1997) to sample dihydrogen. We set up a gas collection bottle through which groundwater flows at a rate greater than 250 mL/min, injected 30 mL of ultra pure nitrogen gas and let the gas equilibrate with the flowing groundwater for 30 min. We withdrew the equilibrated gas using a syringe and injected it into two 10 mL evacuated vials. We later determined the methane concentration in the gas using gas chromatography and a flame ionization detector. The detection limit for methane by this method is about 10 nM.

We collected all samples on a single field trip and therefore cannot report on the possibility of temporal variation in groundwater composition, and might occur, for example, from year to year. When we collected duplicate samples at a single wellhead, the concentrations measured for the samples agreed within the uncertainties cited above.

4. Results

Table 1 and Fig. 2 show the results of analyzing the groundwater samples. At the recharge area, represented by the Bishopville well, the Middendorf aquifer is exposed to the surface (Aucott and Speiran, 1985) and groundwater is saturated with atmospheric dioxygen. The groundwater quickly becomes anoxic as it migrates downgradient. Nitrate was low in concentration (4 μM) in the Bishopville sample, and was not detected at the other wells. The measured Eh was 440 mV near the recharge area and steadily decreased along the flow path, except at Hemingway. The pH of the groundwater at Bishopville was 4.9 and increased downgradient in non-marine sediment to 8.4 near the transition to marine sediment, then changed little. The dissolved inorganic carbon (DIC) concentration increased monotonously along the flow path in non-marine sediment, then more rapidly in the marine sediment.

Methane concentrations gradually increased along the flow path in the non-marine sediment, then more rapidly in the marine sediment. The sulfate concentration was about 10 μM at Bishopville, and near 90 μM for all samples but one (Kingstree) downgradient. Sulfide occurred at concentrations less than 0.4 μM in all samples and there was little regional trend in its value. The ferrous iron concentration was less than 0.2 μM at Bishopville and initially increased to as much

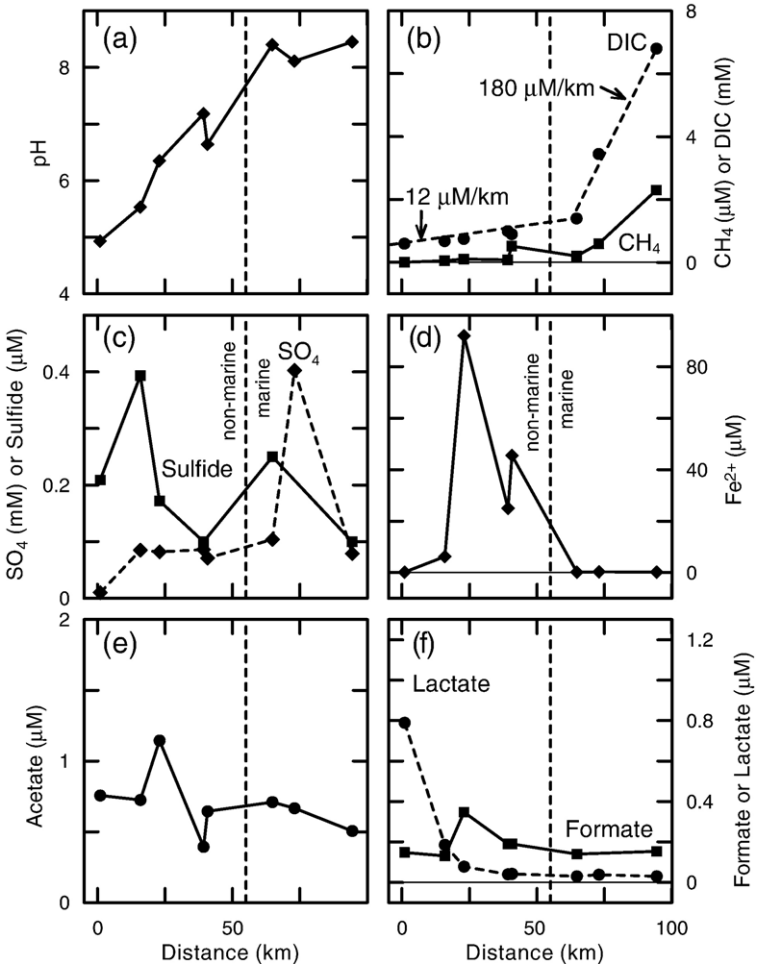

Fig. 2. Variation in pH and concentrations of dissolved inorganic carbon (DIC), methane, sulfate, sulfide, ferrous iron, acetate, formate, and lactate along the sampling traverse of the Middendorf aquifer.

as 90 µM within the non-marine sediments, before decreasing to less than 0.3 µM in the marine sediments. Ammonium concentrations were less than 1 mM, except at Hemingway.

There was little fluoride, bromide or chloride in water samples from the non-marine sediments, but the concentrations of these ions increased along the aquifer in the marine sediments. The concentrations of acetate, formate, lactate, and dissolved organic carbon (DOC) averaged 1 µM, 0.2 µM, 0.07 µM, and 0.7 ppm, respectively, along the entire flow path, with little variation.

5. Discussion

The chemical evolution of groundwater as it flows along the Middendorf aquifer might be expected to be affected by the activities of various functional groups of microbes, as well as chemical reaction of the water with the aquifer's minerals and organic matter. In the sections below, we consider the microbial and chemical processes that occur in the aquifer, and how they might interact to control the chemical evolution of groundwater there.

5.1. Role of microbial activity

The decay of organic matter in the Middendorf aquifer and its associated fine-grained layers, as already discussed, proceeds by the action of fermenting microbes on complex organic molecules. The fermentation produces simple compounds that are in turn oxidized by respiring microbes, or fermented further by methanogens. The initial fermentation step produces organic compounds (principally acetate and formate), dihydrogen, and dissolved carbon dioxide. We can

represent this process as the superposition of several reactions that consume carbohydrate CH_2O, a proxy for the organic matter. These reactions are

$$2CH_2O \rightarrow CH_3COO^- + H^+ \quad (1a)$$

$$CH_2O + H_2O \rightarrow HCOO^- + H^+ + H_2 \quad (1b)$$

$$CH_2O + H_2O \rightarrow 2H_2 + CO_2(aq) \quad (1c)$$

In addition, a minor fraction of the original complex organic matter degrades to a refractory component, liberating carbon dioxide and water. A fraction of the CO_2 produced by these reactions hydrolyzes and deprotonates

$$CO_2(aq) + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (2)$$

Hence, the initial degradation of organic matter produces carbonate and acid, and works to lower pH in the aquifer.

The acetate, formate, and dihydrogen produced by Reactions (1a), (1b) and (1c) are consumed in the aquifer by respiring microorganisms and methanogens. Table 2 shows the most significant of these reactions, including aerobic respiration, the reduction of iron from several ferric iron-bearing minerals (hematite, goethite, and magnetite), sulfate reduction, and hydrogenotrophic and aceticlastic methanogenesis. Each reaction in Table 2 is a combination of an electron donating half-reaction, such as acetate oxidation

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2(aq) + 7H^+ + 8e^- \quad (3)$$

and an electron accepting half-reaction, such as dioxygen respiration

$$8e^- + 2O_2(aq) + 8H^+ \rightarrow 4H_2O \quad (4)$$

which represents the process of aerobic respiration.

In the Middendorf aquifer, acid–base reactions are controlled by reaction of the carbon dioxide–bicarbonate conjugate pair (Reaction (2)), which serves as a pH buffer. In considering the Middendorf aquifer, then, the reactions in Table 2 are more properly written in terms of Reaction (2) rather than the production and consumption of hydrogen ions. The reaction for the oxidation of acetate by aerobic respiration, for example, is the sum of Reactions (3) and (4), expressed in terms of

Table 2
Chemical reactions and associated standard free energy change $\Delta G°$ for oxygen respiration, iron reduction, sulfate reduction, and methanogenesis, calculated using dihydrogen (H_2), acetate (CH_3COO^-), and formate ($HCOO^-$) as electron donors, in terms of the transfer of eight electrons

	$\Delta G°$ (kJ/mol)[a]
Aerobic respiration	
$4H_2(aq) + 2O_2(aq) \rightarrow 4H_2O$	−184.45
$CH_3COO^- + 2O_2(aq) \rightarrow HCO_3^- + H^+$	−146.74
$4HCOO^- + 2O_2(aq) \rightarrow 4HCO_3^-$	−171.22
Iron reduction (hematite, Fe_2O_3)	
$4H_2(aq) + 4Fe_2O_3 + 16H^+ \rightarrow 8Fe^{2+} + 12H_2O$	−667.59
$CH_3COO^- + 4Fe_2O_3 + 17H^+ \rightarrow 8Fe^{2+} + 2CO_2 + 10H_2O$	−524.94
$4HCOO^- + 4Fe_2O_3 + 20H^+ \rightarrow 8Fe^{2+} + 4CO_2 + 12H_2O$	−737.09
Iron reduction (goethite, FeOOH)	
$4H_2(aq) + 8FeOOH + 16H^+ \rightarrow 8Fe^{2+} + 16H_2O$	−689.52
$CH_3COO^- + 8FeOOH + 17H^+ \rightarrow 8Fe^{2+} + 2CO_2 + 14H_2O$	−546.87
$4HCOO^- + 8FeOOH + 20H^+ \rightarrow 8Fe^{2+} + 4CO_2 + 16H_2O$	−759.02
Iron reduction (magnetite, Fe_3O_4)	
$4H_2(aq) + 4Fe_3O_4 + 24H^+ \rightarrow 12Fe^{2+} + 16H_2O$	−904.24
$CH_3COO^- + 4Fe_3O_4 + 25H^+ \rightarrow 12Fe^{2+} + 2CO_2 + 14H_2O$	−761.60
$4HCOO^- + 4Fe_3O_4 + 28H^+ \rightarrow 12Fe^{2+} + 4CO_2 + 16H_2O$	−973.74
Sulfate reduction	
$4H_2(aq) + SO_4^{2-} + 2H^+ \rightarrow H_2S(aq) + 4H_2O$	−303.12
$CH_3COO^- + SO_4^{2-} + 3H^+ \rightarrow H_2S(aq) + 2CO_2 + 2H_2O$	−160.47
$4HCOO^- + SO_4^{2-} + 6H^+ \rightarrow H_2S(aq) + 4CO_2 + 4H_2O$	−372.62
Methanogenesis	
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−193.76
$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	−51.11
$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O$	−263.26

Notes to Table 2:
[a] Standard free energies calculated for standard conditions (1 atm, 25 °C) using the Lawrence Livermore National Laboratory thermodynamic dataset thermo.com.v8.r6+.dat (Delany and Lundeen, 1990).

the carbonate buffer. The reaction produces acid and is written

$$CH_3COO^- + 2O_2(aq) \rightarrow CO_2(aq) + HCO_3^- + H_2O \quad (5)$$

This reaction will drive pH toward about 6.2, the pH at which CO_2 and HCO_3^- are present at equal concentration. Aerobic respiration can proceed in the Middendorf, of course, only near the recharge area where dissolved dioxygen is present.

By similar reasoning, the reaction for the oxidation of acetate by iron reduction is

$$CH_3COO^- + 8FeOOH(goethite) + 15CO_2(aq) + 3H_2O \rightarrow 17HCO_3^- + 8Fe^{2+} \quad (6)$$

This reaction serves to increase pH by increasing the ratio of HCO_3^- to CO_2(aq) in the aquifer. Similarly, acetate oxidation by sulfate reduction proceeds according to

$$CH_3COO^- + SO_4^{2-} + CO_2(aq) + H_2O \rightarrow 3HCO_3^- + H_2S \quad pH<7 \quad (7a)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad pH>7 \quad (7b)$$

depending on whether sulfide is present dominantly as dihydrogen sulfide or bisulfide. These reactions will increase pH to values no greater than about 8.

Reaction (6) predicts that acetate oxidation by ferric iron (hematite or goethite) produces four moles of ferrous iron per mole of carbonate generated (4:1 ratio). Using magnetite as the oxidant results in a 6:1 ratio. Observations in the Middendorf aquifer (Fig. 2), however, show that whereas carbonate concentrations in the groundwater increase about 400 μM along the non-marine interval, where iron reduction is believed important, ferrous iron concentrations in this section never exceed 90 μM. Even if some of the carbonate is derived from the degradation of complex organic matter to produce refractory matter, less iron accumulates in the aquifer than suggested by Reaction (6).

The relatively small amount of iron relative to carbonate in groundwater from the non-marine sediments can be explained if iron precipitates in either oxide or sulfide form, if sulfate rather than iron reduction accounts for much of the carbonate production, or by some combination of these factors. The iron may be taken up during the precipitation of clay minerals within the aquifer (Chapelle, 2001). We can write

$$Fe^{2+} + 2HCO_3^- \rightarrow FeO(ox) + H_2O + 2CO_2(aq) \quad (8)$$

where FeO(ox) represents the ferrous oxide component of an arbitrary clay mineral, such as from the smectite group, members of which can include up to 17% ferrous iron by weight when they precipitate (Badaut et al., 1985; Bischoff, 1972; Craw, 1984; Craw et al., 1995).

Reaction (8) produces two moles of acid per mole of ferrous iron, counteracting the acid consumed by iron reduction (Reaction (6)). Thus when ferrous iron precipitates as an oxide component in clay, the reaction becomes:

$$CH_3COO^- + 8FeOOH(goethite) \rightarrow 8FeO(ox) + CO_2(aq) + HCO_3^- + 5H_2O \quad (9)$$

This reaction drives pH toward about 6.2, the pH at which CO_2 and HCO_3^- are present at equal concentration.

Reactions (7a) and (7b) suggest sulfate reduction will liberate sulfide, but very little H_2S is found in groundwater from the Middendorf (Fig. 2c). The likely reason for the lack of dissolved sulfide is that it precipitates by reacting with ferrous iron. Mackinawite (FeS), for example, might form by the reaction

$$Fe^{2+} + H_2S + 2HCO_3^- \rightarrow FeS + 2CO_2(aq) + 2H_2O \quad (10)$$

In case of the marine sediment, where little iron reduction is expected, ferrous iron in the sediment might react with sulfide

$$FeO(ox) + H_2S \rightarrow FeS + H_2O \quad (11)$$

to form mackinawite. Mackinawite in nature is converted to pyrite (FeS_2) over time (Wersin et al., 1991),

$$FeS + H_2S \rightarrow H_2 + FeS_2 \quad (12)$$

Combining Reactions (6), (7a), (10), and (12), the combined effects of iron and sulfate reduction to produce pyrite can be represented by

$$17CH_3COO^- + 8FeOOH + 16SO_4^{2-} + 3H_2O + 15CO_2(aq) \rightarrow 8FeS_2 + 8H_2 + 49HCO_3^- \quad (13)$$

where the dihydrogen produced is available to drive a subordinate further amount of microbial activity. Where the sulfide reacts with ferrous oxide in clay minerals, the sulfate reduction reaction can be written

$$16CH_3COO^- + 8FeO(ox) + 16SO_4^{2-} + 8H_2O + 16CO_2(aq) \rightarrow 8FeS_2 + 8H_2 + 48HCO_3^- \quad (14)$$

The reactions add carbonate but not iron to solution.

These reactions not only consume acid but augment the bicarbonate buffer. Since the reaction adds bicarbonate faster than it consumes acid, it drives pH toward about 8. Interestingly, for each electron transferred to reduce iron in Reaction (13), 16 electrons are taken up to reduce sulfate. From the perspective of redox chemistry, as well as from that of carbon oxidation, the sulfate reduction reaction dominates.

Small amounts of methane are observed to accumulate in the marine sediments. The reaction for hydrogenotrophic methanogenesis

$$4H_2 + 2HCO_3^- \rightarrow CH_4 + CO_3^{2-} + 3H_2O \quad (15)$$

increases pH. The reaction for aceticlastic methanogenesis

$$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4 \quad (16)$$

drives pH toward 8. The small concentrations of methane found in Middendorf groundwater may indicate that relatively little methanogenesis occurs, or that the methane produced is subsequently oxidized by an anaerobic mechanism (Grossman et al., 2002; van Breukelen et al., 2003; Zhang et al., 1998), perhaps using sulfate as the electron acceptor. In the latter case, methane is an intermediate in the overall reaction, and the net effect is the same as already described for sulfate reduction. The effect of methanogens on groundwater chemistry, then, is likely subordinate to that of other functional groups of microbes.

Table 3 summarizes acid production during the initial fermentation and subsequent aerobic respiration, iron reduction, sulfate reduction, and methanogenesis in the aquifer system (the combined fine-grained and coarse-grained layers). We assume in this summary that the ferrous iron and sulfide liberated by iron and sulfate reduction precipitate, as already described.

Aerobic respiration produces acid (Table 3), which might explain the low pH of 4.93 observed near the recharge area. If all of the CO_2 observed in water at the recharge area, about 0.6 mM, were derived from aerobic respiration, the respiration would indeed be capable acidifying water from pH 7 to pH 4.9. The low pH there might alternatively be explained by the acid produced when pyrite oxidizes, but the lack of sulfate in the water indicates that this reaction has not proceeded to an extent sufficient to explain the pH observed.

Table 3
Acid production (mol) by fermentation, respiration, and methanogenesis per mole of carbon in the initial organic matter consumed[a]

Electron accepting reaction	Electron donor	Acid produced by fermentation	Acid produced by respiration or methanogenesis	Total acid produced
Aerobic respiration	H_2	1	0	1
	Acetate	1/2	1/2	1
	Formate	1	0	1
Iron reduction[b]	H_2	1	0	1
	Acetate	1/2	1/2	1
	Formate	1	0	1
Sulfate reduction[c]	H_2	1	−1	0
	Acetate	1/2	−1/2	0
	Formate	1	−1	0
1 Iron+16 sulfate reduction	H_2	1	−16/17	1/17
	Acetate	1/2	−15/34	1/17
	Formate	1	−16/17	1/17
Methanogenesis	H_2	1	−1/2	1/2
	Acetate	1/2	0	1/2
	Formate	1	−1/2	1/2

[a] Acid production during initial fermentation calculated according to Reactions (1a–c), and during subsequent respiration and methanogenesis according to the reactions in Table 2, combined with iron oxide or sulfide precipitation reactions (Reactions 8, 10, and 12). Since by Reaction (1b) formate production also yields dihydrogen, the calculations for formate account for the combined effects of formate and dihydrogen respiration. We assume that ferrous iron produced precipitates as oxide or sulfide, and that sulfide generated reacts with ferrous iron to produce pyrite.

[b] Reduction of ferric iron in the form of goethite; calculations include methane produced and subsequently oxidized by iron reduction.

[c] Includes methane produced and subsequently oxidized by sulfate reduction.

The results show that the expected effect in the aquifer system of iron reduction alone, in the absence of sulfate reduction and methanogenesis, is to decrease pH. In contrast, sulfate reduction alone, in the absence of iron reduction, adds bicarbonate to solution, driving pH toward about 8. The combined processes of iron and sulfate reduction to produce pyrite leads to the net generation of only a small quantity of acid (Table 3). The initial fermentation to produce refractory organic matter, as already described, also produces a small amount of CO_2, which works to decrease pH. None of these microbial processes, then, can fully account for the observed increase in pH along the aquifer to values above 8.

5.2. Role of mineral reactions

The various microbial metabolisms can be expected to increase the carbonate content of Middendorf

groundwater, and each process except sulfate reduction in the absence of iron reduction leads to a net production of acid. The CO_2 added to solution promotes the weathering of framework aluminosilicate minerals such as albite ($NaAlSi_3O_8$) to produce silica (SiO_2) and clay minerals such as kaolinite ($Al_2Si_2O_5(OH)_4$)

$$NaAlSi_3O_8 + CO_2(aq) + 1.5H_2O \rightarrow Na^+ + 1/2Al_2Si_2O_5(OH)_4 + 2SiO_2(s) + HCO_3^- \quad (17)$$

Microbial CO_2 production drives Reaction (17) to the right, causing the transformation of framework aluminosilicate to clay minerals. Saturation indices calculated from the observed groundwater chemistry (Table 1) confirm that the feldspars albite and anorthite are undersaturated, whereas the clay minerals kaolinite, beidellite, and montmorillonite are supersaturated across all or most of the aquifer (Fig. 3). As already noted, smectite group minerals such as saponite, beidellite and montmorillonite form can incorporate ferrous iron in their structures and hence may represent a sink for the ferrous iron generated by iron reduction.

In the marine sediments, there is a 1:1 relationship between sodium and bicarbonate (Fig. 4) with little change in pH (Fig. 2a). Speiran and Aucott (1994) suggested calcite dissolution and exchange between calcium- and sodium-clay would lead to this type of relationship. The addition of CO_2 drives the dissolution of calcite ($CaCO_3$)

$$CO_2(aq) + H_2O + CaCO_3 \rightarrow Ca^{2+} + 2HCO_3^- \quad (18)$$

The calcium produced exchanges for sodium by ion-exchange with clay minerals:

$$Na_2 - Clay + Ca^{2+} \rightleftarrows Ca - Clay + 2Na^+ \quad (19)$$

In Reactions (17) and (18), mineral dissolution serves to consume acid, increasing the pH. As these reactions proceed, the concentration of sodium in the groundwater also increases. Other calcium-bearing minerals such as anorthite in the aquifer sediments are undersaturated (Fig. 3a) and likely dissolve across most of the aquifer. Thus, the calcium concentration might similarly be expected to increase. Calcium concentration, however, decreases along the flow path (Table 1). This decrease likely results from ion exchange shown in Reaction (19).

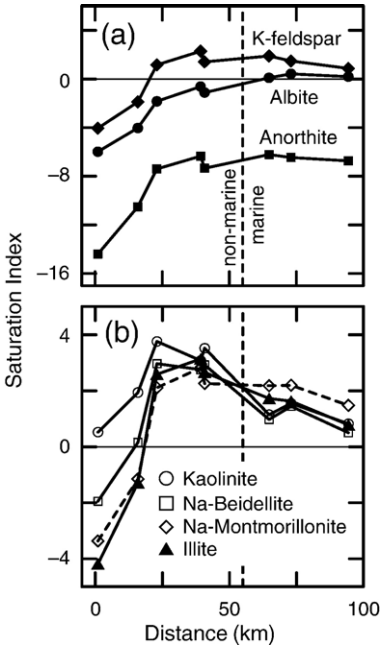

Fig. 3. Saturation indices of (a) feldspars and (b) clay minerals calculated from the chemical compositions of the groundwater samples (Table 1). Calculations made using the Lawrence Livermore National Laboratory thermodynamic dataset "thermo.com.v8.r6+.dat" (Delany and Lundeen, 1990).

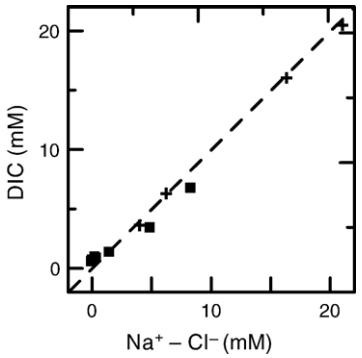

Fig. 4. Relationship of the difference in sodium and chloride concentrations (Na^+–Cl^-) to the dissolved inorganic carbon (DIC) content of groundwater from the Middendorf aquifer. Filled squares are measured data from this study; pluses are data of Speiran and Aucott (1994).

5.3. Availability of energy

To understand the controls on microbial zonation in the Middendorf aquifer, it is instructive to consider the availability of chemical energy to the various functional groups of microbes. The aquifer is generally oligotrophic, and microbes cannot live and grow where the aquifer lacks sufficient energy to support their metabolism. A zonation of energy availability in the aquifer, therefore, might help explain the origin of zoned microbial activity. Furthermore, energy availability represents a possible means for one microbial group to exclude another from its niche. Iron reducing bacteria,

for example, compete with sulfate-reducing bacteria for the same electron donors, but for a given donor species iron reducers derive more energy than sulfate reducers. The iron reducers, then, might be able to preclude growth of sulfate reducing bacteria by keeping donor concentrations too low for the sulfate reducers to derive the energy they need to live.

The minimum energy yield at which a functional group of microorganisms can grow is referred to as the group's threshold energy, and the corresponding amount of electron donors is its threshold concentration. Microbes have been observed in the laboratory and field to metabolize their substrates only as long as the energy available exceeds their threshold (Hoehler et al., 1998, 2001; Lovley and Goodwin, 1988; Westermann, 1994). Typical threshold energies for iron reducers, sulfate reducers, and methanogens have been observed to be about 20 kJ, when the metabolic reaction is written in terms of an eight electron transfer, which corresponds to the consumption of one mole of acetate, four moles of formate, or four moles of dihydrogen. (Hoehler et al., 2001; Liu et al., 2001).

We calculated the free energy available in the Middendorf aquifer to aerobic bacteria, iron reducing bacteria, sulfate reducing bacteria, and methanogens. The free energy change ΔG of a chemical reaction is given by the equation

$$\Delta G = \Delta G^{\circ} + RT\ln\ (\mathrm{IAP}) \qquad (20)$$

where $\Delta G°$ is the free energy change under standard conditions, R is the gas constant, T is absolute temperature, and IAP is the reaction's ion activity product. To calculate the energy liberated by a chemical reaction, then, we need to know the reaction's standard free energy change and the activities of the species appearing in the chemical reaction.

Table 2 shows values of $\Delta G°$ for the different functional groups of microbes, calculated for the consumption of dihydrogen, acetate, and formate, the three most significant electron donors in the Middendorf aquifer (McMahon and Chapelle, 1991). A number of minerals, including hematite, goethite, magnetite, and various ferric oxyhydroxides can serve as electron acceptors for iron reducing bacteria. We balanced the reactions in Table 2 in terms of goethite, which is energetically more favorable than hematite and more likely than magnetite to serve as an important electron acceptor in the Middendorf aquifer. We assumed in the calculation that minerals in the reactions are pure phases, and hence at unit thermodynamic activity.

We used the React speciation model (Bethke, 2004) to calculate at each well the concentrations and activities of a number of species involved as reactants or products in the reactions shown in Table 2, including acetate, formate, bicarbonate, ferrous iron, sulfate, bisulfide, and methane. We assumed dihydrogen concentrations near the recharge area, in the non-marine sediments, at the boundary between non-marine and marine sediments, and in the marine sediments of 0.5, 1, 2, and 3 nM, respectively, which are typical of dihydrogen concentrations measured from groundwater pumped there (Chapelle and Lovley, 1992). We used in our calculations the hydrogen ion activity corresponding to the measured pH. To calculate activity coefficients, the speciation model employed the B-dot equation (Helgeson and Kirkham, 1974), an extended form of the Debye–Hückel equation. From these results, we were able to calculate at each well the free energy changes of each reaction in Table 2, according to Eq. (20).

The most significant factor controlling the variation in energy availability along the flow path is the change in pH, since a unit change in this variable corresponds to an order of magnitude variation in H^+ activity. The concentrations of the electron donors play a smaller role, since these values do not vary as widely. Error in measuring electron donor concentration in our samples, which can safely be assumed to be correct within a factor of two, therefore, little affects the calculations. Reducing hydrogen or formate concentration from 1 nM to 0.5 nM, or increasing it to 2 nM leads to a change in energy availability of only about 7 kJ per eight electron transfer. Halving or doubling the concentration of acetate changes the energy by only about 1.75 kJ.

At Bishopville, the site near the recharge area and the only well producing oxic water, the energies available for aerobic metabolism using dihydrogen, acetate, and formate are 791, 833, and 878 kJ per eight electron transfer, respectively. Fig. 5 shows the energy available along the groundwater flow path to anaerobes: iron reducing bacteria, sulfate reducing bacteria, and methanogens. Energy available to these functional groups in the non-marine sediments decreases along the direction of flow, whereas there is little systematic variation in energy availability in the marine sediments. The calculations suggest that the energy available to iron reducers and sulfate reducers is greater than 35 kJ per 8 electrons transferred everywhere in the aquifer. Sufficient energy is available as well to acetate and formate utilizing methanogens everywhere along the aquifer. Only the hydrogenotrophic methanogens lack sufficient energy to grow.

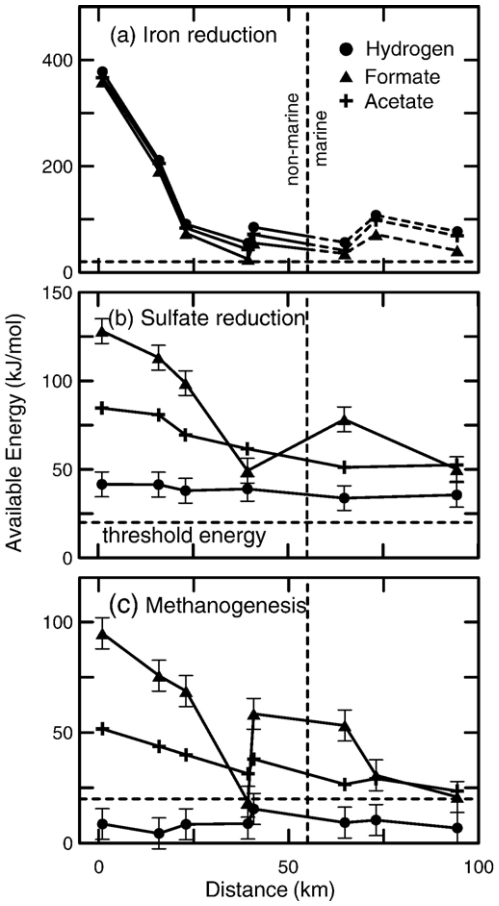

Fig. 5. Calculated thermodynamic energies available to anaerobes using hydrogen, acetate, or formate as an electron acceptor. Plots show results for (a) iron reduction utilizing goethite, (b) sulfate reduction, and (c) methanogenesis. The energies available for aerobic metabolism using dihydrogen, acetate, and formate at the recharge area (Bishopville) are 791, 833, and 878 kJ per eight electron transfer, respectively. The available thermodynamic energy is the negative value of free energy change of a reaction. Corresponding chemical reactions and their standard free energies are listed in Table 2. Error bars show range of values calculated for the hypothetical case in which the concentration measured for the electron donor species is correct only to within a factor of two (some error bars are smaller than marker symbols used and not shown). Horizontal dotted lines represent threshold energy, which is about 20 kJ/mol for the reactions as written in Table 2.

At Bishopville, large amounts of energy are available to drive aerobic respiration and iron reduction, and somewhat lesser amounts for sulfate reduction and methanogenesis. Aerobic respiration is nearly certain to be active here and it is not clear whether iron reduction plays a subordinate role. Iron reducers were found everywhere along the aquifer, including Bishopville, but direct evidence of iron reduction, the accumulation of ferrous iron in the water, cannot be preserved in the presence of dioxygen. Sufficient energy is available for sulfate reducers and the non-hydrogenotrophic methanogens to grow, but this in unlikely since the activity of these functional groups is suppressed by the presence of dioxygen (Mah and Smith, 1981; Pfennig et al., 1981).

In the high-iron zone, there is no evidence that iron reducers suppress sulfate reducing activity by holding the energy available to that group below its energy threshold. This result suggests the possibility that sulfate reduction could be a significant or even dominant process in the high iron zone, contrary to some previous interpretations (e.g., Chapelle and Lovley, 1992). Those authors demonstrated the coexistence of iron and sulfate reducers along the flow path but, on the basis of the distribution of dihydrogen, organic acids, and ferrous iron, preferred to interpret the aquifer as composed of exclusionary microbial zones.

The thermodynamic calculations show that energy available to methanogens is generally less than that available to iron reducing or sulfate reducing bacteria, except locally for hydrogen-utilizing sulfate reducers (Fig. 5c). Insufficient energy is available, furthermore, for hydrogenotrophic methanogens to grow; such growth would require dihydrogen levels roughly four times as high as observed in the aquifer. These results are consistent with the small amount of methane (2 μM at Hemingway) observed to accumulate in Middendorf groundwater. Methane concentration along the flow path increases more rapidly in the marine than the non-marine sediment. Better preservation of organic matter in the marine than the non-marine sediments (Hartog et al., 2004, 2005) may have allowed local zones relatively rich in electron donors, but depleted in sulfate, to form. Methanogenesis would be favored in these isolated areas, explaining the methane production observed.

The distribution of energy in the aquifer as a whole lends little support to the idea that functional groups of microbes are sequestered into segregated zones by competitive exclusion, i.e., by holding energy levels too low for other groups to grow. The only exception noted is the hydrogenotrophic methanogens, which are effectively excluded from much of the aquifer by the absence of available energy due to competition from other functional groups.

5.4. Electron donor concentrations

In many cases, the concentration of electron donors in groundwater has been found to correspond to the terminal electron accepting process (or TEAP), such as aerobic respiration, iron reduction, or sulfate reduction, inferred to be predominant in the aquifer (Lovley and Chapelle, 1995). The correspondence between the

concentration of species such as acetate, formate, and dihydrogen to the predominant TEAP is believed to be related to the energetic yield of the electron transfer reaction. For TEAPs such as aerobic respiration and iron reduction that yield abundant energy, microbes can drive electron donor concentrations to low levels. For less energetically favorable TEAPs such as sulfate reduction, however, substrate concentrations must remain at higher levels in order to maintain a sufficient energy yield (Hoehler et al., 1998).

Where the availability of electron donors is limited, microbial activity may drive their concentrations toward threshold values characteristic of the predominant TEAP. This simple relationship, a commonly cited and useful means for rapidly characterizing subsurface microbiological communities, is not always observed to hold. For example, acetate concentration in groundwater does not show a clear relationship to the associated functional group of microorganism (Christensen et al., 2000; Hansen et al., 2001; Kuivila et al., 1989; Vroblesky et al., 1997; Westermann, 1994).

In the Middendorf aquifer, Chapelle and Lovley (1992) reported that acetate and formate concentrations in pore water extracted from sediment samples are lower in the high-iron zone, where they found about 1 μM of acetate and 2 μM of formate, than in the low-iron zone, which contained about 2.5 μM acetate and 11 μM formate. They noted that these concentrations might represent threshold concentrations for iron and sulfate reduction. In contrast to their results, our measured concentrations of acetate, formate, and lactate in groundwater vary little along the aquifer, regardless of zone (Table 1; Fig. 2). As well, we found acetate and formate at somewhat lower concentrations, generally less the 1 μM, than they did.

This discrepancy may simply reflect differences in sampling. We sampled groundwater from water supply wells, whereas Chapelle and Lovley (1992) collected pore water by squeezing cored sediments. The squeezing was powerful enough to extract water from the clay portion of the sediment, and the composition of this water might differ from groundwater in the coarse grained sediments. Chapelle and Bradley (1996), for example, reported that the concentration of organic acids in pore water squeezed from a sediment sample was considerably higher than in water sampled from a nearby well. Water from the clay fraction of a sediment is likely to be richer in organic acids than the sand fraction, and since marine sediments in the Middendorf aquifer contain more clay than the non-marine sediments (Speiran and Aucott, 1994), it is not surprising that Chapelle and Lovley's (1992) results are not reflected in our measurements.

The concentrations of acetate, formate, and lactate in our samples do not vary systematically from zone to zone along the flow path, as might be expected if each zone were dominated by a single functional group of microbes. The similarity in organic acid concentrations between the high and low iron zones of the Middendorf can be explained in two ways. As already noted, organic acid concentrations may poorly reflect the microbial activity in the aquifer. Or, the various zones may not be dominated by a single functional group of microbes, but instead host a blend of metabolisms. The fact that organic acids do not accumulate along the flow path, furthermore, suggests that the rate at which the initial fermentation supplies electron donors to the aquifer controls the rate of microbial respiration there. Initial fermentation has been identified as a factor limiting the rate of microbial respiration in other environments as well (Boudreau and Ruddick, 1991; Middelburg, 1989; Postma and Jakobsen, 1996; Westrich and Berner, 1984).

5.5. Rates of microbial respiration

Since microbial respiration except hydrogenotrophic methanogenesis produces dissolved inorganic carbon (DIC) (Reactions (6), (7a) and (7b)), the rate of accumulation of biogenic DIC in the Middendorf allows us to calculate the overall rate of microbial activity. In the non-marine sediments, the $\delta^{13}C$ of the DIC indicates that major source of carbonate is the decay of organic matter (Chapelle and Lovley, 1992; Speiran and Aucott, 1994). The DIC concentration increases monotonously along the flow path at a rate of about 15 μM/km (Fig. 2b). The velocity of groundwater in the aquifer is about 0.4 to 1 m/year (Aucott, 1988; Chapelle and Lovley, 1990), which gives a biogenic rate of DIC production in the non-marine sediments in the range 6 to 15 nM/year.

The rate of DIC accumulation in the aquifer increases sharply near the boundary between the non-marine and marine sediments (Fig. 2b), apparently due to a greater rate of DIC production in the marine section. The $\delta^{13}C$ of DIC here indicates that carbonate is derived from both the dissolution of carbonate minerals and the decay of organic matter (Chapelle and Lovley, 1992; Speiran and Aucott, 1994). According to Reaction (18), when biogenic carbon dioxide reacts with calcite, the resulting bicarbonate is one-half biogenic and one-half inorganic. The $\delta^{13}C$ of DIC, however, suggests that somewhat more than half the bicarbonate in the aquifer comes from inorganic sources (Speiran and Aucott, 1994),

apparently as the result of isotopic exchange between bicarbonate in groundwater and shell material in sediment (Clark and Fritz, 1997; Gonfiantini and Zuppi, 2003). Assuming that half of the DIC added to groundwater in the marine sediment was originally of biogenic origin, biogenic DIC increases at a rate of about 90 μM/km and rate of biogenic DIC production is in the range 35 to 90 nM/year.

Chapelle and Lovley (1990), for comparison, estimated the rate of biogenic DIC production in the Middendorf aquifer using a somewhat more involved inverse geochemical modeling approach. They estimated the production rate from an interval spanning non-marine and marine sediments as 30 nM/year, a value similar to our estimate. In the marine sediment, their estimated rate of DIC production ranged from 6.6 to 29 nM/year, somewhat lower but similar in magnitude to our estimate.

According to our estimates, the rate of biogenic DIC production in the marine sediment is about 7.5 times higher than in the non-marine sediment. This ratio may be somewhat of an overstatement, since flow velocity in the aquifer likely diminishes gradually along the flow path, as the result of groundwater leakage through the upper confining layer. A possible explanation for the apparently higher rate of biogenic carbonate production in the marine section may be that organic matter there has been better preserved during sedimentation and diagenesis there than in the non-marine sediments, and hence is more readily degradable (Hartog et al., 2004, 2005). In this case, the initial fermentation might proceed more rapidly in the marine sediments, allowing more rapid respiration of the fermentation products in the aquifer. McMahon et al. (1990), for example, reported that DIC production in their incubation experiments using marine sediment was about six times higher than those using non-marine sediment.

5.6. The electron gap

Since the net rate of methanogenesis is relatively small in the aquifer, we can estimate the number of electrons donated to microbial respiration from the increase of biogenic DIC along the flow path, because each carbonate species has lost about four electrons during the oxidation of carbon atoms from the sediment's organic matter. In making this estimate, we assume that relatively little of the carbonate in the groundwater was produced directly during the initial fermentation. If the ferrous iron and sulfide produced by iron and sulfate reduction were to remain in solution, we could similarly calculate from their concentrations the number of electrons accepted (one electron acceptance per ferrous iron species, eight per sulfide species). In this case, we would expect the number of electrons donated to balance the number accepted.

When the biogenic DIC, ferrous iron, and sulfide concentrations in the Middendorf groundwater are plotted in terms of electrons donated or accepted (Fig. 6), we see that only a small proportion of the electrons donated during carbon oxidation can be accounted for by the reaction products of iron and sulfate reduction. The difference in the accounting between electrons donated and accepted is the "electron gap". The ferrous iron content of the groundwater, in fact, can account for only about 15% of the increase in biogenic DIC, or 10% of the increase, if magnetite is the source of ferric iron; the groundwater's sulfide content represents a negligible fraction of the electron transfer inferred. Most of the ferrous iron produced during respiration likely precipitated within clay minerals or as iron sulfide, and whatever sulfide was generated apparently precipitated as sulfide minerals, as discussed earlier.

The major ion chemistry of the groundwater, therefore, records an estimate of the net amount of microbial respiration that has occurred, but provides little direct information about which TEAPs were involved. In the high-iron zone, for example, we can explain the groundwater chemistry there by iron reduction alone, with most of the resulting ferrous iron precipitating within clay minerals. Alternatively, since sulfate reduction can oxidize up to 16 times more carbon than iron reduction without producing excess sulfide, as discussed in Section 5.1, we can explain the geochemistry in terms sulfate reduction accompanied by iron reduction sufficient to precipitate the sulfide produced.

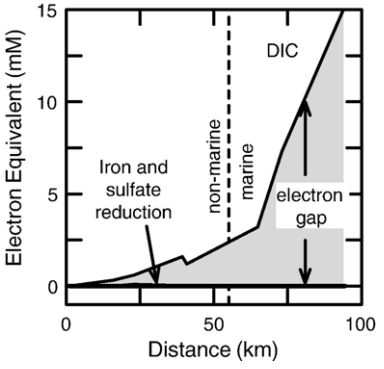

Fig. 6. The "electron gap" between the number of electrons donated, as determined from biogenic DIC content of Middendorf groundwater, and those accepted, as accounted for by the ferrous iron and sulfide in solution.

The iron-rich groundwater in the non-marine sediments, therefore, may represent the product of microbial respiration in which sulfate reduction accounts for a little as none or as much as about 90% of the total electron transfer. We might reasonably consider sulfate reduction to account for 90% of the total electron transfer in the high iron zone, and 100% in the low iron zone. In this case, there would not be any significant difference in sulfate reducing activity between the zones, despite a marked difference in groundwater composition.

5.7. Controls on microbial zonation

Some controls on the origin of the zonation of microbial activity in the Middendorf aquifer, as noted by Chapelle and Lovley (1992), are clear. Aerobic respiration can proceed only until the groundwater's supply of dioxygen is depleted, near the recharge area. Iron reduction, similarly, can proceed freely only in the non-marine section of the aquifer, since the marine section contains little ferric iron. It is clear from the results of this paper, furthermore, that the distribution of microbial activity is not controlled by the amount of free energy liberated by the functional groups' metabolic reactions, since across the aquifer almost all the reactions provide adequate energy for microbial metabolism. Therefore, one functional group does not seem to restrict the activity of other groups thermodynamically, by competitive exclusion.

With respect to the sulfate reducing bacteria, however, the nature of the zonation is not necessarily obvious. Is this functional group excluded from the high-iron zones, as suggested by Chapelle and Lovley (1992)? Or does this group coexist with iron reducers? In the high-iron zone, the supply of easily accessible ferric iron might be exhausted in areas with large influxes of electron donors, creating local environments favorable to sulfate reducers. Brown et al. (1999), for example, studied such a local environment near lignite deposits associated with clay lenses within the Magothy aquifer of New York.

There is some evidence that iron reducers effectively dominate the high-iron zone. In microcosm experiments using the non-marine sediments, Chapelle and Lovley (1992) found that acetate oxidation was not inhibited by molybdate, which suppresses sulfate reduction. The dihydrogen concentration measured by Chapelle and Lovley (1992) in the non-marine sediments is lower than expected for predominant sulfate reduction. These results are suggestive but not conclusive, since microcosm experiments are too small in scale to represent the heterogeneity in the aquifer, or the possibility of sulfate reducing subenvironments, and dihydrogen from such subenvironments might be consumed during subsequent flow through iron-reducing areas. In this study we also found no evidence that the concentrations of other electron donors–acetate and formate–differ between the high-iron and low-iron zones.

The evidence presented in this paper provides little support for the concept of exclusive zonation, either in terms of the availability of energy, the concentrations of electron donors, or the accumulation of metabolic products (ferrous iron and sulfide) in the Middendorf groundwater. Kirk et al. (written communication) recently found evidence that the populations of neither sulfate nor iron reducing bacteria in the Middendorf change significantly along the flow path. We can explain this result if adaptive microorganisms reduce iron in the high iron zone and sulfate in the low iron zone (Coleman et al., 1993). Alternatively, it can be explained if both iron and sulfate reducing activities coexist in the aquifer. Direct information about the distribution of microbial strains and their activities in the aquifer remains elusive and, hence, the exact nature of the zonation there remains unclear.

6. Summary and conclusions

In considering the evolution of groundwater chemistry in the Middendorf aquifer, we reach the following conclusions:

- Certain functional groups of microbes, most notably aerobic bacteria and iron reducers, lead to a net production of acid as organic matter in the sediments is degraded to CO_2 and small amounts of methane. This acid is consumed, however, by mineral dissolution, which drives up pH and accounts for the evolution of the aquifer's major ion chemistry.
- The thermodynamic energy available to each of the primary functional groups of microbes in the aquifer, except for the hydrogenotrophic (H_2+CO_2) methanogens, is sufficient to provide for microbial growth along the entire flow path studied. The energy available to microbial life decreases along flow in the non-marine sediments, then remains about constant in the marine sediments. There is no evidence that microbial zoning is influenced by the availability of energy, or that one functional group suppresses another by competitive exclusion.
- The concentrations of acetate, formate, and lactate do not vary systematically from zone to zone along the flow path, as might be expected if each zone were

dominated by a single functional group of microbes. The organic acid concentrations may poorly reflect the type microbial activity occurring in the aquifer, or the microbial activity may vary less from zone to zone than previously thought.

- The rate of microbial respiration seems to be limited by the rate of initial fermentation of organic matter in the sediment.
- We found no compelling evidence indicating that one functional group of microbes excludes others, except for the hydrogenotrophic methanogens, from any of the aquifer's zones. Sulfate reduction, for example, may be entirely inactive in the high-iron zone association with iron reduction, or may account for up to 90% of the respiration occurring there.

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