Stable Hydrogen and Carbon Isotopic Compositions of Biogenic Methanes

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STABLE HYDROGEN AND CARBON ISOTOPIC COMPOSITIONS OF BIOGENIC METHANES

by

Roger Allen Burke, Jr.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Marine Science in the University of South Florida

December, 1985

Major Professor: Dr. William M. Sackett
This is to certify that the Ph.D. Dissertation of

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with a major in Marine Science has been approved by the Examining Committee on August 14, 1985 as satisfactory for the dissertation requirement for the Ph.D. degree.

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STABLE HYDROGEN AND CARBON ISOTOPIC COMPOSITIONS OF BIOGENIC METHANES

by

Roger Allen Burke, Jr.

An Abstract

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Marine Science in the University of South Florida

December, 1985

Major Professor: Dr. William M. Sackett
Stable hydrogen and carbon isotopic compositions of biogenic methanes collected from the sediments of several deep-sea, nearshore marine-estuarine, and freshwater environments were determined. The isotopic compositions of methane samples from eight different DSDP Sites (mean δD-CH₄ = -185°/oo, std. dev. = 7°/oo, n = 75; mean δ¹³C-CH₄ = -71.3°/oo, std. dev. = 6.3°/oo, n = 44) are generally typical of methane formed via CO₂ reduction in deep-sea sediments.

Methane collected from several freshwater environments was D-depleted (mean δD-CH₄ = -300°/oo, std. dev. = 26°/oo, n = 20) and ¹³C-enriched (mean δ¹³C-CH₄ = -60.1°/oo, std. dev. = 6.1°/oo, n = 20) compared to the deep-sea methane. Normally, acetate dissimilation is thought to account for about 60 to 70% of the total methane production in freshwater sediments.

Nearshore marine-estuarine methanes appear to be isotopically intermediate (mean δD-CH₄ = -258°/oo, std. dev. = 23°/oo, n = 46; mean δ¹³C-CH₄ = -61.8°/oo, std. dev. = 3.1°/oo, n = 46) between deep-sea and freshwater methanes. Variation in the relative importance of the two main methanogenic pathways, acetate dissimilation and CO₂ reduction, is probably the single most important factor responsible for the differences in methane isotopic compositions among these three different types of environments. Other factors that probably contribute to the methane isotopic differences are temperature, sedimentation rate, organic matter type and amount, concentration of alternate electron acceptors, rate of methane formation and possibly
postgenerative isotopic equilibration.

Shallow aquatic sediments are thought to be an important source of methane to the atmosphere; the methane produced in these systems, including the ones sampled in this study, is generally substantially more $^{13}$C-depleted than expected based on the $\delta^{13}$C of atmospheric methane and the isotopic fractionation associated with the atmospheric sink process. Too few $\delta D$ data are available to allow evaluation of the role of shallow aquatic sediments in determining atmospheric $\delta D$-$CH_4$.

Abstract approved: William M. Sackett

Major Professor: William M. Sackett

Professor, Marine Science

11/27/85
Date of approval
INTRODUCTION

The commercial production of natural gas, of which methane is usually the predominant constituent, accounted for about 27% of the total domestic energy production of the United States in 1975 (Natl. Acad. Sci. 1980). Natural gas is projected to account for about 20% of global energy consumption from 1990 to 2000 (Anonymous 1983). The origin of virtually all commercially important accumulations of natural gas is thought to be the degradation of biologically derived organic matter in aquatic sediments, mediated either by bacteria or by the combined effects of time, temperature, and catalysis after sediment burial. In this study, gas produced directly by bacteria is referred to as biogenic gas, and gas resulting from the effects of time, temperature and catalysis on organic matter is referred to as thermogenic gas. Rice and Claypool (1981) recently estimated that roughly 20% of the world's discovered gas reserves were formed by biogenic processes. In view of the fact that biogenic methane occurs in geologically understood circumstances, Rice and Claypool (1981) predict that even more than 20% of future gas discoveries will be of biogenic origin.

Recent studies (Craig and Chou 1982; Khalil and Rasmussen 1982) of polar ice cores indicate an apparent doubling of atmospheric methane concentrations over the past few hundred years. Recent high precision measurements (Khalil and Rasmussen 1983) indicate that the atmospheric
methane concentration has been increasing by 1-2% per year during the last several years. Because methane strongly absorbs infrared radiation within the atmospheric window (700 to 1400 cm\(^{-1}\)) that transmits most of the thermal radiation from the earth's surface to outer space (Wang et al. 1976), the atmospheric methane increase is a potential contributor to global warming. Various investigators (Wang et al. 1976; Lacis et al. 1981; Craig and Chou 1982) estimate the contribution of methane to increasing global temperatures to be about 20-40% of that attributed to the carbon dioxide increase. Microbial decomposition of complex organic matter in environments such as aquatic sediments and the intestines of ruminants is thought to be the most important source of methane to the atmosphere, accounting for an estimated 75 to 87% of the total input (Sheppard et al. 1982; Hameed and Cess 1983). Improved understanding of the geochemistry of biogenic methane may be of practical significance to future energy exploration and climatic predictions.

Two useful parameters characterizing natural gas are the stable hydrogen and carbon isotopic compositions of methane. The natural abundances of the stable hydrogen isotopes H and D are 99.9844% and 0.0156%, respectively (Hoefs 1980). The stable isotopes of carbon \(^{12}\text{C}\) and \(^{13}\text{C}\) have natural abundances of 98.89% and 1.11%, respectively (Hoefs 1980). Stable isotopic compositions are usually reported as a parts-per-mil (‰) deviation (\(\delta\)) from a standard with a known isotopic ratio. The definition of the \(\delta\) value is:

\[
\delta = \left( \frac{R(\text{sample})}{R(\text{standard})} - 1 \right) \times 1000 \quad (1)
\]

where \(R\) is \(D/H\) and \(^{13}\text{C}/^{12}\text{C}\), for hydrogen and carbon isotopic compositions, respectively. Values reported in this study are referred
to the Vienna Standard Mean Ocean Water (V-SMOW; absolute D/H = 155.76 ± 0.05 \times 10^{-6}; Hagemann et al. 1970; Gonfiantni 1978) for hydrogen and to the belemnite standard from the Cretaceous Peedee Formation of South Carolina (PDB; absolute $^{13}$C/$^{12}$C = 0.0112372; Craig 1953, 1957) for carbon.

**Natural Hydrogen Isotopic Compositions**

Isotope effects are fundamentally the result of mass differences. Because hydrogen isotopes have such large relative mass differences, large variations of $\delta$D in nature are expected. Figure 1 shows approximate isotopic ranges for various types of hydrogen containing substances found in nature. The deuterium concentration of continental surface waters is determined primarily by the temperature at which local precipitation takes place; temperature is, in turn, influenced by latitude and altitude (Friedman et al. 1964). In arid regions the process of evaporation can lead to significant deuterium enrichment in standing water (Friedman et al. 1964). The $\delta$D of the organically bound hydrogen of plants is largely determined by the $\delta$D of moisture absorbed during growth (Schiegl and Vogel 1970) and by the pathway of photosynthetic carbon metabolism ($C_3$ (Calvin-Benson), $C_4$ (Hatch-Slack) or CAM (Crassulacean Acid Metabolism)) operative in the plant (Ziegler et al. 1976). The total organically bound hydrogen is usually D-depleted relative to the water that the plants use for growth (Schiegl and Vogel 1970) with additional deuterium depletion during the formation of lipids (Smith and Epstein 1970; Estep and Hoering 1980). When CAM plants are deprived of water their organically bound hydrogen can become D-enriched, apparently due to enhanced evaporation of their tissue water (Ziegler et al. 1976).
Based on their early measurements of the $\delta D$ of coal, Schiegl and Vogel (1970) suggested that there is some deuterium depletion in the conversion of plant material to coal and that some of the variability noted in coal $\delta D$ might be attributable to climatic conditions at the time of plant growth. Based on the results of a previous study by Redding et al. (1980), which indicated that coal $\delta D$ is not greatly changed by the one-step formation of methane, and their own data, which indicated an inverse correlation between coal $\delta D$ and maturity, Smith et al. (1983) concluded that coal $\delta D$ is primarily source-controlled and that maturational effects are unimportant. Smith et al. (1983) assumed that there is essentially no isotope effect in the conversion of plant material to coal and that, following application of an average water-plant fractionation factor, their coal $\delta D$ data could be used to estimate the paleolatitude of coal deposition.

Based on analyses of over 100 crude oil samples and preliminary results from laboratory experiments, Yeh and Epstein (1981) concluded that the $\delta D$ of oil is primarily source controlled and that maturity and chemical composition have little effect on the $\delta D$ of normal crude oils. Yeh and Epstein (1981) based their conclusions on (1) the observation that there was no correlation between $\delta D$-oil and API gravity in their sample set, (2) the range of $\delta D$-oil of their samples was only $100^\circ/oo$ compared to a $\delta D$ range of more than $250^\circ/oo$ for oil reservoir formation waters and, (3) the lack of detectable change in the $\delta D$ of an oil sample after it had been in contact with water with a $\delta D$ of about $2000^\circ/oo$ at $180^\circ C$ for two months. Laboratory and field studies by other authors, however, indicate that there is likely to be significant isotopic exchange between water and at least some of the fractions of
Field (Schiegl and Vogel 1970; Schoell 1980; 1983) and laboratory pyrolysis (Chung 1976; Sackett 1978) studies indicate that methane formed from the thermal degradation of precursors (i.e., kerogen, coal, oil) is D-depleted relative to the precursor and that the magnitude of the depletion decreases with increasing precursor maturity. Field and laboratory studies (Nakai et al. 1974; Schoell 1980; Woltemate et al. 1984) indicate that biogenic methane is D-depleted relative to the water in which the methanogenic bacteria grew and that δD-CH₄ is affected by δD-H₂O, δD of the precursor organic matter, and the pathway used in methane generation. Available measurements (Bainbridge et al. 1961; Begemann and Friedmann 1968; Ehhalt 1973) indicate that atmospheric methane is greatly D-enriched compared to its known sources. There appears, however, to be a large kinetic hydrogen isotope effect associated with the destruction of methane in the atmosphere by reaction with hydroxyl radicals (Senum and Gaffney 1985).

Lyon and Hulston (1984) listed four possible origins for the methane in hydrothermal systems: (1) primordial or abiogenic methane from the mantle (i.e., Welhan and Craig 1979; Gold and Soter 1980); (2) the high temperature decomposition of organic matter (i.e., Welhan 1981; Burke et al. 1981); (3) chemical reaction between carbon dioxide and hydrogen (Fisher-Tropsch reaction, i.e., Hunt 1979; Giggenbach 1980) and (4) biological production (i.e., Burke et al. 1981; Baross et al. 1982; Baross and Deming 1983). Due to rapid isotopic equilibration between hydrogen-water and hydrogen-methane, any δD differences in
methane and hydrogen formed by these processes are obscured, and \( \delta D-H_2 \) and \( \delta D-CH_4 \) reflect the temperature (100-1200°C) of the water at the time of sampling (Gunter and Musgrave 1971; Welhan 1981).

**Natural Carbon Isotopic Compositions**

The distribution of the stable carbon isotopes in natural substances has been the subject of many reviews (i.e., Craig 1953; Fuex 1977; Deines 1980; and others) to which the interested reader is referred for more detail than will be presented here. Marine limestones typically have the greatest relative concentrations of \(^{13}C\), with \( \delta^{13}C \) values generally between -5 and +5 ‰ (Fuex 1977).

Terrestrial plants that utilize the \( \text{C}_3 \) pathway are generally \(^{13}C\)-depleted (-32 to -22‰) relative to terrestrial plants fixing carbon dioxide via the \( \text{C}_4 \) pathway (-18 to -9‰; Deines 1980).

Terrestrial plants that are known to use the CAM pathway exhibit \( \delta^{13}C \) that span the range of \( \text{C}_3 \) and \( \text{C}_4 \) plants (-32 to -11‰) as a result of their unique ability to accomplish net carbon dioxide fixation via either the \( \text{C}_3 \) or \( \text{C}_4 \) pathway (Deines 1980). Most marine phytoplankton from tropical and temperate waters have a \( \delta^{13}C \) of about -20‰ (Sackett in press).

The \( \delta^{13}C \) of coals, crude oils, and kerogens are largely inherited from their precursors and are less apt to change as a result of maturational processes than are \( \delta D \) (Rigby et al. 1981; Yeh and Epstein 1981). Crude oils (mode -27 to -30‰) are generally \(^{13}C\)-depleted relative to coal (mode -23 to -26‰) and kerogen, probably as a result of preferential retention of isotopically light lipid components during oil formation (Deines 1980). Methane produced from the thermal decomposition of organic matter generally has a \( \delta^{13}C \) ranging from -60
to \(-25^\circ/oo\), whereas biogenic methane is generally more 13C-depleted (-90 to -50°/oo; Fuex 1977). The $\delta^{13}C$ of the abiogenic methane emitted from the East Pacific Rise ranges from -17.6 to -15°/oo (Welhan 1981).

**Origin of Natural Methanes**

Interest in primordial or abiogenic methane has increased recently as a result of observations that methane, along with other mantle-derived gases ($^3$He, CO$_2$, H$_2$), is injected into the deep sea from active spreading centers (Welhan and Craig 1979; Welhan 1981). These authors stated that it is uncertain whether the methane is an intrinsic mantle component that is extracted from the basalts by the circulating seawater or whether it represents a chemical equilibrium with CO$_2$ and H$_2$ (Fisher-Tropsch reaction) under high pressure and decreasing temperatures (Welhan and Craig 1979; Welhan 1981). Gold and Soter (1980) suggested that degradation of complex hydrocarbons, formed at the time of earth's accretion under conditions of high temperature and pressure, is responsible for this deep-earth gas. Gold and Soter (1980) further suggested that the quantity of methane produced in this manner is enormous and that methane from this source is responsible for most of the gas and oil (through polymerization) in commercial accumulations.

Most geochemists, on the other hand, think that thermal and biological decomposition of photosynthetically derived organic matter in marine or lacustrine sediments accounts for virtually all significant accumulations of natural gas. Early diagenetic (sed. T < 50°C) reactions, primarily mediated by microorganisms, convert labile chemical groups to gases (i.e., CO$_2$, CH$_4$) and cause the remaining organic matter to be converted into a complex polymeric form termed
kerogen. At temperatures between 50 and 200°C (catagenesis) the combined effects of time, temperature, and catalysis lead to the formation of gas and oil or coal from the kerogen (Hunt 1979).

Between temperatures of about 60 and 150°C oil and some gas is formed by catalytic and thermal cracking of kerogen (Hunt 1979). Peak methane generation from kerogen, oil, and coal occurs between about 150 and 200°C, with only a small amount of methane generation at higher (metamorphic) temperatures (Hunt 1979). In catalytic cracking, which is believed to be the dominant process in petroleum generation at temperatures of up to about 125°C (Hunt 1979), positively charged, highly reactive, carbonium ions are generated by hydride removal from paraffin molecules at Lewis acid centers of clay minerals (Sackett 1978). Carbonium ion reactions can then give rise to a large variety of products. In thermal cracking, which becomes increasingly important at temperatures above 125°C (Hunt 1979), the reactive intermediate is a free radical that arises from the homolytic cleavage of a carbon to carbon bond in which each carbon retains one of the two shared electrons. As with carbonium ion reactions, a wide spectrum of products is possible when free radicals are created.

Biogenic methane is produced by a specialized group of obligately anaerobic bacteria that are limited to a narrow range of low molecular weight substrates for growth and energy production. Known methanogenic substrates include carbon dioxide and hydrogen, acetate, methanol, and methylamines (Zehnder et al. 1982). Methanogenic bacteria thus rely on other bacterial species to decompose complex organic compounds to substrates suitable for their growth.

**Anaerobic Decomposition of Complex Organic Matter**
The microbial degradation of complex organic matter to carbon dioxide and methane in anaerobic environments such as organic-rich sediments and sewage sludge digestors is a multi-step process requiring the participation of at least three different trophic groups of bacteria (Zehnder et al. 1982). In environments in which carbon dioxide and protons are the only available inorganic electron acceptors, this process is believed to proceed as illustrated in Figure 2. In the first step, biological polymers such as polysaccharides, proteins, and lipids are digested by extracellular enzymes to soluble materials that are then fermented to organic acids and alcohols by the acid-forming or chemoheterotrophic fermentative hydrogen-producing bacteria (Brock 1974; Mah 1982). In the next step organic acids larger than acetate and alcohols larger than methanol are converted to acetate, carbon dioxide, and hydrogen by the chemoheterotrophic non-fermentative obligate proton reducers (Mah 1982). The reducing equivalents (hydrogen) and acetic acid generated during the first two steps are disposed of in the final step by the methane-producing bacteria.

The pathways for oxidation of organic compounds and ATP generation under anaerobic conditions can be divided into two major groups, fermentation and anaerobic respiration (Brock 1974). In fermentation a compound at an intermediate oxidation level is disproportioned both to compounds that are more oxidized and compounds that are more reduced than the starting substrate in the absence of an external electron acceptor. For example, the fermentation of glucose by *Ruminococcus albus* produces ethanol, acetate, hydrogen, and carbon dioxide (Wolin 1979). Fermentation releases only a small part of the available
Figure 2. The anaerobic decomposition of complex organic matter to methane in sulfate-depleted environments. Percentages represent the flow of energy from the organic and inorganic compounds to methane (after Zehnder et al. 1982).
substrate energy; e.g., in the fermentation of glucose by R. albus about 90% of the glucose energy remains in the end-products (Mah 1982). In anaerobic respiration, an external electron acceptor other than oxygen (i.e., nitrate, sulfate, protons, or carbon dioxide) is involved in the oxidation of organic compounds for energy production (Brock 1974). Examples of anaerobic respirations involved in the degradation of complex organic matter (Figure 2) are the production of acetate, hydrogen, and carbon dioxide by the obligate proton reducers, and methane production from carbon dioxide and hydrogen.

Without the anaerobic respiration of the obligate proton reducers a significant portion of the complex organic matter would be only partially degraded to higher fatty acids and alcohols (Figure 2). Because degradation of these higher fatty acids and alcohols requires the use of enzymes that are strongly inhibited by hydrogen (Wolin and Miller 1982), the obligate proton reducers can operate only if methanogens, or some other hydrogenotroph, efficiently consume the hydrogen they produce. This process, referred to as interspecies hydrogen transfer (Iannotti et al. 1973; Wolin and Miller 1982), may also ensue when chemoheterotrophic fermentative hydrogen-producing bacteria are cultured with hydrogenotrophs (Wolin 1982). In this case interspecies hydrogen transfer is not obligatory because the fermentative organism has an alternate means of electron disposal. In monoculture, Selenomonas ruminatum fermented lactate to propionate, acetate, and carbon dioxide (Chen and Wolin 1977). Addition of S. ruminatum to a pregrown culture of the methanogen Methanobrevibacter smithii (formerly Methanobacterium ruminatum) by a sequential transfer procedure caused a shift in lactate fermentation products to more
acetate and less propionate with concomitant production of methane from carbon dioxide and hydrogen (Chen and Wolin 1977). Thus, interspecies hydrogen transfer is important to the anaerobic degradation process (Figure 2) not only because it creates an environment that is conducive to the growth of the obligate proton reducers, but also because it can cause a shift in the electron and carbon flow (Mah 1982). Electron flow is diverted away from the soluble reduced electron sink products (i.e., propionate) to proton reduction (hydrogen formation) with subsequent methane production, and carbon is channeled into the production of methane and the more oxidized soluble end products (i.e., acetate) at the expense of the more reduced soluble compounds such as propionate (Mah 1982). This is advantageous to the hydrogenotrophs (especially methanogens) because they gain energy sources (hydrogen, acetate), and often to the hydrogenogens because they may obtain more ATP from their substrates due to the relatively more complete oxidation of the starting substrate (Wolin 1982).

Other electron acceptors such as nitrate, iron and manganese oxides, and oxidized sulfur compounds are preferentially used (compared to protons and carbon dioxide) in anaerobic respirations (Zehnder et al. 1982), and when they are present in sufficient quantities carbon and electron flow (Figure 2) may be diverted away from methane production. Because of its high concentration in seawater, sulfate is the predominant inorganic electron acceptor in upper, anoxic marine sediments (Fenchel and Blackburn 1979; Reeburgh 1983). Consequently, many studies (e.g., Martens and Berner 1974; Claypool and Kaplan 1974; Nikaido 1977) found that optimum rates of methane production and near-saturation methane concentrations are not encountered in marine
sediments until sulfate is largely (80-90%) depleted. This inhibition of methane production by high concentrations of dissolved sulfate is apparently due to the ability of sulfate-reducing bacteria to outcompete methanogens for substrates such as hydrogen and acetate (Winfrey and Zeikus 1977). Claypool and Kaplan (1974) proposed that sulfate reducers gain competitive advantage by virtue of the fact that sulfate reduction yields relatively more energy than methane production; however, McCarty (1972) argued that if two reactions are energetically feasible, then from a strictly thermodynamic viewpoint, one reaction can not inhibit the other simply because it is energetically more rewarding. Recent studies (Kristjansson et al. 1982; Schonheit et al. 1982) have shown that sulfate reducing bacteria have higher substrate affinities (lower $K_a$) by factors of 5 and 15 for hydrogen and acetate, respectively, than do methanogens. This difference in substrate affinities does not imply that methane production and sulfate reduction from these substrates are mutually exclusive (Kristjansson et al. 1982; Schonheit et al. 1982), but it does provide a satisfactory explanation for the vertical separation of the sulfate-reducing zone from the methane-producing zone that is generally observed in marine sediments (Claypool and Kvenvolden 1983).

**Methanogenic Pathways**

In sulfate-depleted marine sediments, as well as freshwater sediments, the dominant terminal process of anaerobic organic matter decomposition is methane production. Methanogenesis is generally thought to occur mainly via two pathways, CO$_2$ reduction (2) and acetate dissimilation (3):

$$\text{CO}_2 + 8\text{e}^- + 8\text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (2)$$
The results of $^{14}$C labeling studies implied that acetate normally accounts for about 60 to 70% of the total methane production in sewage sludge digestors (Jeris and McCarty 1965; Smith and Mah 1966), paddy soils (Koyama 1963; Takai 1970), and some freshwater lake sediments (Cappenberg and Prins 1974; Winfrey and Zeikus 1979; Lovley and Klug 1982), with CO$_2$ reduction responsible for the remainder. In other freshwater lake sediments, however, the CO$_2$ reduction pathway is apparently predominant, accounting for about 90% of the total methane production in Russian Lake Kuznechikha (Belyaev et al. 1975) and about 75% of the methane generated in the sediments of Blelham Tarn in the English Lake District (Jones et al. 1982). Incubation of sediment samples from Cape Lookout Bight, North Carolina, a small, nearshore marine basin with rapidly accumulating, organic-rich sediments, with (1,2-$^{14}$C) sodium acetate indicated that more than 50% of the Cape Lookout Bight summertime methane flux could be accounted for by acetate dissimilation (Sansone and Martens 1981). If sediments receive a fairly specific type of organic matter input or if methanogens are competing with sulfate-reducing bacteria for H$_2$ and acetate, a significant fraction of the total methane production can result from other substrates such as methanol and trimethylamine (Oremland et al. 1982; King et al. 1983).

All of the aforementioned studies used $^{14}$C labeled substrates as tracers to estimate the relative contributions of the methanogenic pathways to total methane production. An alternate method was proposed for estimating relative pathway contribution based on measurements of

$$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \quad (3).$$
δD-CH₄ and the δD of the associated water (δD-H₂O; Schoell 1980; Woltemate et al. 1984). Estimates obtained using this method for methane generated from sewage sludge (Schoell 1980) and freshwater lake sediments (Woltemate et al. 1984) are in good agreement with some of those obtained with ¹⁴C tracers.

Methane Oxidation

As mentioned previously, two useful parameters in natural gas characterization are δD-CH₄ and δ¹³C-CH₄. Consequently, processes that alter these compositions and confuse interpretation are of interest. Based on results obtained from a laboratory study by Coleman et al. (1981) it can be calculated that aerobic oxidation of 40% of an aliquot of methane would leave the remaining 60% about 12.5‰ and 125‰ less ¹³C- and D-depleted, respectively. Further, methane oxidation can be important in limiting the flux of methane to the atmosphere from freshwater lakes (Rudd and Hamilton 1978), shallow, nearshore marine sediments (Martens and Berner 1977), and periodically inundated swamp soils during periods of dryness (Harriss et al. 1982).

Two microbial methane oxidation processes have been shown to be important sinks for methane in nature. An assimilatory, microaerophilic process has been observed in freshwater lakes (Rudd et al. 1976) and coastal marine waters (Sansone and Martens 1978). The microaerophilic process is inhibited by high oxygen concentrations (>31 µM) when dissolved inorganic nitrogen (DIN = NH₄⁺ + NO₂⁻ + NO₃⁻) concentrations are low (<3µM), but not when DIN concentrations are above 15 to 16µM (Rudd et al. 1976; Sansone and Martens 1978). The aerobic oxidation process may require relatively high methane concentrations. For example, methane concentrations <5µM limited the
rate of methane-oxidizer population development in Lake Mendota, Wisconsin (Harrits and Hanson 1980), and Sansone and Martens (1978) proposed that aerobic methane oxidation in Cape Lookout Bight waters should be limited by other factors at methane concentrations >0.5µM.

Pure cultures of methanogenic bacteria (Zehnder and Brock 1979), freshwater lake sediments, and sewage sludge (Zehnder and Brock 1980) anaerobically converted $^{14}$CH$_4$ to $^{14}$CO$_2$ while producing methane; however, net methane production was not observed and the methane oxidation process was inhibited by the same conditions that inhibited methane production (i.e., presence of oxygen) implying that methanogenic bacteria were responsible for the methane conversion.

Profiles of methane concentration in anoxic marine sediments frequently show extreme upward concavity in the zone of sulfate reduction (Barnes and Goldberg 1976; Reeburgh 1976; Martens and Berner 1977; Bernard 1978; Alperin and Reeburgh 1984). Barnes and Goldberg (1976) and Reeburgh (1976) suggested that net consumption of upward diffusing methane in the sulfate-reducing zone is required to explain the extreme upward concavity. Further, successful fits of diagenetic models to methane profiles from various anoxic marine sediments were obtained only if the models included a methane consumption term (Martens and Berner 1977; Bernard 1978; Alperin and Reeburgh 1984). Reeburgh (1976, 1980) proposed that methane consumption in anoxic marine sediments primarily takes place in a relatively thin layer (estimated to be about 10 cm in Cariaco Trench sediments) at the base of the sulfate-reducing zone where the profiles of methane, sulfate, and CO$_2$ all typically exhibit slope changes. Several recent studies (Reeburgh 1980; Devol 1983; Alperin and Reeburgh 1984; Iversen and
Jorgensen (1985) demonstrated the conversion of $^{14}\text{CH}_4$ to $^{14}\text{CO}_2$ in relatively thin layers (about 15 cm to 40 cm thick) near the base of the sulfate-reducing zone of various marine sediments. The location of the anaerobic methane consumption layer at depths where the concentrations of both sulfate and methane are appreciable, the demonstrated ability of the sulfate reducer *Desulfovibrio desulfuricans* to co-metabolize $^{14}\text{CH}_4$ under anaerobic conditions (Davis and Yarbrough 1966), and the co-occurrence of peaks in methane oxidation rate and sulfate reduction rate (i.e., Iversen and Blackburn 1985), have been cited as evidence that anaerobic methane consumption is coupled to sulfate reduction, either directly (i.e., Reeburgh 1976) or indirectly via reactions coupled to other substrates (i.e., Devol 1983).

The anaerobic conversion of $^{14}\text{CH}_4$ to $^{14}\text{CO}_2$ in minimally disturbed marine sediments from the main anaerobic consumption zone of Skan Bay, Alaska was not inhibited by specific inhibitors of either methanogenesis (2-bromoethanesulfonic acid) or sulfate reduction (molybdate), implying that anaerobic methane consumption in marine sediments is not directly mediated by either methanogenic bacteria or sulfate-reducing bacteria (Alperin and Reeburgh 1985). Alperin and Reeburgh (1985) suggested that their results are consistent with two possibilities: anaerobic methane consumption in marine sediments is either mediated by an unknown organism, which possibly uses iron oxides, manganese oxides, or reduced sulfur compounds as an external electron acceptor, or methane consumption is accomplished by a bacterial consortium that includes an unknown organism capable of converting methane to carbon dioxide and hydrogen and sulfate-reducing bacteria that consume the hydrogen. Alperin and Reeburgh (1985)
pointed out that anaerobic methane consumption would not be inhibited by molybdate if other hydrogen-consuming organisms replaced sulfate-reducers in the consortium. It is not clear, however, why this modified consortium does not function at greater depths where methane concentrations are even greater. The confinement of substantial methane consumption to a thin layer implies that some component of the sulfate-reduction zone, perhaps some trace product of the sulfate reduction process, is directly involved in anaerobic methane consumption. The importance of iron and manganese oxides in anoxic sediments to the microbial decomposition of organic matter in general and methane in particular, is unknown because these metal oxides may be used as an electron acceptor directly by organisms or by metabolic products (i.e., sulfide) of organisms (Burdige and Nealson 1985), including a variety of organic substances (Stumm and Morgan 1981).

**Natural Gas Classification**

Isotopic and molecular compositional variations have been used frequently to develop models that describe the origin and subsurface history of natural gas. All of these models assume that the observed isotopic and compositional variations of natural gases predominantly reflect processes (primarily kinetic isotope effects and source maturity) that occur during gas formation (Schoell 1983), although this is not always explicitly stated.

Galimov (1968) presented data that indicated methane becomes less $^{13}\text{C}$-depleted with increasing depth in the sediment, with most of the change occurring during the initial stages of burial. To explain his data, Galimov (1968) developed a model in which the sediment column was divided into three zones: (I) the biochemical zone ($T < 25^\circ\text{C}$) in which
bacteria mediate the production of methane and its isotopic equilibration with carbon dioxide, (II) the catalytic zone (25°C < T < 200°C) in which methane is ¹³C-depleted relative to its precursor due to temperature-dependent kinetic isotope effects but is not in isotopic equilibrium with carbon dioxide because temperatures are too low for sufficient inorganic CO₂-CH₄ isotopic exchange and bacterial activity is practically absent and (III) the thermal zone (200°C < T < 300°C) in which temperatures are sufficient for control of δ¹³C-CH₄ by inorganic CO₂-CH₄ isotopic equilibration.

Stahl (1974) presented a model in which three separate stages of gas formation are apparent from a plot of δ¹³C-CH₄ versus C₁/ΣCₙ (ratio of methane over all saturated gaseous hydrocarbons). In the initial stage the gas is formed predominantly by bacterial action and is characterized by a δ¹³C-CH₄ ranging between about -80 and -68‰ and a C₁/ΣCₙ near unity. The second stage represents thermocatalytic alteration of marine source material as it is subjected to higher temperatures and time during deeper burial. Gas formed during the second stage maturational trend is characterized by progressive increases in δ¹³C-CH₄ from about -68 to -42‰ and progressive decreases in the C₁/ΣCₙ ratio from about 1.0 to about 0.8. Upon further burial the source material and its hydrocarbon products are subjected to increasing thermal stress; this results in gas that continually becomes more methane- and ¹³C-enriched. Gas formed in this third stage is typified by δ¹³C-CH₄ between -42 and -35‰ and a C₁/ΣCₙ ratio of 0.8 to 1.0 (Stahl 1974). The results of Stahl (1974) also indicate that δ¹³C-CH₄ is dependent upon the type of source material from which it originates. Methane produced from predominantly
terrestrial source material during coal formation displays the same trends as noted above for marine source material, but with less $^{13}\text{C}$-depletion ($\delta^{13}\text{C-CH}_4$ as high as $-20^\circ/oo$; Stahl 1974). The apparent decrease in $C_1/IC_n$ with increasing thermal maturity during the second stage observed by Stahl (1974) is at odds with the laboratory pyrolysis results of Sackett (1978) that indicated the opposite trend, i.e., methane initially accounts for a small (<10%) fraction of volatile pyrolysis products and becomes increasingly more important with longer pyrolysis. Sackett's (1978) experiments were carried out at higher temperatures (400-500°C) than would be found under natural conditions and, as pointed out by Bernard (1978), may not accurately simulate natural processes. Also, Hoering (1984) argued that because water is ubiquitous in sediments, dry pyrolysis experiments may be unrealistic in that some hydrogen transfer reactions in which water plays a role under natural conditions may be blocked. On the other hand, the carbon and hydrogen isotopic compositions of several natural methanes associated with crude oils were qualitatively comparable to compositions that would be predicted from the results of Sackett (1978) implying that the dry pyrolysis process bears a close resemblance to gas formation in nature (Schoell 1980).

Bernard et al. (1977) proposed that natural gases could be efficiently characterized by a model in which $\delta^{13}\text{C-CH}_4$ was plotted against the ratio $C_1/(C_2 + C_3)$ (methane divided by the sum of ethane and propane). Bernard (1978) pointed out that molecular ratios expressed in this way vary by more than four orders of magnitude for natural samples, so this parameter should yield greater resolution than the $C_1/IC_n$ ratio of Stahl (1974). Bernard et al. (1977) suggested
that, in general, biogenic gas has a $C_1/(C_2 + C_3)$ ratio greater than 1000 and a $\delta^{13}C$-$CH_4$ more negative than $-60^\circ/oo$, whereas thermogenic gases generally have $C_1/(C_2 + C_3)$ ratios of less than 50 and $\delta^{13}C$-$CH_4$ greater than $-50^\circ/oo$. The Bernard et al. (1977) model also takes into account the isotopic and compositional changes, with concomitant loss of resolution, that may result from substrate depletion during biogenic methane formation, the mixing of biogenic and thermogenic gases, and subsurface migration of thermogenic gas.

Schoell (1980, 1983) developed a more extensive gas characterization model in which $\delta^{13}C$-$CH_4$ is separately correlated with the three parameters: $\delta D$-$CH_4$, $\delta^{13}C$-$C_2H_6$, and $C_2^+$ (concentration of saturated gaseous hydrocarbons larger than methane). Use of the two new parameters $\delta D$-$CH_4$ and $\delta^{13}C$-$C_2H_6$, made possible in part by recently improved analytical capabilities, allows a clearer distinction to be made between different genetic types of gas in some cases. For example, Schoell (1980, 1983) suggested that measurement of $\delta D$-$CH_4$ might allow differentiation of biogenic gas produced under marine conditions from biogenic gas produced in a terrestrial system when $\delta^{13}C$-$CH_4$ alone is insufficient to make the distinction. The isotopic and compositional variations that may result from secondary processes such as oxidation, mixing, and migration are also indicated in the Schoell (1983) model. For example, migration may appreciably change the $C_2^+$ concentration but would generally not be expected to alter significantly the isotopic composition of methane (Schoell 1983).

Objectives

My research had the following two objectives: (1) to add to the meager $\delta D$-$CH_4$ data base by measuring methane samples from a variety of
aquatic environments such as deep-sea, nearshore marine-estuarine, and freshwater lake sediments. The resulting enhanced understanding of natural $\delta^{13}$C-$\text{CH}_4$ variations should then be of use in the improvement of natural gas characterization models and, in conjunction with concurrent $\delta^{13}$C-$\text{CH}_4$ measurements, eventually aid in gaining a clearer understanding of the relative contributions of the major methane sources to the atmosphere; (2) to use $\delta^{13}$C-$\text{CH}_4$ measurements to estimate the relative importance of acetate dissimilation and CO$_2$ reduction to methane production in various aquatic sediment types. In the past, pathway importance was generally estimated from the results of $^{14}$C radiotracer studies.

To accomplish these objectives, it was necessary to construct a preparation system for the separation of methane from other gases and its conversion to products suitable for stable isotopic measurement. The details of such a system have not been previously published and few investigators have made $\delta^{13}$C-$\text{CH}_4$ measurements.
EXPERIMENTAL METHODS

Preparation of Methane for Isotopic Analysis

Methane gas samples were prepared for isotopic analysis with the system illustrated in Figure 3. Preparation involves gas chromatographic (GC) separation of methane from other gases followed by combustion to CO₂ and water in a vacuum line. The GC used is a Hewlett-Packard 5710A equipped with a thermal conductivity detector and two 6mm OD stainless steel columns (3m grade 12 silica gel and 2m molecular sieve 5A) connected in series through a Valco ten port switching valve configured for column sequence reversal and backflush of the silica gel column to the detector. This GC configuration allows baseline separation of H₂, O₂, N₂, CH₄, and CO₂. The helium carrier gas flow rate is 50ml/min and the oven temperature is held constant at 90°C. Methane is combusted in a cupric oxide furnace (850°C), to which 5-10 torr of oxygen is added before each run, as it is swept through the furnace by the GC carrier gas. Addition of oxygen to the system before each run is necessary to assure complete combustion of the methane. The isotopic composition of incompletely combusted methane was found to be significantly lighter than the actual composition; δD-CH₄ and δ¹³C-CH₄ deviations of up to 60‰ and 3.5‰, respectively, were observed. The CO₂ and water resulting from the combustion of methane are condensed by liquid nitrogen (-196°C) in the trap immediately downstream from the combustion oven, the excess oxygen
Figure 3. Schematic of the system used for the preparation of methane for isotopic analyses.
and helium are pumped away, and the CO\textsubscript{2} and water are cryogenically (-89°C) separated. The δ\textsuperscript{13}C of the CO\textsubscript{2} is then measured in a Finnigan Varian MAT 250 isotope ratio mass spectrometer (IRMS) by comparison with the NBS-21 standard (δ\textsuperscript{13}C = -28.16‰; Schoell et al. 1983).

**Reduction of Water and δD Determination**

The water from the combustion of methane is then collected in a sample tube containing approximately 0.25g of treated zinc shot and reduced to H\textsubscript{2} gas by reaction with the zinc at 450°C for 30 minutes (Coleman et al. 1982). The sample tube consisted of either a 15cm length of 9mm OD Pyrex tubing that was sealed at one end, or a high vacuum, teflon plug, 90° pattern stopcock (Model 8195-47, Ace Glass Inc., Vineland, NJ) with one arm sealed. The reducing agent used is AnalaR zinc shot (BDH Chemicals Ltd., Poole, England; presently obtainable from Gallard/Schlesinger Chemical Mfg. Corp., New York) that was sieved so that it passes through size 14 mesh but is retained by size 60 mesh. After sieving, the dull, gray oxide coating is removed from the zinc shot by reaction with dilute nitric acid until the zinc becomes quite shiny. This activated zinc is then washed several times with distilled water, dried and outgassed under vacuum overnight at about 300°C. When not in use, the treated zinc is stored under vacuum.

The δD of H\textsubscript{2} gas is measured in the IRMS by comparison with a H\textsubscript{2} gas of known δD. Through the assistance of Dr. Thomas Hoering of the Geophysical Laboratory, Carnegie Institution of Washington, several high pressure cylinders of H\textsubscript{2} gas of differing δD were prepared for use as routine working standards. Deuterium-depleted methane samples were always compared to Tank Standard-C (TS-C), which was assigned a δD of -220.4‰ based upon comparison with the water reference sample.
Greenland Ice Sheet Precipitation (GISP) and Tank Standard-A (TS-A) which was assigned a δD of -31.9‰ from comparison with the NBS-1 water reference sample (Table 1). Comparison of these two tank standards with a third (TS-B) indicated that at least the isotopic difference between TS-A and TS-C is correct (Table 1). When isotopically heavier water samples were run, TS-A was used as the working standard. Each day that samples were run, the tank standard serving as the working standard was used in determining the δD of at least one of the other tank standards. The isotopic differences (TS-A vs. TS-C (242.4‰) and TS-C vs. TS-A (-194.1‰) were not quite internally consistent (Table 1) implying that H₂ gas admitted to the mass spectrometer through the sample-side of the inlet system is fractionated by about 0.6‰ relative to gas flowing in through the standard-side capillary. Because the magnitude of this apparent fractionation is comparable to the uncertainty involved in determining the δD of H₂ gas with the IRMS and is small compared to the overall uncertainty involved with δD analysis of water (Table 1) and methane (see below) and would have no effect on any interpretations made here, no correction for this effect has been applied to the data presented here.

H₃⁺ Correction

When mixtures of H₂ and HD gas are ionized in the mass spectrometer source, H₂⁺ and HD⁺ ions are formed. Reaction of H₂⁺ with H₂ also results in the formation of H₃⁺ ions that cannot be resolved from HD⁺ ions by the mass spectrometer used in this study. Because determination of the ratio HD⁺/H₂⁺ is desired, the contribution of H₃⁺ to the mass-3 signal must be removed. In this study the H₃⁺ correction
Table 1. Intercomparison of working standards and comparison of working standards with international reference samples (in °/oo).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tank Standard A</th>
<th>Tank Standard C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^D_{TS-A}$</td>
<td>$\delta^D_{SMOW}$</td>
</tr>
<tr>
<td>TS-A</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS-B</td>
<td>-83.1</td>
<td>-112.4</td>
</tr>
<tr>
<td></td>
<td>($\sigma=0.1$, $n=7$)</td>
<td>($\sigma=0.6$, $n=30$)</td>
</tr>
<tr>
<td>TS-C</td>
<td>-194.1</td>
<td>-220.4</td>
</tr>
<tr>
<td></td>
<td>($\sigma=0.5$, $n=42$)</td>
<td></td>
</tr>
<tr>
<td>V-SMOW</td>
<td>31.3</td>
<td>-1.6</td>
</tr>
<tr>
<td></td>
<td>($\sigma=1.2$, $n=21$)</td>
<td></td>
</tr>
<tr>
<td>NBS-1</td>
<td>-15.7</td>
<td>-47.1</td>
</tr>
<tr>
<td></td>
<td>($\sigma=1.1$, $n=16$)</td>
<td></td>
</tr>
<tr>
<td>GISP</td>
<td>----</td>
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</tbody>
</table>
was determined by the method of Nier (1947) using software supplied with the HP 9815A calculator that controls operation of the IRMS. Because the $\text{H}_3^+$ rate of production is proportional to the square of the hydrogen partial pressure, whereas the rates of generation of the $\text{H}_2^+$ and $\text{HD}^+$ ions are directly proportional to the hydrogen partial pressure, measurement of the mass-3/mass-2 ratio ($i_3^+/i_2^+$) at different pressures (proportional to $i_2^+$; Friedman 1953) yields a straight line (Nier 1947). The product of the slope of this line and $i_2^+$ at the pressure at which an isotopic analysis is made is the contribution to the ratio $i_3^+/i_2^+$ resulting from the formation of $\text{H}_3^+$ and is simply subtracted out to yield the desired $i^+(\text{HD})/i^+(\text{H}_2)$ ratio. Determination of this slope or $\text{H}_3$-factor requires a minimal investment of time (five minutes) and operator effort; this makes periodic redetermination practical if it is suspected that IRMS operating conditions have fluctuated. The $\text{H}_3^+$ contribution is large (about 30\%) at typical operating pressures employed here (mass-2 signal $> 5V$ or sample size $> 200\text{cc}(\text{STP}) \text{H}_2$) but is fairly constant (within about 1\%). It can be calculated that a 1\% fluctuation in the $\text{H}_3$-factor should cause an uncertainty of about $0.5\%/00$ when a sample-standard pair differing in $\delta D$ by about $200\%/00$ is compared. This is close to the long term uncertainty that was observed for the comparison of TS-A to TS-C (Table 1), further implying that the $\text{H}_3^+$ contribution remains constant to within 1\% under typical operating conditions. Because the sample-standard pairs measured in this study were generally much closer in $\delta D$ than $200\%/00$, measurement uncertainty due to $\text{H}_3$-factor fluctuation should generally be less than $0.5\%/00$. At very low operating pressures the Nier (1947) relationship may deviate from
linearity (Whelhan 1981) and may not be applicable when samples much smaller than the smallest measured here (about 2cc(STP) H₂) are analyzed.

Sample Measurement Reproducibility

The GC-combustion system (Figure 3) described here is a flow-through system in which methane is combusted as it is swept through the furnace by the GC carrier gas. There is no Toepler pump or bellows to cycle the gas repeatedly through the furnace. As a result, it is reasonable to expect that the time the methane spends in the oven, which is determined by the GC carrier gas flow rate, could be an important variable. To test this, a methane working standard was analyzed several times using carrier gas flow rates of 25ml/min and 50ml/min. Isotopic values obtained at 50ml/min (mean δD-CH₄ = -164°/oo, std. dev. = 1.0°/oo, n = 12; mean δ¹³C-CH₄ = -44.2°/oo, std. dev. = 0.14°/oo, n = 12) were not significantly different from those obtained at a GC flow of 25ml/min (mean δD-CH₄ = -164°/oo, std. dev. = 1.7°/oo, n = 6; mean δ¹³C-CH₄ = -44.1°/oo, std. dev. = 0.17°/oo, n = 10). Repeated analyses of a second methane working standard, analyzed daily during the course of this work, yielded standard deviations (mean δD-CH₄ = -189.3°/oo, std. dev. = 1.7°/oo, n = 36; mean δ¹³C-CH₄ = -39.6°/oo, std. dev. = 0.21°/oo, n = 32) that are similar to those obtained above for the GC flow rate test and are comparable to those reported by Schoell (1980).

The combustion efficiency of the GC-combustion system (Figure 3) was tested by comparing the methane concentration of the gas atmosphere in the vacuum line resulting from the injection into the GC of 2 ml of methane under normal operating conditions (three separate trials) to
the methane concentration of the vacuum line gas atmosphere after injection of 2 ml of methane into the GC with the combustion furnace turned off (three separate trials). The trap immediately downstream from the combustion oven was immersed in a Dewar flask containing an isopropyl alcohol slush (-89°C). The resulting methane concentrations, which were measured with a GC equipped with dual flame ionization detectors, indicated a combustion efficiency >99%.

Water samples were prepared for δD determination by the zinc metal method (Coleman et al. 1982). In this case, water samples were simply injected into the sample tubes with a syringe as described by Coleman et al. (1982). The long term accuracy and precision of δD-H₂O determination are indicated by the analyses of water reference samples shown in Table 1. The δD-H₂O value obtained here for NBS-1 by comparison with TS-A is essentially identical to that listed by Gonfiantini (1978). The GISP δD value of -189.4‰ determined by comparison with TS-C in this study is within analytical uncertainty of the value (-189.8‰) reported for GISP by Gonfiantini (1981). The water reference sample V-SMOW was prepared from distilled Pacific Ocean water (Gonfiantini 1978) such that its δD would match the SMOW standard proposed by Craig (1961) as closely as possible. According to Hagemann et al. (1970) V-SMOW is about -0.1‰ (relative to SMOW), whereas Coplen and Clayton (1973) obtained a δD of -1.2 ± 0.4‰ (relative to SMOW) for V-SMOW and stated that the reason for the discrepancy is not known. Due to this apparent uncertainty in the δD of V-SMOW, the good agreement of TS-A and TS-C with NBS-1 and GISP, respectively, and the uncertainty (σ = 1-2‰) involved with determining δD-H₂O, I have elected not to redefine the δD of TS-A such that the δD of V-SMOW
measured here (-1.60/oo; Table 1) becomes 00/oo. Instead, the $\delta$D of water samples analyzed by comparison with TS-A and with a $\delta$D near zero were corrected by adding 1.60/oo to their measured $\delta$D. Possible errors associated with this method of correction are relatively small and have no significant effect on any interpretations made in this study.

**Determination of Ancillary Parameters**

Preparation of carbon dioxide gas for analysis by IRMS involved cryogenic separation of the CO$_2$ from the other gases. This was accomplished by injecting an aliquot of the sample gas through a rubber septum into a vacuum line with a trap cooled by liquid nitrogen (LN$_2$) and then simply pumping away the LN$_2$ non-condensable gases (O$_2$, N$_2$, CH$_4$). Water vapor was then removed cryogenically (-89°C) from the CO$_2$. Sedimentary organic matter was prepared for $\delta^{13}$C analysis by IRMS in a Craig type combustion apparatus (Craig 1953). The methane and carbon dioxide concentrations (mol%) of the gas samples were calculated from the GC-integration units (HP-3390A Integrator) following application of appropriate T. C. Weight Factors as given by McNair and Bonelli (1969).
ISOTOPIC COMPOSITIONS OF METHANES FROM FRESHWATER SEDIMENTS

Biogenic methane is a common constituent of anoxic sediments of freshwater environments such as lakes and swamps when large quantities of organic matter are vigorously decomposed (Figure 2). When methane production rates are high enough to supersaturate the pore waters of shallow sediments, bubbles may form and be released to the overlying water and then to the atmosphere.

Few measurements of the isotopic composition of biogenic methane formed in freshwater sediments are available in the literature. Oana and Deevey (1960) determined the $\delta^{13}C$ of methane and carbon dioxide obtained in the gaseous state following agitation of the sediments of several Connecticut lakes. The methane was $^{13}C$-depleted ($\delta^{13}C-CH_4$ ranged from -80 to -57‰) relative to both the gaseous carbon dioxide ($\delta^{13}C-CO_2$ ranged from -25 to -4‰) and the sedimentary organic matter ($\delta^{13}C-SOM$ ranged from -33 to -27‰; Oana and Deevey 1960). Stevens and Rust (1982) reported that methane collected from the bottom of a small C_3-plant dominated slough in Northern Illinois had a $\delta^{13}C$ of -57.8 to -54.7‰, whereas that collected at the surface was somewhat less $^{13}C$-depleted ($\delta^{13}C-CH_4$ ranged from -51.3 to -49.4‰) implying that there was partial oxidation of the methane during its transfer from the bottom to the surface. Fuex (1977) measured 17 methane samples obtained from marsh sediments in Minnesota and reported $\delta^{13}C$ ranging from -70.2 to -55.6‰. Methane collected by agitating the
sediments of Würmsee, a shallow West German lake, was $^{13}$C-depleted ($\delta^{13}$C-CH$_4$ ranged from -64 to -52°/oo) relative to the coexisting carbon dioxide gas ($\delta^{13}$C-CO$_2$ ranged from -21 to -3°/oo) and was D-depleted ($\delta$D-CH$_4$ ranged from -338 to -294°/oo) relative to the pore water ($\delta$D-H$_2$O ranged from -28 to -20°/oo; Woltemate et al. 1984).

In this section, measurements of $\delta$D-CH$_4$, $\delta$D-H$_2$O, $\delta^{13}$C-CH$_4$, $\delta^{13}$C-CO$_2$, and ancillary parameters from several freshwater environments and from a few locations within the Tampa Bay estuary are presented. Using these measurements and the model proposed by Woltemate et al. (1984), the relative importance of the two primary methanogenic pathways, acetate dissimilation and CO$_2$ reduction, to methane production in these systems can be estimated.

Sample Collection

Gas samples were obtained by agitating the sediment with a rod and funneling the released gases into 125ml serum bottles (Wheaton Scientific, Millville, NJ) that were initially filled with lake water. The bottles were filled as completely as possible with gas and stoppered with a black rubber stopper (no. 2048-11800, Belco Glass, Inc., Vineland, NJ) that was then secured with an aluminum crimp seal (Wheaton). The samples were stored on ice during transit back to the laboratory where they were kept in a freezer until analysis. Water samples for $\delta$D determination were collected in screw cap vials, the mouths of which were covered with parafilm to retard evaporation. Sediment samples were obtained by scraping surficial material into Whirlpak plastic bags and were frozen until analysis.

Model of Biogenic Methane Formation

Figure 4 is a model consisting of lines that describe predicted
Figure 4. Model of methane formation. Lines represent the percentage mix of acetate dissimilation (AD) and carbon dioxide reduction (R) (after Woltemate et al. 1984).
$^{6}\text{D-}H_2O/^{6}\text{D-CH}_4$ isotopic pairs resulting from varying the relative contributions to methane production of the acetate dissimilation and CO$_2$ reduction pathways. This model was proposed by Woltemate et al. (1984) and used in that study to estimate that methyl group transfer (from acetate or other methyl group donors such as methanol) was responsible for about 76% of the total methane production in the sediments of Würmsee, a shallow lake near Hannover, FRG. Lovley and Klug (1983) reported that methanol and methylamines were the precursors for less than 5 and 1%, respectively, of total methane production in the sediments of eutrophic Lake Wintergreen, Michigan. The likely explanation for this is the low abundance of methanol and methylamine precursors relative to H$_2$ and acetate precursors in the organic matter input to the sediments (Lovley and Klug 1983). Low rates of methane production from methanol and methylamines in near-surface, sulfate-rich marine sediments have been reported recently (King et al. 1983; King 1984a). Although methane production from these "noncompetitive" substrates (King 1984a) is of interest for several reasons, I would argue that it is likely to generate large quantities of methane (e.g., a significant fraction of that required to achieve saturation) only under relatively unusual conditions. Many marine organisms use methylamines or methylamine precursors (King 1984b) in osmoregulation (Yancey et al. 1982). In response to salt stress, the halophyte _Spartina alterniflora_ concentrated up to 30% of its total leaf nitrogen in glycine betaine (Cavalieri and Huang 1981), which can be metabolized to acetate and trimethylamine in marine sediments (King 1984b). Oremland et al. (1982) reported that metabolism of methanol and trimethylamine could account for the bulk of methane produced in salt
marsh sediments containing 8ml of methane per liter of wet sediment. The experiments that yielded these results were conducted in flasks containing 60ml of San Francisco Bay water, 40ml of sediment, and 10ml of homogenized *Spartina foliosa* materials (Oremland et al. 1982). Although known (macroalgae; Blunden et al. (1982)) and potential (mangroves, seagrasses) contributors of methylamine precursors (unknown concentrations) account for some organic input, sediments as rich in organic matter as those used above (Oremland et al. 1982) are rarely encountered (Sackett et al. in press) in the Tampa Bay estuary. From all of the preceding, I conclude that methane production from methanol and methylamines is quantitatively unimportant in the samples analyzed during this study; therefore, in applying the model (Woltemate et al. 1984) to the data presented in this section, I consider acetate to be by far the major source of methyl groups to the methanogens active in these sediments.

In constructing this model, Woltemate et al. (1984) assumed that for the CO₂ reduction pathway all four methane hydrogens are supplied by the environmental water (Schoell 1980; Daniels et al. 1980), and that for the acetate dissimilation pathway only one hydrogen comes from water with the remaining three coming from the methyl group of acetate (Pine and Barker 1956). As a result, the slopes of the 0:100 (acetate dissimilation: CO₂ reduction) and the 100:0 lines are 1 and 0.25, respectively. The slopes of the intermediate lines are obtained by multiplying the relative amount of each pathway by the appropriate slope (1 or 0.25) and summing. For example, the slope of the 80:20 line would be: 0.80(0.25) + 0.20(1) = 0.4. The y-intercept of the 80:20 line was obtained from the sewage sludge incubation experiment of
Schoell (1980). In that experiment, aliquots of sewage sludge were incubated in plastic bottles spiked with different amounts of D₂O. The results indicated a linear correlation between δD-H₂O and δD-CH₄, that was described (Schoell 1980) by the equation:

\[ \delta D-CH_4 = 0.4 \delta D-H_2O - 323^{oo} \]

The y-intercept of the 0:100 line resulted from the observation that δD-H₂O/δD-CH₄ pairs obtained from the measurement of natural samples, in which methane was presumably formed via CO₂ reduction (Schoell 1980) fit the relationship (Schoell 1980; Nakai et al. 1974):

\[ \delta D-CH_4 = \delta D-H_2O - 160 (±10)^{oo} \]

The equations of the remaining lines (Woltemate et al. 1984) are obtained by assuming that all of the lines pass through the point of intersection of equations (4) and (5). Equations (4) and (5) are based on analyses of methane formed under widely different environmental conditions. Variations in the magnitude of isotopic fractionation, due to variation in parameters such as rate of reaction and time (degree of isotopic equilibration), may introduce error into predictions of relative methanogenic pathway selection obtained with the model. It appears, however, that the model yields predictions that are generally valid (e.g., Woltemate et al. 1984), and that application of the model to data presented in this study should yield useful interpretation.

**Results and Discussion**

Measurements of δD-H₂O/δD-CH₄ pairs from various freshwater and estuarine sediments presented in Figure 5 imply that about 50 to 80% of the total methane production in these sediments can be attributed to acetate dissimilation. This agrees with some earlier studies (Capenberger and Prins 1974; Winfrey and Zeikus 1979; Lovley and Klug
Figure 5. Plot of methane hydrogen isotopic composition versus environmental water hydrogen isotopic composition for several freshwater environments and the Tampa Bay estuary. The lines are the same as in Figure 4. Symbols: • Crescent Lake, FL.; ▲ Mirror Lake, FL; ▲ Lake Dias, FL; ▲ Kilmer Pond, SC; ■ Mississippi River Delta, LA; and • Tampa Bay estuary, FL.
1982; Woltemate et al. 1984), but is in conflict with others that indicated CO₂ reduction dominates methane production in other freshwater lake sediments (Belyaev et al. 1975; Jones et al. 1982). The variation in pathway importance between individual lakes and even between different locations within the same lake (Figure 5) may be attributable to natural qualitative and quantitative variations in the organic matter and bacteria occurring in these sediments (i.e., Belyaev et al. 1975). The predominance of the acetate dissimilation pathway under nearshore marine-estuarine (S = 6-34°/oo; Table 2) conditions indicated in Figure 5 was also found in other nearshore-marine circumstances (Sansone and Martens 1981; Mountfort et al. 1980). In contrast, the biogenic methane found in deep-sea sediments is thought to originate almost exclusively from CO₂ reduction (Schoell 1980; Claypool and Kaplan 1974) and is isotopically distinguishable (δD = -170 to -190°/oo; Schoell 1983) from the marine methane measured in this study (Figure 5). The δD-CH₄ values reported here support the idea that with respect to methane production, highly productive brackish and marine sediments can bear a greater resemblance to freshwater than deep-sea sediments if sulfate is depleted near the sediment-water interface due to high rates of respiration (Rudd and Taylor 1980; King 1983).

As discussed previously, partial oxidation of a quantity of methane, mediated by aerobic bacteria, can leave the remaining methane markedly less ¹³C- and D-depleted (Coleman et al. 1981). If the percentage of methane oxidation and the degree of isotopic fractionation varied depending upon location within a given freshwater lake, for example, isotopic variation (Figure 5) could occur. The
Table 2. δD-CH₄, δ¹³C-CH₄, and ancillary data for several freshwater environments and the Tampa Bay estuary.

<table>
<thead>
<tr>
<th>LOCATION/DATE</th>
<th>δD (‰)</th>
<th>δ¹³C (‰)</th>
<th>%C</th>
<th>Sed.</th>
<th>T(°C)</th>
<th>aDb</th>
<th>aDc</th>
<th>Conc. (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/31/84 A</td>
<td>1 -297 ±1.0</td>
<td>-7 ±0.9</td>
<td>61.3 ±0.1</td>
<td>-7.2</td>
<td>31</td>
<td>1.058</td>
<td>1.413</td>
<td>59.8 6.5</td>
</tr>
<tr>
<td></td>
<td>3 -304 ±0.5</td>
<td>-58.8 ±0.1</td>
<td>-6.9</td>
<td>26</td>
<td>1.061</td>
<td>1.427</td>
<td>55.4 2.6</td>
<td></td>
</tr>
<tr>
<td>10/02/84</td>
<td>1 -316 ±0.6</td>
<td>-59.8 ±0.1</td>
<td>-11.6</td>
<td>22</td>
<td>1.057</td>
<td>50.5 2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/17/84</td>
<td>1 -298</td>
<td>-62.0</td>
<td>-22.9</td>
<td>23</td>
<td>1.058</td>
<td>1.405</td>
<td>46.2 2.5</td>
<td></td>
</tr>
<tr>
<td>12/21/84</td>
<td>1 -296</td>
<td>-11 ±0.7</td>
<td>-62.9</td>
<td>-8.8</td>
<td>1.073</td>
<td>1.349</td>
<td>50.9 7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 -257 ±0.4</td>
<td>-75.0 ±0.3</td>
<td>-7.2</td>
<td>16</td>
<td>1.072</td>
<td>1.343</td>
<td>59.1 4.3</td>
<td></td>
</tr>
<tr>
<td>01/23/85</td>
<td>1 -263</td>
<td>-10 ±0.5</td>
<td>-71.4</td>
<td>-5.0</td>
<td>-27.9</td>
<td>3.5</td>
<td>14</td>
<td>1.048</td>
</tr>
<tr>
<td></td>
<td>2 -263</td>
<td>-75.0</td>
<td>-58.8</td>
<td>-9.0</td>
<td>1.068</td>
<td>1.351</td>
<td>49.0 7.2</td>
<td></td>
</tr>
<tr>
<td>04/23/82</td>
<td>2 -335</td>
<td>-48 ±0.2</td>
<td>-60.3</td>
<td>-3.7</td>
<td>-27.5</td>
<td>11.1</td>
<td>21</td>
<td>1.060</td>
</tr>
<tr>
<td></td>
<td>5 -346</td>
<td>-60.0</td>
<td>-66.7</td>
<td>-3.7</td>
<td>-28.0</td>
<td>33.6</td>
<td>14</td>
<td>1.067</td>
</tr>
<tr>
<td>10/14/84</td>
<td>1 -315</td>
<td>-53.6</td>
<td>-53.6</td>
<td>-10.9</td>
<td>1.432</td>
<td>63.9 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 -319</td>
<td>-51.5</td>
<td>-50.0</td>
<td>-26.9</td>
<td>1.456</td>
<td>61.0 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/12/84</td>
<td>1 -311</td>
<td>-16 ±0.6</td>
<td>-55.3</td>
<td>1.042</td>
<td>34.0 1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table contains data on δD and δ¹³C values for methane (CH₄) and ancillary data such as %C, sediment (Sed.), temperature (T), and concentrations (Conc.). The values are given for different dates and locations, with additional columns for ancillary data such as δ¹³C for CO₂ (SOMa), SOM (T), T(°C), and concentrations in moles per liter (mol%).
Table 2. (Cont'd).

<table>
<thead>
<tr>
<th>LOCATION/DATE</th>
<th>θ</th>
<th>CH₄</th>
<th>H₂O</th>
<th>δ²⁷⁻¹³C (‰)</th>
<th>δ¹³C (‰)</th>
<th>%C Sed.</th>
<th>Conc. (mo1L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CH₄</td>
<td>CO₂</td>
<td>SOM</td>
<td>SOM</td>
<td>T(°C)</td>
<td>αC</td>
</tr>
<tr>
<td>09/16/84</td>
<td>1</td>
<td>-323±1.2</td>
<td>+11</td>
<td>-70.8±0.1</td>
<td>29</td>
<td>1.493</td>
<td>54.9</td>
</tr>
<tr>
<td>10/11/84</td>
<td>2</td>
<td>-296</td>
<td>+11</td>
<td>-63.3</td>
<td>28</td>
<td>1.436</td>
<td>79.3</td>
</tr>
<tr>
<td>12/03/84</td>
<td>3</td>
<td>-288</td>
<td>+7±0.4</td>
<td>-64.7</td>
<td>24</td>
<td>1.414</td>
<td>70.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-291</td>
<td>0±0.2</td>
<td>-63.8</td>
<td>1.6</td>
<td>1.410</td>
<td>74.1</td>
</tr>
</tbody>
</table>

**TABLE LEGEND**

A - Crescent Lake, 12ha, fresh, St. Petersburg (SP), FL
B - Mirror Lake, 7ha, fresh, SP, FL
C - Lake Dias, 300ha, fresh, Volusia County, FL
D - Mississippi River Delta, fresh, LA
E - Kilmer Pond, fresh, near Columbia, S.C.
F - Tampa Bay estuary, FL
   1 - Ft. DeSoto Pk., S = 34°/oo
   2 - Bishop Harbor, S = 32°/oo
   3 - Lake Seminole, S = 20°/oo
   4 - Feather Sound, S = 6°/oo

a - SOM = Sedimentary organic matter
b - αC = (CO₂/CH₄) = δ¹³CO₂ + 1000 / δ¹³C-CH₄ + 1000
c - αD = (H₂O/CH₄) = δD-H₂O + 1000 / δD-CH₄ + 1000
generally negative correlation between $\delta^{13}$C-CH$_4$ and $\delta^{18}$D-CH$_4$ indicated in Figure 6 implies that the isotopic variation is not solely due to methane consumption. The negative correlation does not mean that these samples were totally unaffected by in situ methane oxidation, but it does imply that some additional process (i.e., variation in pathway) contributed to the isotopic variation. Methane concentration profiles in the upper 10 to 15 cm of anoxic freshwater sediments are generally linear, implying that production and transport are of greater importance than anaerobic methane consumption in controlling the methane distributions (Reeburgh and Heggie 1977). Methanogenic bacteria are strict anaerobes and produce methane only under highly reducing conditions (Mah 1982). Methane produced under these conditions could only be oxidized by the aerobic process if it either migrated (as a bubble or in solution) to a less reducing location or if the depth of oxygen penetration increased. A bubble rising from the depth at which it was formed would likely exit the sediments entirely, because the distance between the methane production zone and the sediment-water interface is short (50 cm or less), and would not be sampled by the methods employed here. Likewise, methane that dissolved would not be sampled, and because the process of partial dissolution itself would not be expected to induce much isotopic fractionation (Bernard et al. 1976), any methane remaining in bubble form should maintain its isotopic signature. Deeper penetration of oxygen into the sediments because of a temperature-induced decrease in oxygen consumption might allow partial oxidation of trapped bubbles. This could be responsible for the slight increase (Table 2) in the isotopic compositions of methane sampled from Crescent Lake (#2) on 1/23/85.
Figure 6. Plot of methane hydrogen isotopic composition versus methane carbon isotopic composition for several freshwater environments and the Tampa Bay estuary. Symbols the same as in Figure 5.
compared to that sampled at the same location on 12/21/84. This slight
difference has very little effect on any interpretations, and when
considered along with the other discussion advanced above, argues
against extensive isotopic fractionation of samples reported here
(Figures 5, 6) by methane consumption.

All of the isotopic and ancillary data considered in this section
are listed in Table 2. Concentrations of methane and carbon dioxide
ranged from 33 to 86% and from 4 to 13%, respectively, in the samples
that were analyzed. The carbon isotopic composition of sedimentary
organic matter (SOM) ranged from -26.9 to -28.0°/oo for all but one of
the freshwater sediment samples (Table 2); this implies that higher
land plants (C\textsubscript{3} pathway) and low \textsuperscript{13}C/\textsuperscript{12}C aquatic plants (Smith and
Epstein 1971) dominate organic matter input to these sediments. For
instance, an unidentified emergent aquatic macrophyte from Lake Dias
(#3) yielded a δ\textsuperscript{13}C of -28.1°/oo. A δ\textsuperscript{13}C of -22.5°/oo was determined
for the SOM of Mirror Lake (Table 2); this is substantially heavier
than the other freshwater values. Possible explanations for this
include a relatively greater contribution of isotopically heavy C\textsubscript{4}
pathway plant material (i.e., domestic Bermuda grass; Smith and Epstein
1971), and utilization of isotopically heavier source carbon by the
submerged aquatic plants (Osmond et al. 1981). There is no obvious
relationship between either δ\textsuperscript{13}C-SOM or %C-SOM and δ\textsuperscript{13}C-CH\textsubscript{4} for the
samples reported here (Table 2). For example, although the δ\textsuperscript{13}C-CH\textsubscript{4} of
Crescent Lake #2 is 8-12°/oo lighter than the δ\textsuperscript{13}C-CH\textsubscript{4} of Crescent Lake
#1, there is no substantial difference between the two sites with
respect to δ\textsuperscript{13}C-SOM or %C-SOM. Factors other than amount and δ\textsuperscript{13}C of
the source organic matter, such as pathway selection (Figure 5), must
also be involved in determining $\delta^{13}\text{C-CH}_4$. Fractionation factors $\alpha_C(\alpha(\text{CO}_2/\text{CH}_4))$ and $\alpha_D(\alpha(\text{H}_2\text{O}/\text{CH}_4))$ of the freshwater samples, calculated from measured isotopic compositions, ranged from 1.042 to 1.073 and from 1.343 to 1.456, respectively (Table 2). These values are similar to those determined for German lake Würmsee (Woltemate et al. 1984), although the ranges reported here are greater. The greater ranges (higher $\alpha_C$, lower $\alpha_D$) probably reflect the larger relative contribution of the CO$_2$ reduction pathway in some of these samples (Figure 5) compared to the Würmsee samples (Woltemate et al. 1984). The fractionation factors calculated from typical deep-sea isotopic compositions (Rice and Claypool 1981; Claypool and Kaplan 1974; Schoell 1980) are significantly larger ($\alpha_C \sim 1.08$) and smaller ($\alpha_D \sim 1.25$) than the factors listed in Table 2; this implies that both isotopes are affected by methanogenic pathway and indicates the potential applicability of these parameters (particularly together) to the genetic characterization of biogenic methane deposits.

As discussed earlier, recent studies (Craig and Chou 1982; Khalil and Rasmussen 1982) indicated a doubling of atmospheric methane concentrations during the past few hundred years. Furthermore, during the last 3 to 4 years, atmospheric methane concentrations have increased at rates of 1 to 1.9% per year (Khalil and Rasmussen 1983). Decomposition of organic matter in water-covered soils, intestinal fermentation in ruminants, biomass burning, and direct anthropogenic input (i.e., leakage of fossil fuels) are thought to be the major sources of methane to the atmosphere contributing approximately 46, 22, 5, and 7% of the total flux, respectively (Khalil and Rasmussen 1983). The major sink of atmospheric methane is thought to be reaction with OH
radicals in the troposphere (Khalil and Rasmussen 1983). A recent study by Harriss et al. (1982) indicated that periodically inundated soils may also be a site of atmospheric methane removal during dry periods.

Recent measurements indicate that the $\delta^{13}C$ of atmospheric methane is about $-47.0 \pm 0.3^\circ/oo$ (Stevens and Rust 1982). The average isotopic fractionation associated with the sink process is $-2.5 \pm 1.5^\circ/oo$, and there is a $+0.3^\circ/oo$ isotope effect resulting from the nonsteady state increasing methane concentrations (Stevens and Rust 1982). This implies that the average $\delta^{13}C$-CH$_4$ for all sources is $-49.2 \pm 1.5^\circ/oo$; this is significantly less $^{13}C$-depleted than most known biogenic methane sources (Stevens and Rust 1982), and is about $10^\circ/oo$ less negative than the average $\delta^{13}C$-CH$_4$ of all sources ($-61 \pm 3^\circ/oo$) calculated by Senum and Gaffney (1985). Biomass burning and fossil fuels are the only known significant methane sources more $^{13}C$-enriched than $-49^\circ/oo$ (Stevens and Rust 1982), but they apparently account for a relatively small fraction of the total input.

Bubble ebullition is the dominant mode of methane input to the atmosphere from some shallow freshwater (Robertson 1979; Cicerone and Shetter 1981) and marine (Martens and Klump 1980) environments. All of the gas samples analyzed for this section were collected as bubbles from sediments covered by very shallow (1m or less) water. Three of the freshwater lakes (Crescent Lake, Mirror Lake, and Kilmer Pond) have been observed to release gas as bubbles from their sediments and it is very likely that active bubbling also occurs in the other environments. There is a good deal of variation ($-24^\circ/oo$) in the $\delta^{13}C$-CH$_4$ data presented in Table 2; however, most of the samples are substantially
lighter than \(-49^{0}/oo\), in agreement with other data from shallow aquatic environments (Stevens and Rust 1982). Though limited in number, these data imply that ebullition from these sediments is unlikely to provide an atmospheric input of methane as \(^{13}\text{C}\)-enriched as the source average.

Based on the results of a study by Rust (1981), which demonstrated that the \(^{13}\text{C}\) of methane produced by ruminants depends on the \(^{13}\text{C}\) of the plants in their diet, Stevens and Rust (1982) proposed that the anaerobic decomposition of isotopically heavy C\(_4\) plant debris in large wetlands, such as the Sudd marshes and the Florida Everglades, might be a significant source of isotopically heavy methane to the atmosphere. As mentioned before, no such simple relationship between the \(^{13}\text{C}\)-CH\(_4\) and \(^{13}\text{C}-\text{SOM}\) of aquatic sediments is indicated by the data presented in Table 2. According to Wolfe (1979), four well-defined groups of bacteria are involved in the conversion of complex organic matter to methane in sediments, whereas in the rumen only two of the groups are involved and there is no significant production of methane from acetate in the rumen. Also, substrate input, product output, and environmental conditions are more closely controlled in the rumen than in sediments (Wolin 1982). Thus, in addition to \(^{13}\text{C}\)-SOM, other factors such as pathway selection and possibly kinetic effects are probably important in setting the \(^{13}\text{C}\)-CH\(_4\) of aquatic sediments. Escape of dissolved methane across the air-water interface, rather than ebullition, may be the more likely means of transferring isotopically heavy methane from shallow aquatic environments to the atmosphere. Dissolved methane should be more readily available for aerobic methane oxidation (Rudd and Taylor 1980) and isotopic fractionation (Coleman et al. 1981); however, assessment of the potential contributions of ebullition and
dissolved methane to atmospheric $\delta^{13}$C-$\text{CH}_4$ is hindered by a lack of relevant data.

Using data obtained in 1960 and 1970 (Bainbridge et al. 1961; Begemann and Friedman 1968; Ehhalt 1973), Senum and Gaffney (1985) noted an apparent decrease in atmospheric methane $\delta^D$ with time that they extrapolated to the present to yield an estimate of $-104 \pm 4^\circ/oo$ for the $\delta^D$ of present-day atmospheric methane. The extrapolated $\delta^D$ value was corrected for kinetic isotopic fractionation resulting from the reaction of methane with hydroxyl radicals (the magnitude of the fractionation was attributed to Gorden and Mulac 1975) in the troposphere to yield an estimate of $-322^\circ/oo$ as the present-day average $\delta^D$ of all methane sources to the atmosphere (Senum and Gaffney 1985). Analyses reported in Table 2 and by Woltemate et al. (1984) indicate that methane as D-depleted as $-322^\circ/oo$ is produced in some freshwater sediments. Organic matter decomposition in sewage sludge may also yield methane as D-depleted as $-322^\circ/oo$ (Schoell 1980). According to the genetic characterization model of Schoell (1983), which is based on approximately 500 natural gas analyses, thermogenic methane is generally more D-enriched than $-300^\circ/oo$. To my knowledge, there are no estimates of the $\delta^D$ of methane produced from either the intestinal fermentation in ruminants or biomass burning. Although an important source of atmospheric methane with a $\delta^D$ near the estimated source average (Senum and Gaffney 1985) has been identified (freshwater sediments), there are presently too few measurements of the $\delta^D$ of atmospheric methane and its sources available to determine whether or not this apparent agreement has any real significance in regard to evaluating the role of shallow aquatic sediments in the atmospheric
methane budget and in regard to determining the δD of atmospheric methane.
METHANE ISOTOPIC COMPOSITIONS IN CAPE LOOKOUT BIGHT SEDIMENTS

Cape Lookout Bight is a small, partially enclosed marine basin located on the Outer Banks of North Carolina (Figure 7). The bight interior is somewhat protected from high energy winds and waves, and serves as an efficient trap of fine-grained sediments and organic debris that exit the biologically productive barrier island lagoonal system to the north through Barden's Inlet in response to tidal flushing and winter storms (Martens and Klump 1980, 1984). Organic matter in bight sediments comes from plankton, salt marsh and seagrass detritus, eroding shoreline peat deposits (Martens and Klump 1984) and terrigenous materials washed into the barrier island lagoonal system by freshwater runoff.

Rapid remineralization of organic carbon in the rapidly accumulating (8.4 to 11.8 cm/yr.; Chanton et al. 1983), organic-rich (3.3 to 5% organic carbon) sediments of the bight interior (Station A-1; Figure 7) creates anoxic conditions at or near (0.5 to 1.0 cm subbottom) the sediment-water interface (Martens and Klump 1984). The anoxic conditions prevent inhabitation of the sediments by macrobenthic infauna (Menzies et al. 1968), which pump sulfate-rich seawater into their burrows (Martens 1976), except during winter months (Bartlett 1981 as cited by Chanton et al. 1983; Martens and Klump 1984). As a result, dissolved sulfate is quickly depleted (about 1 mM) and methane is produced rapidly enough to supersaturate the interstitial waters.
Figure 7. Cape Lookout Bight, North Carolina, USA. The sampling site was Station A-1 (after Martens and Klump 1980).
near (about 8-15 cm subbottom) the sediment-water interface during warm (16-29°C) summer months (late May to mid November; Martens and Klump 1980; Sansone and Martens 1982; Kipphut and Martens 1982). Reduction of hydrostatic pressure associated with ebb tide (tidal range about 1 m) triggers expansion and upward migration of trapped gas bubbles (82 to 90% methane, mean 86%) through sedimentary "bubble tubes" (Martens 1976) to the water column and, finally, to the troposphere (Martens and Klump 1980). An estimated 70 to 80% of the yearly flux of methane ejected to the atmosphere from the Cape Lookout Bight system comes from direct bubble transport, with the remaining 20 to 30% contributed by escape of dissolved methane from the waters across the air-sea interface (Martens and Klump 1980). Blair et al. (1984) reported that methane bubbles produced in bight sediments display a seasonal δ^{13}C variation (δ^{13}C-CH$_4$ ranging between -64 and -58‰), with ebullition of the more δ^{13}C-enriched methane occurring during the summer when bubbling rates are maximal.

In this section, measurements of the δD and δ^{13}C of methane gas samples from Cape Lookout Bight sediments collected on fifteen different dates from 16 June 1983 to 17 November 1984, and δD measurements of three pore water samples from various times and depths are presented. All of the samples described here were collected by the research group "CH$_4$AO$_2$S" under the direction of Dr. Christopher S. Martens of the University of North Carolina, Chapel Hill. These measurements provide an opportunity to compare the estimates of relative methanogenic pathway importance obtained with the Woltemate et al. (1984) model to those derived from 14C labeling studies reported by Sansone and Martens (1981) and Crill and Martens (in press).
Further, the large range of temperatures (7 to 29°C) at which these samples were collected allows assessment of seasonal influences on methanogenic pathway selection.

Sample Collection

Trapped gas bubbles were displaced by physical disturbance from different depths within the upper 20 to 40 cm of the sediments into a large inverted cone placed about 1 m above the bottom (water depth about 8 m), and transferred at depth from the cone to 125 ml (except for the 19 September 1984 samples (30 ml)) Wheaton serum bottles that were initially filled with water. The serum bottles were filled as completely as possible with gas, stoppered, and crimped with an aluminum seal. During sample collection, gas was continuously released from the sediment to the cone as gas was withdrawn from the cone. As a result, replicate samples, particularly during summer when up to five 125 ml samples were taken, did not all come from exactly the same aliquot of sedimentary gas. The samples were kept in a freezer until analysis, except during transit between North Carolina and Florida.

Results and Discussion

Methane isotopic compositions over the course of a year and sediment temperatures from several years (Martens and Klump 1980; Martens et al. 1980; Crill and Martens 1983; Martens unpublished data) are plotted versus sampling date in Figure 8. The data (Figure 8, Table 3) indicate great isotopic variability among replicate sample bottles during the summer, and indicate that δD-CH₄ and δ¹³C-CH₄ vary in an opposite manner in response to change in season, with methane more D-depleted and more ¹³C-enriched (also Blair et al. 1984) during summer months (July to September; mean δD-CH₄ = -269‰, std. dev. =
Figure 8. Seasonal variations of temperature, δD-CH₄, and δ¹³C-CH₄ in Cape Lookout Bight sediments. The temperature data are from Martens and Klump (1980), Martens et al. (1980), Crill and Martens (1983) and Martens (unpublished).
Table 3. δD-CH₄, δ¹³C-CH₄, and δD-H₂O from Cape Lookout Bight.

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<th>δ¹³C-CH₄ (‰)</th>
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Pore water δD

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$9^\circ/oo$, $n = 21$; mean $\delta^{13}C-CH_4 = -59.5^\circ/oo$, std. dev. = $1.9^\circ/oo$, $n = 21$) when bubbling rates are maximal (Martens and Klump 1980), as compared to winter months (December to April; mean $\delta^{13}C-CH_4 = -236^\circ/oo$, std. dev. = $4.4^\circ/oo$, $n = 6$; mean $\delta^{13}C-CH_4 = -63.9^\circ/oo$, std. dev. = $0.5^\circ/oo$, $n = 6$) when Cape Lookout Bight sediments are not observed to bubble (Martens and Klump 1980, 1984).

The indicated seasonal fluctuation of $\delta^{13}C-CH_4$ (Figure 8) probably results from seasonal variation in methanogenic pathway selection, with acetate dissimilation accounting for a greater proportion of the total methane production during summer than during winter. Evaluation of these data with the Woltemate et al. (1984) model (Figure 9) implies that acetate dissimilation accounts for about 45 to 60% of the total methane production during summer months, and about 25 to 40% of the total during winter months. Sansone and Martens (1981) measured the production rates of carbon dioxide and methane from $^{14}C$-labeled acetate in the 5 cm depth interval immediately below the 1 mM sulfate isopleth of bight sediments at various times of the year, and estimated that over 50% of the summertime methane flux could be accounted for by acetate dissimilation. In contrast, measurements made from January to May indicated that the rate of methane production from acetate in this layer was near zero during the winter (Sansone and Martens 1981).

Crill and Martens (in press) measured methane production rates from $^{14}C$-labeled bicarbonate and acetate at various depths in Cape Lookout Bight sediments during the summer of 1983, and found that the relative importance of the acetate dissimilation pathway to total methane production, integrated over the upper 30 cm of the sediments, increased over the course of the summer from 23% on 21 July to 30% on 30 August.
Figure 9. Plot of methane hydrogen isotopic composition versus pore water hydrogen isotopic composition for samples from Cape Lookout Bight and the samples plotted in Figure 5. Symbols:
• Cape Lookout Bight; all other the same as in Figure 5.
In the sulfate-reducing zone of bight sediments the acetate methyl group is oxidized only to carbon dioxide (Sansone and Martens 1982). Although appreciable rates of methane production from carbon dioxide reduction were measured in Cape Lookout Bight sediments at all depths except the top 2 cm (including some in which high rates of sulfate reduction were measured), acetate was a significant precursor of methane only in sulfate-depleted (< 1 mM) layers; further, acetate dissimilation accounted for an increasing percentage of the total methane production with increasing depth to a maximum of 48% in the 20 to 22 cm layer on 7 August 1983 (Crill and Martens in press). Net methane production rates as determined by the tube incubation technique of Crill and Martens (1983) were nearly equivalent to the sum of the methane production rates from $^{14}$C-labeled bicarbonate and acetate during the summer of 1983, implying that nearly all of the methane production in Cape Lookout Bight sediments during the summer results from carbon dioxide reduction and acetate dissimilation (Crill and Martens in press). Measurements of in situ concentrations of alternate methanogenic substrates, such as methanol and methylamines, and $^{14}$C radiotracer studies will be required for a more accurate assessment of the quantitative significance of alternate methanogenic pathways in bight sediments.

The interpretation presented here (Figure 9) that acetate dissimilation is relatively less important during the winter in Cape Lookout Bight sediments qualitatively agrees with the aforementioned results of Sansone and Martens (1981). Further, if significant methane production from acetate occurs only when sulfate concentrations are < 1 mM (Crill and Martens in press), then acetate dissimilation would be
expected to be of less importance during the winter because penetration of the 1 mM sulfate isopleth is 20 to 22 cm in winter and 7 to 10 cm in summer (Sansone and Martens 1982). Because more than 98% of the calculated total remineralization of metabolizable organic matter takes place within three years in Cape Lookout Bight sediments (Martens and Klump 1984), which corresponds to about 30 cm depth (Chanton et al. 1983), the microbes living at depths where acetate dissimilation to methane should be possible during winter would have to subsist on the more refractory organic matter fractions. Lowered rates of methane production from acetate during winter would be expected due to the combination of lowered temperatures (Zeikus and Winfrey 1976) and poorer nutrition. The logical prediction that acetate dissimilation is relatively less important during the winter and the general agreement of the estimates of summer Cape Lookout Bight methanogenic pathway selection presented in Figure 9 with those from \(^{14}\)C radiotracer studies (Sansone and Martens 1981; Crill and Martens in press) imply that interpretations made using the model (Woltemate et al. 1984) employed in this study are reasonable.

Variation of the relative importance of acetate dissimilation with depth in the sediment (Crill and Martens in press) is probably the factor most responsible for the great isotopic variability observed among replicate samples (Figure 8, Table 3) taken during the summer (19 August 1983 and 31 August 1984) when bubbling rates are maximal (Martens and Klump 1980, 1984). Because the method of sample collection employed in this study did not assure that all "replicates" sampled exactly the same aliquot of sedimentary gas, any isotopic inhomogeneity (i.e., with depth) of gas bubbles trapped within the
sediment could express itself in the replicates analyzed here. Kipphut and Martens (1982) observed $^{222}$Rn profiles in Cape Lookout Bight sediments during early and late summer that exhibited a sharp $^{222}$Rn maximum at 6 to 8 cm depth and a $^{222}$Rn deficit below. The unusual shape of the $^{222}$Rn profiles was attributed to stripping of $^{222}$Rn by methane bubbles at depth followed by partial dissolution of the bubbles at 6 to 8 cm depth, particularly during times of the year when bubbling rates are relatively low and the residence time of the bubbles in the sediment should be longer (Kipphut and Martens 1982). The results of Bernard et al. (1976) indicate that very little isotopic fractionation occurs when methane gas is partially dissolved in water; therefore, it is unlikely that variation in the extent of bubble dissolution with depth in the sediment or season would induce much isotopic variability.

If all of the methane produced in Cape Lookout Bight sediments had the same isotopic composition and was then subjected to varying degrees of bacterial oxidation, depending upon season and/or depth, differential isotopic fractionation (Coleman et al. 1981) and isotopic variability could result. The zone of oxic conditions is very thin (0.5 to 1.0 cm; Martens and Klump 1984) in bight sediments during the winter and essentially absent during the summer, greatly decreasing the possibility of extensive oxidation of sedimentary methane bubbles by the assimilatory, microaerophilic process (Martens and Klump 1980). The potential significance of dissimilatory anaerobic methane oxidation in Cape Lookout Bight sediments is difficult to assess as there are no direct radiotracer measurements of the process (i.e., Alperin and Reeburgh 1984) available for this system. Because appreciable anaerobic methane oxidation in marine sediments is apparently confined
to a thin layer at the base of the sulfate-reducing zone (Reeburgh 1980; Devol 1983; Alperin and Reeburgh 1984; Iversen and Jorgensen 1985), methane bubbles should not be subject to extensive oxidation during entrapment below the sulfate-reducing zone. The linear to convex-up pore water methane concentration profiles that are observed during summer and winter (Martens and Klump 1980; Kipphut and Martens 1982) can be interpreted to mean that the rate of anaerobic methane oxidation is significantly less than the rate of methane transport out of the sediment (Martens and Klump 1984). The negative linear correlation between Cape Lookout Bight $\delta^D$-CH$_4$ and $\delta^{13}$C-CH$_4$ (all data):

$$\delta^D$-CH$_4 = -6.16^{13}$C-CH$_4 - 626^{0/oo} \ (r = 0.90, \ n = 42) \ (6)$$

indicated in Figure 10 is good evidence that the isotopic variability is not simply the result of differential oxidation of a single isotopic end-member, as partial bacterial consumption would likely enrich the residual methane with respect to both isotopes (i.e., Coleman et al. 1981). Thus, it is unlikely that any secondary process such as partial dissolution or bacterial oxidation could account for all of the seasonal and replicate sample isotopic variability indicated in Figure 8.

Linear regression of $\delta^D$-CH$_4$ and $\delta^{13}$C-CH$_4$ data obtained during the main Cape Lookout Bight bubbling period (1 July to 1 October, sediment $T \geq 24^{0}C$, Martens and Klump 1984) yields the equation:

$$\delta^D$-CH$_4 = -4.46^{13}$C-CH$_4 - 528^{0/oo} \ (r = 0.92, \ n = 21) \ (7)$$

With the exception of the 31 May 1984 samples, the methane isotopic compositions of winter samples taken from February to mid-June (Table 3) deviate significantly from the line (7) derived from the summer data. Partial bacterial oxidation of trapped methane bubbles resulting
Figure 10. Plot of methane hydrogen isotopic composition versus methane carbon isotopic composition for the samples from Cape Lookout Bight. The equation: $\delta D = -6.1\delta^{13}C - 626$ is a linear regression of all the data considered in this study. The equation: $\delta D = -4.4\delta^{13}C - 528$ is a linear regression of the data obtained for $T \geq 24^\circ C$. 
from deeper penetration of sulfate during the winter (Sansone and Martens 1982) would be expected to shift the methane isotopic compositions in the direction required to explain the deviation. Blair et al. (1984) suggested that near-surface methane oxidation could account for the variation in pore water methane $\delta^{13}C$ with depth (-61.0 ± 0.6 $^0/oo$ at 2-7 cm depth to -65 ± 0.1°/oo at 35-47 cm depth) that they observed during February of 1984. The $\delta^{13}C$-CH$_4$ of the winter samples discussed here range from -65.1 to -63.0°/oo, implying that they originated in deeper layers where methane oxidation is probably of less significance. The disagreement of the winter isotopic compositions with equation (7) probably results from the greater relative importance of the carbon dioxide reduction pathway to methane production during the winter as discussed above. The biogenic methane found in deep-sea sediments, which is thought to originate almost exclusively from carbon dioxide reduction (Claypool and Kaplan 1974; Schoell 1980), is generally significantly less D-depleted ($\deltaD = -190$ to -170°/oo, Schoell 1983) than the methane produced during the summer in Cape Lookout Bight sediments (mean $\deltaD = -269^0$/oo). It is reasonable to expect that methane produced by carbon dioxide reduction in Cape Lookout Bight sediments would be less D-depleted than -269°/oo, although it might be more D-depleted than the methane produced in deep-sea sediments as a result of environmental differences (i.e., rate of organic matter input and remineralization, sedimentation rate, methane production rate, extent of isotopic exchange) between the two systems.

The $\delta^{13}C$ of methane produced by carbon dioxide reduction depends upon the $\delta^{13}C$ of the substrate carbon dioxide and the degree of
isotopic fractionation induced by the bacteria mediating the reaction. Laboratory studies (Games et al. 1978; Belyaev et al. 1983) indicate that the isotopic fractionation associated with carbon dioxide reduction decreases with increasing temperature, as would be expected for temperature-dependent isotopic equilibration of methane and CO$_2$. The results of laboratory studies of bacterial sulfate reduction (Jones and Starkey 1957; Kaplan and Rittenberg 1964) imply that the sulfur isotope effect associated with this process is dependent upon the rate of reduction, and that temperature influences fractionation only by affecting the rate of reduction. The rate of methanogenesis is known to vary greatly in response to temperature fluctuations (Zeikus and Winfrey 1976), so the isotope effect associated with CO$_2$ reduction (Games et al. 1978; Belyaev et al. 1983) may also be rate-dependent. Extrapolation of the results of these laboratory studies (Games et al. 1978; Belyaev et al. 1983), which were conducted at T $\geq 37^\circ$C and may not be representative of the naturally occurring process, to Cape Lookout Bight winter temperatures (7 to 23$^\circ$C) implies that temperature (or rate) induced $\delta^{13}$C-CH$_4$ variations of about 10 to 30$^\circ$/oo are possible. Blair et al. (1984) reported that $\delta^{13}$C-CO$_2$ in Cape Lookout Bight sediments during February of 1984 ranged from $-9.5 \pm 0.1^\circ$/oo in the sulfate reduction zone to $+6.1 \pm 0.1^\circ$/oo in the methane production zone, indicating that variation in substrate isotopic composition could also be responsible for significant $\delta^{13}$C-CH$_4$ variations.
METHANE ISOTOPIC COMPOSITIONS IN DEEP-SEA SEDIMENTS

Methane associated with present day deep-sea sediments is formed by both microbial and thermal decomposition of buried organic matter. Microbial processes account for most of the methane production in rapidly accumulating (sedimentation rate > 10 m/10^6 years), near-surface (< 1 km depth) deep-sea sediments subjected to normal geothermal gradients ( < 50°C/ km depth; Claypool and Kvenvolden 1983). Only in areas of abnormally high heat flow, such as subduction zones, active spreading centers, or near hot igneous intrusions (i.e., Schoell 1982), are near-surface deep-sea sediments likely to attain sufficiently high temperatures (80 to 150°C) to allow significant thermal decomposition of complex organic matter to methane and other light hydrocarbons (Claypool and Kvenvolden 1983).

Biogenic methane production in deep-sea sediments is thought to occur almost exclusively via CO₂ reduction (Claypool and Kaplan 1974; Whiticar et al. in press). The principal evidence for this is that deep-sea profiles of δ¹³C-CH₄ and δ¹³C-ΣCO₂ are generally parallel, presumably as a result of bacterial reduction of CO₂ with relatively constant kinetic carbon isotopic fractionation (Claypool and Kaplan 1974). Further, available deep-sea δD-H₂O/δD-CH₄ pairs (Whiticar et al. in press) fit rather well the equation (5) mentioned earlier (Nakai et al. 1974; Schoell 1980) that describes methane production from CO₂. As discussed previously, the slope of unity in equation (5)
can be interpreted to mean that all four methane hydrogens are supplied by the environmental water, a condition satisfied only by CO₂ reduction (Schoell 1980; Daniels et al. 1980).

When methane is produced in deep-sea sediments in sufficient quantities to supersaturate the pore waters under appropriate pressure-temperature conditions, it may combine with water and other gases to form gas hydrates (Claypool and Kaplan 1974). Gas hydrates are a type of clathrate compound in which gas molecules are trapped within and stabilize a framework of hydrogen bonded water molecules. The ice-like gas hydrates form at temperatures 10 to 15°C above the freezing point of water when certain gases and water are combined at elevated pressures. With respect to pressure-temperature conditions, about 93% of the world's ocean sediments are suitable for methane hydrate formation (Trofimuk et al. 1973). Most currently known or suspected hydrate zones occur in continental slope regions and are correlated with bottom-simulating reflectors that result from changes in reflection properties at the gas hydrate-free gas boundary at the base of the gas hydrate zone (Shipley et al. 1979). The presence of gas hydrates in continental slope sediments along the east coast of the U.S., the western Gulf of Mexico and along the Pacific side of Central and South America was inferred from the presence of bottom-simulating reflectors in seismic profiles by Shipley et al. (1979). Water depths overlying the suspected hydrate zones range from 700 to 4,400 m with pressure-temperature conditions for hydrate stability satisfied between 100 and 1,100 m subbottom (Shipley et al. 1979).

In this section, measurements of δD-CH₄, δ¹³C-CH₄, and δD-H₂O from several Deep Sea Drilling Project (DSDP) Sites, including some from
which gas hydrates were recovered, are presented. The samples described here were provided by Dr. James M. Brooks of Texas A&M University and Dr. George Claypool of the United States Geological Survey, Denver.

Sample Collection

Samples of gas from expansion pockets, which developed within the cores upon retrieval, were transferred to vacutainers using a needle and septum device with a valve to minimize atmospheric contamination (Baltuck et al. 1985). Interstitial waters were obtained by squeezing (Manheim and Sayles 1974), and collected in glass ampoules (Site 618 only), or in plastic syringes that were sealed with silicone caulking to minimize interaction of the sample with the atmosphere.

Results and Discussion

DSDP Leg 84 - Middle America Trench Region

Leg 84 of the DSDP sampled landward slope sediments of the Middle America Trench off Costa Rica (Site 565) and Guatemala (Sites 566-570). Geophysical studies indicate that during the Miocene and Pliocene undeformed sediments covering the descending Cocos Plate were not scraped off during subduction but continued into the subduction zone making the Middle America Trench a tectonic type end-member (Auboin and von Huene 1985). Gas hydrates had been encountered in the continental slope sediments of this region during drilling on Legs 66 and 67 in 1979.

Isotopic analyses of methane and pore water samples collected during Leg 84 are presented in Table 4. Also listed in Table 4 are the locations where gas hydrates were visually observed in Leg 84 sediments by Kvenvolden and McDonald (1985). The paucity and uneven geographical
Table 4. Isotopic analyses of methane and water from DSDP Leg 84 (in °/oo). Location of hydrates and % org. C data from Kvenvolden and McDonald (1985); Site locations, water depths, and sed. rates from von Huene, Aubouin et al. (1985).

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<td>Site 568 (90°48.0'W, 13°04.3'N; water depth = 2031; sed. rate (0-200 m) = 117 m/m.y., (240-400 m) = 33-66 m/m.y.; % org. C (0-180 m) &gt; 2.6, (180-280 m) = 0.6-2.6, (280-400 m) = 0.6-1.3)</td>
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<td>1 (±1)</td>
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Table 4 (Cont'd).

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<th>Sample (core-section)</th>
<th>Depth (m)</th>
<th>δD-CH₄</th>
<th>δD-H₂O</th>
<th>δ¹³C-CH₄</th>
</tr>
</thead>
</table>

**Site 569** (90°50.4'W, 12°56.3'N; water depth = 2800 m; sed. rate (100 - 240 m) = 25 m/m.y.; % org. C (100-240 m) = 0.4-2.4)

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<td>----</td>
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<td>26-5</td>
<td>238</td>
<td>-181</td>
<td>----</td>
<td>-69.2</td>
</tr>
</tbody>
</table>

**Site 570** (91°23.6'W, 13°17.1'N; water depth = 1718 m; sed. rate (0-230 m) = 130 m/m.y., (220-260 m) = 13 m/m.y., (260-330 m) = 66 m/m.y.; % org. C (0-275 m) = 1.1-3.2, (275-350 m ) = 0.1-3.0)

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<td>-188</td>
<td>----</td>
<td>-71.6</td>
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<tr>
<td>11-1</td>
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<td>-190</td>
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<td>-68.8</td>
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<tr>
<td>15-2</td>
<td>136</td>
<td>-197 (±1.5)</td>
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<td>-53.0</td>
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<td>-42.4</td>
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<td>-41.6</td>
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<td>-188 (±0.4)</td>
<td>----</td>
<td>-41.9</td>
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<td>-40.9</td>
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<td>Hydrate</td>
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<td>----</td>
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<tr>
<td>37-1</td>
<td>346</td>
<td>-189</td>
<td>----</td>
<td>-41.6</td>
</tr>
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</table>
and depth distribution of samples available for analysis in this study make detailed interpretation difficult; however, it appears that the methane recovered from the Sites where gas hydrates were either not visually observed at all (567A, 569) or were only observed near the bottom (568) is generally slightly less D-depleted (mean $\delta^{13}$CH$_4$ = $-179.5^{\circ}$/oo, std. dev. = $7.6^{\circ}$/oo, n = 24) than the methane recovered from the Sites (565, 570) where hydrates were visually observed (Kvenvolden and McDonald 1985) at relatively shallower subbottom depths (mean $\delta^{13}$CH$_4$ = $-190.5^{\circ}$/oo, std. dev. = $4.8^{\circ}$/oo, n = 24;Table 4).

Claypool et al. (1985) found that significantly more deuterium enrichment in methane occurs at depth at Site 568, from which gas hydrates were recovered only in the deepest part of the section penetrated, than in the methane collected at comparable depths at Site 570 where gas hydrates are massively (3 to 4 meters thick) developed at intermediate (250 m) depths. Galimov and Kvenvolden (1983) suggested that, although the $\delta^{13}$C of CH$_4$ and CO$_2$ in gas hydrates should not be directly influenced as a result of their hydrated state, methane in zones of gas hydrate might be slightly $^{13}$C-enriched compared to methane from non-hydrated regions because escape of isotopically light methane might be inhibited due to encagement by the water-clathrate structure.

If this line of reasoning is extended, it might be expected that methane sampled from gas hydrate deposits could also be relatively more D-depleted. This is apparently observed (Table 4, Claypool et al. 1985). Confinement within the clathrate structure might also reduce the magnitude of possible isotopic fractionation induced by rapid depressurization during sampling. The methane sampled from Sites 567A, 568, and 569 shows no $^{13}$C-enrichment relative to that found at Sites
565 and 570, however, reducing the strength of any argument for light-isotope enrichment in gas hydrates that might be made from the δD data. Further, two separate laboratory studies in which naturally formed gas hydrates were sampled intermittently during controlled decomposition revealed no obvious carbon isotopic fractionation during hydrate decomposition (Brooks et al. 1983; Kvenvolden et al. 1984). Large differences in parameters such as sedimentation rate and percent organic carbon (Kvenvolden and McDonald 1985) among Leg 84 Sites are more likely causes of the slight differences in δD-CH₄ noted above.

The δ¹³C of methane (as heavy as -41.00/oo) sampled in the lower parts of Sites 568 and 570 (Claypool et al. 1985; Jeffrey et al. 1985; Table 4) implies that the methane was formed from thermal decomposition of complex organic matter (Bernard et al. 1977). Profiles of δ¹³C-CH₄ and δ¹³C-ΣCO₂ are generally parallel and δ¹³C-ΣCO₂ as heavy as +35.7° and +37.5°/oo were measured in Sites 568 and 570, however, implying that the anomalously ¹³C-enriched methane was formed from the reduction of very ¹³C-enriched CO₂ (Claypool et al. 1985). Further, the δ¹³C difference between the anomalously heavy CH₄ and CO₂ sampled at Sites 568 and 570 is essentially the same as found at other DSDP Sites where biogenic methane exhibits a more typical δ¹³C (Claypool et al. 1985).

**DSDP Leg 87 - Nankai Trough**

Site 583 of DSDP Leg 87 sampled mildly deformed sediments on the lowest structural terrace of the landward slope of the Nankai Trough, which is located off Honshu, Japan. In contrast to the Middle America Trench, most or all of the trench fill covering the descending plate is scraped off and accreted in response to the low rate of subduction (~2 cm/yr.) in the Nankai Trough (Karig, Kagami et al. 1983).
Isotopic analyses of methane and pore water from Site 583 are presented in Table 5. Both δD-CH₄ and δD-H₂O are constant with depth, within the analytical precision of the measurements. The δD-CH₄ is typical of values normally exhibited by biogenic methane formed from CO₂ reduction in deep-sea sediments (Schoell 1983) and the δD-H₂O is within the range found in other DSDP pore waters (Lawrence and Gieskes 1981). Claypool et al. (in press) presented depth profiles of δ¹³C-CH₄ and δ¹³C-ΣCO₂ at Site 583 that exhibited similar shapes with most of the variation in these parameters occurring in the upper 30 m of the sediments. They interpreted these results to mean that methane was formed via CO₂ reduction and that methanogenic activity probably ceased below depths of 30 to 50 m (Claypool et al. in press). The organic carbon content of these sediments is about 0.4 to 0.6% (Claypool et al. in press), approximately the amount thought necessary to support methane production (Rice and Claypool 1981). The apparently limited extent of methane production in these sediments combined with the great water depth (4618 m) make gas hydrate formation at Site 583 unlikely (Claypool et al. in press).

**DSDP Leg 96 - Orca Basin**

Orca Basin is a 400 km² depression on the Louisiana continental slope located in a region where salt diapirism and slumping have created a complex seafloor morphology (Lehner 1969; Shokes et al. 1977). Dissolution by seawater of a near-surface salt deposit on the basin flank apparently accounts for the 200 m thick layer of anoxic, hypersaline (250 g/kg) water that occupies the bottom of the basin (Shokes et al. 1977; Wiesenburg 1980). The brine is near saturation with respect to NaCl and is sulfate-enriched (47.2 mM) relative to deep
Table 5. Isotopic analyses of methane and water from DSDP Site 583 (in °/oo).

<table>
<thead>
<tr>
<th>Sample (Hole-core-section)</th>
<th>Depth (m)</th>
<th>δD-CH₄</th>
<th>δD-H₂O</th>
<th>δ¹³C-CH₄</th>
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<td>100</td>
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<td>-70.6</td>
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<td>D-9-2</td>
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<td>-70.6</td>
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mean

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<th>Depth (m)</th>
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<th>δD-H₂O</th>
<th>δ¹³C-CH₄</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>-188</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

std. dev.

| | 2.2 | 0.9 |
Gulf of Mexico seawater (Sheu et al. in press). The dense brine is highly stable and mixes very slowly with the overlying seawater. As a result, methane produced in the underlying sediments (Sackett et al. 1979) has accumulated in the brine to a concentration nearly six orders of magnitude greater than in the overlying deep Gulf seawater (Wiesenburg 1980).

From consideration of seismic reflection profiles, geotechnical and clay mineralogical properties, and high sedimentation rates (−1 m/1000 yr.), Tompkins and Shephard (1979) concluded that slumping of basin flank sediments, both from within and from outside the area covered by brine, is the primary mechanism of sediment accumulation in the central part of the basin, and that the amount and origin of slumped material varies spatially within the basin. Near-surface basin sediments originally deposited under anoxic conditions are enriched in total organic carbon over normal slope sediments by a factor of 2 to 3 and contain large pieces of undecomposed Sargassum (Sackett et al. 1979; Northam et al. 1981). These observations imply that organic matter in Orca Basin sediments is relatively well-preserved, as a result of reduced rates of biodegradation caused by the hypersalinity (Sackett et al. 1979) and/or the anoxic conditions (Northam et al. 1981).

Isotopic analyses of Site 618 interstitial methane and water are presented in Table 6. Application of the Woltemate et al. (1984) model (Figure 4, equations (4) and (5)) to these data (Table 6) implies that the acetate dissimilation pathway could account for 15% of the total methane production in Orca Basin sediments. Whelan et al. (in press) report a pore water acetate concentration of about 1 mM at 15 m depth.
Table 6. Isotopic analyses of methane and water from DSDP Site 618 (in °/oo).

<table>
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<th>Sample (Core-section)</th>
<th>Depth (m)</th>
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<th>δD-H₂O</th>
<th>δ¹³C-CH₄</th>
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<td>-72.7</td>
</tr>
<tr>
<td>11-1</td>
<td>90</td>
<td>-----</td>
<td>5</td>
<td>------</td>
</tr>
<tr>
<td>11-2</td>
<td>92</td>
<td>-181</td>
<td>---</td>
<td>-75.7</td>
</tr>
</tbody>
</table>

mean                   | -184      | 7      |
at Site 618. This concentration is about a factor of two higher than the Km (substrate concentration at which the initial reaction velocity is half maximal) for the acetate-decarboxylating methane-producing bacterium *Methanobacterium soehngenii* that was isolated from digested sewage sludge (Zehnder et al. 1980). Recently, isolation of a new acetotrophic marine methane-producing bacterium, *Methanosarcina acetivorans*, from methane evolving sediments was reported by Sowers et al. (1984). If an acetotrophic methanogen inhabits Orca Basin sediments, and if its Km is similar in magnitude to that of *M. soehngenii*, then some production of methane from acetate, at least at 15 m depth, may be possible.

The preceding argument assumes that a significant fraction of the acetate detected is "microbiologically available", a condition that may or may not be met. At 15 m subbottom depth at Site 618 the interstitial salinity was about 115°/oo (Bouma, Coleman et al. in press). *M. acetivorans* exhibits a maximum specific growth rate at about 0.2 M NaCl concentration (about 12°/oo salinity) that decreases rapidly with increase in NaCl concentration (Sowers et al. 1984). Thus, any acetotrophic methanogens living at 15 m depth may be inhibited by the high salinity. This would agree with the suggestion of Sackett et al. (1979) that the rate of overall organic matter destruction may be slowed by the hypersalinity. Although acetate dissimilation may account for a small percentage of the methane production in Orca Basin sediments, most of the methane probably results from CO₂ reduction.

The salinity (Bouma, Coleman et al. in press) and δD-H₂O (Table 6) profiles for Site 618 imply that the Orca Basin brine has a δD of about
4°/oo. This is heavier than present day Gulf Basin Water (δD = 0.7 to 1.1°/oo; Frank 1973) and implies that the Orca Basin brine was formed sometime between the last glacial maximum (18,000 yr. B.P.; McIntyre et al. 1976) and the end of the subsequent deglaciation period (about 14,000 to 5,000 yr. B.P.; Barry et al. 1979) when significant quantities of isotopically light water (Dansgaard and Tauber 1969) were tied up in continental ice sheets. It has been estimated that ocean deep waters were about 1.2 (Dansgaard and Tauber 1969) to 1.65°/oo (Shackleton 1977) 18O-enriched at the last glacial maximum relative to present day deep water. This would correspond to a deuterium enrichment of about 8 to 11°/oo, assuming the deuterium-18O relationship for North Atlantic Surface Water of Craig (1967) applies. If these estimates of the deep ocean isotopic fractionation and the brine δD (4 to 5°/oo) are accurate, then the age of the Orca Brine might be further constrained to the deglaciation period (14,000 to 5,000 yr. B.P.). In support of this, the estimate of Addy and Behrens (1980) of when the brine began to accumulate in the basin (about 8,000 yr. B.P.) falls within this range of time. There is, however, some uncertainty about how much sea level was actually lowered during the last glacial maximum. Blackwelder et al. (1979) estimated that substantially less ice was present from 17,000 to 10,000 yr. B.P. than would be expected from the estimates of minimum sea level employed by Dansgaard and Tauber (1969) and Shackleton (1977).

The interstitial methane δ13C values reported in Table 6 and by Pflaum et al. (in press) are similar to values reported by Sackett et al. (1979) for the δ13C of methane dissolved in the brine (−74.5 to −72°/oo), but quite different from interstitial methane δ13C (−105 to
-85°/oo) reported by Sackett et al. (1979). Very light (δ^{13}C < -90°/oo) methane has not been widely encountered in sediments sampled by DSDP (Claypool and Kvenvolden 1983). When highly ^{13}C-depleted methane is found, it usually occurs at the base of the sulfate-reduction zone, which is generally acknowledged to be the shallowest depth of rapid production and significant accumulation of methane in deep-sea sediments (Claypool and Kvenvolden 1983). In general, very ^{13}C-depleted methane is probably not of great quantitative significance in deep-sea sediments and may result only under certain geochemical and/or sedimentological conditions. Because the amount and origin of slumped sediments varies spatially within the basin (Tompkins and Shephard 1979), the sediments cored by Sackett et al. (1979) could be quite different from those cored at Site 618. Sackett et al. (1979) gave a water depth of 2340 m for their core location; this depth is 80 m more shallow than at Site 618 and may actually be on the basin flank. The probable sediment variability and all of the isotopic data imply that the very light sedimentary methane sampled by Sackett et al. (1979) is probably a localized, near-surface occurrence that is not isotopically representative of the methane component of either the Orca Basin brine or pore waters. Between the brine-sediment interface and about 30 m subbottom depth the interstitial salinity decreases from brine levels to values more characteristic of normal marine sediments (Bouma, Coleman et al. in press). Rapid methane production in sediment layers less influenced by high salinity effects and subsequent upward diffusion probably provides most of the methane dissolved in the brine.

Pflaum et al. (in press) observed small, spherical gas hydrates
between 20 and 40m depth at Site 618. The methane that was released upon decomposition of the hydrate had the same $\delta^{13}C$ as methane sampled from gas pockets in the same core section, indicating that no isotopic fractionation of methane was associated with formation of the hydrates (Pflaum et al. in press).

Deep-sea methane isotopic compositions (mean $\delta D$-$CH_4 = -185^0/oo$, std. dev. = $7^0/oo$, n = 75; $\delta^{13}C$-$CH_4 = -71.3^0/oo$, std. dev. = $6.3^0/oo$, n = 44) determined in this study (Tables 4-6) are, for the most part, within the narrow ranges ($\delta D = -190$ to $-170^0/oo$; $\delta^{13}C = -75$ to $-60^0/oo$) considered by Schoell (1983) to be characteristic of methane formed by $CO_2$ reduction in deep-sea sediments. The mean $\delta^{13}C$ was calculated excluding all data from Sites 568 and 570 because the anomalously heavy methane from those Sites constitutes a relatively much larger fraction of this data set than of the data set containing all known DSDP $\delta^{13}C$-$CH_4$ values. The pore waters measured in this study are somewhat more D-enriched ($\delta D = +3^0/oo$, std. dev. = $3^0/oo$, n = 26) than pore waters from 24 DSDP Sites ($\delta D = -1.8^0/oo$, std. dev. = $5^0/oo$, n = 165; Lawrence and Gieskes 1981; Friedman and Hardcastle 1973; Jenden and Gieskes 1983; Lawrence unpublished data) with a wide geographic distribution. If $CO_2$ reduction is by far the predominant source of methane in deep-sea sediments, as proposed by Claypool and Kaplan (1974), then the mean $\delta D$ values for methane ($-185^0/oo$) and pore water ($\delta D = -1^0/oo$, n = 191) discussed here indicate that equation (5) describing $CO_2$ reduction (Woltemate et al. 1984) should be modified to:

$$\delta D$-$CH_4 = \delta D$-$H_2O - 184(\pm15)^0/oo$$ (8).

Alternatively, deviation of deep-sea $\delta D$-$CH_4$ from equation (5) could be explained by minor production (about 10% of the total) of
methane from acetate as discussed previously for DSDP Site 618. Few
determinations of acetate concentration in deep-sea pore waters have
been made to date. Acetate concentrations that are available for
typical sulfate-depleted deep-sea sediments (DSDP Site 619 –
Mississippi Fan) range from 21 to 131 µM (Whelan et al. in press).
Significant (i.e., 10% of the total) methane production from acetate
concentrations as low as 100 µM would require that the rate constant
for acetate conversion to methane be much higher than the rate constant
for CO₂ reduction because deep-sea pore water ΣCO₂ concentrations are
typically two to three orders of magnitude (10 to 100 mM) higher
(Blaypool and Kvenvolden 1983; Claypool et al. 1985).

Baker et al. (1977) determined the δD of a few biogenic methane
samples from DSDP Leg 41 (eastern tropical Atlantic) and obtained
values that were almost identical to δD-CH₄ from DSDP Site 147 (Cariaco
Trench) measured by Lyon (1974). Based on this similarity, Baker et al.
(1977) suggested that δD-CH₄ in deep-sea pore waters is geographically
invariant and proposed that this could result if the deuterium
concentration of deep-sea pore waters is uniform and either the
isotopic fractionation associated with bacterial methane production is
fairly constant or if methane and the interstitial water undergo
postgenerative isotopic equilibration. Using available
temperature-dependent equilibrium isotope fractionation factors and
average δD-CH₄ (-178°/oo; Friedman and Hardcastle
1973), Lyon (1974) calculated an equilibrium temperature for Cariaco
Trench (Site 147) sediments of -23°C, which is about 40°C lower than
the estimated true temperature. Average δD-CH₄ (-185°/oo) and δD-H₂O
(+3°/oo) obtained in this study also indicate deep-sea sediment
temperatures of $<-20 \degree C$ if evaluated with the equilibrium fractionation factors that were compiled by Friedman and O'Neil (1977). Further, Nakai et al. (1974) found that observed differences in $\delta D$ values between methane and water from biogenic gas deposits in Japan were greater by about 50 to 80$^\circ$/oo than would be predicted for isotopic equilibrium. These results imply that the large differences in $\delta D$ between methane and water observed in these environments is due to a large kinetic isotope effect associated with bacterial methane formation as proposed by Nakai et al. (1974).

Reevaluation of the deep-sea $\delta D$ data with recently published equilibrium fractionation factors (Lyon and Hulston 1984), however, yields predicted temperatures that are generally within about 5$^\circ$C of estimated environmental temperatures (Table 7). Thus, low-temperature (0 to 20$^\circ$C) isotopic equilibration between methane and interstitial water during the great length of time ($10^5$ to $10^6$ years) represented by a deep-sea core may play an important role in determining the $\delta D$ of methane associated with deep-sea sediments. In contrast, equilibrium temperatures predicted with $\delta D$ data from freshwater and nearshore marine-estuarine sediments are substantially lower than measured temperatures (Table 7), implying that low-temperature isotopic equilibration does not occur on the short time scale represented by these young sediments.
Table 7. Comparison of isotopic equilibrium temperatures ($T_{eq.}$) as calculated with the data of Lyon and Hulston (1984) with estimated environmental temperatures ($T_{env.}$).

<table>
<thead>
<tr>
<th>Location</th>
<th>$\delta$CH$_4$ ($^\circ$/oo)</th>
<th>$\delta$H$_2$O ($^\circ$/oo)</th>
<th>$T_{eq.}$ ($^\circ$C)</th>
<th>$T_{env.}$ ($^\circ$C)</th>
<th>$\Delta(T_{eq.}-T_{env.})$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep-sea (DSDP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>147 (Cariaco Trench)</td>
<td>-178$^a$</td>
<td>+8$^b$</td>
<td>12</td>
<td>17</td>
<td>-5</td>
</tr>
<tr>
<td>565 (below 200 m)</td>
<td>-196</td>
<td>0</td>
<td>-1</td>
<td>5</td>
<td>-6</td>
</tr>
<tr>
<td>568 (140-180 m)</td>
<td>-190</td>
<td>-1</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>568 (400 m)</td>
<td>-174</td>
<td>+1</td>
<td>25</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>569 (100-150 m)</td>
<td>-185</td>
<td>+2</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>618</td>
<td>-184</td>
<td>+7</td>
<td>5</td>
<td>6</td>
<td>-1</td>
</tr>
<tr>
<td>Cape Lookout Bight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 June 1983 #5</td>
<td>-222</td>
<td>+3</td>
<td>-33</td>
<td>23</td>
<td>-56</td>
</tr>
<tr>
<td>19 August 1983 #3</td>
<td>-284</td>
<td>+3</td>
<td>-82</td>
<td>27</td>
<td>-109</td>
</tr>
<tr>
<td>2 February 1984 #2</td>
<td>-231</td>
<td>+3</td>
<td>-42</td>
<td>7</td>
<td>-49</td>
</tr>
<tr>
<td>Crescent Lake, FL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 August 1984 #2</td>
<td>-304</td>
<td>-7</td>
<td>-92</td>
<td>31</td>
<td>-123</td>
</tr>
<tr>
<td>23 January 1985 #2</td>
<td>-263</td>
<td>-10</td>
<td>-61</td>
<td>16</td>
<td>-77</td>
</tr>
</tbody>
</table>

$a$ - from Lyon (1974)  
$b$ - from Friedman and Hardcastle (1973)
SUMMARY

Methanogenesis, the final step in the anaerobic decomposition of complex organic matter in natural systems, generally occurs via one of two pathways, CO₂ reduction or acetate dissimilation. Most ¹⁴C radiotracer studies (i.e., Lovley and Klug 1982) performed to date have yielded results indicating acetate dissimilation is the dominant methanogenic pathway in freshwater sediments, accounting for 60 to 70% of the total methane production. In contrast, methane production in deep-sea sediments is generally thought to occur almost exclusively via CO₂ reduction (i.e., Claypool and Kaplan 1974). The data generated in this and other studies (Woltemate et al. 1984; Whiticar et al. in press) indicate that biogenic methane produced in young, freshwater sediments can generally be distinguished from the biogenic methane produced in deep-sea sediments by measurement of δD-CH₄, implying that δD-CH₄ may be of use in the genetic characterization of biogenic methanes (Schoell 1983), and in estimating the relative importance of the two methanogenic pathways in nature (Woltemate et al. 1984).

The isotopic compositions of methane samples collected at eight different DSDP Sites (mean δD = -185°/oo, std. dev. = 7°/oo, n = 75; mean δ¹³C = -71.3°/oo, std. dev. = 6.3°/oo, n = 44) are generally typical of methane formed via CO₂ reduction in deep-sea sediments. The absence of wide variation in deep-sea δD-CH₄ could be the result of either a relatively constant kinetic hydrogen isotope effect associated
with CO$_2$ reduction or of postgenerative isotopic equilibration of methane and pore water during the long subsurface burial of these sediments. Resolution of this question through laboratory studies may be quite difficult, as this approach would require extrapolation of results obtained under conditions that are vastly different from in situ deep-sea conditions because of the great length of time and, presumably, the slow rate of methane production under natural deep-sea conditions. The isotopic compositions of methane samples collected from gas hydrate zones at several DSDP Sites indicated no obvious isotope effects associated with gas hydrate formation. Production of minor amounts (about 10% of the total) of methane via acetate dissimilation in deep-sea sediments may explain the deviation of the $\delta D\text{-H}_2\text{O}/\delta D\text{-CH}_4$ pairs from the previously published relationship for CO$_2$ reduction.

The methane isotopic compositions of gas bubbles collected from the sediments of several freshwater environments (mean $\delta D\text{-CH}_4 = -300^\circ/oo$, std. dev. = $26^\circ/oo$, $n = 20$; mean $\delta^{13}\text{C-CH}_4 = -60.1^\circ/oo$, std. dev. = $6.1^\circ/oo$, $n = 20$) imply that the methane produced in these freshwater sediments is generally more D-depleted and less $^{13}\text{C}$-depleted than methane produced in deep-sea sediments. Evaluation of the $\delta D\text{-H}_2\text{O}/\delta D\text{-CH}_4$ with the Woltemate et al. (1984) model implies that acetate dissimilation is responsible for about 50 to 80% of the methane produced in these freshwater sediments. Methane $\delta^{13}\text{C}$ and $\delta D$ are generally inversely correlated, indicating that the large isotopic variability observed in these freshwater sediments is not solely attributable to bacterial methane oxidation, but also to some other parameter such as variability in methanogenic pathway selection.
Nearshore marine-estuarine methanes are isotopically intermediate (mean $\delta^{13}$C-$\text{CH}_4 = -61.8^0\text{/oo}$, std. dev. = 3.1$^0\text{/oo}$, $n = 46$; mean $\delta^{13}$C-$\text{CH}_4 = -61.8^0\text{/oo}$, std. dev. = 3.1$^0\text{/oo}$, $n = 46$) between deep-sea and freshwater methanes, implying that acetate dissimilation plays an intermediate role in nearshore marine-estuarine methane production. Isotopic analyses of methane samples collected over the course of a year from the rapidly accumulating, organic-rich marine sediments of Cape Lookout Bight, North Carolina indicate that methane isotopic composition and probably, methanogenic pathway selection are seasonally, and in all likelihood spatially variable. Methane is generally D-depleted and $^{13}$C-enriched during warm summer months (mean $\delta^{D} = -269^0\text{/oo}$, std. dev. = $9^0\text{/oo}$, $n = 21$; mean $\delta^{13}$C = -59.5$^0\text{/oo}$, std. dev. = 4.4$^0\text{/oo}$, $n = 21$) as compared to winter months (mean $\delta^{D} = -236^0\text{/oo}$, std. dev. = $4^0\text{/oo}$, $n = 6$; mean $\delta^{13}$C-$\text{CH}_4 = -63.9^0\text{/oo}$, std. dev. = $0.5^0\text{/oo}$, $n = 6$). Evaluation of these data with the Woltemate et al. (1984) model implies that acetate dissimilation accounts for a relatively greater proportion of total methane production during the summer (about 45 to 60%) than during the winter (about 25 to 40%). The reasonably good agreement of the summer estimates obtained in this manner with those obtained from $^{14}$C radiotracer studies (Sansone and Martens 1981; Crill and Martens in press) indicates that application of the Woltemate et al. (1984) model to $\delta^{D}$-$\text{CH}_4$ data can yield useful estimates of methanogenic pathway selection in aquatic sediments. As for the freshwater sediments described above, the $\delta^{13}$C and $\delta^{D}$ of methane produced in Cape Lookout Bight sediments are inversely correlated, implying that both $\delta^{D}$-$\text{CH}_4$ and $\delta^{13}$C-$\text{CH}_4$ are influenced by methanogenic pathway and that bacterial methane oxidation plays a relatively minor role in producing the
methane isotopic variability observed in these sediments.

The nature and extent of post-depositional bacteria-organic matter interactions occurring prior to rapid methane production probably play a role in determining methanogenic pathway selection and methane isotopic composition in aquatic sediments. Factors that likely influence these interactions are amount and/or type of organic matter deposited, sedimentation rate, concentrations of external electron acceptors and temperature. Land-derived higher plant material with its relatively lower H/C and N/C ratios (Stuermer et al. 1978) and relatively higher concentrations of biologically resistant lignins, resins and waxes (Aizenshtat et al. 1973) is probably not as readily degraded as algae-derived organic matter. Several investigators (Jorgensen 1978; Berner 1980; Westrich and Berner 1984) suggested that decomposable organic matter should be divided into several fractions (two to eight) based on relative degradability. Model profiles of the decomposable organic matter indicate that the various fractions successively disappear based on their relative degradabilities with depth in the sediment (time), with the result that the remaining fraction becomes progressively more refractory (Berner 1980). A high rate of sediment deposition would favor preservation of the more easily degraded organic matter fractions because both aerobic remineralization occurring at or near the sediment-water interface and the diffusion of other external electron acceptors into the sediments would be minimized by rapid isolation of organic matter from the overlying water (Berner 1980).

In marine systems, where dissolved sulfate is by far the most abundant (28 mM) external electron acceptor, sulfate reduction may
account for a significant part of the total organic matter degradation that occurs prior to rapid methane production. Jorgensen (1982) reported that bacterial sulfate reduction accounted for as much organic matter degradation as did all aerobic processes in Danish coastal sediments. In general, the ratio of [dissolved sulfate]/[organic matter] should decrease in the order deep-sea > nearshore marine-estuarine > freshwater sediments. Because the consumption of the most labile organic matter fractions by aerobic processes and sulfate reduction should be maximized in sediments with low sedimentation rates and high [dissolved sulfate]/[organic matter] ratios, the methane producing zone of deep-sea sediments would be expected to receive a relatively smaller proportion of the more easily degradable organic matter fractions than the methane zone of freshwater sediments. Rapidly accumulating, nearshore marine-estuarine sediments which are deposited in waters containing appreciable concentrations of dissolved sulfate and receive organic matter of mixed origin, might be expected to fall between deep-sea and freshwater sediments with respect to the relative lability of organic matter reaching the methane zone. In general, nearshore marine-estuarine $\delta^{13}C_{CH_4}$ measured in this study (mean $\delta D = -258^\circ/oo$) are intermediate between the freshwater (mean $\delta D = -300^\circ/oo$) and deep-sea (mean $\delta D = -185^\circ/oo$) methanes implying that the lability of the organic matter reaching the methane zone may affect methanogenic pathway selection and $\delta D_{CH_4}$. There is, however, significant overlap between freshwater and nearshore marine-estuarine $\delta D_{CH_4}$; this probably reflects the great variability in important environmental parameters (i.e., sedimentation rate, organic matter input) that is inherent in these types of aquatic systems.
The results discussed above imply that the δD of methane produced in deep-sea sediments is sufficiently different from the δD of methane produced in shallow aquatic sediments to allow the use of δD-CH₄ in the genetic characterization of commercially significant biogenic methane deposits. Rice and Claypool (1981) argue that shallow aquatic sediments are not normally suitable for the accumulation of biogenic gas because rapid production of methane usually begins near the sediment-water interface and hydrostatic pressures are low. As a result, most of the methane is lost from shallow sediments, either by bacterial consumption or by ebullition and escape to the atmosphere (Rice and Claypool 1981). Deep-sea sediments should be more appropriate for biogenic gas accumulation because rapid methane production usually begins deeper in the sediment and higher hydrostatic pressures allow retention of larger quantities of gas in solution in pore waters until the sediments are compacted and traps and seals are formed (Rice and Claypool 1981). Only a few δD-H₂O/δD-CH₄ pairs from a small proportion of the known commercial accumulations of biogenic gas are available in the literature. Surprisingly, most of the waters measured are substantially more D-depleted than ocean pore waters (Nakai et al. 1974; Schoell 1980); this indicates that the methane may have originated in deep freshwater sediments. The differences in δD values between methane and water from these commercial biogenic gas deposits is about the same as is found in deep-sea sediments; this was interpreted by Schoell (1980) to mean that the methane in these gas deposits was formed by CO₂ reduction. If, on the other hand, the observed differences in δD values between methane and water represent significant post-burial isotopic equilibration in these ancient
deposits, then measurement of $\delta^{18}\text{H}_2\text{O}/\delta^{13}\text{CH}_4$ pairs would probably be of little value in the genetic characterization of commercial biogenic methane deposits.

The microbial decomposition of organic matter in water-covered soils is one of the major sources of methane to the atmosphere (Khalil and Rasmussen 1983; Senum and Gaffney 1985), and bubble ebullition is the dominant mode of methane input to the atmosphere from some shallow freshwater (Robertson 1979; Cicerone and Shetter 1981) and marine (Martens and Klump 1980) environments. The results of this and other studies, indicate that methane bubbles formed in shallow aquatic sediments are generally significantly more $^{13}\text{C}$-depleted than the calculated average (Senum and Gaffney 1985) of all methane sources to the atmosphere. Some of the freshwater sediments studied here and by Woltemate et al. (1984) produce methane bubbles that have a $\delta^{18}$D near the estimated source average (Senum and Gaffney 1985); however, there are presently too few measurements of the $\delta^{18}$D of atmospheric methane and its sources available to allow evaluation of the role of shallow aquatic sediments in determining the $\delta^{18}$D of atmospheric methane. Future measurements of: (I) the fluxes (bubble and diffusive) and isotopic compositions ($\delta^{18}\text{D}$ and $\delta^{13}\text{C}$) of methane emitted from various aquatic sediments and other major sources (i.e., ruminants), (II) the isotopic composition of atmospheric methane and, (III) various ancillary parameters (i.e., temperature, salinity, organic matter content) that may affect methane production rate and/or isotopic composition are necessary to allow accurate assessment of the importance of shallow aquatic environments to the global atmospheric methane budget.
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