

Microbial and biogeochemical processes in Big Soda Lake, Nevada

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SUMMARY: Meromictic, alkaline lakes represent modern-day analogues of lacustrine source rock depositional environments. In order to further our understanding of how these lakes function in terms of limnological and biogeochemical processes, we have conducted an interdisciplinary study of Big Soda Lake. Annual mixolimnion productivity (ca. 500 g m^{-2}) is dominated by a winter diatom bloom (60% of annual) caused by upward transport of ammonia to the epilimnion. The remainder of productivity is attributable to chemoautotrophs (30%) and photosynthetic bacteria (10%) present at the oxic-anoxic interface from May to November. Studies of bacterial heterotrophy and particulate fluxes in the water column indicate that about 90% of annual productivity is remineralized in the mixolimnion, primarily by fermentative bacteria. However, high rates of sulphate reduction ($9\text{--}29 \text{ mmol m}^{-2} \text{ yr}^{-1}$) occur in the monimolimnion waters, which could remineralize most (if not all) of the primary productivity. This discrepancy has not as yet been fully explained. Low rates of methanogenesis also occur in the monimolimnion waters and sediments. Most of the methane is consumed by anaerobic methane oxidation occurring in the monimolimnion water column. Other bacterial processes occurring in the lake are also discussed. Preliminary studies have been made on the organic geochemistry of the monimolimnion sediments. Carbon-14-dating indicates a lower depositional rate prior to meromixis and a downcore enrichment in ^{13}C of organic carbon and chlorophyll derivatives. Hydrous pyrolysis experiments indicate that the sediment organic matter is almost entirely derived from the water column with little or no contribution from terrestrial sources. The significance of the organics released by hydrous pyrolysis is discussed.

Introduction

Big Soda Lake is an ideal environment to study microbial reactions occurring in aquatic environments and to determine the impact of these reactions on geochemical processes. Because it is meromictic, the lake's monimolimnion provides a deep and unchanging anoxic water column where anaerobic bacterial processes may be quantified under conditions of elevated pH and salinity, as well as low redox potential. This greatly facilitates the study of microbial reactions occurring under anoxic conditions or at oxic-anoxic interfaces. In addition, because certain lacustrine petroleum deposits appear to have been derived from the sediments of alkaline meromictic lakes (Demaison & Moore 1980), the study of bacterial processes in this lake should be of importance to our understanding of the early diagenetic reactions related to oil and gas formation. Indeed, a detailed knowledge of the nature and function of water column microbial flora, as well as its downward flux into the sediments, should aid in deciphering the organic geochemistry of lacustrine source rocks (Didyk *et al.* 1978). This paper summarizes the findings of our interdisciplinary investigations of Big Soda Lake.

Study site hydrological properties

Big Soda Lake is located in the vicinity of the Carson Sink near Fallon, Nevada (Fig. 1). The lake became meromictic in this century as a consequence of irrigation practices (Kimmel *et al.* 1978). It occupies a closed-basin crater having a narrow (about 10–15 m) littoral zone and steep bottom slope. Surface area is 1.6 km^2 , mean depth is 26 m, and maximum depth near the lake centre (the site of most studies described here) is 65 m. Salinity is 26 g l^{-1} in the surface layer (Kharaka *et al.* 1984), pH is 9.7, and the very sharp pycnocline-chemocline has persisted at a depth of 34.5 m throughout our studies (1981–1985). Salinity below the chemocline is 88 g l^{-1} and this vertical density gradient inhibits mixing between the lower monimolimnion and the upper mixolimnion.

The monimolimnion is permanently anoxic and has very high sulphide concentrations (ca. 7 mM), in addition to other reduced sulphur compounds (ca. 7 mM), ammonia (2.8 mM) and dissolved organic carbon (60 mg l^{-1} ; Kharaka *et al.* 1984). Sediments of the pelagic zone are characterized by an overall green colour interspersed with numerous coloured laminations (see section on organic geochemistry of the pelagic

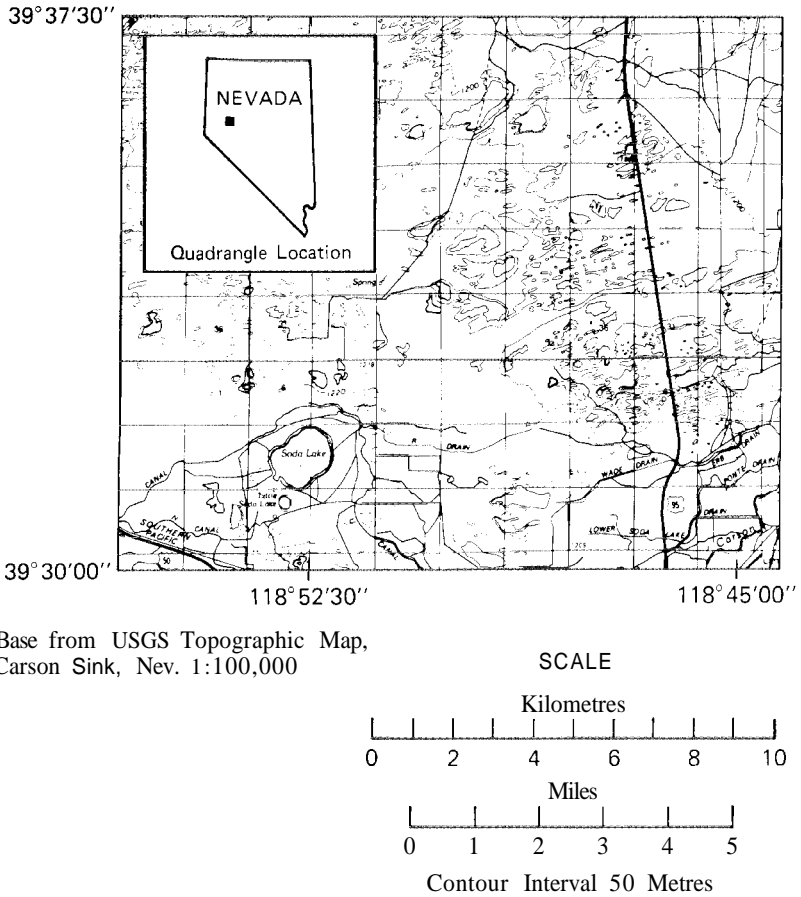


FIG. 1. A map of the location of Big Soda Lake.

sediments). Although the monimolimnion is relatively static (for example, temperature is constant at 12°C), the mixolimnion has large seasonal changes in microbial processes and the distribution of solutes (primarily nutrients) that result from seasonal mixing. From spring through autumn the mixolimnion is thermally stratified and partitioned into three distinct vertical zones (Fig. 2): the epilimnion, the aerobic hypolimnion, and the anaerobic hypolimnion. Thermocline depth is typically at 10–15 m during summer–autumn (Table 1), and the oxycline (separating the aerobic and anaerobic hypolimnion) is found at 20 m. However during winter, following surface cooling and wind mixing from winter storms, the thermocline falls almost to the depth of the chemocline (Table 1) and the mixolimnion becomes an aerobic well-mixed layer with a shallow (2–5 m) anaerobic zone. Hence the annual cycle is characterized by an alternation between thermal stratification in summer and

rapid vertical mixing in winter (Fig. 2), with short transition periods between these two states.

Production and vertical fluxes of organic matter in the mixolimnion

The annual cycle of mixing controls the distribution of nutrients, dissolved gases, autotrophic bacteria, phytoplankton, and rates of production, all of which vary markedly between seasons of stratification and mixing (Cloern *et al.* 1983a, b; Cloern *et al.* 1987).

Summer

During the period of thermal stratification, dissolved inorganic nitrogen becomes depleted in the epilimnion and phytoplankton biomass is low (<30 mg m⁻² chlorophyll *a*; Table 1). Bioassays confirm that phytoplankton are nitrogen limited during summer, although trace metal (Fe) limi-

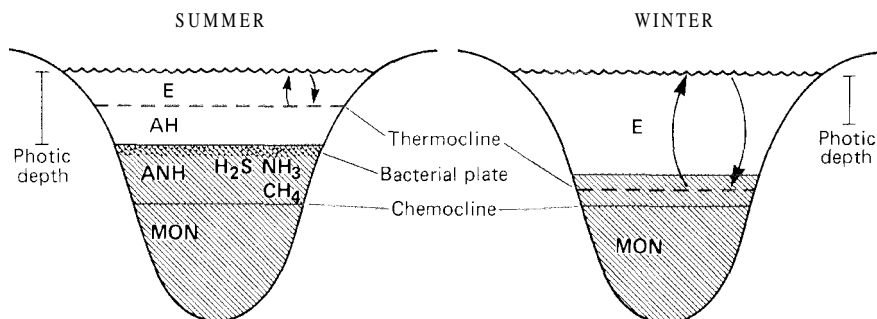


FIG. 2. Seasonal variation in the limnological properties of Big Soda Lake. E = epilimnion; AH = aerobic hypolimnion; ANH = anaerobic hypolimnion; Mon = monimolimnion.

tation is also important (Axler *et al.* 1978, Prisco *et al.* 1982). Oxygen disappears at the compensation depth of phytoplankton photosynthesis (=20 m) and large gradients of other solutes coincide with the oxycline, the anaerobic hypolimnion has detectable concentrations of reduced sulphur compounds (ca. 0.2–1 mM), ammonia (2 μM), and methane (1–5 μM). Low light levels (<1% surface irradiance) penetrate to the depth of oxygen disappearance and sustain anoxygenic photosynthesis by purple sulphur bacteria (*Chromatium* sp. and *Ectothiorhodospira cacuolata*) that are confined to a narrow zone (Fig. 2). Biomass of the photosynthetic bacteria is very high (500–1000 mg m^{-2} bacteriochlorophyll *a*, Table 1), and bacterial productivity (both anoxygenic photosynthesis and chemoautotrophy) exceeds phytoplankton productivity by about a factor of

six. When production is dominated by bacteria, vertical fluxes of particulate organic matter (measured with sediment traps just above the chemocline; Cloern *et al.* 1987) are small, ranging from 45–110 $\text{mg C m}^{-2} \text{d}^{-1}$ and 12–20 $\text{mg N m}^{-2} \text{d}^{-1}$, Table 1). These small vertical fluxes (about 10% of daily productivity) are presumably the result of slow sinking rates of autotrophic bacteria, and suggest that most new organic matter is mineralized in the water column before it sinks to the monimolimnion (see later section on microbial heterotrophy).

Winter

Vertical distributions of solutes and autotrophs are radically different in winter when the mixolimnion is isothermal to below 30 m. Erosion of

TABLE 1. Properties of the Big Soda Lake mixolimnion contrasting the summer–autumn period of thermal stratification with the winter–spring period of mixing

	Summer–Autumn	Winter–Spring
Thermocline depth (m)	10 ^c –16 ^e	26 ^g –33 ^h
DIN (= $\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$) in photic zone (μM)	< 1 ^c	15 ^f
Phytoplankton biomass (mg m^{-2} chl <i>a</i>)	11 ^a –26 ^b	100 ^h –950 ^f
Photosynthetic bacteria biomass (mg m^{-2} bacteriochlorophyll <i>a</i>)	500 ^d –1040 ^b	90 ^h –130 ^g
Productivity ($\text{mg C m}^{-2} \text{d}^{-1}$):		
Phytoplankton	90 ^{b,c}	2800 ^f
Bacteria	570 ^b –620 ^c	30 ^f
Total	660 ^b –710 ^c	2830 ^f
Vertical fluxes to the chemocline:		
$\text{mg C m}^{-2} \text{d}^{-1}$	45 ^e –110 ^d	400 ^h
$\text{mg N m}^{-2} \text{d}^{-1}$	12 ^e –20 ^d	47 ^h

^a July 1981; ^b November 1981; ^c July 1982; ^d July 1984; ^e October 1984; ^f February 1982; ^g February 1985; ^h May 1985.

Data from Cloern *et al.* (1987).

the thermocline allows vertical mixing of NH_4^+ and other constituents from the anaerobic hypolimnion to the photic zone, and increased concentrations of DIN (Table 1) stimulate phytoplankton growth and lead to a bloom dominated by the pennate diatom *Nitzschia palea*. Phytoplankton biomass increases by a factor of 10–100 and phytoplankton productivity increases about thirty-fold (Table 1). Conversely the biomass and productivity of autotrophic bacteria decline. Vertical mixing disperses the plate of autotrophic bacteria, and it brings oxygen well below the photic zone (Fig. 2) so anoxygenic photosynthesis is not sustained in winter. Hence the winter period is characterized by increased community productivity (by a factor of four) that is dominated by planktonic diatoms, and greatly diminished significance of autotrophic bacteria. Sediment trap measurements following the decline of the winter bloom in 1985 show that vertical fluxes of particulate organic matter are also enhanced then. For example, vertical fluxes of particulate

carbon and nitrogen increased about four-fold during May 1985, reflecting both the faster sinking rates of diatoms relative to bacteria and the higher rates of production during the bloom. As a consequence, there is seasonal variability in the sinking flux of biogenic materials (C, N, Si) to the monimolimnion, and the increased vertical flux during the winter bloom is a potential mechanism of layer formation in the pelagic sediments.

Water column microbial biomass

Profiles of microbial biomass (cell protein, adenosine triphosphate, cell counts, and turbidity) in the lake's water column during autumn and spring are shown in Fig. 3. The most apparent difference between these two seasons was the presence of a dense bacterial layer ('plate') at 21 m depth during October but not during May. The plate harboured a population of purple sulphur photosynthetic bacteria (as well as other

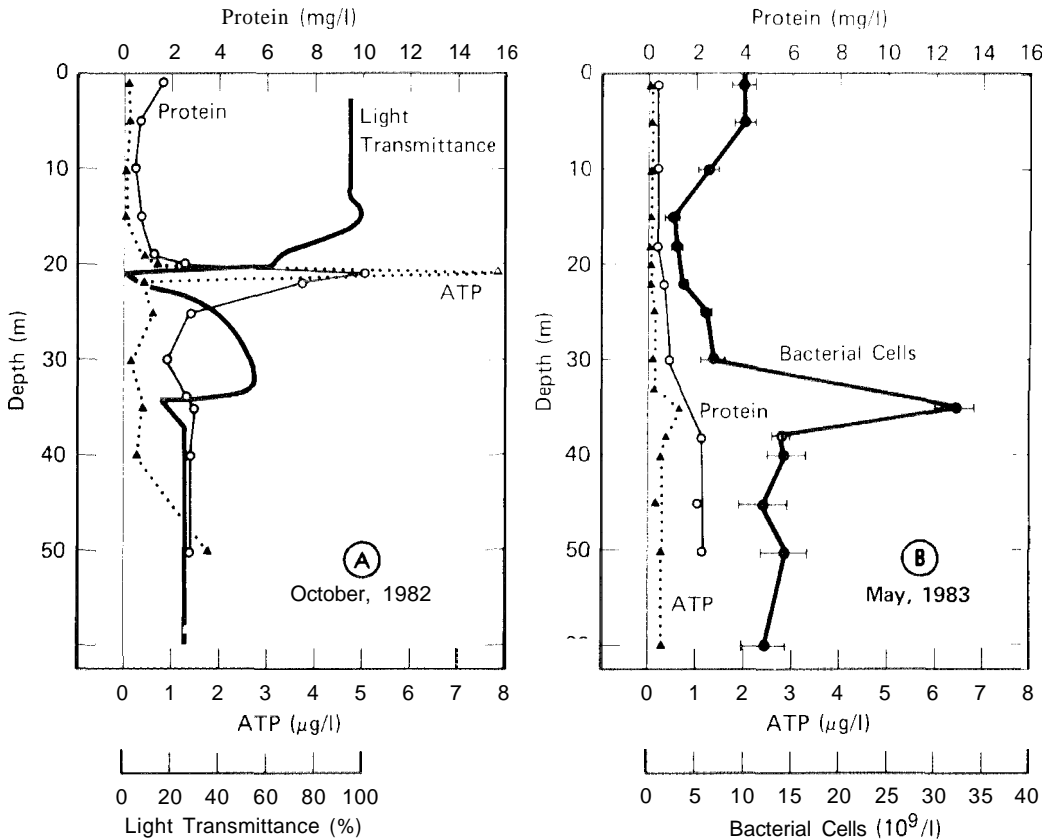


FIG. 3. Depth distributions of cell protein (○); adenosine triphosphate (Δ); bacterial cells (●) ± 1 standard deviation; and light transmittance determined during (A) October 1982, and (B) May 1983 in Big Soda Lake. The water column became anoxic at depths greater than 19 m in October and 16 m in May.

bacteria), which was reflected in high values of bacteriochlorophyll *a* and low values of light transmittance (Cloern *et al.* 1983a, b). Values of cell protein and adenosine triphosphate in the monimolimnion were similar for both seasons (Fig. 3). Direct counts of bacterial cells were made using acridine orange epifluorescence techniques (Harvey 1987). Bacterial density in the mixolimnion ranged from 2.5×10^9 to 10×10^9 cells l^{-1} . Cell density increased to 30×10^9 cells l^{-1} at the chemocline (35 m) and decreased to constant values (about 14×10^9 cells l^{-1}) in the water column of the monimolimnion (Fig. 3). The monimolimnion seasonally contained most of the total water column microbial biomass (ca. 60%), while lesser amounts were found in the anoxic mixolimnion (ca. 24–33%) and aerobic mixolimnion (ca. 15–17%) (Zehr *et al.* 1987).

Microbial heterotrophy

Uptake of 3H -glucose by microbial assemblages in the water column of Big Soda Lake was linear at all depths studied during the course of a 5-day experiment. Incorporation rates were 20–60 times higher in the mixolimnion than in the monimolimnion (Fig. 4). Seasonal uptake profiles for either ^{14}C -glutamate or 3H -thymidine exhibited maxima just beneath the photosynthetic plate (Zehr *et al.* 1987). Very low rates were usually observed in the monimolimnion. These results

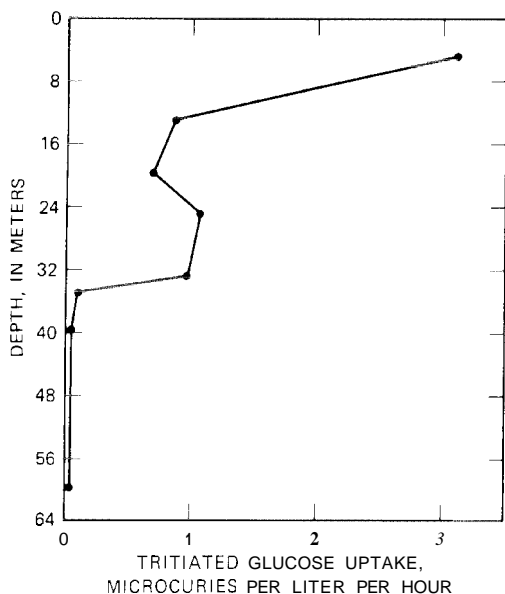


FIG. 4. Uptake of tritiated glucose by microbial assemblages in the water column of Big Soda Lake. Experiments were performed during May 1983.

indicate that the lower uptake rates observed at or beneath the 35-m chemocline were due to a physiological response of the bacterial flora to the harsh chemical environment of the monimolimnion. Thus, although the monimolimnion harbours most (ca. 60%) of the water column microbial biomass, these cells exhibit little activity and are probably mainly derived from sinking out of the mixolimnion.

Chemical factors which may retard bacterial heterotrophic activity in the monimolimnion include high salinity and free sulphide levels. In contrast, the mixolimnion (especially the anoxic region) appears to be the zone in which most (ca. 90%) of the primary productivity is mineralized (Cloern *et al.* 1987; Zehr *et al.* 1987). The bulk of this activity appears to be linked to fermentative reactions, because of the low rates of sulphate reduction, methanogenesis and undetectable denitrification in this region.

Chemoautotrophy

From spring to autumn, high rates of chemoautotrophy (dark ^{14}C CO₂ fixation) were evident just beneath the oxycline (21 m). This activity coincided with the depth interval occupied by the bacterial plate (Cloern *et al.* 1983a). Addition of chemical inhibitors of nitrifying bacteria (nitrapyrin or acetylene) decreased dark CO₂ fixation by 40–80%. Addition of thiosulphate to washed cell suspensions taken from 21 m stimulated dark CO₂ fixation. These results indicated that oxidation of ammonia and reduced sulphur compounds at the oxic–anoxic interface (21 m) was responsible for the observed bacterial chemoautotrophic fixation of CO₂. Chemoautotrophy accounted for 30% of annual water column productivity (Cloern *et al.* 1983a). Estimates of rates of sulphide and ammonia oxidation, however, have not as yet been measured.

Hydrocarbons and $\delta^{13}CH_4$

Most of the methane formed in Big Soda Lake originates in the sediments of the monimolimnion at a depth of more than 1 m below the lakebed. However, geochemical evidence also suggests that small quantities of methane are produced by bacteria in the anoxic portion of the water column (Oremland & Des Marais 1983). Methane concentrations beneath 1 m in monimolimnion sediments were as high as $418 \mu\text{mol kg}^{-1}$ and dissolved methane concentrations in the monimolimnion waters were 50–60 μM . Methane concentrations decreased markedly (about ten-fold) above the chemocline and again above the oxycline. Surface waters were supersaturated

with respect to the atmosphere and contained $0.2 \mu\text{M}$ methane. Methane efflux from the lake's surface was estimated to be about $36 \mu\text{mol m}^{-2} \text{d}^{-1}$ (Iversen *et al.* 1987). Ethane, propane and both normal and iso-butane were abundant in the monimolimnion and displayed maximum concentrations of 260, 80, 22 and 23 nM, respectively. A bacterial origin either in the monimolimnion water or surficial sediments was indicated for these gases (Oremland & Des Marais 1983), perhaps via mechanisms similar to those in estuarine sediments (Oremland 1981; Vogel *et al.* 1982).

Isotopically 'light' values of $\delta^{13}\text{CH}_4$ (-70 to -74‰) were encountered in the deeper (> 1 m) sediments of the monimolimnion. However, values became enriched in ^{13}C at the surface of these sediments (-55‰) and in the monimolim-

nion water column (-55 to -60‰). A further enrichment of water column $\delta^{13}\text{CH}_4$ values in ^{13}C was evident in the anoxic monimolimnion. In this zone, 92% of the samples had values between -48 and -20‰ . Bacterial processes were cited as the cause of the ^{13}C enrichment (Oremland & Des Marais 1983). These processes include anaerobic methane oxidation and methanogenesis from isotopically 'heavy' substrates. A typical profile of $\delta^{13}\text{CH}_4$ is shown in Fig. 5.

Methanogenesis

Methanogenic activity was detected both in the monimolimnion sediments (Oremland *et al.* 1982a) and anoxic mixolimnion waters (Oremland & Des Marais 1983). Methanogenic substrates in these and other high sulphate

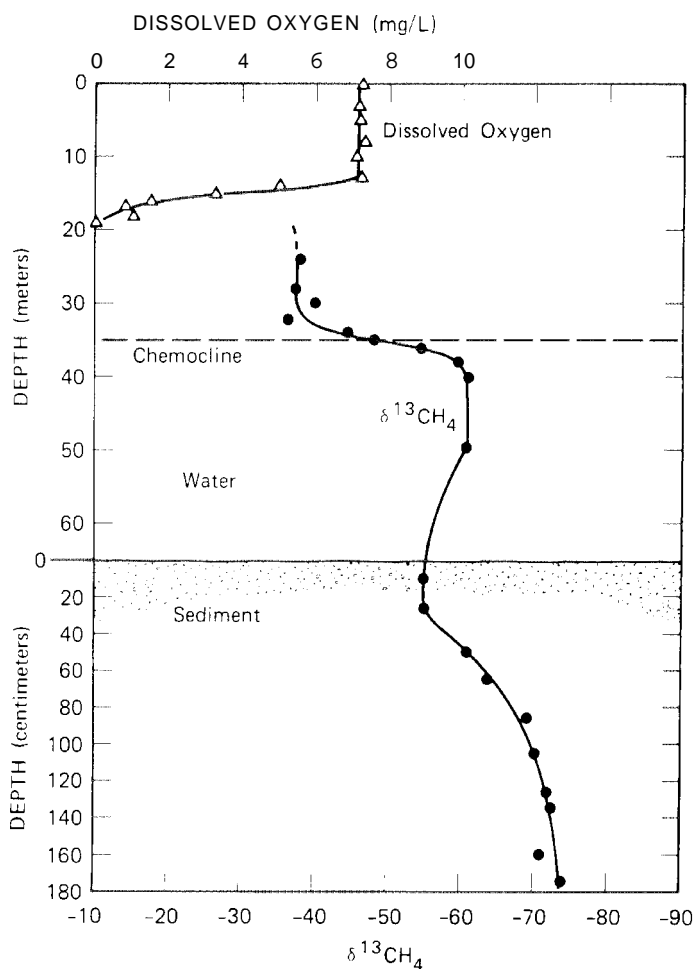


FIG. 5. Stable carbon isotopic composition of methane ($\delta^{13}\text{CH}_4$) determined in the anaerobic water column of Big Soda Lake during October 1982. Data is from Oremland & Des Marais (1983).

environments appear to be compounds such as methanol and methylamines rather than acetate or hydrogen (Oremland *et al.* 1982a, b; Oremland & Polcin 1982). This phenomenon is caused by the channelling of acetate and hydrogen to the metabolically more efficient sulphate-reducing bacteria. However, because sulphate reducers have relatively little affinity for methanol or methylated amines, methanogenic bacteria can metabolize these compounds, thereby allowing for both methanogenesis and sulphate reduction to occur simultaneously. Recently, dimethylsulphide was identified as another possible methane precursor (Kiene *et al.* 1986).

In a preliminary study of methanogenesis in the water column of Big Soda Lake, lake water from 40 m depth was incubated in the laboratory. The collected water samples were stored for 3 months prior to the experiment at 12°C in completely filled, 4-l glass bottles fitted with ground glass stoppers. Water was degassed of methane and dispensed into serum bottles which contained a small headspace of N₂ (Iversen *et al.* 1987).

The results for some samples are shown in Fig. 6. Methanogenesis occurred in the unsupplemented bottles and was stimulated by methanol. Water containing BES, a specific inhibitor of methanogens (Gunsalus *et al.* 1978), formed much less of the gas, as did water supplemented

with Ni²⁺. Nickel addition caused an initial stimulation (< 10 days), after which time no further methane was produced. The results for all conditions after 83 days incubation are shown in Table 2. Only methanol and trimethylamine enhanced methanogenesis, while BES and Ni²⁺ inhibited the process.

The stimulation of methanogenesis by methanol and trimethylamine, as well as the absence of stimulation by acetate, was consistent with earlier observations with sediment slurries (Oremland *et al.* 1982a), as was the inhibition achieved by BES. Methanogenic bacteria have nutritional requirements for Ni²⁺, Co²⁺, and Fe²⁺ (Daniels *et al.* 1984). Because the chemistry of the lake water is such that these metals are probably present only at extremely low levels (Kharaka *et al.* 1984), these substances were added in an attempt to see if methanogenic bacteria in the monimolimnion were limited by the availability of trace metals. However, with the exception of slight initial stimulation by Co²⁺ and Ni²⁺, no long-term enhancement was observed. Kaolinite was added as a control to determine if the presence of increased surface area would enhance activity, since addition of metals resulted in the formation of sulphide precipitates. Since no obvious enhancement occurred, additional surface area did not enhance methanogenesis.

Methanogenic activity in the water column of

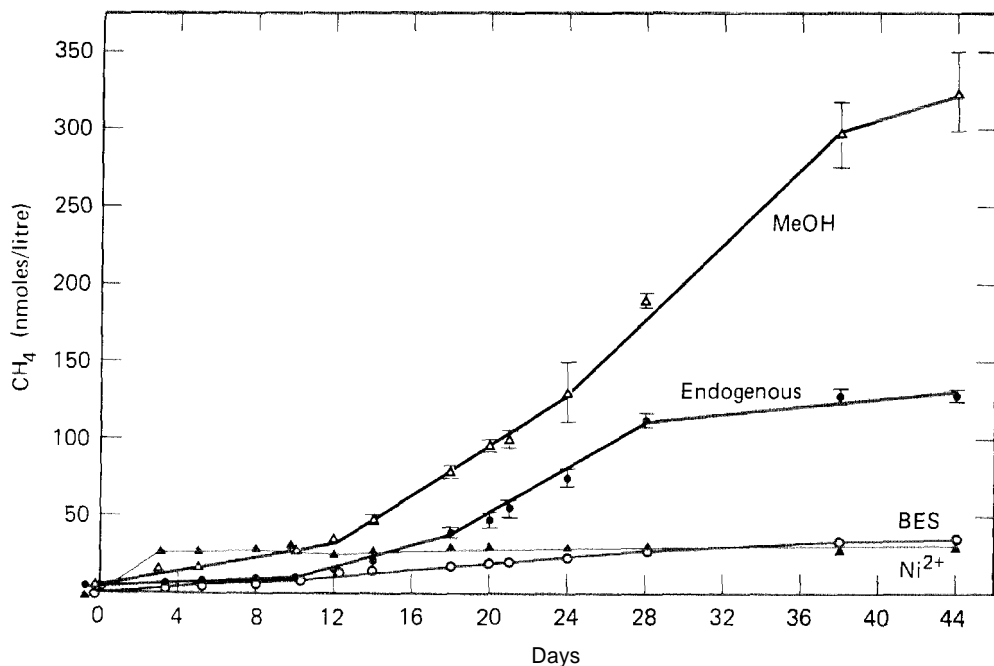


FIG. 6. Methane production by monimolimnion water. MeOH = methanol; BES = 2-bromoethanesulfonic acid. Results represent the mean of three bottles and bars = 1 standard deviation.

TABLE 2. Production of methane by monimolimnion water (40 m) after 83 days of a laboratory incubation (12°C)

Addition	Concentration	Methane (nmol l ⁻¹)
None	—	794 (84)
BES	5 mM	79 (11)
Methanol	50 μM	5129 (1331)
Trimethylamine	50 μM	6325 (2122) ^a
Acetate	50 μM	897 — ^b
FeCl ₂ ·H ₂ O	100 mg l ⁻¹	938 (116)
NiCl ₂ ·6H ₂ O	120 mg l ⁻¹	30 (6.6)
CoCl ₂ ·6H ₂ O	100 mg l ⁻¹	915 (127)
Kaolinite	1 g l ⁻¹	1119 (287)

Values represent the mean of the three samples with a standard deviation indicated within brackets.

^a represents average and range of two samples.

^b represents one sample (due to breakage). After 20 days, triplicate acetate-amended samples were 49.5 ± 5.5 nmol l⁻¹ and methanol-amended were 93.9 ± 4.0 nmol l⁻¹.

Big Soda Lake was measured with freshly recovered samples during October 1983 and July 1984 (Iversen *et al.* 1987). Methanogenic activity ranged between 0.1 and 1.0 nmol l⁻¹ d⁻¹ in the anaerobic mixolimnion and between 1.6 and 12 nmol l⁻¹ d⁻¹ in the monimolimnion. The experiments conducted under *in situ* conditions differed from the laboratory experiments in two respects. First, no enhancement of methane formation was observed when 40-m water samples were supplemented with either methanol or trimethylamine (each 50 μM). This suggests that these substances were not limiting methanogenic bacteria in the field experiments, although they were in the preliminary, long-term laboratory experiments. Second, although 5 mM BES inhibited methanogenesis in the preliminary, long-term laboratory experiments (Fig. 6), no inhibition was achieved using 10 mM BES at any of the eleven depths tested during October 1983. In the July 1984 experiments 37 mM BES caused a partial inhibition in mixolimnion samples, but did not inhibit monimolimnion samples (Fig. 7). These mixed results with inhibitor concentrations may have been caused by differential susceptibility by the methanogenic flora to BES. For example, acetoclastic methanogenesis in digestors was blocked by 1 mM BES, but that formed via H₂ reduction of CO₂ required 50 mM (Zinder *et al.* 1984). The fact that 37 mM BES worked in the mixolimnion, but not in the monimolimnion (Fig. 7) indicates that a situation of ineffective concentrations occurred in these experiments. Similar difficulties with BES (1.4–14 mM) were

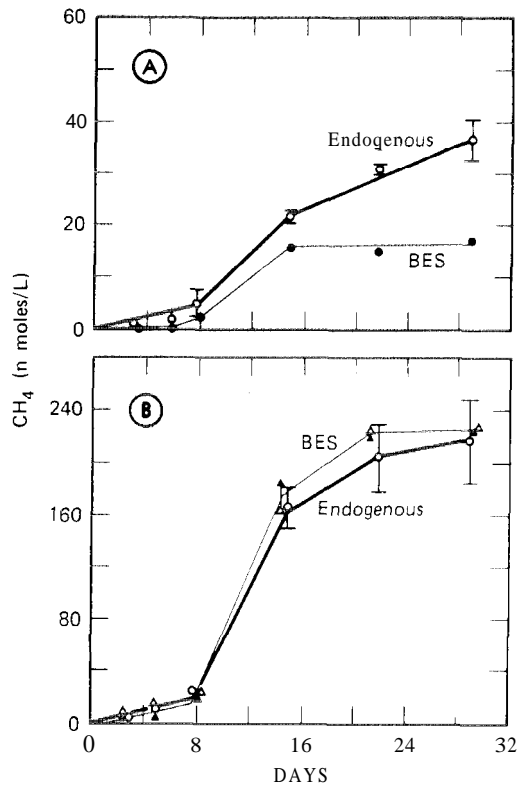


FIG. 7. Effect of BES (37 mM) on methanogenic activity in water samples taken from (A) the mixolimnion (28 m); and (B) the monimolimnion (40 m) of Big Soda Lake. Experiments were conducted during May 1984.

evident in some of the earlier reported Big Soda Lake sediment slurry-incubations (Oremland *et al.* 1982a).

Methane oxidation

Incubation of lake water under *in situ* conditions with ¹⁴CH₄ demonstrated the production of ¹⁴CO₂ with time (Iversen *et al.* 1987). Rates in the aerobic mixolimnion were very low (0.2–1.3 nmol l⁻¹ d⁻¹) and accounted for only 0.04% of the bacterial methane oxidation occurring in the water column. Therefore, anaerobic methane oxidation was the process which accounted for over 99% of the water column methane consumption. Rates were first order with respect to methane and were higher in the monimolimnion (49–85 nmol l⁻¹ d⁻¹) than in the anoxic mixolimnion (2–6 nmol l⁻¹ d⁻¹). Rates of anaerobic oxidation always exceeded those of production, thus indicating a net consumption of the gas occurred in the anoxic water column. Anaerobic

oxidation could be blocked by filter-sterilization, but was uninfluenced by Na_2WO_4 (an inhibitor of sulphate-reducing bacteria). Although a net consumption of methane occurred in the lake's anoxic zone, the daily rate of oxidation was about 1000-fold lower than the ambient levels of dissolved methane. Thus, a decrease in the dissolved methane content of incubated water samples could not be observed over a 97 h time period. However, anaerobic methane oxidation was probably responsible for the isotopically 'heavy' CH_4 detected in the anoxic mixolimnion (Fig. 5).

Sulphate reduction

Sulphate reduction is the apparent source of the high sulphide levels in the anoxic mixolimnion (ca. 0.7 mM) and monimolimnion (ca. 7 mM) as well as for the observed $\delta^{34}\text{S}$ sulphate values in the monimolimnion (-34‰), mixolimnion (-6‰) and monimolimnion $\delta^{34}\text{S}$ sulphide values (-26‰) (Kharaka *et al.* 1984). Estimates of the rates of water column sulphate reduction were achieved by performing *in situ* incubations with ^{35}S -sulphate (Smith & Oremland 1987). Rates of $^{35}\text{S}^{2-}$ production were linear over 5 days. Five depths sampled in the monimolimnion (35, 38, 40, 50 and 60 m) all had seasonal rates between 0.9 and 3 $\mu\text{mol SO}_4^{2-}$ reduced $\text{l}^{-1} \text{d}^{-1}$. This agrees well with the overall estimate of 6.6 pmol SO_4^{2-} reduced $\text{l}^{-1} \text{d}^{-1}$ deduced by analysis of past and current chemical data (Kharaka *et al.* 1984). Sulphate reduction rates decreased to ca. 1.4 and ca. 0.6 $\mu\text{mol l}^{-1} \text{d}^{-1}$ at 33 and 30 m respectively. Rates in the anoxic mixolimnion were below 0.025 $\text{pmol l}^{-1} \text{d}^{-1}$. Annual monimolimnion sulphate reduction was estimated to be 9–29 $\text{mmol m}^{-1} \text{d}^{-1}$ (Smith & Oremland 1987). This extrapolated to a monimolimnion sulphate turnover of 60–270 years, not accounting for oxidation of sulphide. Equivalent carbon mineralized was 210–700 $\text{g m}^{-2} \text{yr}^{-1}$, which could account for most, if not all, of the mixolimnion productivity. The apparent discrepancy between the carbon mineralized by monimolimnion sulphate reduction and that occurring via fermentative reactions in the mixolimnion has not been fully reconciled. Possibilities include downward flux of additional carbon from the littoral zone and the steeply-graded mixolimnion sediments. Sulphate reduction in monimolimnion waters was stimulated two-fold by addition of FeS (in contrast to methanogenesis) or H_2 . Acetate or lactate caused only 28 and 16% enhancement, respectively. No stimulation occurred with MnS , methanol or kaolinite. Sulphate reduction was inhibited by about 67% when

samples were incubated with Na_2WO_4 (20 mM). Rates of sulphate reduction were not influenced by either the removal or addition of methane to the samples. Results indicate that sulphate reduction is not directly coupled to anaerobic methane oxidation.

Nitrogen cycle

Attempts were made to measure nitrogen fixation and denitrification in the mixolimnion of the lake using the acetylene reduction and acetylene block assays, respectively. Seven depths (1, 5, 10, 15, 20, 25 and 30 m) were routinely assayed over the course of the four seasons during 1981–1983. Bottles containing 200 ml of lake water and ca. 50 ml N_2 (or air) gas phase were incubated *in situ*. Acetylene (10 ml) was added to the gas phase of all but the control bottles. Selected bottles were amended with NaNO_3 (1 mM) and/or glucose (1 g l^{-1}) to enhance denitrification, or with NH_4Cl (1 mM) to inhibit N_2 fixation. After 24 h incubation, headspace analyses revealed only background level of C_2H_4 ($<0.1 \text{ nmol ml}^{-1}$) and N_2O ($<0.6 \text{ pmol ml}^{-1}$) in all the bottles, including the controls lacking C_2H_2 . Therefore neither denitrification nor N_2 fixation were detected in the mixolimnion during the four seasonal sampling intervals. In addition, amendment of anoxic mixolimnion water with nitrate and/or glucose did not elicit any detectable denitrification (N_2O accumulation). It is preliminarily concluded that these two processes do not occur in the lake's water column. The aerobic mixolimnion of Big Soda Lake is severely nitrogen-limited for much of the year (Priscu *et al.* 1982; Cloern *et al.* 1983a).

Extremely high rates of nitrogen fixation ($\sim 100 \mu\text{mol m}^{-2} \text{h}^{-1}$) were detected in the littoral zone. Fixation was caused by cyanobacterial epiphytes (*Anabaena* sp.) colonizing the abundant macrophyte (*Ruppia* sp.) present in the littoral zone. These cyanobacterial aggregates also evolved H_2 (6.8 $\text{pmol m}^{-2} \text{h}^{-1}$), however this activity was caused by fermentative bacteria living within the matrix and occurred only in the dark (Oremland 1983). Other littoral zone sites of nitrogen fixation include anaerobic, shallow 'cul-de-sacs' which are infested with purple sulphur photosynthetic bacteria. Areal rates of fixation (about 100 $\mu\text{mol m}^{-2} \text{h}^{-1}$) were equivalent to the high rates exhibited by the cyanobacteria (J. Duff *et al.* in prep.). The extent to which this littoral-zone-fixed nitrogen is transported to the pelagic region of the lake is not yet known. In addition, it is not entirely clear why the mixolimnion is devoid of cyanobacterial N_2 -fixation, although possibly a trace element limitation could exist.

Active denitrification was found to occur in some littoral zone sediments. Sediments of the *Ruppia* beds had the greatest activity; however high concentrations of added nitrate ions (1 mM) were required to elicit such a response. Thus, the 'potential' for denitrification was about $100 \mu\text{mol N}_2 \text{m}^{-2} \text{h}^{-1}$, which was equivalent to inputs via nitrogen fixation. However, the apparent K_m for nitrate was estimated to be $87 \mu\text{M}$. Since nitrate + nitrite levels in the interstitial waters are $5 \mu\text{M l}^{-1}$ (or less), it is clear that in *situ* rates of denitrification are well below the 'potential' rate estimate (S. Paulsen *et al.* unpublished data). Estimates of littoral zone denitrification were also made using a novel N_2O reductase assay (Miller *et al.* 1986). A rate of $6 \mu\text{mol N}_2 \text{m}^{-2} \text{h}^{-1}$ was arrived at using this technique.

Nitrification occurs at the oxic-anoxic interface (21 m) of the mixolimnion (Cloern *et al.* 1983a). However, ammonia oxidation rates have as yet not been measured. In addition, a dissolved N_2O maximum that usually accompanies the zone of water column nitrification (McElroy *et al.* 1978) was not evident in Big Soda Lake (Fig. 8). It is possible that N_2O is not released by nitrifying bacteria under the highly alkaline conditions (pH = 9.7) characteristic of the lake.

Other processes

Anaerobic decomposition of oxalate was studied in the sediments of both the littoral and pelagic zones of Big Soda Lake (Smith & Oremland 1983). Although the total oxalate content of these sediments was similar (ca. $100 \mu\text{M}$), degradation rates were about fifty-fold higher in the littoral zone. This lower activity in the monimolimnion sediments may also be a response to the harsh chemistry of the bottom waters.

Uprooted *Ruppia* sp. plants along with their associated decomposing cyanobacterial epiphytes eventually are forced into shallow, littoral-zone 'cul-de-sacs'. This rotting plant material can be as thick as 50 cm deep. Bubbles rich in methane (ca. 40%) are associated with this plant matter, and both ethane and propane are present as trace constituents. The bubbles have a distinct, obnoxious mercaptan odour. Anaerobic incubation of this material results in the formation of methane and traces of ethane. Methane formation was stimulated by addition of methanethiol, dimethylsulphide and dimethyldisulphide (Kiene *et al.* 1986), while stimulation of ethane production (traces) was achieved with diethylsulphide (Fig. 9) or ethanethiol. Because the formation of these gases was blocked by BES, methanogenic bacteria are responsible for the conversion of methylated and ethylated sulphur

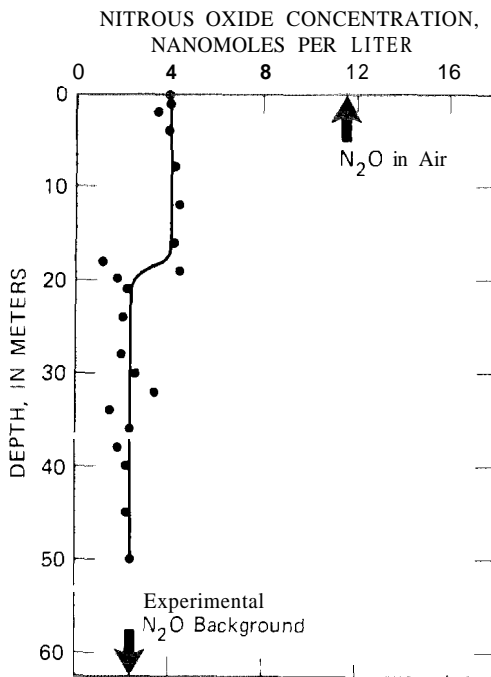


FIG. 8. Dissolved N_2O profile determined during November 1981. At this time of the year, the water column became anoxic at depths greater than 21 m. Arrows indicate the N_2O concentrations in the atmosphere (top of graph) as well as values of N_2O indistinguishable from background contamination (bottom).

gases to methane and ethane (Kiene *et al.* 1986; R. Oremland *et al.* in prep.).

Organic geochemistry of the pelagic sediments

General characteristics

Only preliminary investigations have been conducted upon the monimolimnion sediments; nonetheless several interesting aspects are apparent. These sediments are unusual in that they have a distinctively green colour (from preserved chlorophylls and their degradation products). Usually, sediments in a highly sulphidic environment are black due to the presence of amorphous iron sulphides. However, because of the low iron content of the lake, iron sulphides are not present in sufficient abundance to mask the 'organic' colouration. Pennate diatoms, derived from the winter bloom (see pp. 61–62) are abundant in the upper 30 cm. Multicoloured layers are also evident within the sediments and occur from the surface to at least 2 m depth (Plate 1). A very low

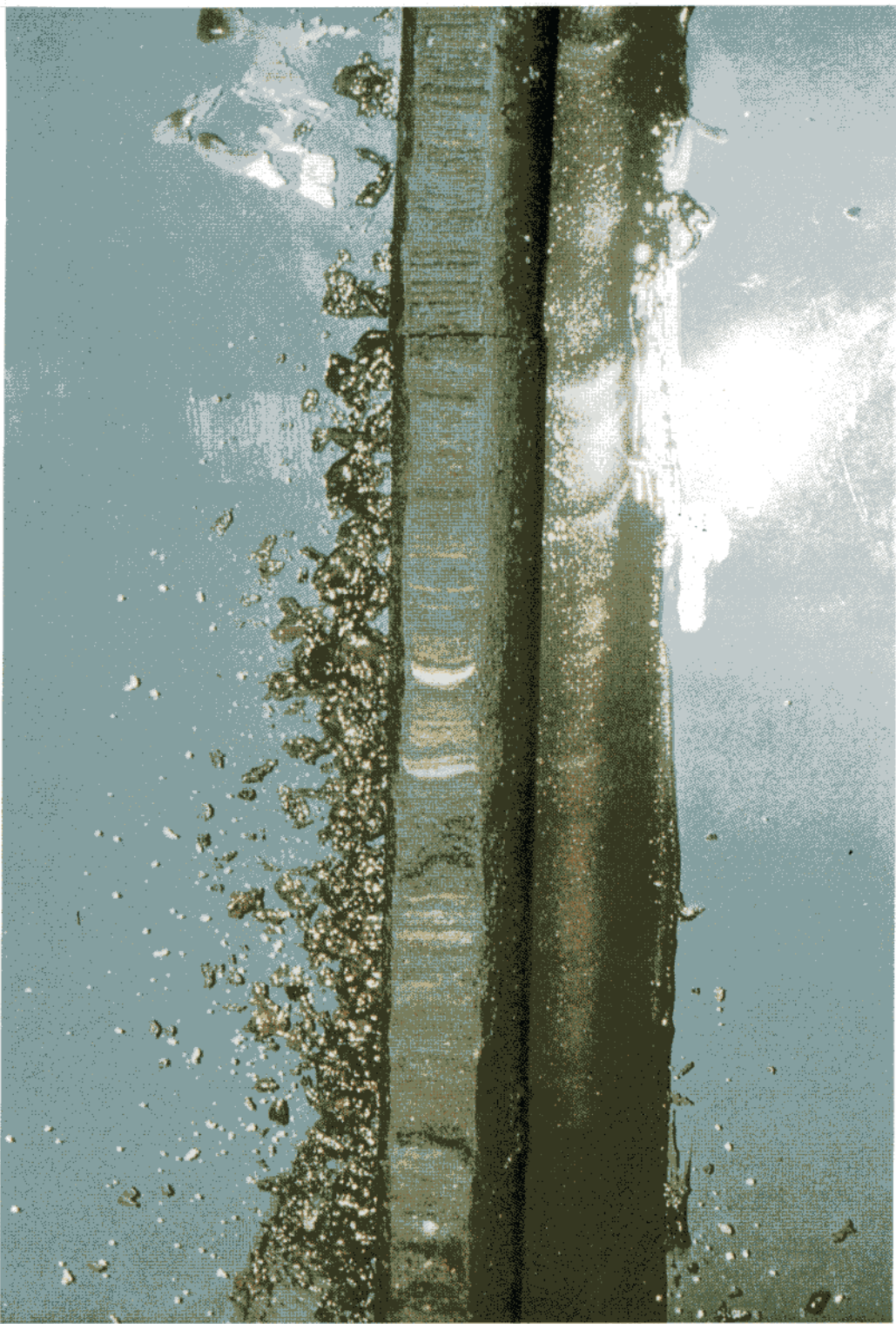
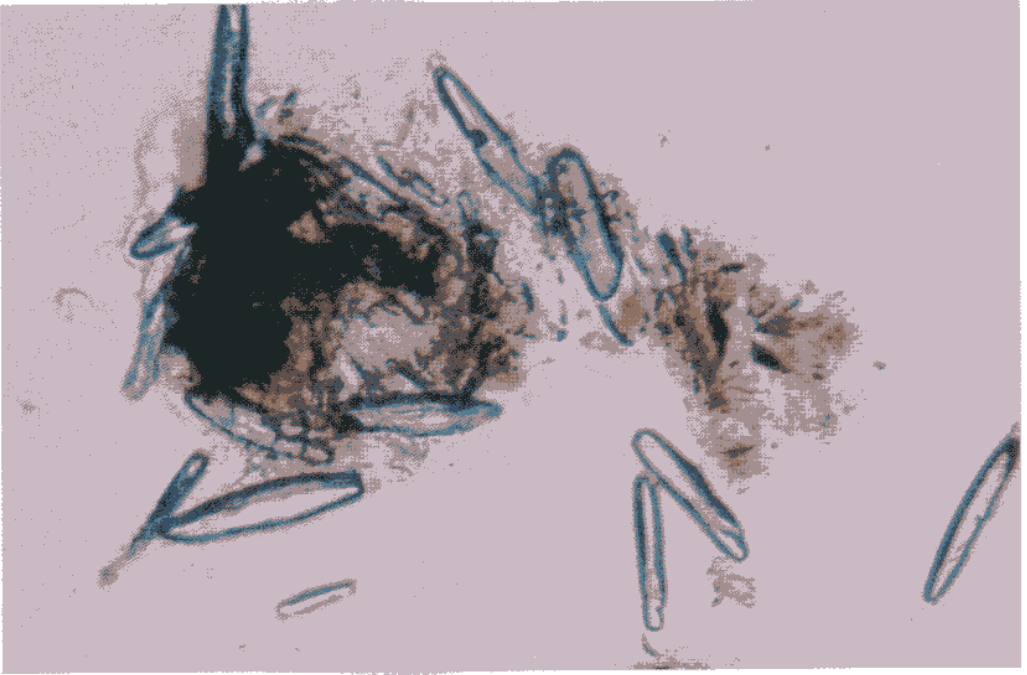
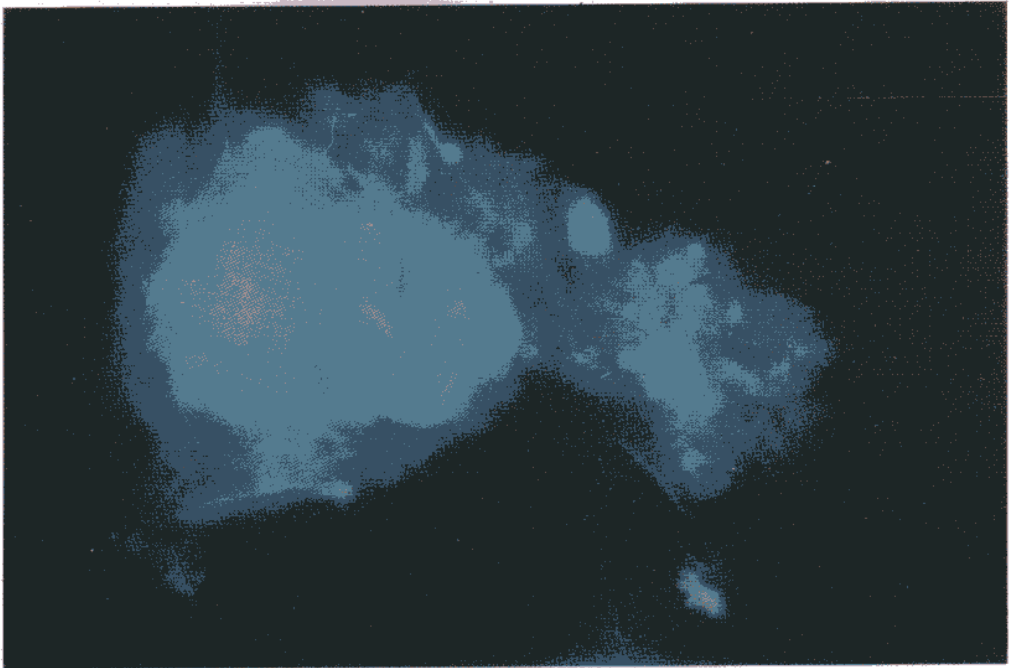


PLATE 1. Core from monimolimnion (depth interval = 95–150 cm) used in the hydrous pyrolysis experiments.

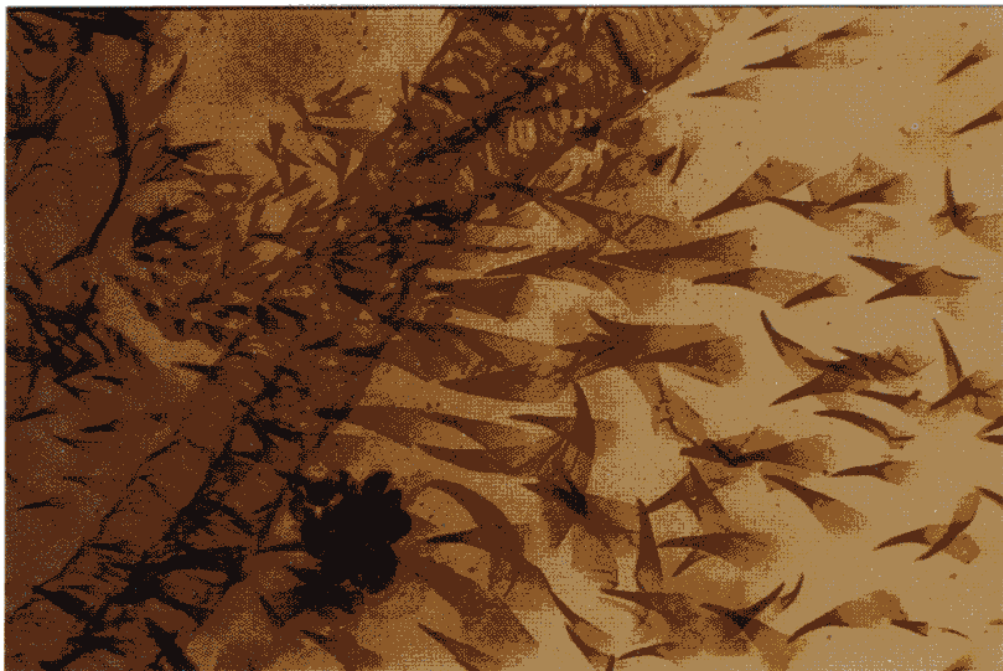


(a)

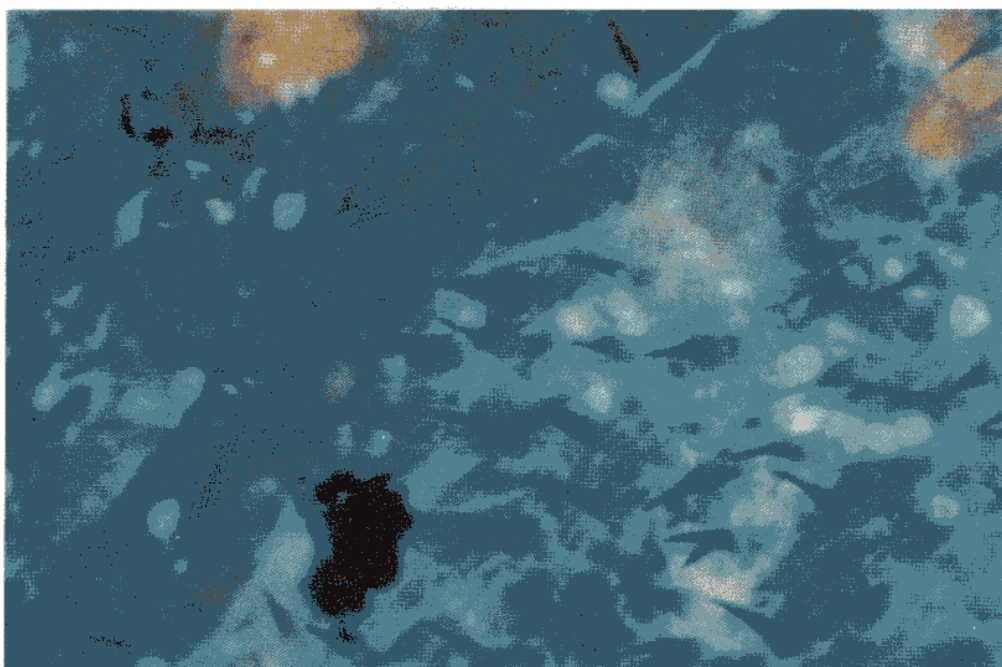


(b)

PLATE 2. Particulate organic matter in untreated lake sediments (scale: 1 cm = 25 μ). Note pennate diatoms and amorphous organic matter under transmitted light (a) and UV light (b).



(a)



(b)

PLATE 3. Chitinous remains of zooplankton and sheet-like amorphous organic matter after removal of mineral matter by acid treatment (scale: 1 cm = 25 μ). (a) transmitted light; (b) UV light.

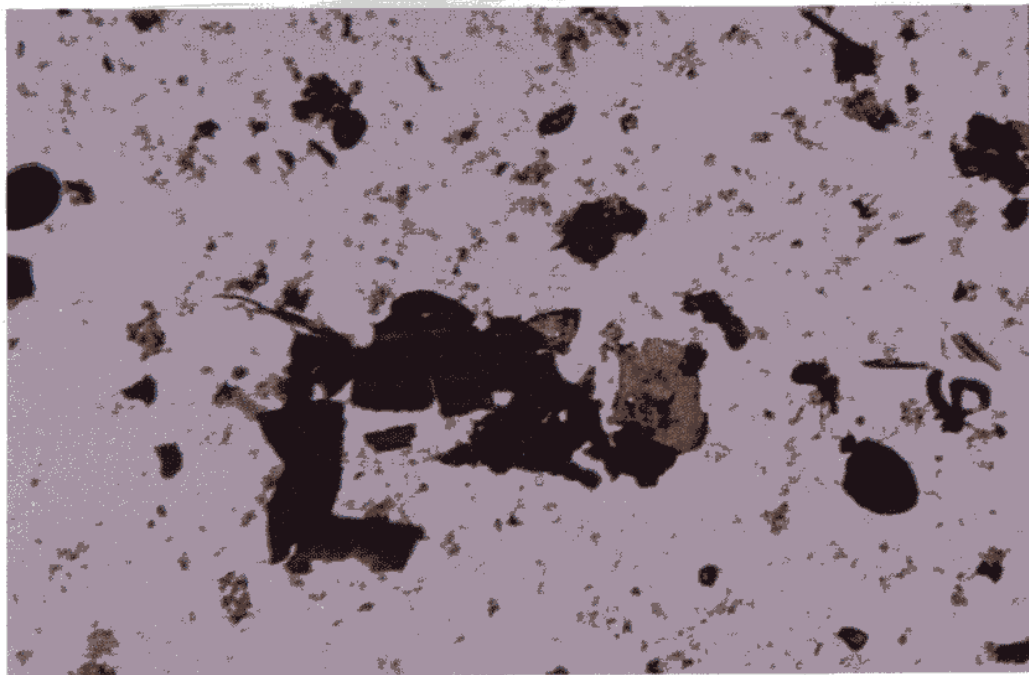


PLATE 4. Lake sediments after hydrous pyrolysis, solvent extraction and mineral matter removal (1 cm = 25 μ). The relative amount of woody plant fragments and possibly altered chitinous material appear to increase in samples after hydrous pyrolysis. The fine grained nature of the amorphous material may be a result of thermal alteration. No fluorescence was observed after hydrous pyrolysis.

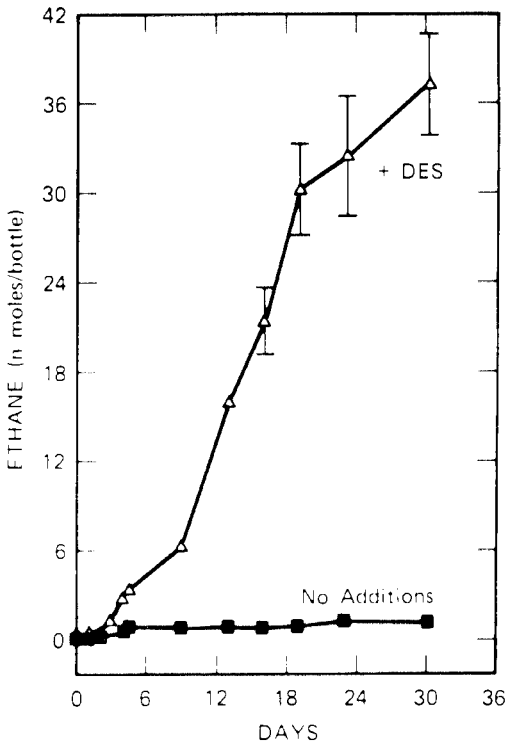


FIG. 9. Formation of ethane from diethylsulphide (10 mM) in Big Soda Lake littoral zone sediments. Results represent the mean of three bottles and bars indicate ± 1 standard deviation.

pre-meromixis sedimentation rate (ca. 0.25 mm yr^{-1}) was indicated by performing ^{14}C -dating on core organic matter (Fig. 10). Stable carbon isotope values ($\delta^{13}\text{C}$) of total organic carbon as well as chlorophyll degradation products exhibit a downcore shift from relatively heavy values at the surface to significantly lighter values at 34-cm depth (Fig. 11). Because the surrounding water-

shed excluded significant input from terrestrial plant material (a fact reinforced by the organic chemistry data below), such isotopic variations must reflect changes in processes internal to the lake. Some possibilities include: (1) changes in phytoplankton communities and associated preferential isotope abundance during carbon fixation (e.g., Wong & Sackett 1978); (2) alteration in phytoplankton isotope abundance induced by changes in epilimnetic temperature (e.g., Degens *et al.* 1968) or salinity; or (3) other possible effects (Deines 1980).

Nature of the organic matter (Z. Sofer)

The bottom sediments in the lake are highly reducing resulting in preservation of abundant organic matter. A portion of the sediment (55 cm representing the 95–150-cm interval below the sediment–water interface) was homogenized, freeze-dried, and studied microscopically and chemically. Based on the microscopical study (Plate 2) the organic matter is primarily amorphous; abundant pennate diatom frustules are also present and indicate that, along with bacteria, they are a major contributor to the organic matter. Table 3 shows that the dried sediment contains 2.65% organic carbon. After removal of the mineral matter from the sediment (by treatment with HCl and HF), the isolated organic matter consists of abundant amorphous material, spores, pollen, woody plant fragments and large, tan fragments, possibly the chitinous remains of crustaceans (Plate 3).

The freeze-dried sediment was soxhlet-extracted with chloroform:methanol (9:1) and the extractable matter was quantitatively separated into C_{15+} aliphatics, aromatics, heteroatomic (NSO) compounds, and asphaltenes (i.e., large heteroatomic compounds insoluble in cold pentane). As seen in Table 3, about 0.6% of the organic matter is soluble, and most of it is in the

TABLE 3. Organic and isotopic composition of Big Soda Lake sediments before and after hydrous pyrolysis

	% Organic carbon		C_{15+} Organic extract (ppm)				
	Total	Insoluble	Alkanes	Aromatics	NSO	Asphaltenes	Total C_{15-}
Organic composition							
Before HP*	2.65	2.04	32	278	1601	6937	8847
After HP	1.63	0.28	2253	2205	4458	34421	43337
Carbon isotopic composition ($\delta^{13}\text{C} - \text{PDB} \text{ ‰}$)							
Before HP	-23.80	-22.92	-26.48	-26.26	-26.26	-24.98	
After HP		-24.34	-25.76	-24.97	-24.42	-24.13	

*HP = hydrous pyrolysis.

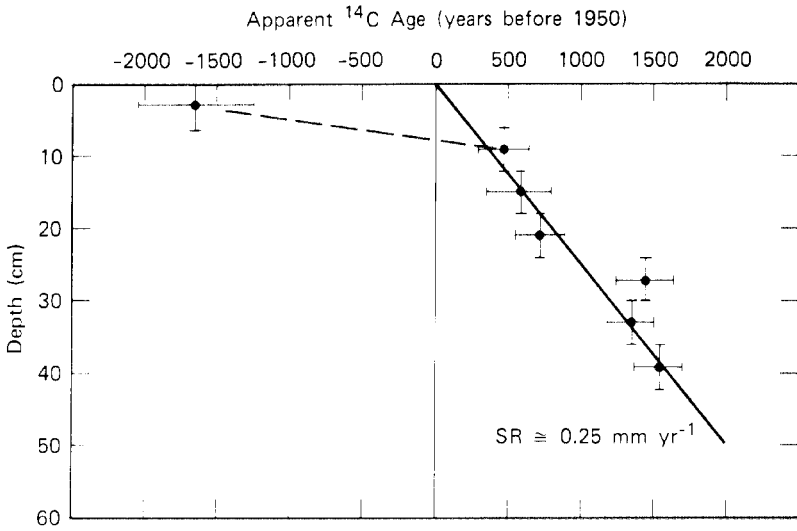


FIG. 10. Apparent ^{14}C age of organic carbon in monimolimnion sediment core.

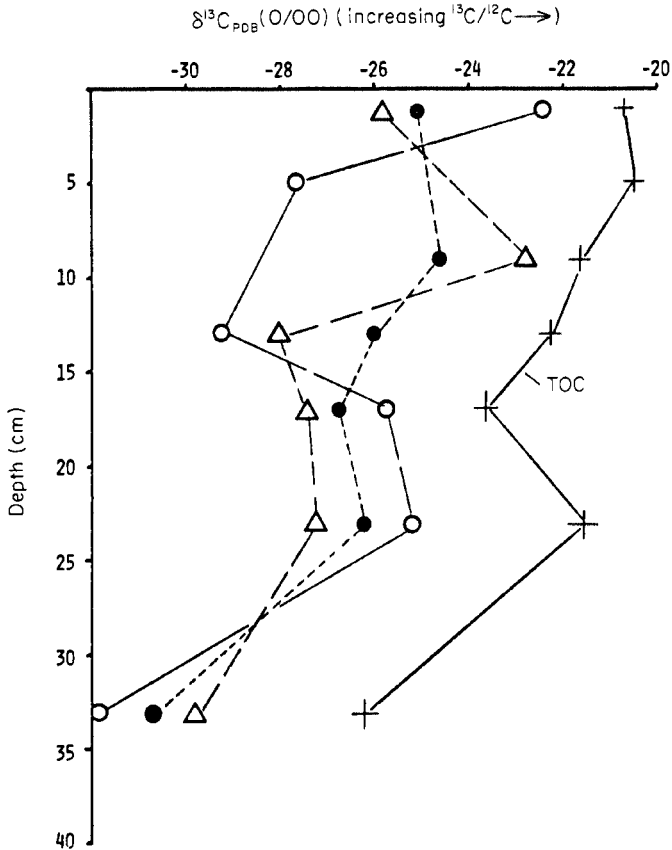


FIG. 11. Stable carbon isotope values ($\delta^{13}\text{C}$) of total organic carbon (+), phaeophytins (Δ , \circ) and phaeophorbides (\circ) in sediment core. Compounds were extracted with acetone/methanol solvent and separated by HPLC.

form of asphaltenes. In addition, the freeze-dried sediments were subjected to a mild thermal alteration by means of hydrous pyrolysis (300°C for 72 h, as described by Schiefelbein, 1982), in order to evaluate the 'petroleum-like' material that such sediments would generate.

After hydrous pyrolysis the total organic carbon in the lake sediments was reduced from 2.65 to 1.63% due to the formation of carbon dioxide, and hydrocarbon gases which are not accounted for in the total organic carbon. The resulting insoluble organic matter (kerogen) is reduced, as shown in Table 3, to approximately 1/7 of the original amount (from 2% to 0.3%). The thermally altered 'kerogen' (Plate 4) appears to be composed mainly of woody matter. The observed decrease in the amount of the amorphous and chitinous material suggests that these materials were transformed into organic soluble matter. Hydrous pyrolysis of the sediment resulted in a five-fold increase in the extractable C_{15+} compounds relative to the precursor material (see Table 3: aliphatics increased 70×, aromatics 8×, NSO 2.8×, and asphaltenes 5×). The amount of extractable matter relative to total organic carbon after hydrous pyrolysis is about 4.3-fold larger than what was observed in sediments from Mud Lake (Florida) where the dominant organic matter is peat derived from higher plant fragments (Schiefelbein 1982). This suggests that

organic matter derived from vascular plants contributes only a small fraction to the total organic carbon in Big Soda Lake.

The gas chromatogram of the aliphatic fraction in the lake sediment (Fig. 12a) shows a very strong predominance of the odd numbered higher plant *n*-alkanes (Eglinton & Hamilton 1963) indicating some contribution of land-derived organic matter. The gas chromatogram of the aliphatic fraction after thermal alteration (Fig. 12b), shows a waxy *n*-alkane distribution maximized at *n*- C_{24} to *n*- C_{25} and a slight even-over-odd *n*-alkane predominance has often been observed in carbonate environments (Dembicki *et al.* 1976); however, the waxy *n*-alkane distribution was unexpected in a depositional environment that is dominated by aquatic organisms such as bacteria and algae. A low Pr/Ph ratio is in agreement with a highly reducing depositional environment (Powell & McKirdy 1973). Another interesting feature in the aliphatic gas chromatogram of the hydrous pyrolysed sediment (Fig. 12b) is the presence of a homologous series of C_{21} to C_{35} branched alkanes which were positively identified by GC/MS mass spectra (Fig. 13) as 10-methylalkanes. The origin of these branched alkanes (i.e., the organism in which the precursors are synthesized) is not yet clear.

GC/MS analysis of the di- and triterpanes ($m/z=191$) and steranes ($m/z=217$) in the ali-

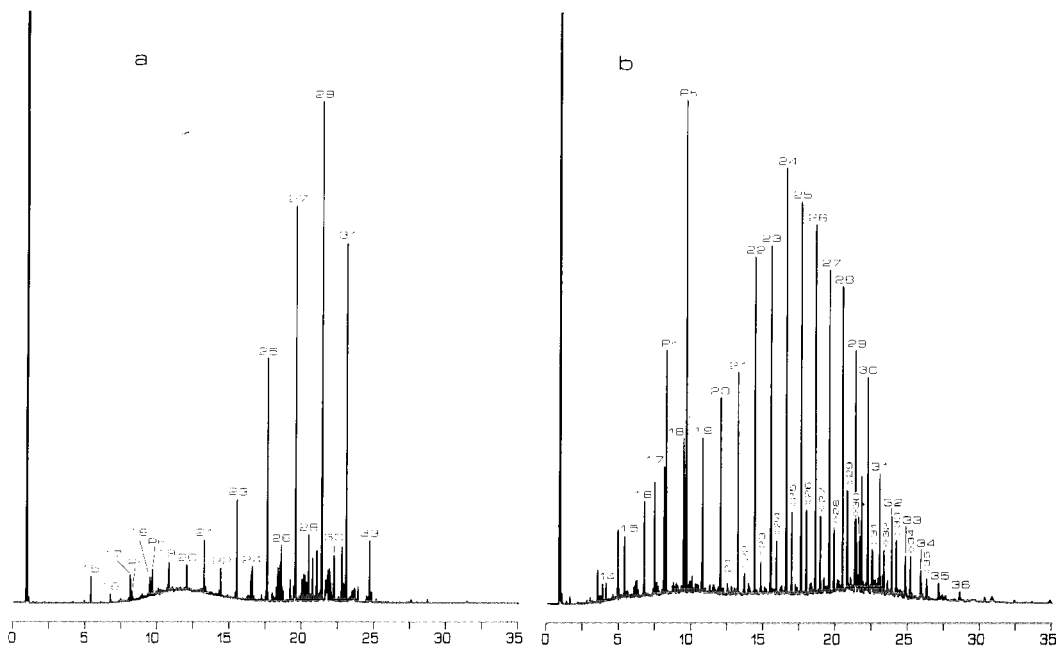


FIG. 12. Gas chromatograph of the aliphatic fraction of (a) lake sediments, and (b) hydrous pyrolysed lake sediments. Peaks with asterisk in (b) indicate 10-methylalkanes (see Fig. 5).

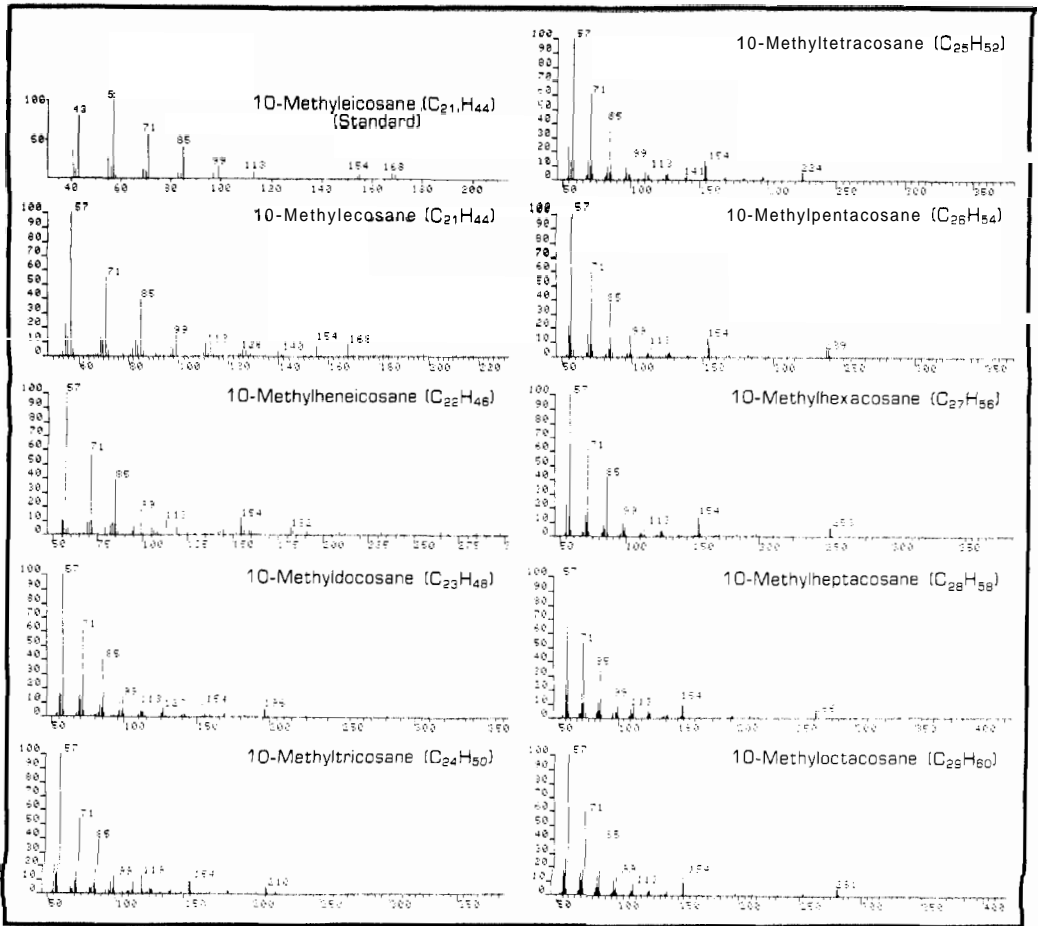


FIG. 13. Mass spectra of 10-methylalkanes (peaks with asterisks in Fig. 12b).

phatic fraction (Fig. 14) of the lake sediments shows only few of the compounds commonly observed in more mature sediments. The less thermally stable terpenoid compounds (such as the $17\beta(H)C_{30}$ triterpane and other unidentified compounds) are diminished after hydrous pyrolysis; however, under the conditions of this experiment, the remaining terpanes (triterpanes and steranes) are still typical of low maturity. Because C_{28} sterols are predominant in diatoms (Lee *et al.* 1980) it is very likely that the $5\alpha(H)$ and $5\beta(H)C_{28}$ steranes seen in the GC/MS patterns represent the abundant diatoms observed in the Soda Lake sediments. Small amounts of gammacereane were identified by mass spectra analysis of the pyrolyzate. The diterpanes after hydrous pyrolysis show a predominance of the C_{20} compounds. This predominance has also been observed in sediments

containing non-marine organic matter as well as in non-marine oils (unpublished data).

The carbon isotopic composition of the various fractions (aliphatic, aromatic, etc.) before pyrolysis range from -26.5 to -24.9‰ and -25.8 to -24.1‰ after pyrolysis (Table 3). The isotopic composition of the total untreated organic matter is -23.8‰ . This value is more positive (by about 4.5‰) than values reported for other recent non-marine organic matter (e.g., Schiefelbein 1982; Scalán & Morgan 1970) and again indicates that higher plant organic matter contributes only a small fraction to the total organic carbon. Mass balance calculations that include the TOC and the insoluble organic matter before hydrous pyrolysis indicate that the extractable organic matter should have an isotopic composition of -26.7‰ . This is in good agreement with the measured isotopic composition of the individual

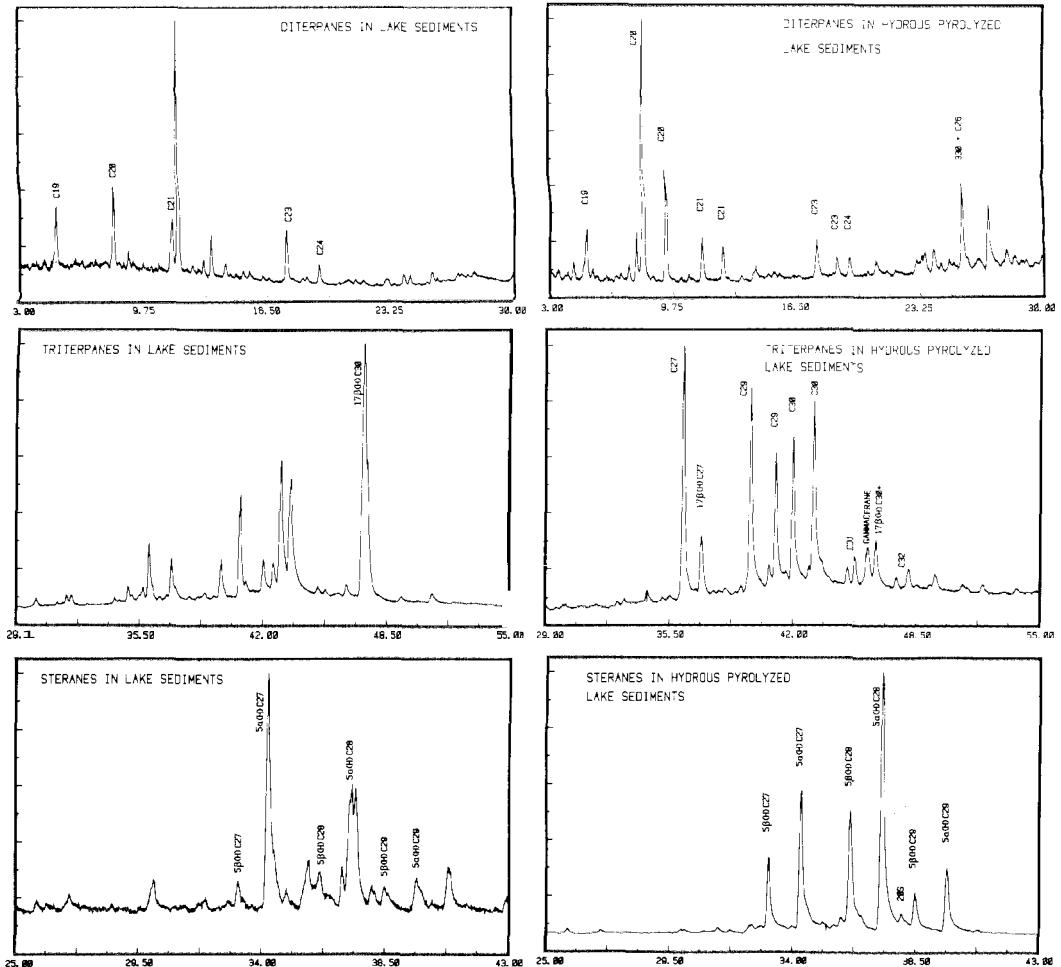


FIG. 14. GC/MS traces of di- and triterpanes ($m/z=191$) and steranes ($m/z=217$).

C_{15+} fractions (see Table 3). The isotopic composition of that extractable organic matter is *more negative than the composition of the total* organic carbon, suggesting that it contains larger proportions of higher-plant-derived organic matter, mainly in the aliphatic, aromatic, and NSO fractions. After hydrous pyrolysis the different C_{15+} fractions become isotopically more positive, indicating the addition of (soluble) compounds that were previously bonded to the (isotopically more positive) insoluble organic matter.

Summary and future work

Work on Big Soda Lake to date represents the first detailed data set conducted on modern day analogues of lacustrine oil source rocks as identified by Demaison & Moore (1980). Studies

on the microbial geochemistry of Big Soda Lake have already made an important impact on our *interpretation of $\delta^{13}C_H$ values found in nature* (Oremland & Des Marais 1983) and upon mechanisms of methanogenesis in high-sulphate environments (Oremland *et al* 1982a, b, Oremland & Polcin, 1982) The lake has now been well-characterized in terms of its hydrogeochemistry, nutrient dynamics, mixing and productivity. Recent investigations have centered upon bacterial decomposition processes occurring seasonally in the water column of the lake, and how this relates to inputs of carbon derived from primary productivity. However, although methane oxidation in the water column has been investigated, estimates of sulphide and ammonia oxidation have not been made. Thus, the lake's carbon budget is the most comprehensive, while

the sulphur and nitrogen budgets are as yet incomplete. All of these budgets require further studies. The contribution of the littoral zone to the C, N and S budgets must also be assessed.

In terms of the lake's sediments, only a preliminary data set exists with regard to its microbial carbon mineralization reactions, biogeochemistry and organic geochemistry. This

aspect should be stressed in future investigations. It would be of particular interest to isolate alkalophilic anaerobes from the different zones of the lake because little is known about the physiology, ecology or bioenergetics of these types of bacteria (Horikoshi & Akiba 1982). This work is being pursued currently.

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