Volatile organic acids and microbial processes in the Yegua formation, east-central Texas

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Abstract

Geochemical and microbiological evidence indicates that viable microorganisms produce and consume volatile organic acids (VOA) in the Yegua formation. Acetic and propionic acid concentrations in mudstones range from 200 to 1270 and 20 to 38 $nmol \cdot gdw^{-1}$ respectively, whereas concentrations in sands are 50–200 and less than 20 $nmol \cdot gdw^{-1}$. VOA concentrations in sediments and in laboratory incubations suggest net production of VOAs by microorganisms in mudstones, and net consumption of VOAs by SO_4 reducing bacteria (SRB) in sands. Notably, SRB activity is mostly confined to aquifer sands.

Vertical diffusion and advection were modeled to estimate acetic acid transport from aquitard to aquifer. Assuming that SRB completely respire the acetic acid transported into the aquifer (3.2 $\mu mol \cdot l^{-1} \cdot m \cdot a^{-1}$), the CO_2 production rate in the aquifer sands is 5.3 $\mu mol \cdot l^{-1} \cdot a^{-1}$. This slow mineralization rate of in situ organic matter is within the range for deep aquifers, and probably accounts for the long-term survival of microorganisms in oligotrophic environments. Finally, the microbial communities in Yegua sediments appear to exhibit a loose commensalism, with microorganisms in aquitards providing VOAs for respiratory processes (i.e., SO_4 reduction) in aquifers. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

The discovery of diverse microbial communities in the terrestrial subsurface has important ramifications for bioremediation and sediment diagenesis (Ehrlich, 1996). Microbes in the soil-zone rapidly consume soluble nutrients, rendering much of the recharge waters oligotrophic (Madsen and Ghiorse, 1993). Therefore, in situ sedimentary organic matter (OM) is most likely the primary source of nutrients (electron donors) for microbial activity in the deep subsurface. Under anoxic conditions typical of subsurface environments, microbes degrade large polymeric compounds in a sequence of oxidation and reduction reactions. The fermentation product acetic acid is a key intermediate in these processes, serving as a substrate for SO_4 reducing bacteria (SRB) and methanogens (Sansone and Martens, 1982).

Volatile organic acids such as acetic, propionic, *iso*-butyric, *n*-butyric, *iso*-valeric, and *n*-valeric acids are

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hydrophilic, short-chain carboxylic acids, and range in concentrations from less than 1 mg·l^{-1} in groundwater to thousands of mg·l^{-1} in oil-field brines (Leenheer et al., 1974; Carothers and Kharaka, 1978). VOAs are important in nature because of their role as: (1) substrates for a variety of microorganisms (Balba and Nedwell, 1982; Monetti and Scranton, 1992), (2) complexing agents of metals and radio-nuclides (Giordano, 1994), (3) diagenetic agents affecting secondary porosity (Surdam et al., 1984), and (4) precursors for hydrocarbons (Carothers and Kharaka, 1978). VOAs in the soil-zone and in shallow marine and continental sediments are mostly generated by biotic processes. In contrast, VOA sources in deeply buried sediments and formation fluids are considered to be mostly abiotic (Lundegard and Kharaka, 1994). The nature of VOA production in sedimentary basins, however, is still in question. For example, Kawamura and Ishiwatari (1985) note that abiotic production of VOAs in sedimentary basins at low temperature is problematic and difficult to verify in laboratory experiments. In addition, many researchers question whether high temperature and short-duration experiments provide a reasonable simulation for VOA generation at low temperatures (Lewan and Fisher, 1994; Chapelle and Bradley, 1996).

The role of VOAs in microbial processes and OM mineralization in soils and marine sediments has received much attention (Sansone et al., 1987; Shaw and McIntosh, 1990; Fox and Comerford, 1990; Lundström, 1993; among others), but few researchers have studied their role in aquifer systems. Jones et al. (1989) noted acetic acid production and consumption in deep Atlantic Coastal Plain sediments, and Liu and Suflita (1993) further demonstrated the presence of acetogenic microorganisms in these sediments. The first comprehensive study of the relationship between VOA distribution and subsurface microbial activity in aquifer systems was reported by McMahon and Chapelle (1991). Also studying Atlantic Coastal Plain sediments, these authors presented evidence that microbial production of VOAs in aquitards and consumption in aquifers results in diffusive transport of VOAs from aquitards to aquifers. Calculations of diffusive flux indicated that rates of organic acid transport are sufficient to account for observed rates of microbial respiration in the aquifers. Chapelle and Bradley (1996) confirmed the higher concentrations of VOAs in fine-grained sediments compared with sands in Atlantic Coastal Plain sediments. More importantly, these authors found and characterized viable acetogenic microorganisms within fine-grained sediments in the deep subsurface. Except for these few studies, little is known about the relation between VOAs and microbial activity in the subsurface.

The authors studied the VOA distribution in Yegua

sediments (∼40 Ma old) from a Gulf Coast aquifer in east-central Texas. To understand the microbial and geochemical processes controlling the distribution of these compounds in the subsurface, VOA concentrations and microbial processes (fermentation and SO$_4$ reduction) were quantified in different lithologies. Fine-grained sediments (aquitards) were also tested for whether they serve as VOA sources for microorganisms living in sands (aquifers). This was verified by laboratory incubation for fermentation in fine-grained sediments, and quantifying VOA concentrations across the aquifer-aquitard boundary. Finally, to evaluate the components of this groundwater system which govern the in situ microbial activities, VOA fluxes and CO$_2$ production rate in the formation were calculated.

2. Study area

Sediment and groundwater samples were collected from the Yegua formation on the northwestern extremity of the Texas A&M University campus (Fig. 1). The Yegua formation is the uppermost unit of the Eocene Claiborne Group and has proven oil and gas reserves in Louisiana and Texas (Fisher, 1990). The formation is bounded above and below by the glauconitic and marine claystones of the Caddell and Cook Mountain formations. The lithology consists of interbedded fine-grained to medium-grained sand, silt, sandy and carbonaceous clay or mudstone, and lignite. Interpretations regarding the depositional environment of the Yegua formation differ. Berg (1970) concluded
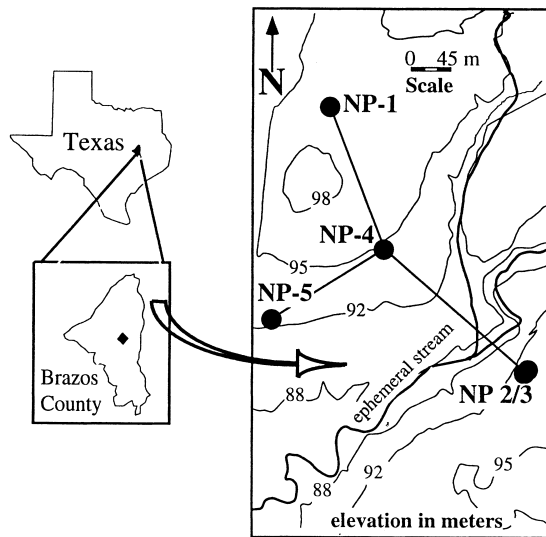

Fig. 1. Yegua formation stratigraphy and location map. NP-4 and NP-5 have well clusters at the site.

that the Yegua at Turkey Creek in Bryan represents a fluvial meander belt sequence, implying a mostly terrestrial origin. On the other hand, Yancey et al. (1993) interpreted the same outcrop as a bay deposit whose deposition was controlled by tidal currents, implying a marine origin.

The Yegua aquifer system provides moderate quantities of fresh to somewhat saline waters. Groundwaters from the well clusters at NP-4 and NP-5 (Fig. 1) are confined and under artesian pressure. The aquifer becomes anaerobic at shallow depths (< 30 m) because the low hydraulic conductivity of the unit (10^{-4}–10^{-3} cm·s^{-1}; Martino et al., 1998) results in slow recharge rate, restricting the supply of dissolved O_2. Previous study suggests horizontal groundwater velocities of 10^{-5}–10^{-6} cm·s^{-1}, and vertical velocities (< 30 m depth) of roughly 10^{-8} cm·s^{-1} in the downward direction (Martino et al., 1998). Yegua groundwater ^{14}C contents, modeled to correct for the addition of "dead" C (Fontes, 1992), yield groundwater ages of 2.7–10.5 ka (Martino et al., 1998; Ulrich et al., 1998).

3. Materials and methods

To date sediments have been collected from four 19–31 m deep boreholes drilled with hollow-stem augers, and eight water wells have been installed in clusters at the four borehole sites (Fig. 1). This paper will concentrate on the NP-4 and NP-5 sites. To check for possible contamination during drilling, carboxylated fluorescent microspheres (Polysciences Inc., Warington, Pennsylvania) were used as microbiological tracers. Samples showing levels of microsphere contamination of less than 10^3 microspheres·g^{-1} (detection limit) were designated as aseptically attained and used in this study. Cores were removed from the split-spoon sampler, wrapped in plastic, stored on ice, and brought back to the laboratory within 3 h. The oxidized and contaminated core exteriors were pared with sterile knives in an Ar-filled glove bag and discarded, and samples from core interiors were stored in Nalgene® centrifuge tubes. 3–5 ml of 7.4 mmol·l^{-1} HgCl$_2$ ($\sim$20% wt/v) were added to the sediment parings to inhibit microbial activity and the samples were stored at -20°C.

Because of the difficulty in extracting pore waters from the compacted Yegua sediments, an aqueous extraction technique was used. The poisoned sediments were thawed and dried in a N_2-filled glove bag at room temperature. From the dried sediment mass, 10 g of sediment was lightly crushed with a mortar and pestle, and slurried with 25 ml of distilled deionized water in Nalgene® centrifuge tubes. The sediment slurries were kept on a shaker (150 rpm) for 24 h followed by centrifugation for 10 min at 12,000 g. Later 2 ml of the extract was filtered (0.25 μm Dionex filters) and

transferred to chromatographic vials. The remaining sediment slurry was again shaken, and another set of sample was extracted in similar manner after 24 h to test if shaking time (24 vs 48 h) affected VOA concentrations.

The sediment extracts were analyzed on a gas-chromatograph equipped with a flame ionization detector (HP 6890) and a free-fatty-acid-phase column (HP-FFAP; 0.53 mm I.D. $\times$ 30 m) within 48 h of extraction. The advantages of VOA analyses using the HP-FFAP column include: (1) small sample capability (1–2 μl), (2) rapid analysis (25 min/sample), (3) high sensitivity, (4) specificity in separating VOA isomers, and (5) elimination of derivitization steps commonly involved with other gas chromatography techniques. Typical working conditions were He carrier gas at 15 ml·min^{-1}, injection temperature 100°C, detector temperature 220°C, and 1.0 μl sample/injection. C_2–C_{20} organic acids can be analyzed by this column, but not formic acid (C_1). The GC oven was programmed with an initial temperature of 98°C for 3 min followed by a temperature program of 15°C·min^{-1} to a final temperature of 200°C for 10 min. The detection limit was 250 μg·l^{-1} for acetic and propionic acids. For the method, this equates to a detection limit in sediments of 10 and 8 nmol per gram dry weight (gdw^{-1}) for acetic and propionic acids, respectively. With only minor exceptions, the VOA concentrations in sediment slurries extracted after 24 and 48 h are similar. The precision for VOA analyses was ±10%, as determined by analyses of standards (multi-fatty acid standard from Alltech) and duplicate samples after every five runs. Reproducibility for higher VOAs was poor in some samples because their concentration was near the detection limit of the instrument.

Grain size was determined by wet sieving. Percent moisture was determined from the weight loss of 2 g samples after heating at 100°C for 24 h. Total organic C (TOC) in sediments was analyzed using the sealed tube combustion method (Boutton, 1991) with pretreated CuO and Cu granules. Carbon dioxide from sediment combustion was manometrically measured on a vacuum line. Pyrite was determined by using a sequential extraction technique and dissolved SO_4 was analyzed by an ion chromatograph. Details of these analyses are provided in Ulrich et al. (1998). In addition, groundwater samples were collected quarterly for a 2-a period for various inorganic geochemical analyses including temperature, pH, dissolved O_2, SO_4, and alkalinity.

Microbial activities were evaluated in a mudstone and lignite-rich sediment layer in the NP-4B core at 18.38 m. Separate mudstone and lignite samples and a 1:1 mixture of both lithologies were slurried with NP-4B groundwater (40% wt:vol) in 25-ml serum bottles sealed with butyl rubber septa and placed under a N_2

headspace. The slurries were incubated at 22°C in the dark for 270 d. Liquid and gas samples were periodically withdrawn from the incubations using N_2-flushed syringes and needles. VOA and SO_4 contents were determined using high pressure liquid chromatography. Methane was monitored by flame-ionization gas chromatography. SRB activity was determined for several NP-4 sediments using radiotracer methodology (Ulrich et al., 1998). Additional experiments were performed to evaluate the possibility that VOAs were produced during bacterial SO_4 reduction. For these experiments the slurries were prepared with NP-3 groundwater and sand from 27–30 m because high SRB activity was previously reported in this interval (Martino et al., 1998). Autoclaved incubations served as sterile controls. Sodium molybdate (1 mM) was added as an inhibitor of SO_4 reduction. Sulfate and VOAs were determined during the 270 d incubation period.

4. Results

4.1. Geochemistry

The Yegua aquifers are most likely isolated sand bodies between mudstones with limited lateral continuity, and range in thickness from 0.9 to 8.2 m. Abrupt vertical and lateral changes in water chemistry (Table 1) and lithology made correlation of these sand bodies and tracing of flowpaths difficult (Figs. 2(A) and 3(A)). The sediments in NP-cores ranged from fine- and medium-grained sand to silt and clay (Figs. 2(A) and 3(A)). Widespread Fe-sulfide minerals (mostly pyrite) associated with sediments suggest early SO_4 reduction, probably during marine flooding. A color change from tan to gray at 6.1 and 4.5 m for NP-4 and NP-5 cores, respectively indicated a shift from oxidizing to reducing conditions. Previous studies indicated that SO_4 reduction is likely the major terminal electron accepting process in deeper sediments at the site because: (1) dissolved O_2 is generally low ($< 15 \ \mu\mathrm{mol} \cdot \mathrm{l}^{-1}$), (2) easily reducible Fe-oxyhydroxides

are absent at depth, (3) dissolved NO_3 is near the detection limit, (4) dissolved SO_4 concentrations in groundwater range from 0.8 to 28 $\mathrm{mmol} \cdot \mathrm{l}^{-1}$, (5) dissolved CH_4 is near the detection limit, and (6) SRB and SO_4 reduction were detected (Martino et al., 1998; Ulrich et al., 1998). Pyrite concentrations were lower in shallow sediments relative to deeper sediments (Fig. 2(E)). In contrast, SO_4 occurred in higher concentrations in shallow unsaturated sediments relative to deeper sediments.

TOC and VOA concentrations in Yegua sediments were high in mudstones, but low in sands (Table 2; Figs. 2(B) and (C) and 3(B) and (C)). TOC content in aquifer sands was $< 0.25\%$, whereas in mudstones TOC content is $> 0.50\%$. Acetic acid was the dominant organic acid measured followed by propionic, *iso*-butyric, *n*-butyric, *iso*-valeric, and *n*-valeric acids. VOAs greater than C_8-carboxylic acids were below the detection limit. Acetic and propionic acid concentrations in mudstones ranged from 200 to 1270 and 20 to 38 $\mathrm{nmol} \cdot \mathrm{gdw}^{-1}$ respectively, whereas in sands they were 50–200 and less than 20 $\mathrm{nmol} \cdot \mathrm{gdw}^{-1}$. Typically, unpoisoned Yegua sediments yielded VOA concentrations below the detection limit or at the low end of the concentration range. Occasionally, lignite stringers associated with sands (e.g., NP-4 at 17.95 and NP-5 at 23.27, 27.49 and 30.27 m) significantly increased the TOC and VOA concentrations in these samples (Table 2). Excluding these samples, TOC and % sand in the NP-4 and NP-5 cores showed a negative correlation ($r = 0.73$, Fig. 4). Consistent with this, acetic acid and % sand also correlated negatively ($r = 0.71$, Fig. 5).

4.2. Microbiology

Incubation experiments indicated that acetic and propionic acids were produced by the biological degradation of endogenous C in mudstone and lignite-rich sediments. These acids accumulated slowly in lignite and mudstone slurries during a 270 d incubation (inset of Fig. 6), but were not produced in autoclaved con-

Table 1
Inorganic chemistry of Yegua groundwaters

Sample	Screened depth (m)	pH	Diss O_2 ($\mu\mathrm{mol} \cdot \mathrm{l}^{-1}$)	Alkalinity (mM)	Sulfate (mM)
NP-1	18.2 ± 1.5	6.2	38	4.3	2.1
NP-2	15.8 ± 1.5	5.7	9	1.1	14.2
NP-4A	7.0 ± 0.8	3.7	112	0.0	28.1
NP-4B	13.0 ± 1.5	6.4	35	2.0	2.3
NP-5A	10.0 ± 0.4	6.4	54	2.5	4.7
NP-3	30.3 ± 1.5	7.1	7	2.6	0.39
NP-4C	26.5 ± 1.5	7.0	11	4.0	1.3
NP-5C	26.5 ± 1.5	7.2	14	4.3	1.1

trols. A low level of acetic acid was detected in the sterilized incubations at the beginning of the incubation period. The acetic acid in the sterile controls was presumably liberated from the sediments while autoclaving the incubations. Interestingly, when mudstone and lignite-rich sediments were mixed, the acetic acid production rate increased 30-fold. Sodium molybdate (a SO_4 reduction inhibitor) did not prevent the production of acetic and propionic acids suggesting that production of these VOAs is not associated with SO_4 reduction (Fig. 7). Sulfate reduction activity, as measured using $^{35}SO_4$ as a tracer, was detected in the sands throughout the NP-4 depth profile (Fig. 2(D)). In fact, SO_4 reduction was significant only in sediments with greater than 60% sand (Table 2).

5. Discussion

Fermentation and SO_4 reduction are important mi-crobial processes which influence the distribution of VOAs in the subsurface through production and consumption reactions. Fermentation involves breakdown of complex OM under anoxic conditions and forms VOAs, alcohols, hydroxy acids, and molecular H_2 (Winfrey, 1984). In reducing environments such as those of the Atlantic Coastal Plain aquifers and shallow marine sediments, SRB and methanogens consume the fermentation products (McMahon and Chapelle, 1991; Novelli et al., 1988). McMahon and Chapelle (1991) linked fermentation in aquitards to SO_4 reduction and CO_2 production in aquifers.

5.1. VOA concentrations

With one exception, acetic acid concentrations in Yegua sediments ranged from 50 to 694 $nmol·gdw^{-1}$, equivalent to 200–2780 $\mu mol·l^{-1}$. These values are slightly higher than those obtained by Chapelle and Bradley (1996) for Atlantic Coastal Plain sediments
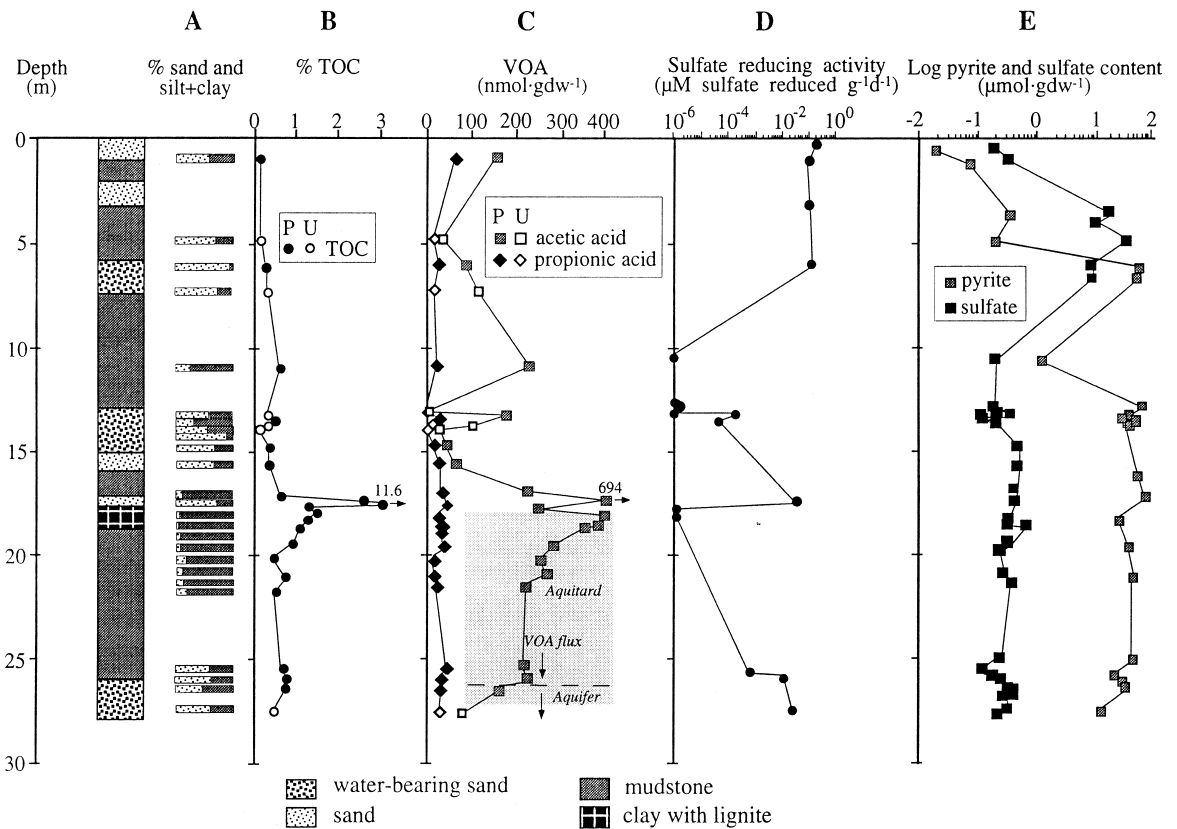

Fig. 2. Sediment geochemistry and microbiology of NP-4 core. P = poisoned, U = unpoisoned. A = grain size, B = %TOC, C = VOA concentration, D = SO_4 reducing activity (Ulrich et al., 1998), and E = pyrite and SO_4 concentration (Ulrich et al., 1998). Stippled area in 2(C) represents VOA concentrations used in calculations of fluxes from aquitard to aquifer. Grain size analyses of discrete intervals may differ from the lithology of the overall unit because of lithologic heterogeneity.
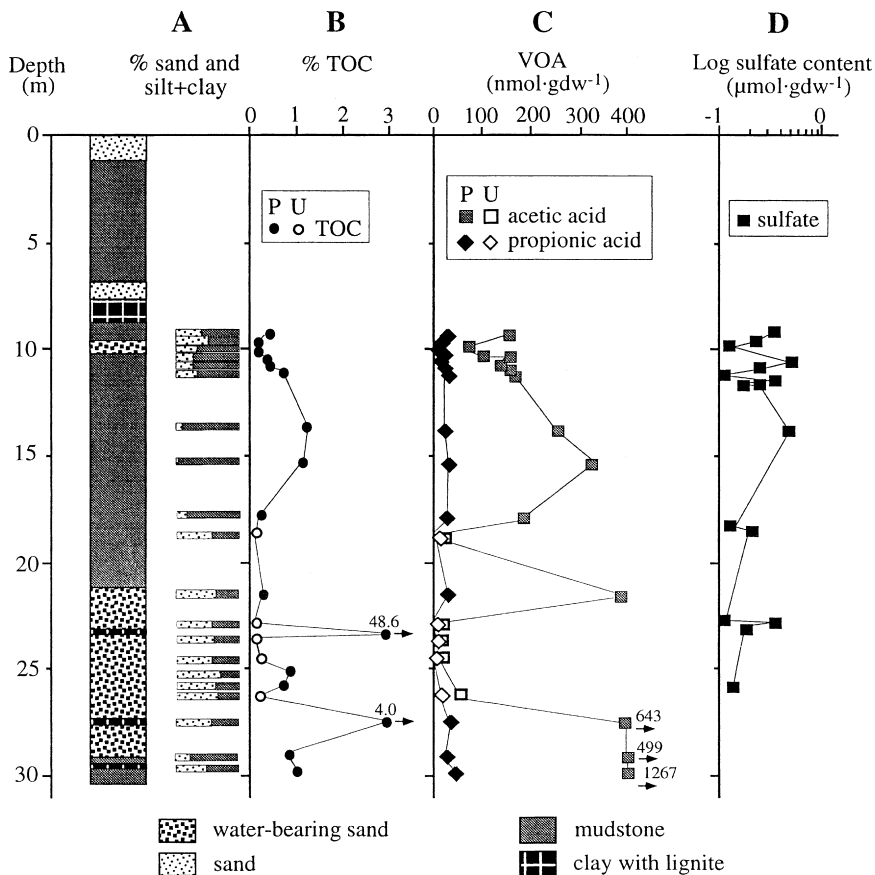
Fig. 3. Sediment geochemistry of NP-5 core. P = poisoned, U = unpoisoned. A = grain size, B = % TOC, C = VOA concentration, and D = SO$_4$ concentration (Ulrich et al., 1998).

(2–1800 μmol·l^{-1}). However, these authors suggested that contamination by drilling fluids may have diluted some of the VOA concentrations. In the Yegua for-

mation, acetic acid concentrations are higher than concentrations in anoxic marine pore waters (1–
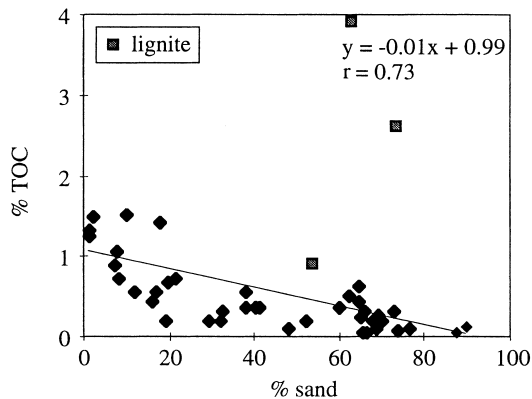

Fig. 4. Total organic C vs % sand in NP-4 and NP-5 sediments. (Regression does not include samples with visible lignite laminae.)
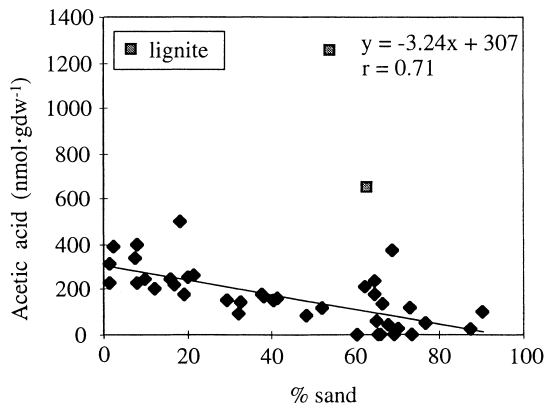

Fig. 5. Acetic acid concentrations vs % sand in NP-4 and NP-5 sediments. (Regression does not include samples with visible lignite laminae.)

Table 2
Total organic C (TOC), grain size, volatile organic acid concentrations, and SRB activity in Yegua sediments. SRB activity data from Ulrich et al. (1998)[a]

Depth (m)	TOC (%)	Sand (%)	Silt + clay (%)	Acetic (24 h) (nmol·gdw^{-1})	Acetic (48 h) (nmol·gdw^{-1})	Propionic (24 h) (nmol·gdw^{-1})	Sulfate reduction (μmole g^{-1} yr^{-1})
NP-4							
0.6 (soil zone)							0.20
1.21	0.05	67	33	133	79	24	0.08
4.26[b]	0.20	70	30	23	58	14	0.13
6.70	0.11	90	10	98	99	21	0.14
7.31[b]	0.30	73	27	115	12	13	†
10.97	0.55	17	83	222	†	16	0.00
13.01[b]	0.36	60	40	bdl	bdl	bdl	0.00
13.13	0.37	38	62	177	176	30	0.00
13.21	x	x	x	x	x	x	0.00
13.44[b]	0.20	52	48	123	bdl	13	†
13.48	x	x	x	x	x	x	0.00
13.72[b]	0.05	88	12	23	bdl	bdl	0.00
14.78	0.18	68	32	46	52	15	†
15.12	0.25	65	35	58	68	17	†
17.07	0.71	8	92	225	234	26	†
17.95	2.60	74	26	x	x	x	0.04
17.95	11.6	x	x	694	757	42	†
18.38	1.24	1	99	233	261	28	0.00
18.56	1.48	2	98	392	496	37	0.00
18.62	1.06	8	92	395	418	30	†
18.78	0.89	7	93	339	285	28	†
19.93	0.73	22	79	262	286	23	†
20.54	0.44	16	84	247	238	20	†
21.49	0.67	20	80	253	259	19	†
22.13	0.56	12	88	201	212	29	†
25.60	0.51	38	63	210	159	21	0.00
26.09	0.63	35	65	240	469	29	0.01
26.63	0.54	62	38	170	144	24	†
27.52[b]	0.44	65	35	80	†	20	0.05
NP-5							
9.66	0.35	42	59	163	148	23	†
10.00	0.10	48	52	86	90	19	†
10.94	0.19	32	68	92	95	19	†
10.91	0.20	29	71	149	153	19	†
11.28	0.31	32	68	147	149	22	†
11.31	0.35	41	59	153	153	24	†
14.02	1.51	10	90	247	254	27	†
15.70	1.32	2	98	315	310	29	†
18.50	0.18	19	81	178	190	27	†
18.60[b]	0.30	66	34	bdl	6	bdl	†
21.46	0.09	69	31	374	523	32	†
22.95[b]	0.05	66	35	bdl	bdl	bdl	†
23.27	48.6	x	x	x	x	x	†
23.47[b]	0.06	74	26	bdl	bdl	bdl	†
24.87[b]	0.27	69	31	bdl	bdl	bdl	†
26.02[b]	0.10	77	23	54	61	13	†
27.49	3.96	63	37	643	507	43	†
29.05	1.41	18	82	499	799	35	†
30.27	0.93	54	46	1267	1270	27	†

[a] bdl — below detection limit; † — not analyzed; x — samples unavailable.
[b] Unpoisoned samples kept frozen or stored anaerobically.

44 μmol·l^{-1}; Shaw and McIntosh, 1990; Novelli et al., 1988), but are at least an order of magnitude lower than those commonly found in deep (>1800 m) formation fluids (>50,000 μmol·l^{-1}; Carothers and Kharaka, 1978). Carothers and Kharaka (1978) suggested that these hydrocarbon-associated VOAs form through catagenetic processes during sediment diagenesis.

Extraction procedures and analytical techniques can significantly affect the concentrations and distribution of VOAs. Parkes and Taylor (1983) suggested that high salt content in samples may cause interference with chromatographic columns. However, according to the manufacturer (Hewlett Packard), dissolved solutes do not significantly affect the sensitivity of the HP-FFAP column unless brines or concentrated seawaters are analyzed without desalination (A. Folk, pers. commun., 1995). VOA analyses of desalted and untreated groundwater samples from the Yegua boreholes are within ±10% (data not shown), the precision of the method.

The hydrophilic character of most VOAs permits them to be removed from sediments through aqueous extraction (Herbert and Bertsch, 1995). Some workers have used acid extraction techniques to enhance the recovery of organic acids from sediments (Takijima, 1964; David et al., 1989), especially when studying high molecular weight organic acids (Kawamura and Ishiwatari, 1985; Mendoza et al., 1987). However, the authors refrained from using such methods because studies indicate increases in water soluble OM produced through acid-hydrolysis of large macromolecules and mineral dissolution (Herbert and Bertsch, 1995; David et al., 1989). For example, David et al. (1989) indicated significant variation in the hydrophobic/hydrophilic acid ratio after treating soils with inorganic acids under different pH conditions. Moreover, use of

strong inorganic acids during extraction may result in volatilization of low molecular weight organic acids (Yamane and Sato, 1967).

Another consideration is sample handling. Shaw and McIntosh (1990) noted variation in acetic acid concentrations obtained by squeezing, centrifuging, and dialysis. Squeezing sediments resulted in higher acetic acid concentrations than other techniques. Centrifugation of sediments gave results which varied irregularly with the centrifugal force. Recently, Monetti and Scranton (1992) suggested that slurrying sediments could yield higher VOA concentrations. However, other researchers argue that slurrying does not increase VOA concentrations in sediments (C. Martens, pers. commun., 1997), and use the technique in their studies (e.g., Chapelle and Lovley, 1990). Unfortunately, extraction of pore waters from all Yegua lithologies is difficult, making slurrying the most practical technique for VOA extraction.

5.2. Origin of VOAs

It is difficult to prove that subsurface VOAs form solely by biotic or abiotic processes. Evidence for abiotic formation of VOAs at low temperature is based on extrapolation of high temperature experiments to low temperature, using the Arrhenius relationship applied to experimentally determined reaction rates (Lewan and Fisher, 1994). However, others have suggested that microbial processes produce VOAs in sedimentary basins at low temperature (Jones et al., 1989; Chapelle and Bradley, 1996). There is both geochemical and microbiological evidence that VOAs (mainly acetic and propionic acids) in Yegua sediments are produced by fermentation of endogenous OM. Consistent with this, evidence from Routh et al. (1999) argues against significant abiotic production of VOAs in Yegua sedi-
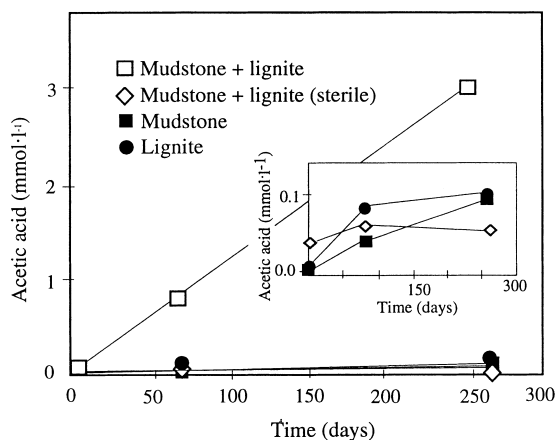

Fig. 6. Production of acetic acid in NP-4 Yegua sediments (18.38 m).
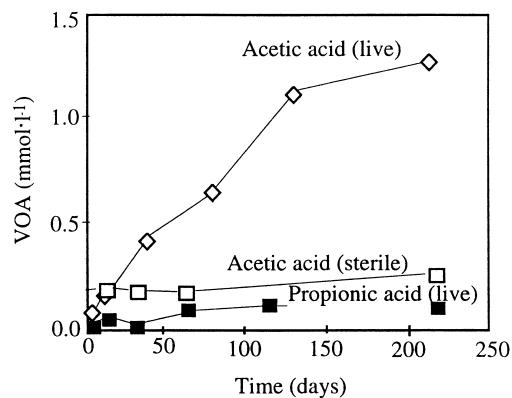

Fig. 7. Production of volatile organic acids in microbial cultures from Yegua sediments treated with sodium molybdate (sample NP-4 at 18.38 m).

ments because sedimentary OM is immature (Type III OM with low thermal alteration index), and has not undergone sufficient burial for catagenetic degradation. While it is impossible to rule out some catagenetic VOA production during the 40 Ma history of these sediments, biological production of acetic and propionic acids in sediment incubations over relatively short time periods strongly suggests that microorganisms are actively involved with VOA production in the Yegua formation.

Non-sterilized, anaerobic incubations of mudstone and lignite-rich samples produced acetic and propionic acids, but sterilized slurries did not. The identity of the microorganisms responsible for VOA production is not known. However, the results indicate that SO_4 reduction is not directly responsible since VOAs are produced in clay and lignite-rich strata where SO_4 reduction apparently does not occur. Furthermore, the inhibition of SO_4 reduction did not prevent the production of acetic and propionic acids (Fig. 7). The presence of acetogenic bacteria in subsurface sediments combined with potential activity measurements (Liu and Suflita, 1993; Chapelle and Bradley, 1996; Krumholz et al., 1997) suggest that these microorganisms may be involved in the formation of acetic acid. However, the relative role of acetogenesis and fermentation in subsurface acetic acid production is not clear.

5.3. Sulfate reduction

Lack of culturable methanogens and dissolved CH_4 in groundwater, as well as the absence of other electron acceptors, suggest that SO_4 reduction is the dominant terminal electron accepting process in deeper reducing sediments. SRB activity in NP-4 sediments correlates positively with % sand and negatively with VOAs, implying net VOA consumption in sands (Fig. 2(D); Ulrich et al., 1998). This is consistent with previous work on deep subsurface sediments which reported high SRB activity in aquifer sands (Jones et al., 1989; Sinclair and Ghiorse, 1989; McMahon and Chapelle, 1991; among others).

The underlying reasons for the stratification of microbial processes (mainly SO_4 reduction in sands and fermentation in aquitards) and the absence of SO_4 reduction in fine-grained sediments is unclear. In mudstones, TOC, VOA, and SO_4 concentrations are high yet SRB activity is negligible, suggesting that some parameter(s) other than availability of electron donors and acceptors limits SO_4 reduction in fine-grained sediments. These parameter(s) may be water potential, pH, pore throat diameter, or availability of nutrients (e.g., Ghiorse and Wilson, 1988; Lovley and Chapelle, 1995). In contrast, enhanced SRB activity in sands or sandstones at aquifer-aquitard interfaces in Yegua and Atlantic Coastal Plain sediments likely reflects the

emanation of electron donors and perhaps electron acceptors from aquitards (McMahon and Chapelle, 1991; Lovley and Chapelle, 1995; Krumholz et al., 1997; Ulrich et al., 1998).

The present results support the contention that OM in lignite and mudstones may be a potential source of electron donors (Fredrickson et al., 1991; Murphy et al., 1992), and may be less refractory than previously suggested. This has important implications for survival of microbial communities in oligotrophic environments where the microorganisms depend upon endogenous C sources.

5.4. VOA fluxes and microbial activity

Cedarstrom (1946) suggested that SO_4 reduction was the principal HCO_3^--producing mechanism in Atlantic and Gulf Coast aquifers. However, no direct evidence for microbial SO_4 reduction was provided until recently (Chapelle et al., 1987; McMahon and Chapelle 1991; Ulrich et al., 1998). McMahon and Chapelle (1991) modeled the diffusive acetic acid flux from aquitards to aquifers and its impact on groundwater chemistry in Atlantic Coastal Plains.

As indicated in the Atlantic Coastal Plain aquifers, net production of VOAs in the Yegua mudstones was

VOA flux from aquitard to aquifer

$$J \ = \ V \cdot C_{aqt} \cdot n$$

Rate of CO_2 production in aquifer

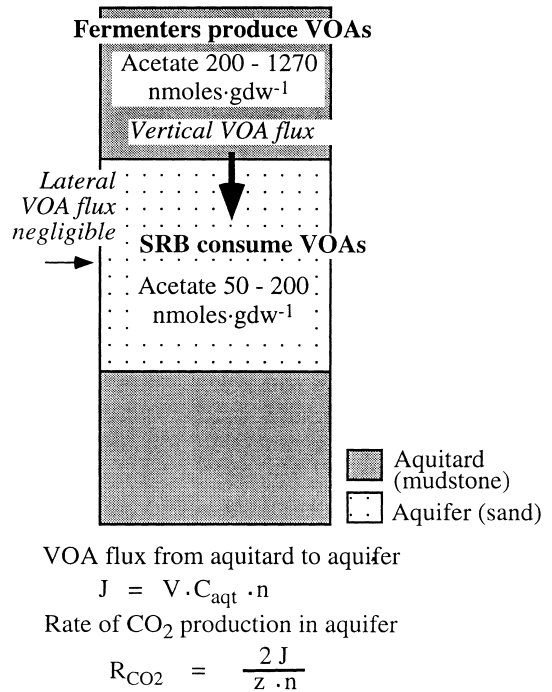$$R_{CO2} \ = \ \frac{2\,J}{z \cdot n}$$

Fig. 8. A cartoon outlining a model for VOA transport in the Yegua formation (not drawn to scale). See text for definition of symbols.

observed. To estimate the impact of this process, vertical diffusive and advective transport of acetic acid from aquitards to aquifers was modeled under steady state conditions (Fig. 8). Diffusive transport was calculated from Fick's first law:

$$J_d = -D \cdot (dC/dz) \cdot n \tag{1}$$

where J_d = diffusive flux into aquifer in $\mu mol \cdot l^{-1} \cdot m \cdot a^{-1}$, D = diffusivity of acetic acid ($8.7 \times 10^{-3} \, m^2 \cdot a^{-1}$; McMahon and Chapelle, 1991), C = concentration in $\mu mol \cdot l^{-1}$, z = depth in meters, and n = effective porosity of aquitard (0.4). The concentrations used were for the transition from aquitard to aquifer in NP-4 well, 960 $\mu mol \cdot l^{-1}$ at 26.09 m and 680 $\mu mol \cdot l^{-1}$ at 26.63 m. Acetic acid concentrations ($nmol \cdot gdw^{-1}$) were converted to $\mu mol \cdot l^{-1}$ by assuming that 1 gdw sediment equals 0.25 gm (20% water by weight) of water. The estimated diffusive flux is 1.8 $\mu mol \cdot l^{-1} \cdot m \cdot a^{-1}$.

The vertical advective flux of acetic acid (J_a) from the overlying aquitard into an aquifer can be estimated from:

$$J_a = V \cdot C_{aqt} \cdot n \tag{2}$$

where V = vertical advective velocity and C_{aqt} = average VOA concentration in the aquitard (1125 $\mu mol \cdot l^{-1}$; Fig. 2(C)). Vertical advective velocity was estimated as 0.003 $m \cdot a^{-1}$ using Darcy's law, hydraulic conductivity estimates from lithology, and water level measurements (Martino et al., 1998). Eq. (2) yields an acetic acid flux of 1.4 $\mu mol \cdot l^{-1} \cdot m \cdot a^{-1}$. Assuming that the diffusively and advectively transported acetic acid is completely consumed by SRB activity, the rate of microbial CO_2 production in aquifers (R_{CO2}) can be estimated by:

$$R_{CO2} = \frac{2(J_d + J_a)}{h \cdot n} \tag{3}$$

where h = aquifer thickness (3 m minimum). The rate of CO_2 production calculated from Eq. (3) is 5.3 $\mu mol \cdot l^{-1} \cdot a^{-1}$.

Lateral advection may be a source of fermentable dissolved organic C and, therefore, acetic acid in aquifers. It is unclear, however, how much of this C survives in groundwater hundreds to thousands of years old. VOA concentrations decrease with depth in lake sediments and the soil-zone because of rapid microbial consumption (Fox and Comerford, 1990; Hordijk et al., 1994), suggesting that only a small fraction of VOAs survive degradation and is potentially available for recharge waters. Moreover, radiocarbon dating of dissolved organic C in the Milk River aquifer suggests that the low molecular weight fraction (including acetic acid) is derived predominantly from sedimentary OM in the aquifer (Murphy et al., 1989; Wassenaar et al., 1990).

McMahon and Chapelle (1991) obtained a close match between the CO_2 production rate calculated from flowpath modeling of HCO_3^- contents, and those calculated assuming diffusive transport of acetic acid and acetic acid oxidation coupled to SO_4 reduction. In the absence of carbonate precipitation, SO_4 reduction in aquifers coupled to fermentation in aquitards should increase HCO_3^- concentrations along the flowpath in the Yegua formation. Thus, for the case of vertical advection, HCO_3^- should increase downward. HCO_3^- concentrations in the study area do not uniformly increase with depth (Table 1), and uncertainty in groundwater flowpaths makes independent mass balance calculation of CO_2 production difficult. Moreover, CO_2 volatilization in shallow acidic intervals (e.g., NP-4A) may cause loss of HCO_3^-.

The CO_2 production rate for the Yegua formation (5.3 $\mu mol \cdot l^{-1} \cdot a^{-1}$) is at the high end of the values for deep aquifers (10^{-3}–10 $\mu mol \cdot l^{-1} \cdot a^{-1}$), and much lower than rates for shallow marine sediments (10^2–10^5 $\mu mol \cdot l^{-1} \cdot a^{-1}$; Table 3). As with other deep aquifers, the metabolic rates in the Yegua formation are comparable to the rate estimates for highly oligotrophic deep ocean waters (Williams and Carlucci, 1976). The low rates of OM oxidation in the Yegua formation could be a consequence of the low OM content in sands or the low reactivity of OM compared with that in recent organic-rich sediments (Westrich and Berner, 1984). However, the present research indicates that there are factors in addition to organic C availability or quality which limit microbial activity. For instance, SO_4 reduction does not occur in fine-grained sediments where ample electron donor and SO_4 are present. Similarly, when sediments from two vertically juxtaposed strata were mixed, the biological production of acetic acid occurred at a rate roughly 1000 times faster than the in situ rates based on flux calculations. This clearly indicates the presence of bioavailable C in Yegua sediments and that some factor(s) prevents its utilization in situ.

6. Conclusions

Microbiological and geochemical evidence indicates that fermentation processes are active in mudstone and lignitic sediments, and result in production of VOAs. In contrast, SRB activity is mostly confined to aquifer sands and likely results in consumption of VOAs. Geochemical modeling suggests that diffusion and vertical advection transports VOAs into the aquifer where SRB consume the VOAs during respiration. CO_2 production rates in Yegua sediments are within the range obtained for deep aquifers, but are still 10^3–10^5 times slower than near-surface microbial processes.

The Yegua sediments support microorganisms that

Table 3
CO_2 production rate in aquifers and marine environments

Environment	Approx. CO_2 production $\mu mol \cdot l^{-1} \cdot yr^{-1}$	Reference
Marine		
Deep seawater	10^{-3}–10^{-6}	Williams and Carlucci, 1976
Abyssal sediments	0.1–100	Reimers et al., 1984
Continental sediments	0.1–10	Jørgensen, 1982
Deep to shallow aquifers		
Middendorf (S. Carolina, USA)	10^{-4}–10^{-5}	Chapelle and Lovley, 1990
Patapsco (Maryland, USA)	10^{-2}–1.0	Chapelle et al., 1987
Hawthorn (S. Carolina, USA)	10	Chapelle et al., 1988
Yegua (Texas, USA)	10	This study

are clearly metabolically active. These microorganisms have developed a loose commensalism; SRB in aquifers benefit from microorganisms in aquitards that provide VOAs produced by sedimentary OM degradation. The authors conclude that these microbial communities have developed these complex associations as an adaptation for survival in subsurface oligotrophic environments.

References

Balba, M.T., Nedwell, D.B., 1982. Microbial metabolism of acetate, propionate and butyrate in anoxic sediment from the Colne Point Saltmarsh, Essex, UK. J. Gen. Microbiol. 128, 1415–1422.

Berg, R.R., 1970. Outcrops of the Claiborne group in the Brazos Valley southeast Texas. In: Geol. Soc. Am., 4th Ann. Meeting of the South-Central Section, p. 79.

Boutton, T., 1991. Stable carbon isotope ratios of natural materials: 1. Sample preparation and mass spectrometric analysis. In: Coleman, D.C., Fry, B. (Eds.), Carbon Isotope Techniques. Academic Press Inc, San Diego, pp. 155–171.

Carothers, W.W., Kharaka, Y.K., 1978. Aliphatic acid anions in oil-field waters—implications for origin of natural gas. Bull. Am. Assoc. Petrol. Geol. 62, 2441–2453.

Cedarstrom, D.J., 1946. Genesis of groundwaters in the coastal plain of Virginia. Econ. Geol. 41, 218–245.

Chapelle, F.H., Bradley, P.M., 1996. Microbial acetogenesis as a source of organic acids in ancient Atlantic Coastal Plain sediments. Geology 24, 925–928.

Chapelle, F.H., Lovley, D.R., 1990. Rates of microbial metabolism in deep coastal plain aquifers. Appl. Environ. Microbiol. 56, 1865–1874.

Chapelle, F.H., Morris, J.T., McMahon, P.B., Zelibor Jr, J.L., 1988. Bacterial metabolism and the $\delta^{13}C$ composition of groundwater, Floridan aquifer South Carolina. Geology 16, 117–121.

Chapelle, F.H., Zelibor Jr, J.L., Grimes, J.D., Knobel, L.L., 1987. Bacteria in deep coastal plain sediments of Maryland: A possible source of CO_2 to groundwater. Water Resour. Res. 23, 1625–1632.

David, M.B., Vance, G.F., Rissing, J.M., Stevenson, F.J., 1989. Organic carbon fractions in extracts of O and B horizons from a New England spodosol: effects of acid treatment. J. Environ. Qual. 18, 212–217.

Ehrlich, H.L., 1996. Geomicrobiology. Marcel Dekker, New York.

Fisher, W.L., 1990. Delta systems and oil-gas occurrence. South Texas Geol. Soc. Bull. 31, 13–26.

Fontes, J.C., 1992. Chemical and isotopic constraints on ^{14}C dating of groundwater. In: Taylor, R.E., Long, A., Kraceds, R.S. (Eds.), Radiocarbon After Four Decades, An Interdisciplinary Perspective. Springer-Verlag, New York, pp. 242–261.

Fox, T.R., Comerford, N.B., 1990. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. Soil Sci. Soc. Am. J. 54, 1139–1144.

Fredrickson, J.K., Balkwill, D.L., Zachara, J.M., Li, S.W., Stewart, D.L., Workman, D.J., 1991. Physiological diversity of heterotrophic bacteria associated with deep Cretaceous sediments of the Atlantic Coastal Plain. Appl. Environ. Microbiol. 57, 402–411.

Ghiorse, W.C., Wilson, J.T., 1988. Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. 33, 107–172.

Giordano, T.H., 1994. Metal transport in ore fluids by organic ligand complexation. In: Pittman, E.D., Lewan, M.D. (Eds.), Organic Acids in Geological Processes. Springer-Verlag, New York, pp. 319–354.

Herbert, B.E., Bertsch, P.M., 1995. Characterization of dissolved and colloidal organic matter: a review. In: Carbon Forms and Functions in Forest Soils. Soil Science Society of America, Madison, Wisconsin, pp. 63–87.

Hordijk, C.A., Kamminga, H., Cappenberg, T.E., 1994. Kinetic study of acetate in freshwater sediments: use of stable isotopic tracers. Geochim. Cosmochim. Acta 58, 683–694.

Jones, R.E., Beeman, R.E., Suflita, J.M., 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. Geomicrobiol. J. 7, 117–130.

Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed — The role of sulfate reduction. Nature 296, 643–645.

Kawamura, K., Ishiwatari, R., 1985. Conversion of sedimentary fatty acids from extractable (unbound + bound) to tightly bound form during mild heating. Org. Geochem. 8, 197–201.

Krumholz, L.R., McKinley, J.P., Ulrich, G.A., Suflita, J.M., 1997. Confined subsurface microbial communities in Cretaceous rock. Nature 386, 64–66.

Lewan, M.D., Fisher, J.B., 1994. Organic acids from petroleum source rocks. In: Pittman, E.D., Lewan, M.D. (Eds.), Organic Acids in Geological Processes. Springer-Verlag, New York, pp. 70–114.

Leenheer, J.L., Malcolm, R.L., McKinley, P.W., Eccles, L.A., 1974. Occurrence of dissolved organic carbon in selected ground-water samples in the United States. J. Res. US Geol. Surv. 50, 361–369.

Liu, S., Suflita, J.M., 1993. H_2-CO_2-dependent anaerobic O-demethylation activity in subsurface sediments and by an isolated bacterium. Appl. Environ. Microbiol. 59, 1325–1331.

Lovley, D.R., Chapelle, F.H., 1995. Deep subsurface microbial processes. Rev. of Geophy. 33, 365–381.

Lundegard, P.D., Kharaka, Y.K., 1994. Distribution and occurrence of organic acids in subsurface waters. In: Pittman, E.D., Lewan, M.D. (Eds.), Organic Acids in Geological Processes. Springer-Verlag, New York, pp. 40–69.

Lundström, U.S., 1993. The role of organic acids in the soil solution chemistry of a podzolized soil. J. Soil Sci. 44, 121–133.

Madsen, E.L., Ghiorse, W.C., 1993. Groundwater microbiology: Subsurface ecosystem processes. In: Ford, T.E. (Ed.), Aquatic Microbiology an Ecological Approach. Blackwell Scientific Publications, London, pp. 167–213.

Martino, D.P., Grossman, E.L., Ulrich, G.A., Burger, K.C., Schlichenmeyer, J.L., Suflita, J.M., Ammerman, J.W., 1998. Microbial abundance and activity in a low conductivity aquifer system in east-central Texas. Microbial Ecol. 35, 224–234.

McMahon, P.B., Chapelle, F.H., 1991. Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry. Nature 349, 233–235.

Mendoza, Y.A., Gülaçar, F.O., Buchs, A., 1987. Comparison of extraction techniques for bound carboxylic acids in recent sediments. Chem. Geol. 62, 307–319.

Monetti, M.A., Scranton, M.I., 1992. Fatty acid oxidation in anoxic marine sediments: the importance of hydrogen sensitive reactions. Biogeochem. 17, 23–47.

Murphy, E.M., Schramke, J.A., Fredrickson, J.K., Bledsoe, H.W., Francis, A.J., Sklarew, D.S., Linehan, J.C., 1992. The influence of microbial activity and sedimentary organic carbon on the isotope geochemistry of Middendorf Aquifer. Water Resour. Res. 28, 723–740.

Murphy, E.M., Davis, S.N., Long, A., Donahue, D., Jull, A.J.T., 1989. Characterization and isotopic composition of organic and inorganic carbon in the Milk River Aquifer. Water Resour. Res. 25, 1893–1905.

Novelli, P.C., Michelson, A.R., Scranton, M.I., Banta, G.T., Hobbie, J.E., Howarth, R.W., 1988. Hydrogen and acetate cycling in two sulfate-reducing sediments: Buzzards Bay and Town Cove, Mass. Geochim. Cosmochim. Acta 52, 2477–2486.

Parkes, R.J., Taylor, J., 1983. Analysis of volatile fatty acids by ion-exclusion chromatography, with special reference to marine pore water. Mar. Biol. 77, 113–118.

Reimers, C.E., Kalhorn, S., Emerson, S.R., Nealson, K.H., 1984. Oxygen consumption rates in pelagic sediments from the central Pacific: First estimate from microelectrode profiles. Geochim. Cosmochim. Acta 48, 903–920.

Routh, J., McDonald, T.J., Grossman, E.L., 1999. Sedimentary organic matter sources and depositional environment in Yegua formation (Brazos County, Texas). Org. Geochem. 30, 1437–1453.

Sansone, F.J., Andrews, C.C., Okamoto, M.Y., 1987. Adsorption of short-chain organic acids onto nearshore marine sediments. Geochim. Cosmochim. Acta 51, 1889–1896.

Sansone, F.J., Martens, C.S., 1982. Volatile fatty acid cycling in organic-rich marine shales. Geochim. Cosmochim. Acta 46, 1575–1589.

Shaw, D.G., McIntosh, D.J., 1990. Acetate in recent anoxic sediments: direct and indirect measurements of concentration and turnover rates. Estuar., Coast. and Shelf Sci. 31, 775–788.

Sinclair, J.L., Ghiorse, W.C., 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol. J. 7, 369–377.

Surdam, R.C., Boese, S., Crossey, L.J., 1984. The chemistry of secondary porosity. In: McDonald, D.A., Surdam, R.C. (Eds.), Clastic Diagenesis, Mem. Am. Assoc. Petrol. Geol., 37, pp. 127–149.

Takijima, Y., 1964. Studies on organic acids in paddy field soils with reference to their inhibitory effects on the growth of rice plants Part 1. Growth inhibitory action of organic acids and adsorption and decomposition to them by soils. Soil Sci. Plant Nutr. 10, 14–21.

Ulrich, G.A., Martino, D., Burger, K.C., Routh, J., Grossman, E.L., Ammerman, J.W., Suflita, J.M., 1998. Microbial interactions in the terrestrial subsurface: sulfur cycling, heterogeneity, and survival. Microbial Ecol. 36, 141–151.

Wassenaar, L.I., Hendry, M.J., Aravena, R., Fritz, P., 1990. Organic carbon isotope geochemistry of clayey deposits

and their associated porewaters, southern Alberta. J. Hydrol. 120, 251–270.

Westrich, J.T., Berner, R.A., 1984. The role of sedimentary organic matter in bacterial sulfate reduction: the G model tested. Limnol. Oceanog. 29, 236–249.

Williams, P.M., Carlucci, A.F., 1976. Bacterial utility of organic matter in the deep sea. Nature 262, 810–811.

Winfrey, M.R., 1984. Microbial production of methane. In: Atlas, R.M. (Ed.), Petroleum Microbiology. MacMillan Publishing, London, pp. 57–77.

Yamane, I., Sato, K., 1967. Method for extracting organic acid from flooded soil. Soil Sci. Plant Nutrition 13, 113–120.

Yancey, T.E., Davidoff, A.J., Donaho, T.S., 1993. Depositional gradient analysis in transgressive systems tracts and highstand systems tracts, mid to late Eocene of the Brazos River Valley, Texas. Gulf Coast Assoc. Geol. Soc. 43, 465–471.