STABLE ISOTOPIC STUDIES OF BACTERIOGENIC METHANE EMISSIONS

BY

SUSAN WALDRON

Department of Geology and Applied Geology,
University of Glasgow,
May 1994.

Thesis submitted for the degree of Doctor of Philosophy.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

DECLARATION

The material presented in this thesis is the result of research carried out between September 1990 and April 1994 in the Department of Geology and Applied Geology, University of Glasgow, under the supervision of Dr. Allan Hall.

This thesis is based on my own independent research and any published or unpublished material used by me has been given full acknowledgement in the text.

Susan Waldron
May 1994

Co-authors declaration:

The paper presented in this research is the product of independent research by Susan Waldron. We have assisted with advice and help of a general, technical, conceptual nature, as would be during the course of normal Ph.D. supervision. Susan Waldron has written the thesis and paper within herself, and is responsible for its content.

Dr. A.J. Hall

Dr. A.E. Fallick

Dr. R. Gilmour

Mrs. M. MacDonald

ACKNOWLEDGEMENTS:

Where do I begin? Without funding from Greenpeace Environmental Trust it is unlikely that this research would have taken place, but without supervision from Allan I am certain that this research would not have taken place. I couldn't have asked for a better supervisor. I would like to thank Tony for introducing me to the wonders of stable isotope analysis and for encouraging my interest by allowing me to carry out my research at East Kilbride. Although registered at Glasgow, my desk was empty for many a month while my seat at the SURRC was always warm!

At the SURRC I would like to thank my many friends in Block C who helped me while away the hours both analytically and socially. There is truly not one person who can be singled out as being more important than others, so here goes. Thank-you to Adrian (for the numerous lifts and veterinary advice); Alison (for eating Gawen's cake); Andrew (for expert technical assistance and interesting tales about Garry); Chris (for picking up my sandwiches from Safeway and GC analysis); Elspbeth (for first teaching me how to carry out hydrogen isotope analysis and for her efforts with the 602); Fred (for helping put up the greenhouse and outstanding glass blowing, with never a grumble!); Garry (for standing on the centipede I don't think - elephants never forget!); Gawen (for acting as chauffeur for three years and helping with the greenhouse, oh and I nearly forgot putting mercury into the mass spectrometer capillary just to make me feel good), Joss (for interesting conversations and unusual reading matter) Julie G. (DIY expert); Julie (another Safeway lifesaver); Kirsten (for her melodic strains around the lab), Margaret (for her efforts with the 602) and Terry (for keeping me company on Sundays, for never saying no when I needed help even though he is obviously up to his eyes with more pressing matters, for helping with the 602, for listening to my moans, for...). Aside I would like to thank Karen (for loading up samples for me) and David and Jim who kept me company in the lab. I really miss hearing about Jim's love life and David's culinary expertise!

I am also grateful to Prof. Clymo and Dr. Pearce for introducing me to Ellergower Moss and, in conjunction with Dr. Claricoates, for allowing me to use their gas sampler design. Thanks to those within the TIGER group who provided me with information from their research on Ellergower Moss: Dr. Pearce for partial pressure and flux data and Ken Hargreaves for climatic data. Scottish Natural Heritage and the Forestry Commission are thanked for allowing me to site the greenhouse on Ellergower Moss. I am indebted to Allan, Fred, Gawen, Ian, Jimmy, Robert and Roger (Mexican hexagons indeed) who spared their time to construct/dismantle a greenhouse on a peat bog during one sunny Scottish summer!

A special thank-you to Irene, who took a chance on me when I wrote to her out of the blue and allowed me to carry out research in her lab, using her facilities and guided by her staff, Terry and Sandra. I really enjoyed this project.

Of course I couldn't miss out the team at Strathclyde University Water and

Environmental Management Unit - Michelle, Bob and Les. A trip to a landfill site without Michelle would have been a boring experience, who else could drag me out of 3ft. of mud when my wellies got stuck or miraculously just miss dropping the car keys down a gas vent! I just wish I had gone on your boat trip.

Writing up in the dept. wouldn't have been so pleasurable without the company of fellow post-graduates and post-doctoral researchers, particularly Farmer Órla for her expert advice on sheep and cattle breeding habits, so thanks to you too. Murdoch, Dougie, Kenny and Roddy are thanked for technical help.

Without financial and emotional support from my parents, Gran and Ian, it is unlikely that I would have completed this project. Ian was also pretty good at turning his hand to isotope modelling equations, driving out to East Kilbride at obscene hours to pick me up from the lab and helping me out in the field, even if the flies got too much and he had to hide in the greenhouse.

TABLE OF CONTENTS

TABLES:

FIGURES:

SYMBOLS AND UNITS

δ	the isotopic composition of a sample
$\delta^{13}C(CH_4)$	the carbon isotopic composition of methane.
$\delta^{13}C(CH_4)_{TF}$	the mass balanced carbon isotopic composition of methane fluxing to the atmosphere.
$\delta^{13}C(CH_4)_A$	the mass balanced carbon isotopic composition of methane in the atmosphere.
$\delta^{13}C(CO_2)$	the carbon isotopic composition of carbon dioxide.
$\delta D(CH_4)$	the hydrogen isotopic composition of methane.
$\delta D(CH_4)_{TF}$	the mass balanced hydrogen isotopic composition of methane fluxing to the atmosphere.
$\delta D(CH_4)_A$	the mass balanced hydrogen isotopic composition of methane in the atmosphere.
$\delta D(H_2O)$	the hydrogen isotope composition of water. In Chapter 4 this refers to the hydrogen isotope composition of the supernatant liquid with the bacterial cultures.
δD(porewater)	the hydrogen isotope composition of the porewater collected from the peat at Ellergower Moss.
δD(organic)	the hydrogen isotope composition of the organic matter.
δ^{13}C(organic)	the carbon isotope composition of the organic matter.
α	the isotopic fractionation factor.
Δ_{x-y}	the difference in δ values between two phases X and Y.
‰	permil - parts per thousand.
%	percent - parts per hundred.
°C	temperature in degrees centigrade.
M	molarity of a solution in mole dm^{-3} ($1000cm^3$).
m^2	measurement of area.
yr^{-1}	per year

ABSTRACT

Methane is produced thermogenically from organic matter, associated with oil and coal production, and bacteriogenically by two main biochemical pathways, CO_2 reduction and acetoclastic methanogenesis (commonly known as fermentation). CH_4 emissions can be an environmental problem on a local scale, due to the explosive potential of CH_4, and on a global scale as it is an effective radiatively forcing greenhouse gas, with continued emissions to the atmosphere contributing to potential global warming. Stable isotopic characterisation of sources can be used, when monitoring the composition of atmospheric CH_4 and its secular trend, to provide constraints on the relative magnitude of fluxes (Stevens, 1988). The overall flux isotopic composition should correspond to the isotopic composition of the gas in the atmosphere, once fractionation associated with atmospheric loss processes has been accounted for (Stevens and Engelkemeir, 1988). At present, $\delta^{13}C$ characterisation of CH_4 sources is well established. By comparison, δD characterisation of global sources of CH_4 is poorly established and the atmospheric chemistry complex. This research utilised stable isotope analyses of bacteriogenic CH_4 to constrain the isotopic signature of **both** carbon and hydrogen, and, where possible, to understand the factors controlling that signature.

Bacteriogenic methane releases are an ubiquitous feature of recent anoxic environments and are well documented both in freshwater environments, such as lakes and swamps, and in marine environments e.g. estuaries and shelf regions (Whiticar et al., 1986). One modern analogy to a 'freshwater' CH_4 producing anaerobic environment may be that of the landfill site.

$\delta^{13}C(CH_4)$ of gas samples from several Scottish landfill sites ranged evenly from -44 to -66‰, mean of -56.5±6‰. $\delta D(CH_4)$ varies from -258 to -352‰, but 80% of the values lie in the narrow range from -294 to -318‰, mean of 302±8‰. Landfill waste is unlikely to be isotopically homogenous for both C and H, and larger hydrogen isotope fractionations are observed in nature; thus at least as large a range in $\delta D(CH_4)$ would have been expected as observed in $\delta^{13}C(CH_4)$. This suggests that $\delta D(CH_4)$ is influenced by a large, isotopically homogenous hydrogen reservoir, most likely to be the water.

A large uncertainty still exists regarding the controls on $\delta D(CH_4)$ produced by acetoclastic methanogenesis (Jenden and Kaplan, 1986, Schoell et al., 1988). Four laboratory based, methane producing, anaerobic enrichment systems were monitored over a period of three months to investigate these controls. Systems 1 and 2, selected for butyrate degrading bacteria and systems 3 and 4 for hexanoate degrading bacteria. The $\delta D(H_2O)$ of systems 1 and 3 differed from that of systems 2 and 4 by 118‰. $\delta^{13}C(CH_4)$ of system 1 ranged from -63 to -48‰, $\delta^{13}C(CO_2)$ ranged from -8.6 to 2.1‰, and $\delta D(CH_4)$ remained constant with a mean of -354±3‰. $\delta^{13}C(CH_4)$ of system 2 ranged from -61 to -47‰, $\delta^{13}C(CO_2)$ ranged from -8.0 to 0.6‰, and $\delta D(CH_4)$ remained constant with a mean of -296±2‰. Furthermore, the difference in $\delta D(CH_4)$ between systems 1 and 2 was 56±4‰. Systems 3 and 4 failed after an taking initial sample. However $\delta D(CH_4)$ showed the same difference in magnitude as between systems 1 and 2. $\delta D(CH_4)$ is strongly influenced by an isotopically homogenous source i.e. the

water, the difference between systems reflecting the isotopically distinct water used. It is evident that more H_2O than has previously been considered is incorporated into the resultant CH_4 molecule during the β-oxidation of fatty acids to acetate. As fatty acid chain length increases, the influence of δD(organic) on $\delta D(CH_4)$ lessens, while the influence of $\delta D(H_2O)$ increases. The trend shown by $\delta^{13}C(CH_4)$ is attributed to changing dominance of methanogenic pathway with time. The initial CH_4 produced is by the reduction of CO_2, but as acetate levels increase, acetoclastic methanogenesis becomes dominant.

Almost 3% of the Earth's land surface is covered by peat and yet this is one environment for which there are few paired $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measurements. The stable isotope ratios, $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ of CH_4 and CO_2 from Ellergower Moss, a raised peat bog in S.W. Scotland were measured from gas samples collected in situ, from two profiles, at 1m intervals, from the surface to a depth of 5m. One of the profiles was sampled underneath a greenhouse, which was erected to consider the impact that global warming might exert on $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$‰ and temperature profiles within the peat. $\delta^{13}C(CH_4)$ had a mean of -75.6±3‰ (n=22); $\delta^{13}C(CO_2)$ has a mean of 3.8±5.3‰ (n=22); $\delta D(CH_4)$ has a mean of -294±39‰ (n=21). In each profile sampled $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ show a general gradation from isotopically heavy at the 5m depth to isotopically light at the surface. A boundary in $\delta D(CH_4)$ is present between 2-3m as the measured value at 3-5m is substantially heavier by 52±18‰, in comparison to 0-2m. Such a difference is also observed with $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ measurements. $\delta^{13}C$ and δD of the peat and of the pore water were also measured to examine the influence of δD(porewater) on $\delta D(CH_4)$. However such a boundary is not observed in δD(porewater). $\delta^{13}C$(peat) has a mean isotopic composition of -27.0±5‰. δD(peat) within the profile and at each measured depth is isotopically heterogeneous with a mean of -92.6±11.4‰, generally becoming isotopically heavier with decreasing depth. δD(porewater) has a mean of -37.7±3.1‰ and showed a gradual gradation with depth becoming isotopically heavier by 8‰ in the greenhouse profile and 6‰ in the control profile. In general δD(porewater) in the control profile is isotopically lighter than the greenhouse profile by 4±2.2‰. The effect that simulating global warming by placing a greenhouse with a $7.06m^2$ base on the peat from May - Oct. 1992 was monitored by continuous measurements of temperature at depth. The greenhouse profile shows a significant increase in temperature down to a depth of 2m with warming by 2°C, which has implications for increased CH_4 production and hence global warming. It may be possible that the increase in ambient peat temperature caused by the erection of the greenhouse may be responsible for the isotopically heavier greenhouse profile samples collected from between 0-2m.

Finally, the isotopic signatures from the above CH_4 sources are used, in conjunction with published isotopic signatures to demonstrate the application of stable isotope analysis in monitoring atmospheric CH_4 and understanding flux budgets. Independently calculated budgets for 1992 were up to 1.6 times higher than the Watt Committee CH_4 budget for 1992.

CHAPTER 1: INTRODUCTION

1.1. Why is methane of interest?

Methane can be an environmental problem on a local scale, due to the explosive potential of CH_4, and on a global scale as it is an effective radiatively forcing greenhouse gas, with continued emissions to the atmosphere contributing to potential global warming. Methane concentrations have increased by 190ppbv (or 11.9%) during the past 13 years. The rate of increase in the first two years was about 20±4 ppbv/yr but in the last two years was 10±2ppbv/yr, suggesting a decline in the concentration increase at northern middle and high latitudes of 1ppbv/yr (Khalil et al., 1993). The reason for this decline is not yet established, although several suggestions, including a reduction in fossil fuel emissions from the former U.S.S.R. or an increase in atmospheric hydroxyl radical concentration, have been offered. Stable isotope measurements of atmospheric CH_4 ($\delta^{13}C(CH_4)$ and $\delta D(CH_4)$) are considered one of the most effective tools for determining the reason for the observed decrease in atmospheric CH_4 (Dlugokencky et al., 1994).

Stable isotopic characterisation of sources can be used, when monitoring the composition of atmospheric CH_4 and its secular trend, to provide constraints on the relative magnitude of fluxes (Stevens, 1988) as the overall flux isotopic composition corresponds to the isotopic composition of the gas in the atmosphere, once fractionation associated with atmospheric loss processes has been accounted for (Stevens and Engelkemeir, 1988). This in turn can provide further information as to the significance of anthropogenic input to a potential greenhouse effect (an important political question), and may provide answers to why the rate at which CH_4 concentrations have been increasing in the atmosphere, has slowed down.

Bacteriogenic methane releases are an ubiquitous feature of recent anoxic environments and are well documented both in freshwater environments, such as lakes and swamps, and in marine environments e.g. estuaries and shelf regions (Whiticar et al., 1986). One natural methanogenic freshwater environment of significance is that of peatlands. These are unbalanced systems in which the rate of production of organic matter by living organisms exceeds the rate at which these compounds are decomposed (Moore & Bellamy, 1974). Permanent water-logging of the majority of the peat body results in anaerobic conditions, and with a high organic content, peatlands provide a suitable environment for bacteriogenic CH_4 production. Almost 3% (Clymo, 1987) of the Earth's land surface is covered by peat and yet this is one environment for which there are few paired $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measurements.

CH_4 production rates from wetland environments have been shown to increase with increased temperature, which could result in a potential positive feedback for global warming (Hameed and Cess, 1983). However, although peatlands are an important source of CH_4, not all CH_4 produced is actually emitted to the atmosphere due to oxidation within the

peat by methylotrophs (Lidstrom and Somers, 1984), postulated to be a major CH_4 sink in peatland environments. This takes place aerobically and possibly anaerobically, although an obligate anaerobic organism living on CH_4 has yet to be isolated (Yavitt et al., 1990).

An anthropogenic analogy to natural freshwater environments in which bacteriogenic methane production prevails may be landfill sites, increasing in size if not in number and thus worthy of consideration as a substantial source of atmospheric CH_4 flux.

The prestigious Dahlem workshop recommended that future environmental research programmes should address several questions including the response of northern ecosystems to expected climatic changes that may be induced by global warming (Schimel et al., 1989). The effectiveness of stable isotope measurements over time as an aid in understanding the biogeochemical cycle of CH_4 was reiterated and it was suggested that such measurements should be included in as many field studies as possible. Establishment of manipulated experiments were recommended in a) greenhouses and b) experimental fields, including sites with the water table lowered by drainage and flooded sites in areas with long meteorological / botanical records.

In this research project stable isotopic fingerprinting has been applied to both field and laboratory environments to characterise more accurately the isotopic composition of bacteriogenic CH_4 produced therein and to understand local environmental controls on the measured signature.

1.2. Background to this research project and research objectives.

This research was funded by Greenpeace Environmental Trust (GET), who could only commit research funding on a yearly basis rather than as one three year grant. As a result each year's project had to be concise enough to be suitable for a one year research project, but broad enough to relate to the future or previous year's research. Thus specific objectives for all three years' research were not clearly defined at the beginning of this project, although common to each was to develop an understanding of, and where possible quantify, the factors most strongly controlling the isotopic signature of methane.

Each research objective evolved with time based on what was deemed to be scientifically worthy of investigation. Such funding style meant care had to be taken to avoid discontinuity within the project. However it also allowed complete freedom (approved of course by GET) to research the areas of interest. Despite this style of funding, several clearly defined objectives evolved, all of which contribute towards understanding of the controls on isotopic composition of bacteriogenic CH_4 and are thus important in constructing properly balanced flux budgets from the changing trend of atmospheric CH_4:

- To develop a cryogenic vacuum system which could be used to measure $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$ from a CH_4 rich air sample (Chapter 2).
- To compile an isotopic database of bacteriogenic CH_4 samples from different sources and use this information to aid identification of an unknown source of CH_4 (Chapter 3 and Appendix 4).

• To investigate the terrestrial biological, geological and chemical constraints that may have a significant influence on the isotopic composition of CH_4 fluxing to the atmosphere (Chapters 4 and 5).

• To confirm that $\delta D(H_2O)$ has a much greater influence on $\delta D(CH_4)$ produced by acetoclastic methanogenesis than previously considered in the published literature and model this influence where possible (Chapter 4).

• To undertake a pilot experiment fingerprinting the isotopic signature of CH_4 and CO_2 produced within a peat bog and to consider the influence that global warming may have on this signature and the methanogenic environment. And to study the isotopic composition and controls on CH_4 produced within a peat bog and thus further constrain the isotopic signature of CH_4 that may be released catastrophically to the atmosphere (Chapter 5).

1.3. Principles of stable isotope analysis.

Hydrogen and carbon, elements intimately associated with the biosphere, hydrosphere and lithosphere, are amongst those especially susceptible to natural isotope fractionation i.e. the selective partitioning of one isotope into a compound. Approximately 98.89% of all carbon in nature is ^{12}C (carbon of mass number 12), and 1.11% of all carbon is ^{13}C. Hydrogen has two stable isotopes whose abundances are ^{1}H= 99.985% and ^{2}H = 0.015%. The ratio of these two isotopes for each element in natural materials varies slightly around these average values as a result of isotopic fractionation during physical, chemical and biological processes. However differences between materials in the range of several parts per thousand can be significant.

Isotope fractionation is a consequence of the fact that certain thermodynamic properties of molecules depend on the masses of the atoms of which they are composed. Bonds formed by the lighter of two isotopes are weaker and therefore more easily broken, making the molecule with the lighter isotope more reactive than a similar molecule containing the heavier isotope. In methane formation, bacterially controlled fractionation processes such as bacteriogenic production from landfill waste or from fermentation in an animal's rumen, result in methane richer in light isotopes than methane formed from an organic substrate as a more direct result of elevated temperatures and pressures, such as occurs in the formation of fossil fuels. Different sources therefore have different isotopic signatures so measurements of the ratios of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ in the methane molecule allow the characterisation of sources.

1.4. Notation and terminology:

1.4.1. The delta notation:

The quantity measured by a stable isotope mass spectrometer is not the absolute isotope ratio of the sample, but the relative difference between the isotope ratios of samples and standard gases. For most geochemical purposes, knowledge of only the difference in

absolute isotopic ratios between two substances is sufficient. In addition, differences can be measured more precisely than absolute ratios (O'Neil, 1986). As a result, a differential notation known as the δ (delta) notation (McKinney et al., 1950) has been adopted for expressing relative differences isotopic ratios between samples and standards and is the quantity actually measured on isotope ratio mass spectrometers. If the absolute ratios of the standards employed are known, the absolute ratio of any sample is readily calculated from its δ value. The δ value is defined as follows:

$$\delta_X = \left[\frac{R_X - R_{STANDARD}}{R_{STANDARD}}\right] \times 10^3‰ \qquad 1.1.$$

where, R is an atomic ratio and by convention is always written as the ratio of the heavy to light isotope. $R_{STANDARD}$ is the corresponding ratio in a standard and in the case of methane $R_X = (^2H/^1H)_X$ or $(^{13}C/^{12}C)_X$. The reference standard for carbon is the Pee Dee Belemnite (PDB) (Craig, 1957), the standard for hydrogen is Standard Mean Oceanic Water (SMOW). The δ value, then, is the difference in isotopic ratio between a sample and a standard, expressed in parts per thousand, or per mil (‰). Negative values indicate that the sample is depleted by that amount relative to the standard, while positive values indicate the standard is enriched in that amount relative to the standard. For example, a sample with a δ value of -10‰ has an isotopic ratio which is 10 parts per thousand (or one percent) lower than the appropriate standard; a sample with a δ value of +1.5‰ has an isotopic ratio 1.5 parts per thousand higher than the appropriate standard.

Further information about the theory behind stable isotope analysis can be gained from O' Neil in Valley et al., 1986.

1.4.2. Carbon isotope standard:

By international convention, $\delta^{13}C$ measurements are always expressed relative to a calcium carbonate standard known as PDB. This standard was a limestone fossil of *Belemnitella americana* from the Cretaceous Pee Dee formation in South Carolina. As the basis of the PDB scale it has been assigned a $\delta^{13}C$ value of 0‰. The PDB standard is no longer available. Its absolute $^{13}C/^{12}C$ ratio (R) has been reported to be 0.0112372 (Craig, 1957).

1.4.3. Hydrogen isotope standard:

The first hydrogen isotope standard was originally a hypothetical water sample with hydrogen and oxygen isotope ratios that were similar to those of an average sample of oceanic water. The original SMOW (standard mean oceanic water) standard was defined by Craig (1961) in terms of NBS-1 (Potomac River Water) as :

$(D/H)_{SMOW} = 1.050\ (D/H)_{NBS\text{-}1}$

Subsequent to this definition, a large quantity of water now called V-SMOW was prepared by R. Weiss and H. Craig for the IAEA as one of the four standard reference waters available to isotope geochemists. By international convention, δD measurements are

always expressed relative to V-SMOW.

1.4.4. The α notation:

The isotope fractionation that occurs during a process is defined by the fractionation factor (α) where:

$$\alpha_{A-B} = \frac{R_A}{R_B} \qquad \text{(O'Neil, 1986) 1.2.}$$

where R_A and R_B are the absolute isotopic ratios in phase A and phase B respectively. In terms of δ values (Eq. 1.1) this expression becomes:

$$\alpha_{A-B} = \frac{\left[\frac{(\delta_A + 1000)R_{STD}}{1000}\right]}{\left[\frac{(\delta_B + 1000)R_{STD}}{1000}\right]} \qquad \text{1.3a.}$$

which simplifies to:

$$\alpha_{A-B} = \frac{1 + \frac{\delta_A}{1000}}{1 + \frac{\delta_B}{1000}} = \frac{1000 + \delta_A}{1000 + \delta_B} \qquad \text{(O'Neil, 1986) 1.3b.}$$

The α value, rather than δ values, is used to represent the fractionation between two phases, for if the isotopes are randomly distributed over all possible sites or positions in the substances A and B, the fractionation factor (α) is related to the equilibrium constant, K, for isotope exchange reactions in the following way :

$$\alpha = K^{\frac{1}{n}} \qquad \text{(O'Neil, 1986) 1.4.}$$

where n is the number of atoms exchanged (normally 1). For the isotope exchange reaction between CH_4 and H_2O:

$$K = \alpha = \frac{\left({}^2H / H\right)_{CH_4}}{\left({}^2H / H\right)_{H_2O}} \qquad \text{1.5.}$$

Kinetic fractionation is associated with fast, incomplete or unidirectional processes such as evaporation, diffusion and dissociation and redox reactions. Fractionation during evaporation and diffusion can be explained by kinetic theory, while kinetic fractionation during dissociation and redox reactions is a result of the molecules containing the heavier isotope having a higher dissociation energy than those containing the lighter isotope (Jenkin, 1988).

Kinetic fractionation occurs during the scavenging of atmospheric $^{13}CH_4$ and $^{12}CH_4$ by hydroxyl radicals, with the relative rates of removal equal to the relative rate constants (k) of this reaction for each isotope. Thus for carbon the kinetic isotope fractionation can be defined as :

$$\alpha = \frac{k_{^{13}C}}{k_{^{12}C}} \qquad \text{(which is <1)} \qquad \text{1.6.}$$

1.5. Thesis structure and chapter summaries.

The thesis comprises four main sections, Chapters 2 to 5, which investigate various aspects of stable isotope analysis of methane. Chapter 2 records the research carried out during the first year, which was dominated by the development of the analytical technique to measure $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ from an air contaminated CH_4 sample. Once the technique was established a sample isotopic characterisation programme was undertaken. All samples analysed during the research are listed in Appendix 1. The bulk of the landfill site isotopic characterisation was carried out during 12-18 months of this research and Appendix 4 consists of a paper, published in a landfill site technology conference proceedings, which stemmed from collaborative links with Strathclyde University Civil Engineering Department, which provided the majority of landfill site samples for the database. The paper demonstrates the application of stable isotopic fingerprinting in provenancing an unknown source of CH_4, a technique which is potentially economically attractive.

Chapter 3 reviews in greater detail the importance of CH_4 in the atmosphere and considers the atmospheric chemistry of methane as this has implications for the isotopic composition. Published flux budgets are considered and compared with an independently calculated budget. The application of stable isotope analysis in monitoring atmospheric CH_4 and understanding flux budgets is demonstrated.

It is clear from Chapter 3, that it is difficult to apply stable isotope analysis to monitor atmospheric fluxes, and understand processes without accurate flux measurements and isotopic signatures. Chapters 4 and 5, therefore report research undertaken to characterise the isotopic signatures of important terrestrial bacteriogenic CH_4 fluxes and understand the controls on $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$.

Chapter 4 investigates, through use of closed culture enrichment systems, the influence that $\delta D(H_2O)$ has on $\delta D(CH_4)$, and the influence of methanogenic pathway on $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$. A brief overview of methanogenesis demonstrating the complexity of the biochemical pathways and the consortium of bacteria involved, provided in this chapter, is also of relevance in Chapter 5. New models are proposed to characterise the influence of $\delta D(H_2O)$ on $\delta D(CH_4)$ produced via acetoclastic methanogenesis from a fatty acid substrate. This is contrasted with previous isotopic modelling of acetoclastic methanogenesis (Whiticar et al., 1986).

Chapter 5 reports the findings of a pilot experiment devised to measure peatland $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ in situ, while manipulating of the ecosystem by use of a greenhouse in the field. The role of the greenhouse was to simulate global warming while a) monitoring the stable isotope composition of CH_4 produced in-situ and b) monitoring the effect on the ambient temperature within the bog. Monitoring the magnitude of this change is important for, at present, methanogenesis is considered to be slow in most peatlands due to suboptimal temperature and pH (Dinel et al., 1988). In this experiment, gas samples were collected at known depths within the peat 'reservoir'. $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$

were measured for each sample, and the temperature at each depth recorded. To investigate the controls on $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$, both $\delta^{13}C$ and δD of the peat and porewater at the corresponding depths were also measured.

Previous research, (encompassing more than stable isotopic studies), is discussed where relevant in each chapter rather than being given as a separate review. It should be noted that the literature is particularly lacking in publications containing paired measurements of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$, and thus there is little to compare with $\delta D(CH_4)$ measured in this research.

The Chapters are presented in the form of self-contained papers with overall conclusions of the individual research topics are presented in Chapter 6.

CHAPTER 2: EXPERIMENTAL TECHNIQUE AND METHODOLOGY

2.1. Introduction.

The first year's objective of this three year project was to develop an analytical system to measure $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ from an air contaminated CH_4 sample. Separation of CH_4 from the impurities is not sufficient as CH_4 is not usually a suitable form to send to the mass spectrometer. Because of instrumental requirements, it is easier to convert carbon to CO_2, and H_2O to H_2 for stable isotope ratio measurements. Any method of conversion must not cause isotopic fractionation and should produce quantitative yields of carbon and hydrogen to permit determination of the percent carbon and hydrogen in the sample. Successful stable isotope analysis of CH_4 (to concentrations as low as atmospheric, 18ppb) had been carried out in the United States, and it was therefore decided to base our system on those documented in the literature.

In this chapter, the technique refined to measure $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$ from a sample containing CH_4 and CO_2 is described and the rationale behind each stage in the process explained. The evolution to this finalised procedure is discussed. Before analyses of preliminary CH_4 standards could begin, all areas where a gas volume measurement was to be made were calibrated. Calibration of the uranium furnace was also necessary (this is the same calibration process applied approximately every two months when renewing the hydrogen mass spectrometer reference gas). Typical examples of these calibrations are presented. A comparison is made between $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ isotopic signatures measured and those supplied with the standards. Factors affecting the isotopic signature of the standards and the resultant criteria applicable to the analysis of non-standard samples are reported. The methodology of other standard carbon and hydrogen techniques used often during this research will be given. Gas sampling techniques are briefly discussed.

2.2. CH_4 analysis technique.

Gas samples were prepared for mass spectrometry at the Scottish Universities Research & Reactor Centre, East Kilbride in a specially constructed and dedicated vacuum line similar to that designed by Stevens of the Argonne National Laboratory, Illinois (Stevens & Rust, 1982). A diagrammatic representation of the vacuum line is given in Figure 2.1. The Pyrex glass vacuum line was evacuated from atmospheric pressure to a working vacuum initially by use of a rotary pump until a vacuum of around 10^{-3} mbar was reached. A cryogenically cooled diffusion pump was then used to reach the working vacuum of 10^{-5} mbar. The cryogenic diffusion pump was effective by condensing, freezing and/or sorption of gas at surfaces cooled to liquid nitrogen temperature thus removing the gases from the gas phase in the vacuum system (Harris, 1989). When re-evaporating, such gases could be removed by the diffusion part of the pump. Here gas transport is achieved by a series of high-velocity vapour jets (in this line mercury, rather

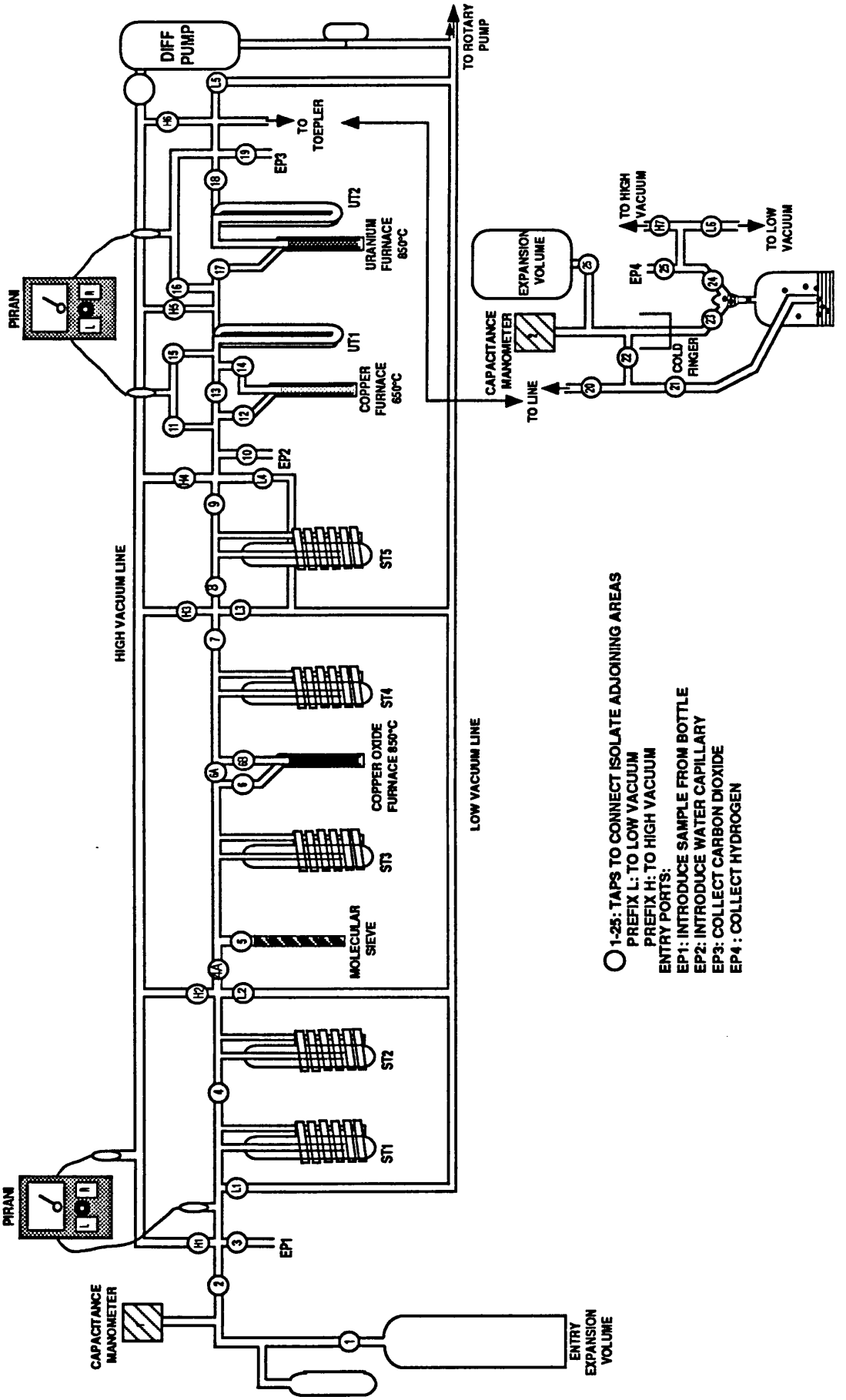


Fig. 2.1 Diagrammatic representation of the vacuum line used to analyse for $\delta^{13}C(CH_4)$, $\delta D(CH_4)$, $\delta^{13}C(CO_2)$, $\delta^{13}C$(organic), δD(organic) and $\delta D(H_2O)$.

than oil vapour, was used to avoid hydrocarbon contamination) emerging from an assembly within the pump body (Harris, 1989). In normal operation a proportion of any gas arriving at the inlet jet is entrained, compressed and transferred to the next stage, which here was removal by the rotary pump.

When not in use, all sections of the vacuum line with the exception of the uranium furnace and the molecular sieve (these are evacuated and then isolated) were kept under a working vacuum by diffusion pumping. As an added precaution before beginning the analysis, the vacuum line and molecular sieve were degassed under high vacuum by flaming with a gas torch. The sample gas was then introduced by a gas-tight syringe from the sampling vessel via a septum into an evacuated area of known volume (between Tap 1 (T1) and T2). If the sample size necessitated multiple injections then the bottle was attached to the line at entry port 1 (EP1) and an aliquot introduced. The volume into which the sample was introduced was connected to a fixed volume capacitance manometer so that the volume of gas could be measured quantitatively. The capacitance manometer is a pressure-measuring instrument that electronically senses the deflection of a diaphragm by means of a change in capacitance between an electrode (or electrodes) and the diaphragm as the diaphragm deflects under forces due to the pressure differential across it. It has a range from atmospheric pressure to 10^{-6} mbar and is attached to a digital display, so that once calibrated (Section 2.3.1.) the volume of gas present is displayed (μM).

Sample purification:

If the sample was small (less than 14μM), liquid nitrogen was placed over the entry port trap. If the sample was large, then at liquid nitrogen temperature the partial pressure of CH_4 within the sample may have been great enough that the CH_4 became condensable. As such the sample was expanded to T4 before placing liquid nitrogen over the entry trap. At liquid nitrogen temperature, atmospheric CO_2, H_2O, H_2S and higher weight hydrocarbons are trapped whilst CH_4 and other non-condensable gases (non-condensable at -196°C i.e. nitrogen, hydrogen, helium, argon, carbon monoxide) remain free.

Once the reading on capacitance manometer 1 had settled at a minimum (indicating all condensable gases had been trapped) liquid nitrogen was then placed over the first spiral trap (ST1) and T2 was opened (if not already opened for a large sample). The sample was then allowed to expand into the vacuum line passing through another liquid nitrogen cooled spiral trap (ST2) as it did so. This was an added precaution in the unlikely event that there were traces of condensable gases still present. At each stage the drop in capacitance manometer reading was noted.

Once the gauge reading was at a minimum and steady, the finger of molecular sieve was cooled to liquid nitrogen temperature and opened up to the line. At this temperature the molecular sieve acted like a pump, trapping on its surface the non-condensable gases, including CH_4, previously non-condensable. Thus all remaining non-condensable gases were drawn through the liquid nitrogen traps and retained on the cold molecular sieve surface. With large gas samples it was important to place the nitrogen at the bottom of the

molecular sieve finger and move it up gradually as the gas condensed. Otherwise the neck of the molecular sieve finger became saturated and was not capable of trapping any more gas. As gas was trapped the gas pressure in the line decreased. With the majority of samples a complete recovery of vacuum was observed on both the Pirani and the capacitance manometer gauges. The line was evacuated and the molecular sieve finger isolated. A change in reading was rarely observed on the Pirani when the line was evacuated, indicating that the molecular sieve was effective in trapping all non-condensable gases.

The advantages of using the molecular sieve to trap all non-condensable gases were three-fold. Firstly, all the sample injected was drawn through a minimum of two liquid nitrogen cooled spiral traps, thus ensuring all condensable gases within the sample were trapped. Secondly, once the gas was trapped, the section of vacuum line to the left of the molecular sieve was isolated thus reducing the volume of line open during the conversion of CH_4 to CO_2 and H_2O. A decrease in available volume was considered to increase the likelihood of the CH_4 molecule entering the furnace and thus the % of CH_4 converted. Thirdly, when the molecular sieve was allowed to warm to room temperature the CH_4 was released but many other non-condensable gases (e.g. N_2) were not. Thus the composition of the gas entering the furnace was either pure CH_4 or mixed with inert gases that would remain stable.

Conversion of CH_4 to CO_2 and H_2O:

Liquid nitrogen was first placed on the adjacent spiral trap (ST3) before removing the liquid nitrogen from the molecular sieve. The molecular sieve was allowed to warm to room temperature and T5 opened to the cooled spiral trap. When the molecular sieve had warmed to room temperature and the sample had equilibrated between furnace and molecular sieve, the sample was passed into an electrically heated quartz furnace containing copper oxide at approximately 750°C by opening T6. Oxygen produced at this temperature by the copper oxide was the oxidant, and CO_2, H_2O and oxides of nitrogen were formed. CO may also have been present in the reaction by-products. After 30 minutes liquid nitrogen was placed on the spiral trap on the right hand side of the furnace, ST4 was opened (by opening T6A) and the gas was allowed to pass through the furnace. The sample was then left for as long as possible (approximately 4 hours) as the % oxidation of CH_4 was thought to be rate limited by the likelihood of the CH_4 molecule entering the furnace. Maximum possible time at the furnace section was considered to increase the volume of gas that would enter the furnace and thus the yield.

Collection of CO_2 for measurement of $\delta^{13}C(CO_2)$:

While the purified sample was reacting in the isolated combustion section of the line (between T4A and T7), CO_2 associated with the sample condensed in the purification section of the line was separated and collected from other trapped condensable gases, and the volume measured, before transferring cryogenically to an evacuated bottle attached at entry port 1 (EP1). $\delta D(H_2O)$, δD(organic) and $\delta^{13}C$(organic) analyses could also be carried out in the right hand side of the line (from EP2 onwards) during this 4 hour period.

Collection of combustion products:

With the exception of carbon monoxide all reactant products were condensed in the liquid nitrogen cooled spiral traps on either side of the furnace. Products formed as a result of incomplete oxidation e.g. CO, OH, nitrogen oxide, were most likely to form if the CuO furnace needed regenerated, generally indicated during the analysis by low yield and poor carbon:hydrogen (C:H) ratio. If substantial in volume, these products can be completely oxidised by passing through a trap containing Schutze reagent (iodine pentoxide on silica wool), a powerful oxidising agent, and similarly collected at liquid nitrogen temperature. Stevens (pers. comm.) found only a 0.1‰ fractionation for $\delta^{13}C(CH_4)$ (no data available for $\delta D(CH_4)$) when Schutze reagent was not used. When testing the application of Schutze reagent for this research project, $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ were erroneous, hence this step was deemed unnecessary.

The furnace was isolated and combustion products in the ST3 were transferred cryogenically to ST4. The final stage in collecting the combustion products was to evacuate remaining undesirable non-condensable gases (e.g. free oxygen and possibly unconverted CH_4) through L3 and H3.

Separation and purification of CO_2 from H_2O:

After the oxidation of the CH_4 to H_2O and CO_2, ST4 containing the reaction products was warmed to -78°C with an acetone-dry ice mixture, which allowed the CO_2 and any oxides of nitrogen to sublime whilst retaining the H_2O. The liberated CO_2 was allowed to circulate in the copper furnace at 650°C for ten minutes. Nitrous oxides were unstable at this temperature and were reduced to free nitrogen, whereas the CO_2 was stable and remained unaffected. The trap on the right hand side of the furnace (UT1) was then cooled to liquid nitrogen temperature, thus condensing the CO_2, before evacuating any free nitrogen. The CO_2 was transferred cryogenically to the cold finger (situated above the Toepler pump) and the volume of CO_2 measured quantitatively, before collection in an evacuated sample bottle attached to EP3. $\delta^{13}C(CH_4)$ was measured on a VG Sira 10 triple collector mass spectrometer.

Reduction of H_2O to H_2:

H_2O was reduced to H_2 using the method of Friedman and Smith (1958). The purified water was allowed to warm to room temperature and the line heated to evaporate any H_2O adsorbed to the glassware. The H_2O was passed through uranium turnings at 850°C followed by another liquid nitrogen cooled trap (UT2). The furnace reduced the H_2O to H_2, and the cooled trap acted as a further precaution to retain any unconverted H_2O. H_2 is a non-condensable gas and thus to avoid fractionation a mercury filled Toepler pump was used to transfer the gas. The Pirani could be seen to drop as the H_2 evolved from the furnace, and rise again when pumped by the Toepler into a chamber of known volume. When the gauge had recovered the glass piping was flamed to release adsorbed water. T18, T13, T14 and T15 were shut and T16 opened. The liquid nitrogen was then removed and the trap warmed to room temperature, recycling any H_2O that may have been trapped

through the uranium furnace. The Pirani gauge was monitored for any drop in vacuum, indicating that not all H_2O had been converted on the first pass. This was rare, but when observed, the uranium furnace was replaced as it indicated that the uranium was becoming too oxidised to function properly. The hydrogen yield was measured with a fixed volume capacitance manometer. Using the Toepler the sample was transferred to an evacuated sample tube, attached to EP4, for analysis on the mass spectrometer.

$\delta D(CH_4)$ was measured using a VG Micromass 602B with a modified inlet system. This inlet system makes use of mercury pistons which enables very small samples of gas (at the present moment <4μM) to be introduced into the mass spectrometer. $\delta^{13}C(CO_2)$ was measured using a VG Sira 10 Mass Spectrometer. The condensable nature of CO_2 allows the use of an inbuilt cold finger making $\delta^{13}C$ measurements of very small samples possible. As with hydrogen, the reference gas is calibrated by running against standards.

Methane analysis are quoted to a precision for $\delta^{13}C$ of ±2.2‰, for δD of ±5.5‰, although as will be shown in section 2.3.3., the precision is often greater than this. Each sample took one day to analyse.

2.3. Development of the analysis technique.

2.3.1. Calibration of fixed volume capacitance manometer.

The volume of gas sample introduced into the line at the beginning of the analysis and the volumes of CO_2 and H_2 yielded at the end of the analysis (whether CH_4, organic carbon and hydrogen or water samples) were measured where possible. Thus the capacitance manometer and connected digital display attached to each measurement areas had to be calibrated. This was a simple procedure that involved the use of pre-calibrated mercury manometer on a 'donor' line.

A large aliquot of CO_2 was released into the donor line and once condensed at liquid nitrogen temperature, the line was evacuated to remove any non-condensable gases. An evacuated sample bottle of a known volume was attached to the line. The CO_2 trap was opened up to both the mercury manometer and the calibrated sample bottle and the liquid nitrogen was removed. As the temperature rose the CO_2 sublimed and expanded to fill the available volume thus displacing the mercury in the manometer. At a given displacement (column 1 and 4, Table 2.1) the sample bottle was isolated, as was the trap containing the CO_2 before recooling to liquid nitrogen temperature. A note was made of the distance which the mercury in the manometer had been displaced by the CO_2 before evacuating the manometer and other areas of the line not isolated. The size of the displacement of the mercury corresponded directly to a gas pressure within the manometer and all areas open to it, e.g. the standard bottle. Thus knowing the volume of the sample bottle and the gas pressure the number of moles of gas in the bottle could be calculated (column 1 and 4, Table 2.1).

The calibrated sample bottle was then removed from the 'donor' line and attached to the evacuated CH_4 analysis line at the port closest to the desired area of calibration. CO_2 in

the bottle was cryogenically transferred to the calibration area, and the trap containing the CO_2 at liquid nitrogen temperature was evacuated and isolated. The liquid nitrogen was removed and the trap warmed to room temperature allowing the CO_2 to sublime. As the gas sublimated the vacuum dropped and the reading on the digital display unit connected to the capacitance manometer rose. Once the trap had reached room temperature the maximum steady value (from an initial reading of zero) displayed was recorded. This could then be directly related to the volume of gas in μM determined to have been introduced from the sample calibration bottle.

The sample calibration bottle was then reattached to the donor line, evacuated and the procedure was repeated until sufficient data had been collected (usually covering the range of the digital display) to construct an accurate calibration line for the entry area. The data set for the large volume Toepler calibration area is given as an example in Table 2.1. A computer aided linear regression was performed on the collected data and a calibration line generated. The graph depicting this line is given in the Figure 2.2. (overleaf)

Hg (cms)	μM of CO_2 n = 14.5p	Baritron reading	Hg (cms)	μM of CO_2 n = 14.5p	Baritron reading
0.50	7.250	0.23	9.85	142.825	4.20
0.80	11.600	0.44	11.15	161.675	4.72
1.05	15.225	0.49	12.10	174.450	5.07
1.20	34.075	0.55	13.60	197.200	5.70
1.55	22.475	0.72	15.00	217.500	6.27
2.35	34.075	1.05	15.80	229.100	6.67
3.50	50.750	1.50	15.90	230.550	6.73
4.05	58.725	1.79	17.25	250.125	7.20
4.80	69.600	2.09	19.70	285.650	8.18
5.80	84.100	2.50	20.55	297.975	8.53
6.90	100.050	2.97	21.35	309.575	8.85/7
7.85	113.825	3.39	23.20	336.400	9.70
9.15	132.675	3.93	24.10	349.450	10.10

Table 2.1. Example of measurements taken for Toepler calibration line.

2.3.2. Uranium furnace and hydrogen mass spectrometer calibration.

The hydrogen mass spectrometer compares the sample H_2 to a reference H_2 gas. The reference gas used is not an international standard, and therefore must be calibrated against international standards. When the reference gas in the mass spectrometer has been renewed or the mass spectrometer has undergone any fine tuning or repair work, the calibration line must be checked. The procedure followed is that each hydrogen analysis line in the lab is used to analyse a series of water standards of known δD, which can then be incorporated into a calibration line to give δD_{SMOW}. This is stored in the computer, thus when the sample

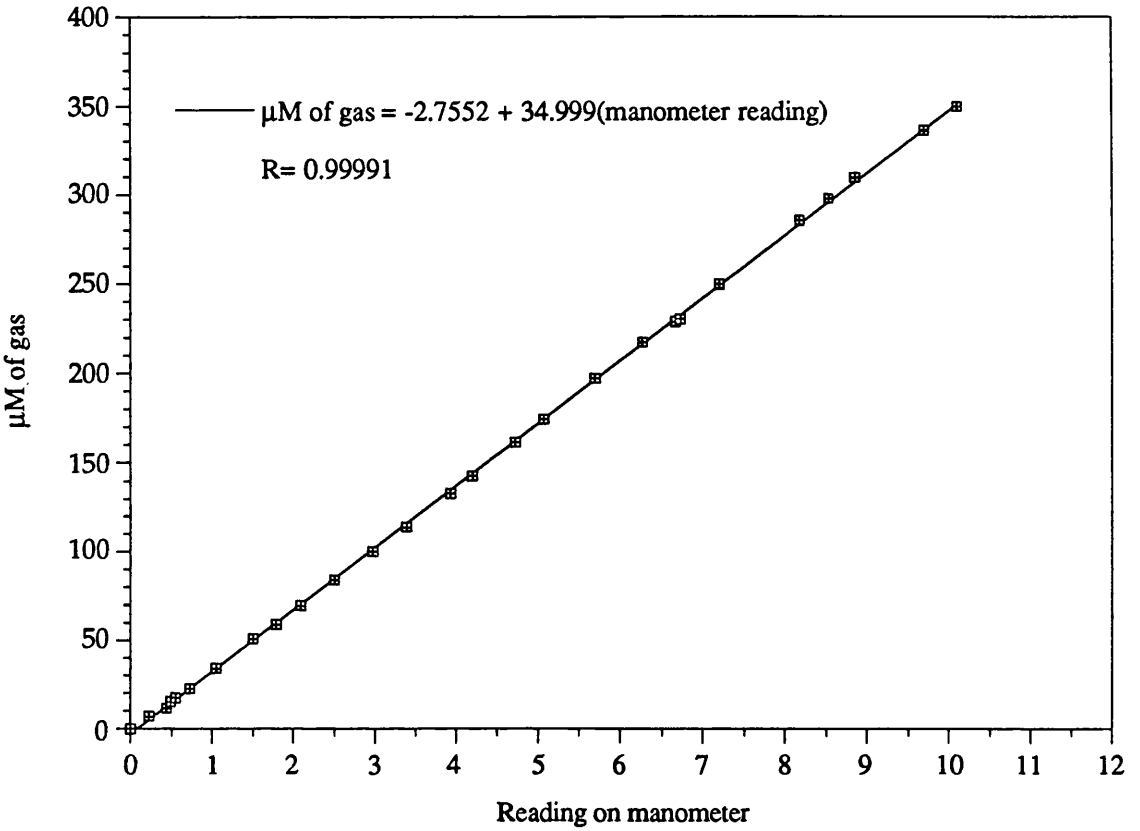


Figure 2.2 Calibration line for Toepler large volume.

is run, all δD values can be reported with reference to SMOW.

Likewise, when a fresh uranium furnace is placed on the line, a similar procedure must be undertaken to ensure that the furnace is functioning properly and that there is no added source of contamination. Firstly the furnace is degassed at slightly above running temperature while under vacuum thus removing any surface water and hydrogen that may be present. A series of standards are then run to ensure that the calibration line, as would be expected, has not changed.

The data points generated and a calibration line for the CH_4 analysis rig, typical of those undertaken in during this research, are given in Table 2.2. and Figure 2.3. respectively. In order to alleviate any memory effect of the previous sample that may be present in the uranium furnace, the furnace can be flushed with the contents of two capillaries of the standard being analysed before collecting H_2 from a further three capillaries for δD measurement. This procedure was not adopted with non-standard samples analysed. The calibration data in Table 2.2 are presented chronologically and flushing did not take place between the standards. There is no apparent memory effect between samples, with the largest discrepancy between three comparable SLAP analyses. It is debatable whether this inaccuracy lies with the first two samples analysed, the last sample analysed, the contents of the capillary or the mass spectrometer.

Standard name	δD_{RAW}‰	δD_{SMOW}‰
SMOW	94.7	0
	95.9	0
	94.6	0
LIGHT STANDARD	-34.6	-93.7
	-35.5	-93.7
	-34.8	-93.7
GISP	-171.7	-190
	-170.4	-190
	-170.8	-190
SLAP	-503.6	-428
	-504.9	-428
	-507.8	-428

Table 2.2. δD_{RAW} and δD_{SMOW} values for a typical calibration line.

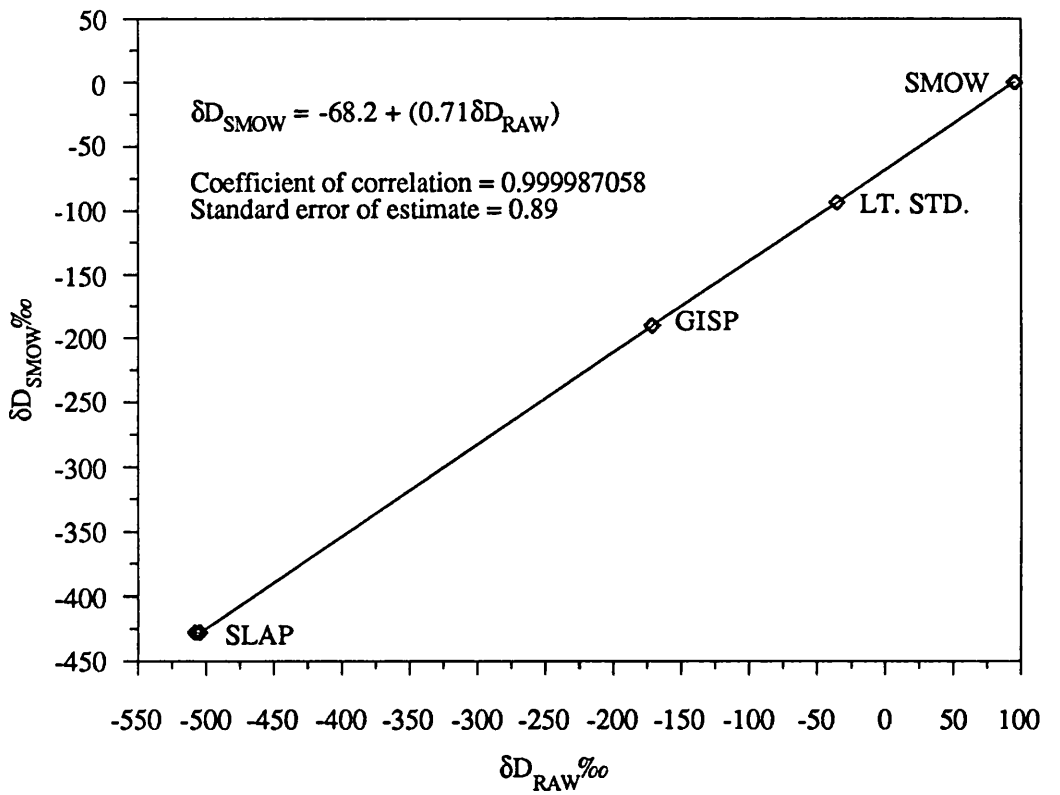


Figure 2.3. Calibration line for hydrogen mass spectrometer / fresh uranium furnace.

2.3.3. Standard gases.

Methane standards were kindly supplied by Martin Miller of the British Geological Survey in Keyworth, to whom they were supplied by the International Atomic Energy Agency (IAEA) after a preliminary inter-laboratory calibration exercise. The three standards supplied were NGS1, NGS2 and NGS3, which were coal related, oil related and

bacteriogenic in origin respectively. It is suggested by $\delta D(CH_4)$ measurements (Table 2.3.) that only three institutions were involved in the IAEA inter-laboratory calibration. CH_4 standards are not currently available commercially from the IAEA. The composition and isotopic signature, $\delta^{13}C$ and δD of each standard is outlined below in Table 2.3.

Standard	NGS1	NGS2	NGS3
	COAL	OIL	BACTERIOGENIC
CH_4	81.238	52.775	98.825
C_2H_6	2.832	2.65	0.042
C_3H_8	0.387	1.29	0.004
N_2	14.265	16.613	1.118
CO_2	0.999	25.143	0.009
$\delta^{13}C(CH_4)‰$	-29±0.2‰	-44.3±0.7‰	-72.3±1.2‰
$\delta D(CH_4)‰$	-133/-141.9‰	-169.4/-173/-175.2‰	-175.6/-176.3‰

Table 2.3. Composition and isotopic composition of NGS standard gases (data as supplied)

Tables 2.4, 2.5 and 2.6 represent a selection of analyses for the standards NGS1, NGS2 and NGS3. Some results have been omitted, for example standards run when 'problem solving'. A representative selection of unacceptable values have been included in the three tables as they demonstrate the evolution of the analytical technique. $\delta^{13}C(CH_4)‰$ and $\delta D(CH_4)‰$ mean values determined by the analysis technique used in this research have been listed at the bottom of each table. In calculating mean $\delta^{13}C(CH_4)‰$ and $\delta D(CH_4)‰$, each sample has been considered as providing two separate pieces of information. Due to the many different stages of processing, an unacceptable $\delta^{13}C(CH_4)‰$ does not imply an unacceptable $\delta D(CH_4)‰$. If the main source of error is during the combustion of CH_4 to CO_2 and H_2O then it is likely that both values will be unaffected. However if the source of error lies somewhere else e.g. in the reduction of H_2O to H_2 or contamination of the combustion products by traces of CO_2, then the complementary measurement will remain unaffected and acceptable. As such, the mean values of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ have been considered separately regardless of the values of the other measurement. Analyses of NGS1, NGS2 and NGS3 have not been presented in standard numerical order, but rather in the order of most commonly processed first.

2.3.3.1 NGS3

Table 2.4. Data collated from analysis of NGS3.

Analysis N°	$\delta^{13}C(CH_4)‰$	% yield	$\delta D(CH_4)‰$	% yield	C:H ratio
CHA 64	-69.9[1]	59	-107	70	1:4.28
CHA 65	-72.1[1]	65	-172[1]	95	1:6.45
CHA 91	-72.4[1]	60	-198	68	1:4.65

.....continued: Table 2.4. Data collated from analysis of NGS3.

Analysis N°	$\delta^{13}C(CH_4)$‰	% yield	$\delta D(CH_4)$‰	% yield	C:H ratio
CHA 94	-67.0	44	-209	48	1:4.36
CHA 95	-71.5[1]	52	-197	61	1:4.70
CHA 104	-70.0[1]	41	-212	48	1:4.76
CHA 105	-66.7	68	-180[1]	73	1:4.29
CHA 106	-71.9[1]	60	-174[1]	69	1:4.60
CHA 110	-72.7[1]	59	-182[2]	64	1:4.36
CHA 114	-71.2[1]	50	-214	52	1:4.36
CHA 115	-68.2[2]	65	-194	72	1:4.45
CHA 116	-70.6[1]	65	-189	69	1:4.24
CHA 117	-55.2	81	-173[1]	131	1:6.47
CHA 118	-72.9[1]	60	-169[1]	70	1:4.65
*					
CHA 120	-72.6[1]	60	n.m.	n.m.	-
CHA 121	-73.0[1]	52	-181[1]	54	1:4.13
CHA 126	-72.6[1]	54	-176[1]	60	1:4.45
CHA 136	-74.0[1]	49	-185	57	1:4.7
CHA 138	-63.5	52	n.m.	n.m.	-
CHA 139	-68.8	57	-173[1]	57	1:4.03
CHA 149	-73.4[1]	47	-176[1]	47	1:3.96
CHA 168	-64.8	30	-117	21	1:2.76
CHA 170	-61.0	28	-153	68	1:9.47
CHA 173	-63.9	16	n.m.	28	1:6.89
CHA 176	-76.4[2]	44	-134	55	1:5.00
CHA 177	-72.0[1]	48	-146	63	1:5.20
CHA 192	-70.4[1]	48	-178[1]	51	1:4.22
CHA 203	-80.8	13	-166	17	1:5.05
CHA 205	-73.2[1]	n.m.	-163	n.m	1:3.47
CHA 227	-60.0	6	-133	12	1:8.04
CHA 231	-67.2[2]	35	-161	55	1:5.43
CHA 268	-75.5[1]	32	-182[1]	35	1:4.36

$\delta^{13}C(CH_4)^1$	-72.0±1.2	n=18
$\delta^{13}C(CH_4)^2$	-71.9±2.2	n=23
$\delta D(CH_4)^1$	-175.8±4.0	n=11
$\delta D(CH_4)^2$	-176.3±4.2	n=12

n.m. not measured.

- not calculable.

* For all subsequent samples the molecular sieve was degassed before analysis.

2.3.3.2. NGS1

Table 2.5. Data collated from analysis of NGS1

Analysis N°	$\delta^{13}C(CH_4)$‰	% yield	$\delta D(CH_4)$‰	% yield	C:H ratio
CHA 75	-29.4[1]	77	-142[1]	81	1:4.39
CHA 81	-28.0[1]	75	-160	82	1:4.37
CHA 108	-29.5[1]	65	-171	71	1:4.34
CHA 109	-37.7	20	-147[2]	41	1:8.26
CHA 119	-34.6	37	-155	41	1:4.38
*					
CHA 122	-30.8[1]	64	-151	70	1:4.40
CHA 123	-30.0[1]	58	-145[1]	62	1:4.29
CHA 124	-32.8[2]	54	-146[1]	58	1:4.08
CHA 125	-30.7[1]	60	-147[2]	62	1:4.20
CHA 140	-31.9[1]	66	-152	73	1:4.42
CHA 171**	-35.2	22	-134[1]	67	1:11.96
CHA 172**	-11.5	57	-136[1]	137	1:7.76
CHA 228	-28.9[1]	38	-142[1]	49	1:5.21
CHA 232	-28.1[1]	46	-135[1]	63	1:3.99
CHA 269	-32.1	65	-153	65	1:5.45
CHA 649	-30.2[1]	91	-148	91	1:4.07

$\delta^{13}C(CH_4)^1$	-29.74±1.2	n=10
$\delta^{13}C(CH_4)^2$	-30.19±1.5	n=12
$\delta D(CH_4)^1$	-140±5.0	n=7
$\delta D(CH_4)^2$	-142±5.5	n=9

* For all subsequent samples the molecular sieve was degassed before analysis.

** Wax free joints on CuO furnace.

At several stages during the development process new techniques were tried, and if successful incorporated into the procedure. From Tables 2.4 to 2.6 it is clear that one of the most effective changes was to degas the finger of molecular sieve, used to trap the non-condensable gases at liquid nitrogen temperature, by heating with a gas torch to red heat while under high vacuum. The memory effect if used without degassing is quite clearly demonstrated by consideration of $\delta D(CH_4)$.

Not degassing the molecular sieve has a far greater effect on $\delta D(CH_4)$ than $\delta^{13}C(CH_4)$, thus $\delta D(CH_4)$ values measured without degassing have not been included in mean calculations. This is most clearly demonstrated when comparing NGS2 $\delta D(CH_4)$ with $\delta^{13}C(CH_4)$, where all $\delta D(CH_4)$ measurements generated when the molecular sieve was not

2.3.3.3. NGS2

Table 2.6. Table collated from analysis of NGS2

Analysis N°	$\delta^{13}C(CH_4)$‰	% yield	$\delta D(CH_4)$‰	% yield	C:H ratio
CHA 76	-45.2[1]	63	-195	67	1:4.25
CHA 78	-45.2[1]	68	-197	79	1:4.63
CHA 80	-45.6[1]	80	-190	81	1:4.02
CHA 82	-43.2[1]	82	-190	95	1:4.65
*					
CHA 127	-44.0[1]	66	-178[1]	n.m.	-
CHA 233	-43.4[1]	32	-173[1]	43	1:5.42
CHA 275	-44.3[1]	50	-174[1]	50	1:4.00

$\delta^{13}C(CH_4)$[1]	-44.4±1	n=7
$\delta D(CH_4)$[1]	-175±2.4	n=3

* For all subsequent samples the molecular sieve was degassed before analysis.

n.m. not measured

- not possible to calculate

degassed are clearly inaccurate, while most $\delta^{13}C(CH_4)$ measurements are within the accepted range. Thus $\delta^{13}C(CH_4)$ values, where considered accurate, have been included. Once the importance of degassing the molecular sieve was established, this was incorporated as the first stage in the analysis procedure (Section 2.2).

Table 2.7. compares the mean $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measured of each standard with that supplied with the standards.

STANDARD	$\delta^{13}C(CH_4)$ Data supplied	$\delta^{13}C(CH_4)$ Data measured	$\delta D(CH_4)$ Data supplied	$\delta D(CH_4)$ Data measured
NGS3	-72.3±1.2	-71.9±2.1	-176.0±0.5	-176.3±4.2
NGS1	-29±0.2	-30.2±1.5	-137.5±6.3	-141.6±5.5
NGS2	-44.3±0.7	-44.4±1.0	-172.5±2.9	-175.2±2.4

Table 2.7. Comparison of supplied and measured $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for standards NGS1, NGS2 and NGS3.

The measured mean $\delta^{13}C(CH_4)$ and mean $\delta D(CH_4)$ for all standards agree well with the data supplied, with the exception of $\delta D(CH_4)$ for NGS1. Only two $\delta D(CH_4)$ values were supplied for NGS1, -133‰ and -141.9‰ (Table 2.3.). Without a third value it is impossible to ascertain which of the latter is more accurate. As such $\delta D(CH_4)$ of -141.6±5.5‰ was considered acceptable as the measured standard value, especially as 1σ is less than that supplied. The precision to which the mean can be quoted tends to be less for measured

$\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ than for supplied values, although the precision quoted on measured $\delta D(CH_4)$ for NGS1 and NGS2 is greater than that for the supplied values.

The precision to which $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ can be quoted appears to relate directly to the frequency with which the standard was run. There is a far wider spread in $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for standard NGS3 than NGS1 than NGS2. This is a function of two factors:

a) NGS3 was used more often than NGS1 than NGS2, particularly during the developmental stage and thus more erroneous measurements are likely to have been made.

b) increasing accuracy of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ may be directly related to lower concentrations of CH_4 within the sample with other gases acting as a carrier gas, 'transporting' the CH_4 into the furnace more efficiently.

Data generated from this project can thus be considered accurate, however the precision (1σ) to which measurements can be quoted is less. $\delta^{13}C(CH_4)$ is accepted to be accurate to ±2.2‰, while $\delta D(CH_4)$ is quoted to a precision of ±5.5‰ maximum.

Standards were run regularly between sample analyses to ensure that the accuracy of the data produced was maintained. However it is still possible that an accurate standard could be followed by an inaccurate sample. Thus two criteria, maximum C:H ratio and minimum % yield, were applied, which a non-standard sample had to meet before it could be considered accurate.

The next stage of this chapter discusses how these criteria were developed.

2.3.3.4. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ as a function of C:H ratio:

One control in accepting $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ as accurate is the C:H ratio. Complete oxidation of a CH_4 sample generates twice the volume of H_2 as CO_2 and thus should give rise to a C:H ratio of 1:4.

Figures 2.4a-2.4f (pp 25-27) show the relationship between $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ and acceptable C:H ratios. Each graph is a plot of either $\delta^{13}C(CH_4)$ or $\delta D(CH_4)$ (x-axis) against C:H ratio (y-axis). For each standard the mean, σ_{n-1} and maximum acceptable range for the standard are shown by vertical lines. The maximum acceptable range for NGS3 $\delta^{13}C(CH_4)$ and NGS1 $\delta D(CH_4)$ is the same as σ_{n-1}. All values that fall within the acceptable ranges (mean ± 2.2‰ for $\delta^{13}C(CH_4)$ and mean ± 5.5‰ for $\delta D(CH_4)$) have been used to calculate the mean acceptable C:H ratio. These are displayed in Table 2.8. as a function of $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and degassing the molecular sieve. The difference in C:H ratio degassing the molecular sieve has on $\delta D(CH_4)$ compared to $\delta^{13}C(CH_4)$ is again clearly demonstrated.

In the standard analyses, there are a few circumstances where the C:H ratio was 1:4, but it was usually higher, with a calculated mean of 1:4.5±0.5 for $\delta^{13}C(CH_4)$ and 1:4.9±1.2 for $\delta D(CH_4)$ for all acceptable standard measurements (Table 2.8.).

The data in this Table have been used to decide the maximum C:H ratio acceptable in each $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ analysis. As degassing the molecular sieve was adopted as

part of the analytical procedure from an early stage of the research, the mean C:H ratios for

	$\delta^{13}C(CH_4)$	$\delta^{13}C(CH_4)$	$\delta^{13}C(CH_4)$	$\delta D(CH_4)$	$\delta D(CH_4)$	$\delta D(CH_4)$
molecular sieve	degassed	not degassed	total	degassed	not degassed	total
NGS1	1:4.5±0.5 (n=8)	1:4.4±0.03 (n=3)	1:4.5±0.5 (n=11)	1:4.4±0.5 (n=4)	1:6.3±2.7 (n=2)	1:5.0±1.6 (n=6)
NGS2	1:4.7±1 (n=2)	1:4.4±0.3 (n=4)	1:4.5±0.5 (n=6)	1:4.7±1 (n=2)	no values acceptable	1:4.7±1 (n=2)
NGS3	1:4.3±0.6 (n=7)	1:4.7±0.6 (n=10)	1:4.5±0.6 (n=17)	1:4.2±0.2 (n=5)	1:5.5±1.2 (n=4)	1:4.7±1.0 (n=9)
Σ(NGS1, 2 and 3)	1:4.4±0.6 (n=17)	1:4.6±0.5 (n=17)	1:4.5±0.5 (n=34)	1:4.4±0.5 (n=11)	1:5.7±1.6 (n=6)	1:4.9±1.2 (n=17)

Table 2.8. Mean C:H ratio for $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ of each standard and the influence of degassing the molecular sieve.

$\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ where the molecular sieve was degassed have been used. From the table, maximum C:H ratio for $\delta^{13}C(CH_4)$ was 1:4.4±0.6 and for $\delta D(CH_4)$ was 1:4.4±0.5. Any sample analysed that had a C:H ratio higher than 1:4.9 was repeated where possible (see CHA 240 and CHA 239). From Appendix 1 (isotopic composition, %yield and C:H ratio of all samples used in the database in Chapter 3) it is clear that the C:H ratio of all samples analysed has mean values of 1:4.3±0.2 (n=65) or 1:4.3±0.4 (n=68), which are both less than the maximum acceptable C:H ratio.

2.3.3.5. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ as a function of % yield.

One possible source of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ fractionation is during the conversion of CH_4 to CO_2 and H_2O. If 100% of the sample is converted, then $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ fractionation will not occur (at this stage). If only a proportion of the sample is converted, and this is not representative of the true isotopic composition, then fractionation will undoubtedly occur. Incomplete oxidation of each molecule within an isotopically representative sample will also result in fractionation. However, complete oxidation of an isotopically representative proportion of the sample injected will not cause isotopic fractionation. Thus 100% of the sample need not be converted to measure $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ accurately, although it is likely that the smaller proportion of the sample converted, the less accurate $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ will be.

Figs. 2.5.a -2.5.f (pp 28-30) illustrate the relationship between % of CH_4 converted to CO_2 and H_2O, and the accuracy of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$. They are laid out in a similar style to Figures 2.4 with the x-axis depicting $\delta^{13}C(CH_4)$ or $\delta D(CH_4)$ and the y-axis depicting % yield. The mean, σ_{n-1} and maximum acceptable range for each standard are again shown by vertical lines. The minimum yield necessary to give rise to accurate $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ has been measured simply by taking the lowest possible value that

falls within the acceptable ranges. Table 2.9. summarises the minimum volume of CH_4 converted (with the molecular sieve degassed) known to provide accurate results, within the limits of precision.

	NGS3	NGS1	NGS2	mean
$\delta^{13}C(CH_4)$	47%	46%	50%	47.6±2.1
$\delta D(CH_4)$	47%	41%	50%	46±4.6

Table 2.9. Minimum yield necessary for accurate $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ of each standard.

In many analyses the volume of CH_4 within the sample was unknown. In this case the %yield was calculated based on the assumption that the sample was composed of 100% CH_4. (This is an immediate over-estimate for samples injected into the vacuum system, for in those cases a few μM of air was also unavoidably injected). Where the sample was assumed to be 100% CH_4, the apparent % yield calculated is likely to be a minimum with the true % yield likely to be higher. The values in Table 2.9. may not be the lowest threshold necessary to give rise to acceptable values, but are the lowest known values at which that % conversion may give rise to acceptable values. Thus the minimum % yield acceptable in running a standard was set at 50%.

When the % of CH_4 in the sample was thought to be very low, a volume of the sample, larger than the capacitance manometer could measure, had to be aliquoted into the line in order to provide sufficient CO_2 and H_2 for mass spectrometric analysis. In such instances, it was not possible to calculate the % yield of C and H, as the volume of sample introduced was unknown. As such, the minimum 50% yield guideline could not be applied and instead an acceptable C:H ratio, combined with accurate standard measurements were considered sufficient.

2.3.4. Line Blank.

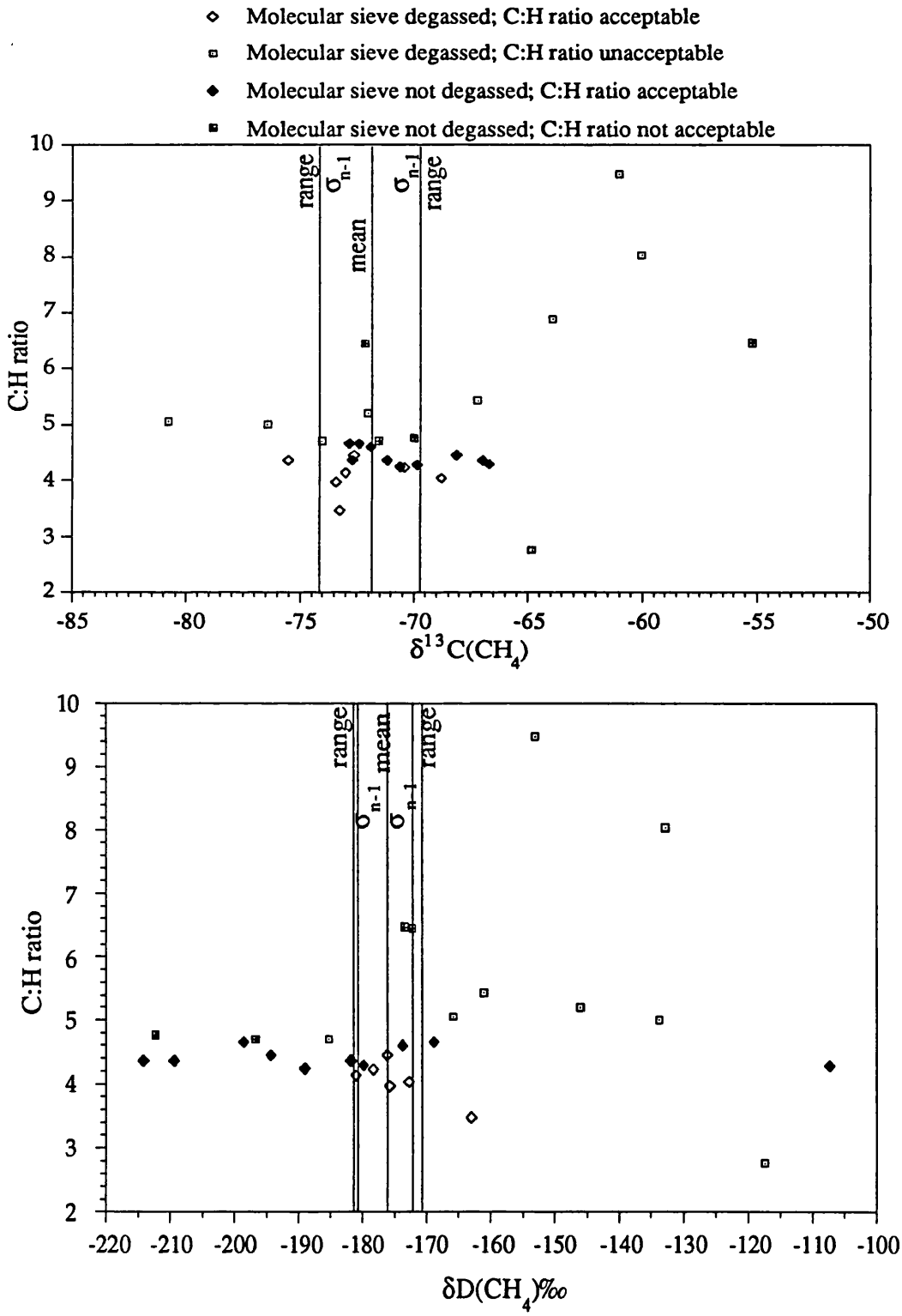
The 'line blank' was measured by processing a sample of N_2 by the normal procedure and measuring quantitatively and isotopically the CO_2 and H_2 liberated. 0.09μM of CO_2 was collected (minimum sample yield normally 15μM) with $\delta^{13}C(CH_4)$ = -25.0‰. 0.9μM of H_2 was collected (minimum sample yield for CH_4 samples was at least 30μM) with $\delta D(CH_4)$ = -42.0‰. Thermodynamic theory predicts that bonds formed by the lighter isotopes are more easily broken by those formed by heavier isotopes. It would therefore be expected that the resultant blank would be isotopically heavier than the average composition of all samples analysed, $\delta^{13}C(CH_4)$ = ~ -60‰ and $\delta D(CH_4)$ =~ -300‰.

Blanks of this size and isotopic composition cause only 0.2‰ error in $\delta^{13}C(CH_4)$ measured from a 15μM CO_2 sample (which is within error), but an 8‰ error in $\delta D(CH_4)$ for a 30μM H_2 sample, which is significant on samples this small. With the majority of samples analysed, H_2 yield was much higher, ~250μM (CO_2 yield of 125μM respectively),

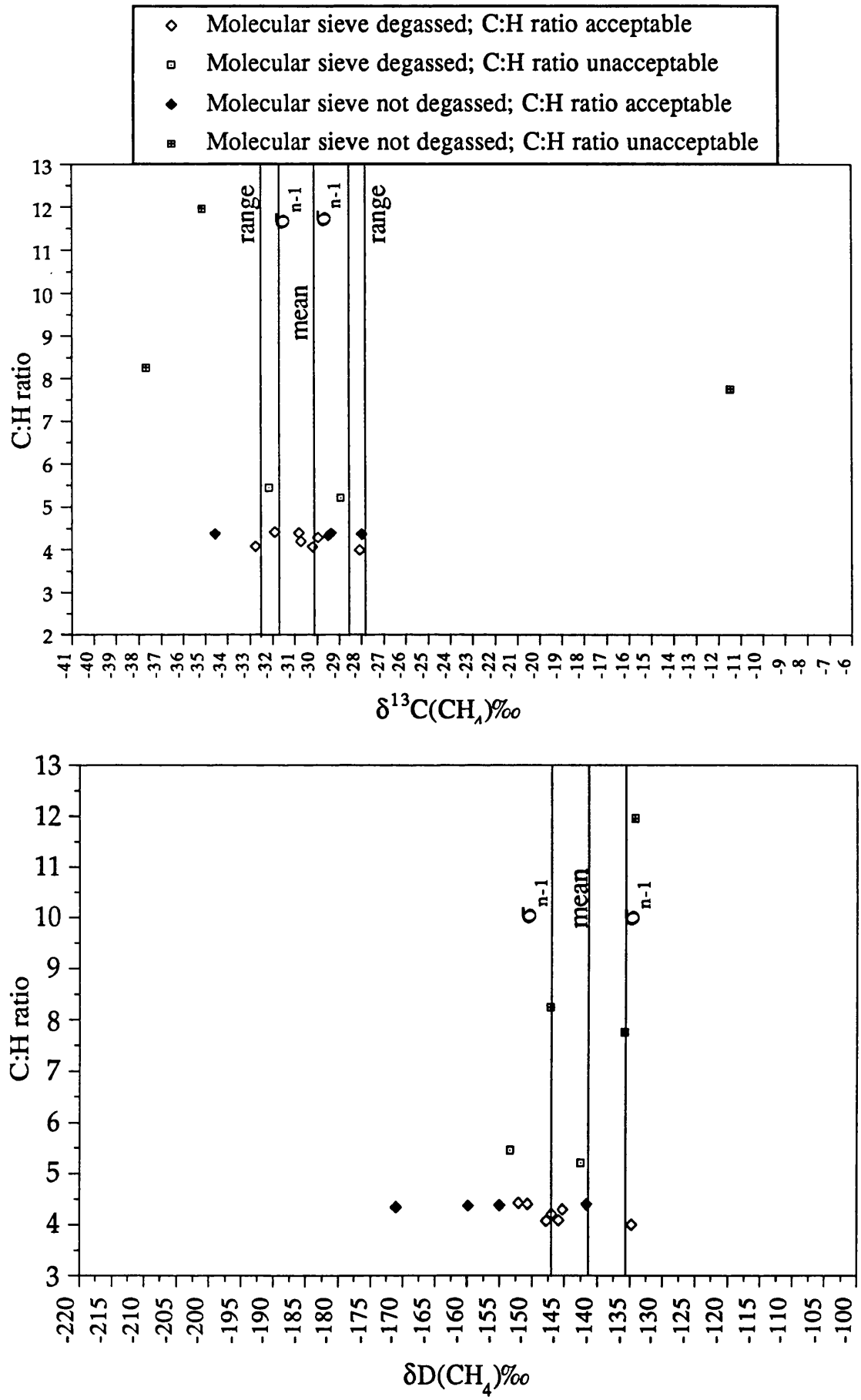
in which case a blank of 0.9μM causes an error in $\delta D(CH_4)$ of 0.9‰, which is negligible.

Degassing the uranium furnace for 5 hours yielded 0.5μM of H_2, too small a volume to measure with confidence on the mass spectrometer. This is more than half of the measured H_2 blank, which suggests that degassing of the furnace contributes significantly towards the H_2 blank. Another likely sources of H_2 may be from water adsorbed to the glass walls of the vacuum line. The most likely source of carbon in the CO_2 blank is from the CuO furnace.

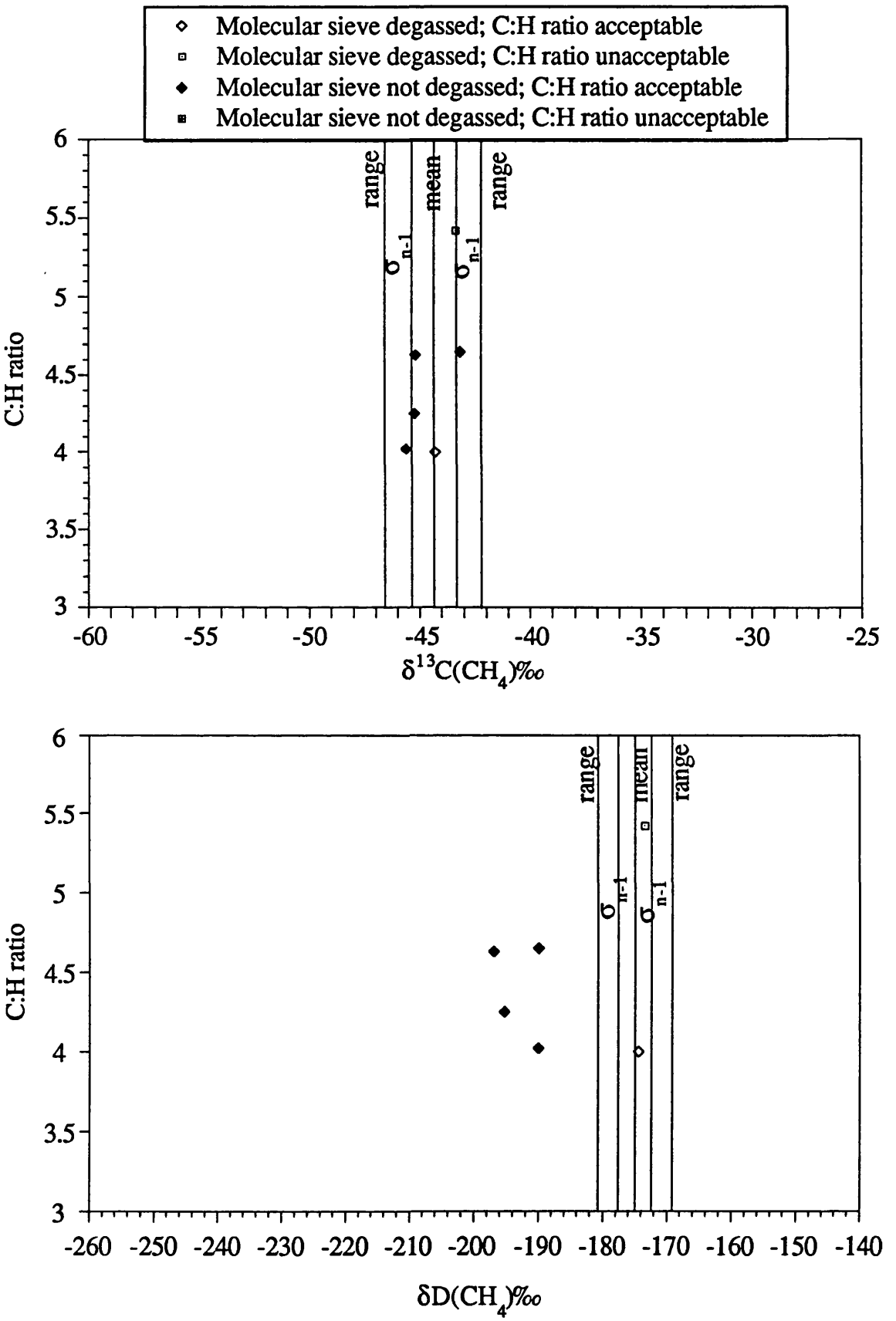
Figure 2.4a and 2.4b. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS3 as a function of C:H ratio.



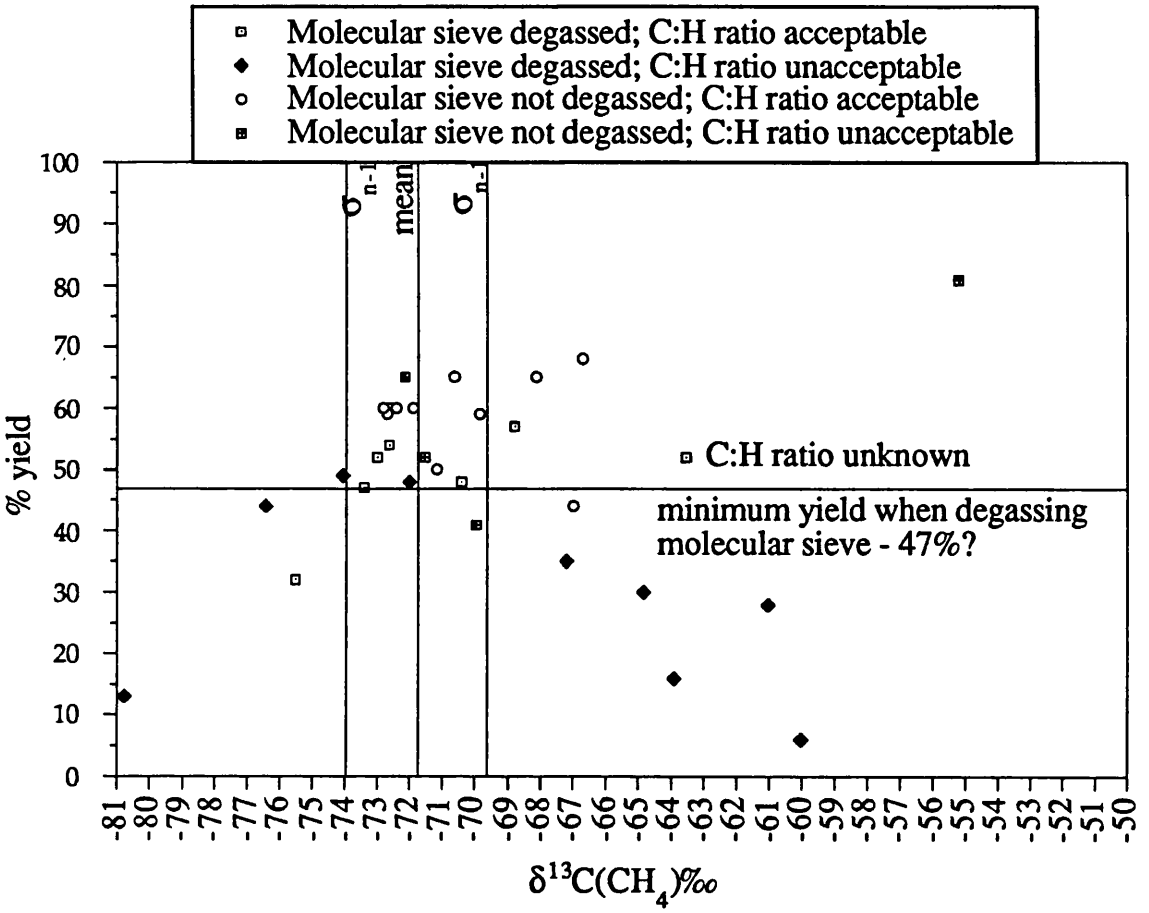
Figures. 2.4c and 2.4d. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS1 as a function of C:H ratio.

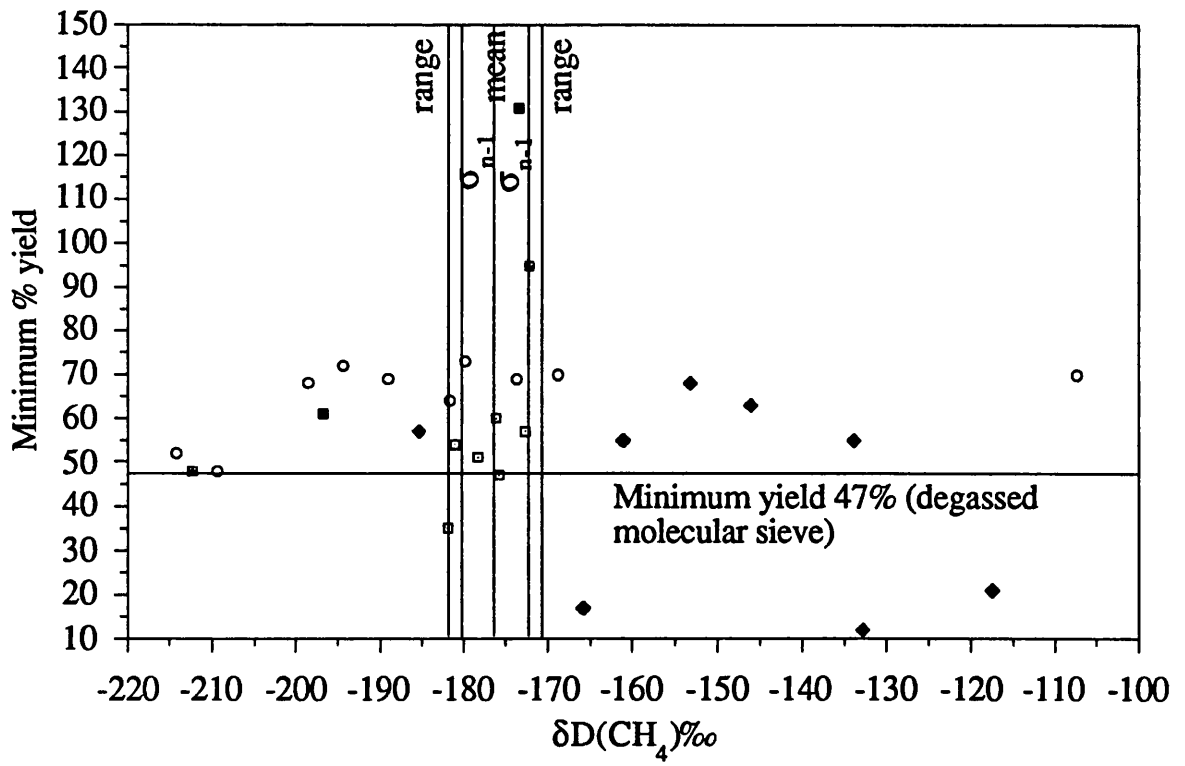


Figs. 2.4e and 2.4f. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS2 as a function of C:H ratio.

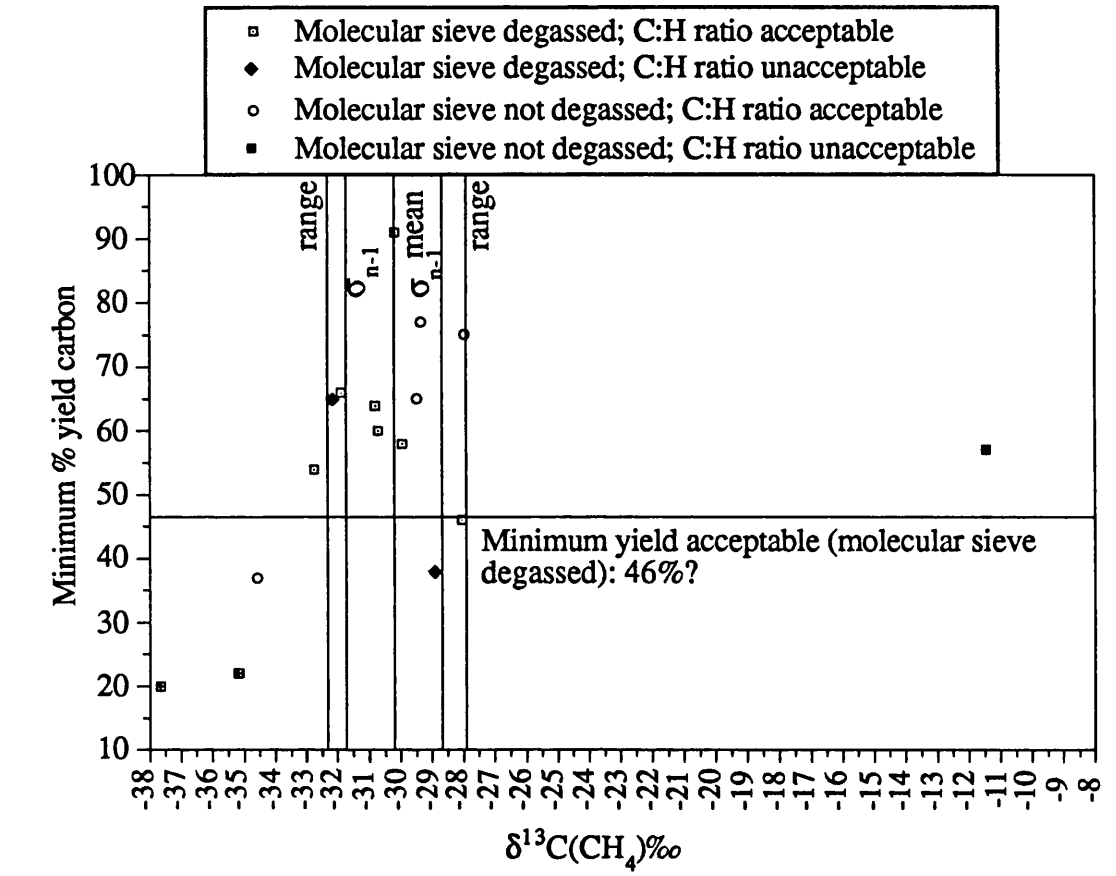


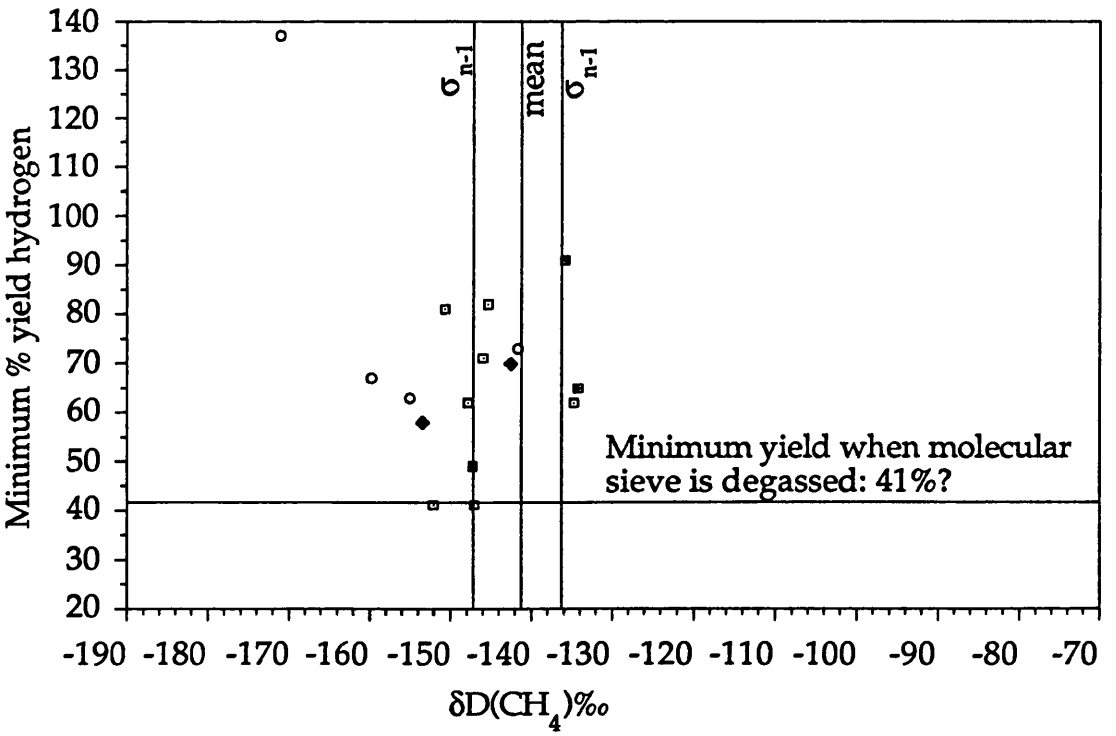
Figs. 2.5a and 2.5b. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS3 as a function of % yield.



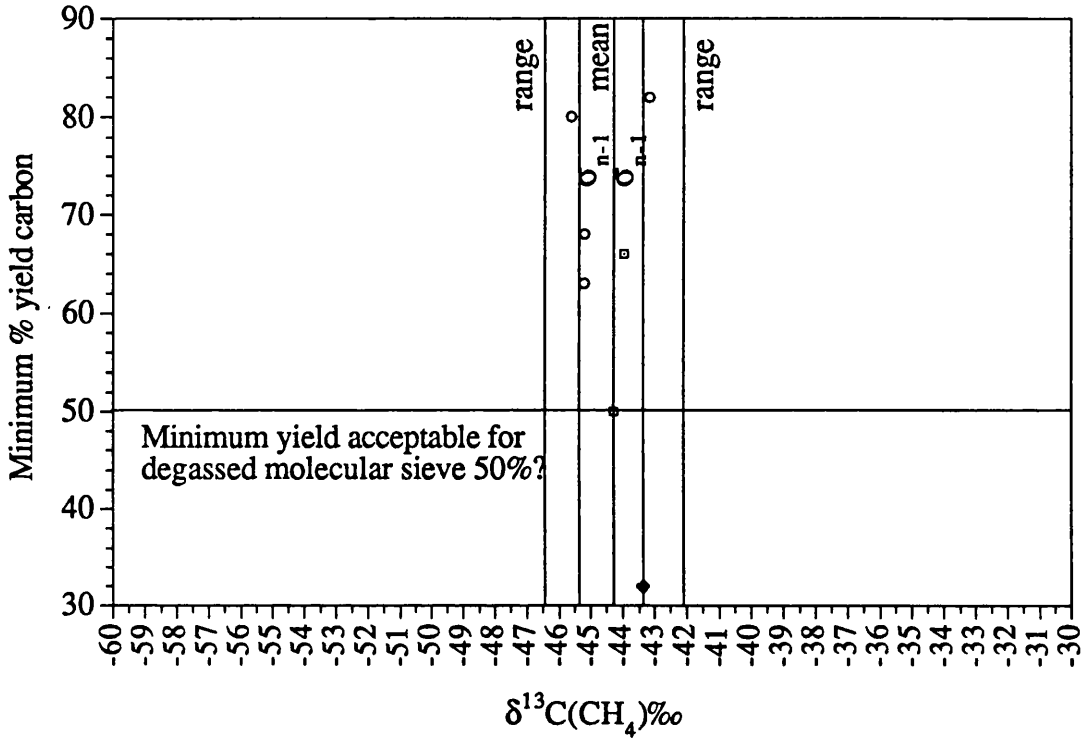


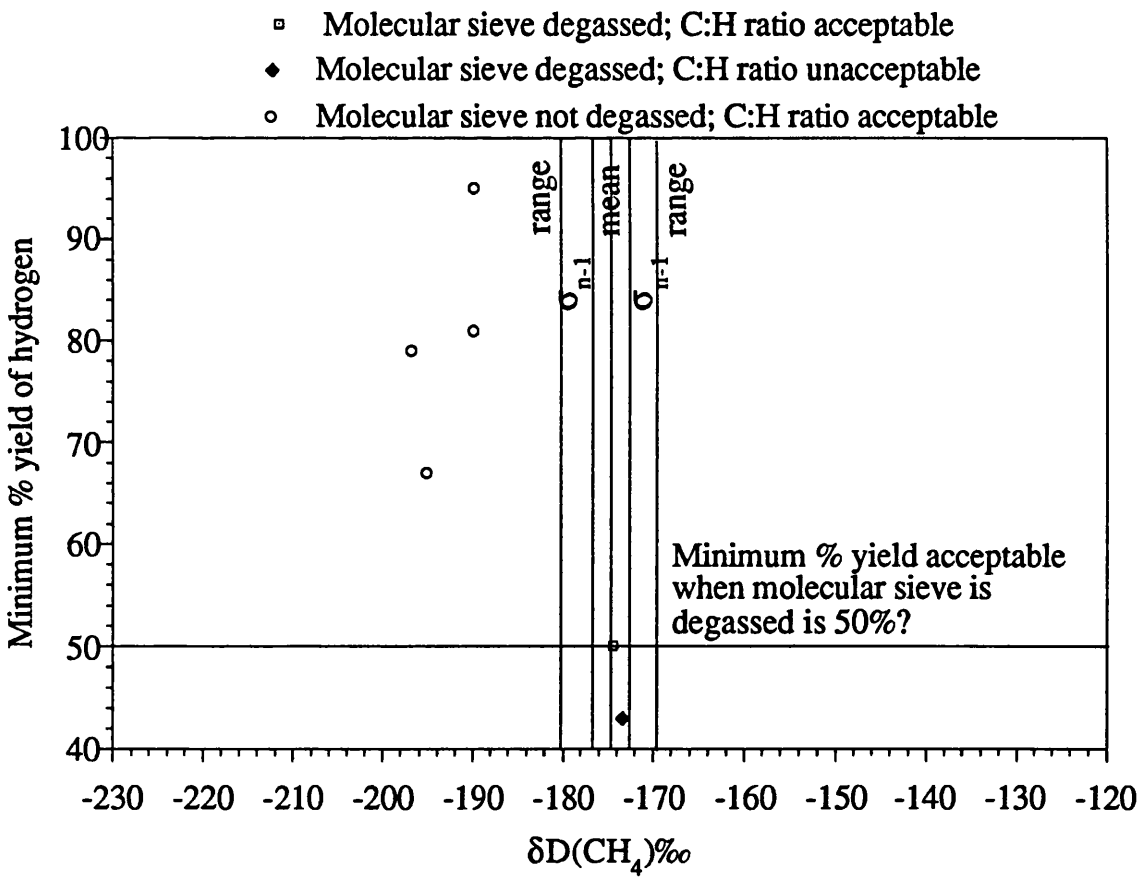
Figs. 2.5c and 2.5d. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS1 as a function of % yield.





Figs. 2.5e and 2.5f. $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for NGS2 as a function of % yield.





2.4. Hydrogen isotope analysis of waters.

All water samples to be analysed were stored in the fridge or freezer (if long term storage was required) in glass bottles, with clingfilm around the seal, in order to avoid potential fractionation by evaporation and leakage. For analysis, 5μl of each water sample was sealed in a capillary, ensuring air bubbles were not trapped within the water sample and that the ends were sealed without boiling, and thus potentially fractionating, the sample. The capillary was then placed in a breaker, attached to the line at EP2 and evacuated. The H_2O was converted to H_2 as described in section 2.2. The volume of hydrogen liberated was measured and the sample transferred to an evacuated sample bottle for δD measurement.

2.5. Carbon and hydrogen analysis of organic matter.

Conversion of organic samples to CO_2 and H_2O (subsequently reduced to hydrogen, section 2.3) for isotopic analysis is accomplished by dry combustion in an excess of oxygen. Although a variety of combustion techniques have been described, the simplest and fastest involve the combustion of individual samples in sealed, evacuated quartz tubes containing CuO as the oxygen source (Buchanan and Corcoran, 1957; Frazer and Crawford, 1963). $\delta^{13}C$ and δD of organic matter was measured using the closed combustion method of Stuermer et al. (1978). Because each sample is prepared in its own container, there is little opportunity for memory effects.

Quartz combustion tubes and a crucible of CuO, the reagent for the combustion, were heated in a muffle furnace overnight at 800°C in order to remove potential organic contaminants and to reoxidise the copper oxide. Samples were dried to constant weight prior to combustion. This was necessary a) to avoid the quartz tubes exploding during combustion due to the generation of large amounts of water vapour and b) in order to avoid contamination when δD(organic) was also to be determined.

When dry, the organic matter was ground finely where possible, using a mortar and pestle, to ensure homogeneity of sample. Before loading the combustion tube, each tube was labelled with an engraving tool. 3 mg of the sample were placed in a quartz combustion tube with an excess of copper oxide. Once loaded, combustion tubes are attached to the manifold with Cajon Ultratorr o-ring fittings, the tubes evacuated and subsequently sealed under high vacuum using a gas/oxygen torch. Goggles with shade 8/9 welding lenses were worn when sealing combustion tubes, as standard glasses or goggles are inadequate for quartz work.

Once sealed, the combustion tubes were roasted in a furnace at 850°C for a minimum of 6 hours, but usually overnight. Inside the muffle furnace, each sealed tube is placed inside a ceramic tube to shield it from the potential shock effects of an exploding tube. At this temperature the copper oxide released free oxygen which can react with the organic to produce CO_2, H_2O, nitrous oxides etc. The furnace and quartz combustion tubes were allowed to cool slowly during which any nitrous oxides formed became unstable and the cooling copper oxide reabsorbed oxygen to free the nitrogen.

After combustion, the CO_2 and H_2O must be separated from other combustion products by cryogenic distillation. The quartz combustion tube was attached to the line in a specially designed breaker at EP2 and the breaker was evacuated. The line and breaker were flamed whilst open to high vacuum to remove any traces of gases adsorbed on the glass. The vacuum taps were shut and a LN_2 dewar placed over UT1. The quartz combustion tube was broken, allowing all condensable gases (CO_2 and H_2O) to be trapped at liquid nitrogen temperature. All non-condensable gases were evacuated. The trap containing CO_2 and H_2O was warmed to -78°C with an acetone-dry ice mixture, which allowed the carbon dioxide to sublime. The volume of CO_2 was measured, and transferred to an evacuated sample bottle for mass spectrometry. Conversion of the H_2O to H_2 was undertaken as described above in section 2.3.

Engel and Maynard (1989) observed that sealed quartz combustion tubes stored for 274 days prior to analysis were depleted by more than 3‰ relative to the average NBS 22 $\delta^{13}C$ values that have been reported in the literature. Statistical analysis revealed that a linear relationship exists between the stable carbon isotope values of the NBS 22 samples and storage time subsequent to combustion (Pearson's correlation coefficient = 0.98). The solution to this problem was simply to reheat the tubes prior to analysis. Most samples analysed in this research project were analysed within 7 days of combustion, with any stored longer than 7 days simply reheated prior to analysis.

2.6 Collection of gas samples.

Gas samples were initially collected in one litre Tedlar™ gas sample bags using a vacuum method designed by Mr. L. Thomson of Strathclyde University. Unfortunately it was later discovered that such bags are not suitable for long term storage of methane samples due to diffusion of the gas through the Tedlar™ membrane. Mixtures of CH_4 in air stored in a Tedlar™ bags for a maximum of fifteen days became 5.5‰ isotopically heavier in $\delta^{13}C$ and 23‰ in δD. The isotopic composition of some samples did not change at all. This is surmised to be a function of the age of the bag. All samples were analysed as soon as possible after collecting. Any change in isotopic composition as a result of leaking from the Tedlar bags, would make little difference to the origin of gas attributed for each landfill site, for if the true composition was isotopically lighter then the landfill samples would have an even more convincing bacteriogenic signature. Gas samples for both the 'greenhouse' project and the 'bacteria' project and the remaining landfill sites samples were collected in evacuated glass bottles.

2.7. Concluding remarks.

The analytical procedure developed during this research allows accurate measurement of $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$ of a CH_4, CO_2 and air sample. The precision to which $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ can be measured is less than was desired, but considered sufficient to undertake the projects outlined in Chapters 4 and 5. One future research aim is

to improve the technique and increase the precision with which $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ can be measured. This would probably be done effectively by increasing the temperature of the CuO furnace where the CH_4 oxidation takes place, and by using an inert carrier gas to transport all the sample into the CuO furnace, ensuring 100% oxidation of the introduced sample and avoiding potential fractionation.

Development of the analytical method took one year and future funds were limited, thus a decision was made to accept the limitations of the analysis procedure and instead use the technique to investigate several aspects of isotopic biogeochemical cycle of CH_4. It is hoped the following chapters will demonstrate that in retrospect, this was a wise choice.

CHAPTER 3: ISOTOPIC STUDIES OF ATMOSPHERIC METHANE

3.1. Introduction

3.1.1. Methane's role in the climate system. Why is it of interest?

The presence of methane in the atmosphere is significant because it can exert influence over Earth's climate both directly and indirectly. The most important infra-red spectral feature of CH_4 molecules is their 7.66μm absorption band; quantitative models of the impact of methane's role in the Earth's energy budget focus on this band. The direct radiative effect of atmospheric CH_4 also extends into the stratosphere for, at altitudes above about 20km, CH_4 molecules act to cool the atmosphere through radiative losses to space (Ramanathan et al., 1985). Thus the interaction with planetary infra-red radiation warms the Earth's surface and near-surface atmosphere, and cools the stratosphere i.e. methane fulfils the role of an effective greenhouse gas. Donner and Ramanathan (1980) calculated that the presence of 1.5ppm in the atmosphere causes the globally averaged surface temperature to be about 1.3K higher than it would be with zero methane, with larger effects applying to polar latitudes.

Indirect effects of increasing atmospheric CH_4 are the chemical production of tropospheric O_3 and increases in tropospheric water vapour, both important greenhouse gases. The latter effect is not proven (Cicerone and Oremland, 1988), but it is a common and plausible assumption in climate model sensitivity with general circulation models predicting that as temperature rises atmospheric relative humidity will remain rather constant. Accordingly, atmospheric H_2O burden would increase. Tropospheric O_3 increases, that can result in the presence of NO_x (refer to section 3.2.1), can also affect climate, especially if ozone concentrations should increase in the upper troposphere where O_3 is a particularly effective greenhouse gas. Finally, even with the complete destruction of the CH_4 molecule, the atmospheric CO_2 produced is about 6% of the direct annual release from anthropogenic sources (Cicerone and Oremland, 1988).

One positive outcome caused by increased CH_4 concentrations in the atmosphere is that in the stratosphere, methane scavenges chlorine atoms, thus helping prevent the destruction of the ozone layer by chlorofluorocarbons and other chlorine containing gases (Cicerone and Oremland, 1988).

3.1.2. Atmospheric methane concentrations

More than a decade ago, automated measurements from Cape Meares on the Oregon Coast (representative of methane concentrations in the northern latitudes from 30°N to 90°N) established that methane was increasing in the atmosphere at a rapid rate (Rasmussen and Khalil, 1981). Between 1979 and 1992, 120,000 measurements of the concentration of atmospheric methane were made. The Cape Meares data have been used in many subsequent analyses of the trends and budgets of methane (Khalil and Rasmussen 1983, 1990). The average concentration during the experiment was 1698 parts per billion by volume (ppbv) (Khalil et al., 1993). Methane concentration increased by 190ppbv (or

11.9%) during the 13 year span of the experiment. The rate of increase in the first two years was about 20±4 ppbv/yr but in the last two years of the experiment was 10±2ppbv/yr, suggesting a substantial decline in trend at northern middle and high latitudes (-1ppbv/yr^2).

Prominent seasonal cycles were observed. During each year, the CH_4 concentration stayed almost constant until May, before falling and reaching the lowest levels in July and August, and then rising rapidly to nearly maximum concentrations in October. The average amplitude of this cycle was about 30±7ppbv and this has increased during the course of the experiment. Interannual variations with small amplitudes of 2-3ppbv occurred with a period of 1.4 and 6.5 years. Mass balance calculations showed that to explain the observed seasonality of concentrations, the emissions must have peaked in late summer and early autumn (August-September).

3.1.3. Decline in the growth rate of atmospheric methane.

Global measurements of atmospheric methane have revealed a sharp decrease in the growth rate in the Northern hemisphere during 1992 (Dlugokencky et al., 1994). The average increase for the Northern hemisphere during 1983-1991 was 11.6±0.2 ppbv/yr, but the increase in 1992 was only 1.8±1.6 ppbv/yr. In the Southern hemisphere, the average increase during 1983-1991 was 11.1±0.2ppbv/yr, and the 1992 increase was 7.7±1.0 ppbv/yr. Determining the reasons for this decrease is difficult, but possible explanations are: increased atmospheric hydroxyl radical (OH), which increases the sink for CH_4 (Prinn et al., 1992); changes in the size of fossil fuel source of CH_4 (Dlugokencky et al., 1994); and a slowing in the growth of some CH_4 sources such as rice agriculture and cattle production (Khalil and Rasmussen 1993).

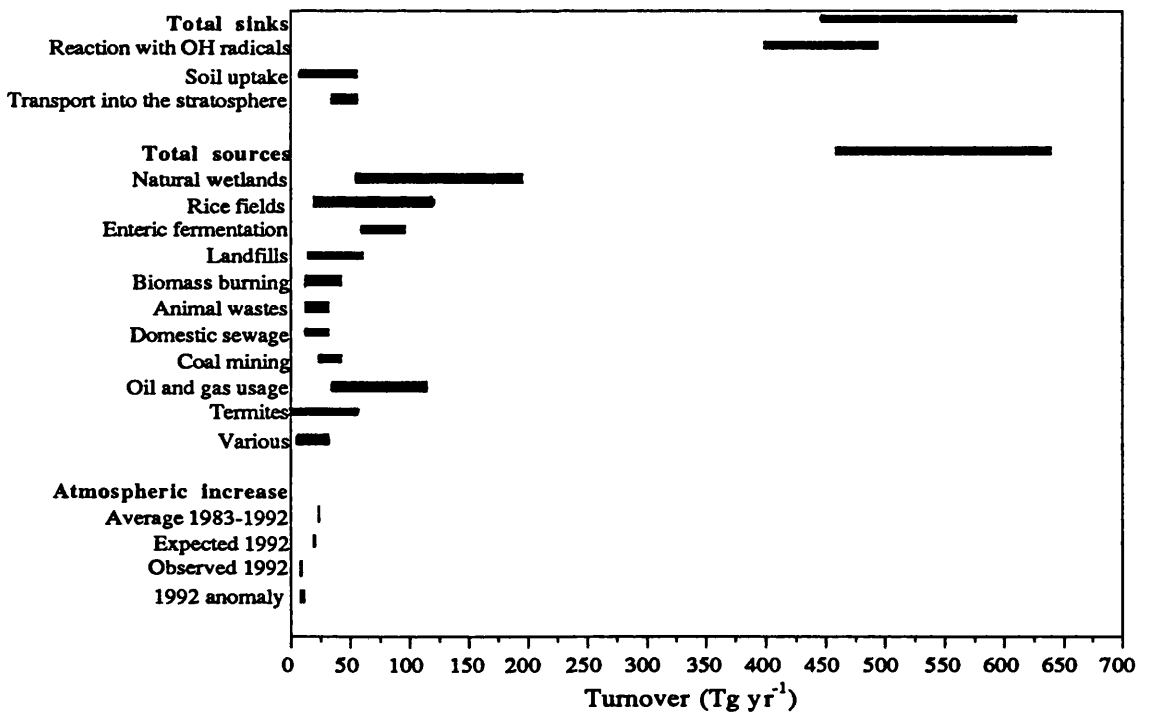


Figure 3.1. Sources and sinks of atmospheric CH_4 flux. The horizontal bars indicate the estimated range of uncertainty. A comparison of the 1992 CH_4 increase with the tropospheric CH_4 budget is included. From Rudolph (1994).

Figure 3.1. shows the sources and sinks of atmospheric CH_4 flux. The horizontal bars indicate the range of uncertainty. The CH_4 growth rate is determined by the imbalance between CH_4 sources and sinks. Since the source / sink imbalance is relatively small, about 31Tg CH_4 yr^{-1} or ~ 6% of the global source (~510 Tg CH_4 yr^{-1} based on a global annual mean of 1714ppbv in 1992 and a CH_4 lifetime of 9.9 years [Prinn et al., 1992]), a small decrease in the source or increase in the sink could lead to the observed result. The largest sources in the global budget are each estimated to be on the order of 80 to 100 Tg CH_4 yr^{1} (e.g. natural wetlands, rice production, fossil fuel sources, oil and gas production and enteric fermentation), so this represents a change of only 20% to one of these source groups, or alternatively, a 2% change to the CH_4 sink (~480 Tg CH_4 yr^{-1}; determined using measurements of methyl chloroform to estimate the globally averaged $OH^{\cdot}$ concentration [Prinn et al., 1992] and the rate coefficient for the reaction of $OH^{\cdot}$ with CH_4 [Vaghjiani and Ravishankara, 1991]).

Dlugokencky et al (1994) suggest several reasons for the decreased growth rate in 1992. The eruption of Mt. Pinatubo in June 1991 resulted in a decrease in surface air temperature (lower troposphere) of 0.7°C in the Northern hemisphere in 1992 (Dutton and Christy, 1992). Based on the temperature dependence of wetland emission (Fung et al, 1991) this temperature anomaly could have decreased CH_4 fluxes from northern wetlands by ~5% or ~2Tg CH_4 (approximately 6% of the source / sink imbalance). Changes in temperature and precipitation patterns between CH_4 emissions and other ecological parameters make it difficult to determine what effect the eruption of Mt. Pinatubo had on net CH_4 emissions from natural wetlands in the northern hemisphere; it is unlikely that this factor alone could explain the decrease in growth rate. Furthermore, a change in tropospheric temperature would also change the rate at which methane is removed in the troposphere (Rudolph, 1994). From the temperature dependence of the reaction rate constant for CH_4 with OH (Vaghjiana and Ravishankara, 1991), it can be calculated that a decrease in 0.5°K would actually reduce the chemical methane sink by about 1%, which corresponds to an increase of 4Tg per year provided the OH radical concentration does not change (Rudolph, 1994). Thus an even larger anomaly in methane flux for 1992 than measured may have been expected.

Unfortunately very little is known about changes of tropospheric OH radical concentrations in 1992. As the removal by this reaction is by far the largest single item in the global methane budget, minor changes in the average tropospheric OH radical concentration could easily explain the reduced methane growth rate. A significant decrease in stratospheric ozone was observed in 1992 (Gleason et al., 1993), the net effect of which can lead to an increase in tropospheric OH and increased CH_4 destruction (Dlugokencky et al., 1994).

Approximately one-third of the world's natural gas reserves are contained in the West Siberian Basin (Grace and Hart, 1986). The rapid economic changes in the Former Soviet Union and Eastern Europe could have produced great changes in the methane emissions

from fossil fuel use. The decrease in CH_4 growth rate may be, in part, due to the decreased CH_4 emissions in the FSU and Eastern Europe resulting from natural gas production level stabilisation and repair of major leaks in pipelines, combined with a reduction in global coal production. The reduction potential of 13-29 Tg/yr from these sources is enough to explain a decrease of 13-29 ppbv/yr, roughly twice the observed anomaly (Dlugokencky et al., 1994). However Rudolph (1994) believes that it is unrealistic that a remedial programme, initiated after an explosion in 1989, may have had a big enough impact by 1991 to reduce leakage by the extent necessary to impact the CH_4 growth rate.

Dlugokencky *et al.* (1994) believe that their measurements are evidence that, due to a relatively small source / sink imbalance for atmospheric CH_4, modest decreases in anthropogenic CH_4 emissions can lead to rapid stabilisation of, or a decrease in, the atmospheric burden of CH_4. Rudolph (1994) believes that while several sources that might have been lower in 1992 can be identified, the evidence for the cause being a reduction in Eastern Block emissions is not conclusive. Rather it was considered significant that the atmospheric carbon dioxide record at Mauna Loa monitoring station also showed an anomalously low increase in 1992. This may be coincidence, but it may indicate changes in factors that influence atmospheric methane and carbon dioxide behave in the same way.

Dlugokencky *et al.* suggest that stable carbon isotope measurements may provide the best means for determining the cause of the observed decrease in CH_4 growth rate. The overall objective of isotopic studies of an atmospheric trace gas, such as CH_4, is to establish constraints on the relative fluxes of isotopically distinct natural and anthropogenic sources. The mass-balanced global flux isotopic composition should correspond to the isotopic composition of the gas in the atmosphere, once fractionation associated with atmospheric loss processes has been accounted for (Stevens and Engelkemeir, 1988). In this chapter, the application of stable isotope analyses to trace gas budgets will be demonstrated by consideration of United Kingdom and global CH_4 budgets. The influence that changes in a given flux source will have on the isotopic composition of atmospheric CH_4 will be demonstrated. To understand the kinetic isotope effects associated with the oxidation of methane in the atmosphere, an overview of the chemical processes responsible for atmospheric CH_4 oxidation will be first provided.

3.2. The chemistry of atmospheric methane.

3.2.1. The destruction of methane in the atmosphere.

The chemical reactions that destructively oxidise atmospheric CH_4 affect the chemical state of the atmosphere through the products of the reactions and through consumption of the reactant species. Methane oxidation produces CO, CO_2, H_2O, H_2 and CH_2O and it consumes OH. The reaction pathways below affect tropospheric and stratospheric ozone amounts, and they produce important quantities of H_2O in the stratosphere. Also stratospheric CH_4 reacts with Cl atoms, forming HCl, a reservoir species for Cl atoms. Finally a portion of hydrogen carried upwards into the stratosphere as CH_4 escapes to

space, mostly as H atoms (Cicerone and Oremland, 1988).

Complete oxidation of CH_4 yields CO_2 and H_2O. Schematically this can be represented by the combustion of CH_4:

$$CH_4 + 2O_2 \longrightarrow 2CO_2 + 2H_2O \qquad 3.1$$

While this seems simple and clear enough, it does not describe the mechanism through which the atmosphere oxidises CH_4. In the atmosphere, the process is initiated by OH radicals, not by O_2, and it requires light.

The single biggest sink of atmospheric CH_4 arises from the destruction of CH_4 in the troposphere by the gas phase hydroxyl radical, OH, a key radical in atmospheric photochemistry.

$$CH_4 + HO^{\bullet} \longrightarrow H_2O + H_3C^{\bullet} \qquad 3.2$$

Perhaps 85% of the CH_4 that is emitted into the atmosphere is destroyed by this reaction in the troposphere (Cicerone and Oremland, 1988). In the stratosphere, almost all the remaining CH_4 is destroyed by OH, by Cl atoms and by oxygen atoms, generally produced by the photo-chemical dissociation of NO_2, or of O_3:

$$CH_4 + O \longrightarrow H_3C^{\bullet} + HO^{\bullet} \qquad 3.3$$

The methyl radical produced reacts rapidly with molecular oxygen to form very reactive peroxyl radicals (in this case methoxyl) which participate in a number of chain reactions (Manahan, 1990):

$$H_3C^{\bullet} + O_2 + M \longrightarrow H_3COO^{\bullet} + M \qquad 3.4$$

The following are additional reactions that are also involved in the overall oxidation of CH_4:

$$H_3COO^{\bullet} + NO \longrightarrow H_3CO^{\bullet} + NO_2 \qquad 3.5$$

$$H_3CO^{\bullet} + O_3 \longrightarrow \text{various products} \qquad 3.6$$

$$H_3CO^{\bullet} + O_2 \longrightarrow CH_2O + HOO^{\bullet} \qquad 3.7$$

$$H_3COO^{\bullet} + NO_2 + M \longrightarrow CH_3OONO_2 + M \qquad 3.8$$

$$H_2CO + h\nu \longrightarrow \text{photodissociation product} \qquad 3.9$$

3.2.3. Destruction of CH_4 in high NO_x concentrations.

It is also known (Cicerone and Oremland, 1988) that the mechanism of CH_4 oxidation and the products formed are very different in the two cases of high concentrations of nitrogen oxides (NO_x) and low NO_x concentrations. In NO_x rich air columns, found in polluted or moderately dirty tropospheric air and all of the stratosphere, CH_4 oxidation produces ozone and hydrogen oxides. The process first involves the production of formaldehyde (CH_2O) :

$$\textit{Net reaction}: CH_4 + 4O_2 + 2h\nu \longrightarrow CH_2O + H_2O + 2O_3 \qquad 3.10$$

All reactions constituting each given net reaction can be found in Cicerone and Oremland (1988). Formaldehyde is then oxidised to CO by either:

$$\textit{Net reaction}: CH_2O + 4O_2 + 3h\nu \longrightarrow CO + 2O_3 + 2OH \qquad 3.11$$

$$\text{or } \textit{Net reaction}: CH_2O + 2O_2 + h\nu \longrightarrow CO + H_2O + O_3 \qquad 3.12$$

The final step, still assumed to occur in the presence of high NO_x concentrations, is the oxidation of CO to CO_2:

$$\textit{Net reaction: } CO + 2O_2 + h\nu \longrightarrow CO_2 + O_3 \qquad 3.13$$

Thus the complete oxidation of CH_4 in the presence of adequate NO_x produces O_3 and depending on the relative fractions of CH_2O oxidised, can produce OH radicals.

For formaldehyde to form from the partial oxidation of CH_4, and then carbon monoxide from the formaldehyde, there must be enough NO present for HO_2 to react preferentially with NO rather than with O_3, and for CH_3O_2 (an intermediate in the partial oxidation of CH_4 to formaldehyde) to react preferentially with NO rather than with HO_2. Present rate data and model results indicate that NO mole fractions must exceed 5 to 10ppt for this to occur (Cicerone and Oremland, 1988).

3.2.3. Destruction of CH_4 in low NO_x concentrations.

In large fractions of the troposphere, NO mole fractions are probably 10ppt or less, especially in the altitude range of 0 to 6km (Cicerone and Oremland, 1988 and references therein). Under these circumstances CH_4 oxidation consumes ozone and it consumes HO_x species (OH and HO_2) species in producing CO_2, H_2O and H_2.

A potentially very important consequence of CH_4 oxidation is that of CH_4, CO and OH concentration perturbations. Because OH is a major sink for atmospheric CH_4 and CO and because these same reactions of OH with CH_4 and CO suppress OH concentrations, there is in principle an instability in the system (Cicerone and Oremland, 1988). Increases in atmospheric CO or CH_4 concentrations can lead to decreases in OH concentrations thereby further increasing CO or CH_4 perturbations. In the context of atmospheric CH_4 it is possible that OH decreases with time are responsible in part for the temporal increase of CH_4 concentrations (Cicerone, 1988)

3.2.4. An overview of isotopic fractionation of atmospheric methane.

Kinetic isotope fractionation plays an important role in the quantitative analysis, by isotopic studies, of the cycle of CH_4 (Stevens and Wagner, 1989). The relationship between the composition of the gas in the atmosphere and the average value of the sources is determined by the fractionation effect of the scavenging processes, particularly the reaction with OH. Thus, knowledge of the fractionation effect is essential to determining the relative distribution of fluxes from isotopically distinct sources. A comprehensive outline of the fractionation effects in atmospheric chemistry that effect methane are documented by Steven and Wagner (1989):

The isotopic composition of an atmospheric trace gas can be significantly different from the average composition of the source fluxes as a result of isotope fractionation in the scavenging mechanisms. This is described by the mass balance equations for two isotopes of mass *a* and *b*, as follows:

$$P_a = N_a \lambda_a \quad 3.14$$

$$P_b = N_b \lambda_b \quad 3.15$$

where

P represents fluxes to the atmosphere

N represents the steady state content (proportional to concentration) in the atmosphere

λ represents the removal rate.

In this study a and b represents the isotopes of C and H. The ratio of the isotopic species in the atmosphere, $R_a = N_a / N_b$, is then

$$R_a = R_S (\lambda_b / \lambda_a) \quad 3.16$$

where $R_S = P_a / P_b$, which is the isotopic ratio of the source fluxes.

For gases scavenged only by reaction with OH radicals, the relative rates of removal are equal to the relative rate constants of the reaction, $k_b/k_a = \alpha$, the kinetic isotope fractionation effect (KIE). The quantity $(\alpha\text{-}1)10^3$ expressed in per mil will be used frequently in this chapter as the KIE. The isotopic signature of the total flux can be expressed as:

$$\delta_S = \delta_A + (\alpha\text{-}1)(1 + [\delta_A \times 10^{-3}])10^3 \quad 3.17$$

$$\delta(\text{source fluxes}) = \delta(\text{atmospheric}) + \text{KIE } (R_A/R_o) \quad 3.18$$

where δ_x is defined as $\delta_x = ([R_x/ R_o]\text{-}1)10^3$ and R_o is the isotopic ratio of an arbitrary standard.

Where a gas undergoes a series of reactions, the isotopic composition for an element at any intermediate state, compared to that of the initial source, is determined only by the KIE of the loss reaction involving that state; the KIE of the intermediate reactions are cancelled out providing there are no competing mechanisms (Stevens and Wagner, 1989). For example, in the case of atmospheric methane, which goes through several reactions leading to CO, the carbon isotope composition of the CO product is

$$\delta_{CO} = \delta_S - (\alpha_{CO} - 1)10^3 \quad 3.19$$

and

$$\delta_{CO} = \delta_{CH_4} + (\alpha_{CH_4} - 1)10^3 - (\alpha_{CO} - 1)10^3 \quad 3.20$$

where α_{CH_4} and α_{CO} are the ratios of the reaction rates of ^{13}C and ^{12}C with OH and CH_4 and CO respectively (Stevens and Wagner, 1989).

In the cases of atmospheric CO and CH_4, the source fluxes are a mixture of natural and anthropogenic emissions of different isotopic compositions. The average isotopic composition of the sources, determined from the isotopic composition measured from gas in the atmosphere for the above relation, provides constraints on the relative distribution of the fluxes of the isotopically different sources, with the fractionation effect (KIE) of the removal mechanism being a key factor in the determination.

The measured KIE values for carbon and hydrogen in the reaction CH_4 + OH are listed in Table 3.1.

Isotopic species	α	Reference
$^{13}CH_4$	0.997±0.001	Rust and Stevens (1980)
	0.990±0.003	Davidson et al. (1987)
	0.9946±0.0009	Cantrell et al. (1990)
CH_3D	0.67	Gordon and Mulac (1975)
CH_2D	0.54	Gordon and Mulac (1975)
CHD_3	0.20	Gordon and Mulac (1975)
CD_4	0.09	Gordon and Mulac (1975)

Table 3.1. Measured kinetic isotope effect values for carbon and hydrogen in the reaction CH_4 + OH. From Stevens and Wagner (1989).

The higher KIE value of 0.990 measured by Davidson et al. (1987) for $^{13}CH_4$, is considered more accurate than that measured by Rust and Stevens, due to a superior experimental method, which produced higher OH concentrations by photolysis of ozone, reacting larger fractions of CH_4, producing consequently greater changes $\delta^{13}C(CH_4)$ of the unreacted CH_4. However the most accurate kinetic isotope effect value is considered to be that determined by Cantrell et al (1990), which has an uncertainty an order of magnitude smaller than that measured by Davidson et al. (1987), thus this will be the KIE value used in any calculations of $\delta^{13}C(CH_4)$.

3.3. Reassessing the UK methane budget. Bigger is not better!

Under the global Convention on Climate Change, signed at the 1992 Earth Summit, the United Kingdom pledged to return its emissions of each greenhouse gas to 1990 levels by the year 2000. Strangely, the official inventory of CH_4 emissions has been subject to bewildering changes over the last few years. In 1991, the estimated United Kingdom total CH_4 emissions for 1989 was 3450kt. In 1992, this figure was revised upwards to 4420 kt, however in 1993 (one year after signing the global Convention on Climate Change), total CH_4 emissions for 1989 had reverted to the lowest estimate yet of 3440kt (ENDS, 1993)!.

The Watt Committee on Energy, an independent organisation and a registered charity, was founded in 1976 by the professional institutions to disseminate knowledge and promote independent discussions of questions concerning all aspects of energy. Their sponsors include all major private electricity companies, the British Coal Corporation, British Gas and the Environmental Division of the Department of Trade and Industry. In 1991, The Watt Committee on Energy was commissioned by the former Department of Energy (now merged with the Dept. of Trade and Industry) to undertake a two year study on methane emissions, with the 1990 United Kingdom CH_4 budget published recently (Tiger Eye, 1992). Department of the Environment (DoE) estimates of trace gas emissions

are published each year by the Governmental Statistical Service. The United Kingdom CH_4 budget for 1992 has recently become available (HMSO, 1994) and is given in Table 3.2.

Accurate flux measurements are necessary to calculate the isotopic signature of atmospheric CH_4 accurately, thus it seemed wise to check the 1992 DoE budget with independent sources where possible, particularly as some sources considered in Watt Committee assessments are not included in the DoE 1992 CH_4 budget e.g. peat wetlands and seepages/oceans. Each source listed in Table 3.2. and additional sources included in other estimations by the Watt Committe are considered in order below, with the revised United Kingdom budget given at the end of section 3.3.

Source	Emission CH_4 kt/yr^{-1}	Emission CH_4 g/yr^{-1}	Emission CH_4 moles/yr^{-1}
Energy production	1226	1.226×10^{12}	7.642×10^{10}
Landfill generation	1919	1.919×10^{12}	1.196×10^{11}
Other waste treatment and disposal.	72	7.200×10^{10}	4.488×10^{9}
Agriculture	1517	1.517×10^{12}	9.456×10^{10}
Total	4734	4.734×10^{12}	2.951×10^{11}
Additional sources considered by the Watt Committee:			
Peat and Wetlands	100	1.00×10^{11}	6.23×10^{9}
Forests / land use biomass	2	2.00×10^{9}	1.25×10^{8}
Seepages / Ocean	5	5.00×10^{9}	3.11×10^{8}
Total	4841	4.841×10^{12}	3.02×10^{11}

Table 3.2. Department of the Environment methane budget for 1992. (HMSO, 1994)

3.3.1. Energy Production

The major sources of energy production are oil and gas production and coal production. Table 3.3. gives the total amount of coal and gas extracted during 1992 (HMSO 1993).

Amount of coal mined (kt)	65800 (deep mined)	18567 (open cast)	84874 *
Amount of CH_4 released (kt)	942.468 (deep mined)	166.212 (open cast)	1108.68
Natural Gas recovered	597854 Gw/hrs#		
Amount of CH_4 released (kt)	1241.11kt		
Total CH_4 released from energy production	2349.79kt		

* The total is not equal to the sum of deep mined and opencast coal as an allowance of 507 kt is made for CH_4 released from the slurry.

The conversion from Gw/hrs to kt is given in Appendix 2.

Table 3.3. Amount of coal and gas mined during 1992.

It is proposed that using natural gas rather than coal in power stations will lead to 0.8 to 3.0 times the emissions of greenhouse gases (Wallis 1990), but this is controversial (James, 1990). According to British Coal, 12.5 m^3t^{-1} of CH_4 is released for coal mined, however Wallis (1990) believes that this is higher with 20 m^3t^{-1} of CH_4 released from deep mined coal. If it is assumed that 12.5m^3t^{-1} of CH_4 is released for opencast mined coal and 20 m^3t^{-1} of CH_4 released from deep mined coal then for 1992, 1108.68 kt of CH_4 was released. This is close to the Department of the Environment figure of 1226 kt of CH_4 but gas leakage during natural gas production and extraction has yet to be considered. 4137.04 kt of natural gas was recovered during 1992. Assuming, an upper limit for leakage of 3% (James, 1990), which is again a controversial figure (Wallis, 1990), then 1241.11 kt of CH_4 is released during natural gas recovery.

Thus the total CH_4 released from energy production during 1992, assessed here by independent means is 2349.79kt, which is more than 1.9 times the volume of CH_4 released from energy production given by the Department of the Environment.

3.3.2. Landfill site emissions.

The Department of the Environment estimate 1919 kt CH_4 yr^{-1} is emitted to the atmosphere from landfill sites. The Environmental Resources Ltd. (ERL) report for the governmental Energy Technology Support Unit (ETSU) studied 75 of the 453 largest landfills in England and Wales and provided the basis for the 1992 Digest of Environmental Protection and Water Statistics (DEPWS) estimate of 1919kt CH_4 (Wallis, 1993). The ERL study was motivated towards estimating the exploitable resource of landfill gas, and thus its suitability for estimating total atmospheric emissions (Aitchison, 1993) from landfill is questionable. It was based on the lower limit of 1.4 Mt/yr. Numerous smaller landfill sites containing biodegradable waste were ignored, a 12.5% reduction was assumed to result from oxidation in surface soils, and capture and combustion of the gas from sites collecting CH_4 (20% of the 453) was assumed 100% effective.

Although ERL calculations give an average of 55% CH_4 capture at existing sites, the individual values range from ~20% to over 100% and so are unreliable (Wallis, 1993). Entrapment and collection technology is at an early stage and relatively inefficient; pipework and valves to flare systems leak; flares themselves allow as much as 15% of the gas unburned (burning at less than optimum levels with fluctuating flow; perhaps 5% leaks on average) (Wallis, 1993). An UK study (AFRC 1988) gave actual capture rates as under 50%. Thus at least 20% of the methane escapes, inclusive of leakage in the collection system and slippage through the flare (Wallis, 1993).

Grossing up the ERL value to include Scotland and N.Ireland and taking into account CH_4 oxidation and the volume of gas flared and utilised Wallis (1993) calculates that > 1600 - 3000 kt CH_4 yr^{-1} is emitted from the United Kingdom. The Department of the Environment figure of 1919 kt CH_4 yr^{-1} is towards the lower end of the scale. Furthermore Environment Minister, David Maclean, acknowledged that emissions of CH_4 from landfills

may be up to five times higher than the figure currently incorporated in the Government's published environmental statistics (Maclean, 1993)

3.3.3. Ruminant emissions (agriculture).

Ruminant animals which include cattle, sheep, buffalo and some wild animals, produce the highest concentrations of CH_4, while smaller concentrations arise from non-ruminant animals such as pigs, deer, horses and camels. Both domesticated and wild animals contribute to the global CH_4 budget, although animals reared as livestock are by far the largest source.

CH_4 is a byproduct of the anaerobic microbial breakdown of carbohydrates, mainly cellulose, in the digestive tracts of herbivores, and the energy required to generate CH_4 may be expressed as a percentage of the gross energy intake consumed when feeding. In general the CH_4 yields were found to depend upon the variety of animal species, the quality (digestibility) of the feed and the feeding level (relative to the maintenance level). On average the CH_4 yield from ruminants varies between 5-9% of the gross energy intake, while non-ruminant animals produce considerably less CH_4, with values in the range of 0.5-3% of gross energy intake (Crutzen et al., 1986). Gases produced during the rumen fermentation are vented to the atmosphere by the animal's belching and typically contain 30-40% CH_4, with the rest composed of CO_2 and traces of N_2, H_2S and H_2 (Cicerone and Oremland, 1988). Gas eructation rates for cows can be as high as 20l/min at 30 minutes after feeding, but decline to 5-10l/min by 4 hours after feeding (Cicerone and Oremland, 1988). Most of the CH_4 formed in the rumen is from the reduction of CO_2, because fatty acids are unavailable to the rumen bacteria. In contrast only one-third of the adult human population has an active flora of methanogenic bacteria present in the colon (Wolin, 1981).

Cattle represent the largest worldwide source of enteric CH_4, thus calculations were carried out for different age classes, cattle type (dairy, beef or range) and feeding levels to give annual CH_4 production rates of 55kg CH_4 yr^{-1} per animal for cattle in the developed world and 35kg CH_4 yr^{-1} per animal in the developing countries (Crutzen et al., 1986). Similar analyses for sheep resulted in yields of 8 and 5kg CH_4 yr^{-1} per sheep in developed and developing countries respectively. Cattle, sheep and buffalo account for approximately 91% of all CH_4 emitted from animals (Anastasi and Simpson, 1993). There are no buffalo in the United Kingdom, thus cattle and sheep only have been considered in this comparative estimate.

Table 3.4. gives important CH_4 producing animal populations. Data for the number of cattle in the United Kingdom was supplied by the Animal Data Centre in Hertfordshire (MMB, 1993), while data for the number of sheep, goats, pigs and deer in Britain and the United Kingdom was supplied by the Ministry of Agriculture (MAFF, 1994). Cattle under 6 months and lambs under 1 year old have not been included in the calculations. The volume of CH_4 emitted per group has been calculated using the figures measured by Crutzen *et al.* (1986) where possible and personal interpretation of likely values for unknown species.

CH_4 yields were found to depend upon the variety of animal species, the quality (digestibility) of the feed and the feeding level (relative to the maintenance level). Adult pigs are likely to consume a similar quantity of feed as ruminant cattle, but as they are not ruminants, produce less CH_4 yr^{-1} each. Thus the volume of CH_4 yr^{-1} produced by each pig, can be estimated to be 0.1 of that produced by ruminants i.e. 5.5kg CH_4 yr^{-1} each. Both adult deer and goats are likely to consume, in general, less feed than ruminant cattle -approximately 60% may be a reasonable figure - thus, each adult deer and goat can be assumed to produce 2.75kg CH_4 yr^{-1} each.

Animal Type	United Kingdom Population	CH_4 emissions per animal	United Kingdom CH_4 emissions per group for 1992 (kt)
Cattle	10.163×10^6	55kg CH_4 yr^{-1}	558.965
Sheep	21.657×10^6	8kg CH_4 yr^{-1}	173.256
Pigs	7.609×10^6	5.5kg CH_4 yr^{-1}	41.8495
Goats	104,000	2.75kg CH_4 yr^{-1}	0.286
Deer	51,000*	2.75kg CH_4 yr^{-1}	0.140
		Total	774.497

* This figure does not include the deer population in N.Ireland.

Table 3.4. Animal methane emissions in the United Kingdom for 1992.

It should be noted that the estimates of animal population in Table 3.4. do not include minor holdings in Scotland. The population of each group may be larger than estimated, thus the corresponding CH_4 emissions size calculated will be a minimum figure.

3.3.4. Peatland and wetland emissions:

14% of the Scottish mainland and 6% of mainland Britain is covered in wetlands. By using published measured emission rate values (mol CH_4 m^{-2}/yr^{-1}) and multiplying by the area of wetland in the British mainland the annual wetland CH_4 flux can be calculated. Table 3.5. lists emission rates measured by two groups involved in the NERC TIGER (Terrestrial Initiative into Global Environmental Research) programme.

Emission rate measurements from Ellergower Moss were made by a team of scientists from Queen Mary and Westfield College, London, using a quadrupole mass spectrometer. Emission rate measurements from Strathy Bog in Caithness and Sutherland, were made by a group of scientists from the Institute of Terrestrial Ecology using both light aircraft to take air samples, and laser diode cuvette techniques. The measurements recorded at Ellergower Moss were given in a personal communication, while those from Strathy Bog are given by Gallagher (1994).

The annual CH_4 flux gained from each is comparable, particularly as aircraft fluxes were typically a factor of 2 to 4 times larger than those measured using cuvette techniques at the site by ITE, which can be attributed to the different spatial averaging scales involved

(Gallagher, 1994). The mean annual efflux from Ellergower Moss was given, however the mean annual efflux from Strathy Bog was not given, thus the lower value of 0.89±0.55 mol CH_4 m^{-2}/yr^{-1} was chosen as representative of the mean annual flux.

Peat Bog	mol CH_4 m^{-2}/yr^{-1}	g CH_4 m^{-2}/yr^{-1}
Ellergower Moss (SW Scotland)		
Lawn	1.0	16.04
Hummock	0.4	6.42
Hollow	0.9	14.44
mean annual efflux (given)	0.59	9.47
Strathy Bog (NE Scotland)		
mean daytime flux	1.79±1.00	28.72±16.04
mean flux from nocturnal boundary layer studies	0.89±0.55	14.28±8.82
mean annual efflux (assumed)	0.89±0.55	14.28±8.82

Table 3.5. Comparison of mean annual efflux from Ellergower Moss, Dumfries and Galloway and Strathy Bog, Caithness and Sutherland.

The area of each country in the British mainland (Table 3.6) covered in wetland was supplied by Scottish Natural Heritage (SNH, 1993). The principal source for this information was from BGS drift 1:50,000 sheets which map peat > 1m thick. It should be noted that peat coverage in Northern Ireland is not included, that Scottish fens are under-represented, that shallow peat soils are also much more extensive than is implied here and that highland peat soils are not included here. Thus the wetland CH_4 flux calculated (Table 3.8) will be a minimum figure.

	Scotland	England	Wales	Σ British mainland
Peatland > 1m deep	10947.43 km^2 1.09 x $10^{10}m^2$	2525.32 km^2 2.53 x 10^9m^2	1629.41 km^2 1.63 x 10^9m^2	15102.16 km^2 1.51 x $10^{10}m^2$
Fenland > 1m deep	12.15 km^2 1.22 x 10^7m^2	1316.72 km^2 1.32 x 10^9m^2	28.67 km^2 2.87 x 10^7m^2	1357.54 km^2 1.36x 10^9m^2
Total per country	10959.58 km^2 1.10 x $10^{10}m^2$	3842.04 km^2 3.84 x 10^9m^2	1658.08 km^2 1.66 x 10^9m^2	16459.7 km^2 1.65 x $10^{10}m^2$

Table 3.6. Area of British mainland covered in wetland (peatland and fenland) greater than 1m deep.

Table 3.7. compares the annual efflux rate from Scotland, England and Wales (calculated by multiplying the emission rate in mol CH_4 $m^{-2}yr^{-1}$ by the area of peatland per country). The efflux from Scotland is almost 3 times higher than the efflux from England and nearly 7 times higher than the flux from Wales. Scotland is clearly the most important

source of wetland CH_4 in the British CH_4 budget.

Wetland type Emissions rate mol CH_4 $m^{-2}yr^{-1}$	Scotland mol CH_4 yr^{-1}	England mol CH_4 yr^{-1}	Wales mol CH_4 yr^{-1}
Peatland			
0.59	6.46×10^9	1.50×10^9	9.61×10^8
0.89	9.74×10^9	2.25×10^9	1.45×10^9
Fenland			
0.59	7.17×10^6	7.77×10^8	1.70×10^7
0.89	1.08×10^7	1.17×10^9	2.56×10^7
Σ Wetland			
0.59	6.47×10^9	2.67×10^9	9.78×10^8
	103.73kt / yr^{-1}	36.37kt / yr^{-1}	15.69kt / yr^{-1}
0.89	9.75×10^9	3.42×10^9	1.48×10^9
	156.48kt / yr^{-1}	54.86kt / yr^{-1}	23.67kt / yr^{-1}

Table 3.7. Comparison of methane emission rates from wetlands in Scotland, England and Wales.

Wetland type Emission rate mol CH_4 m^2yr^{-1}	mol CH_4 yr^{-1}	g CH_4 yr^{-1}	kt CH_4 yr^{-1}
Peatland			
0.59	8.91×10^9	1.43×10^{11}	142.94
0.89	1.34×10^{10}	2.16×10^{11}	215.63
Fen			
0.59	8.00×10^8	1.28×10^{10}	12.85
0.89	1.21×10^9	1.94×10^{10}	19.38
Σ Wetland			
0.59	9.71×10^9	1.56×10^{11}	155.79
0.89	1.46×10^{10}	2.35×10^{11}	235.01
1.79	2.95×10^{10}	4.73×10^{11}	472.66

Table 3.8. Calculated emission rates for British mainland wetlands.

Table 3.8. quantifies the annual CH_4 flux for the British mainland as a function of peatland, a function of fenland and for total wetlands. There are no peatland emission rates included in the Governmental 1992 budget. The Watt Committee on Energy estimates that 100kt of CH_4 was released from peat wetland during 1990. Unless climatic conditions changed considerably to alter favourably the methanogenic evironment, and unfavourably the methylotrophic environment, it is unlikely that emission rates would vary substantially.

It is quite clear that, even assuming the lowest emission rate of 0.59mol CH_4 m^2yr^{-1} to be the most accurate, the volume of CH_4 fluxing from British mainland wetlands to the atmosphere is at least 50% larger than the figure published for 1990 (potentially applicable to 1992) by the Watt Committee. At the higher measurement of 1.79mol CH_4 m^2yr^{-1}, the calculated CH_4 flux becomes more than 4 times greater the figure published by the Watt Committee (Tiger Eye, 1992). The volume of CH_4 fluxed is based on measured site emission rates and thus will have incorporated potential methane sinks, such as methane oxidation. It is not representative of the volume of CH_4 stored in wetlands, which is likely to be much higher.

3.3.5. Seepages and oceans.

A limited number of active seepages have been reported from the North Sea (Hovland and Judd, 1992). However, evidence of current or previous seepage activity in the form of pockmarks is widespread, covering an area estimated at 100,000km^2 (Hovland and Judd, 1992). Much, but not all, of this area overlies the petroliferous sediments of the Viking and Witch Ground Grabens. Seepage from an active pockmark in the UK Block 15/25, has been recorded in 1983, 1985, 1988, 1989 (twice) and 1990 (Hovland and Judd, 1992). The total area drained through this pockmark was estimated from shallow seismic profiles to be 640,000m^2, which represents an average net flux of 26g CH_4 m^2 yr^{-1}. In contrast, in the Tommeliten field of the Norwegian sector, gas seepage to the seabed along sealed faults was an order of magnitude lower with 5.6 x 10^6g CH_4 yr^{-1} escaping. $\delta^{13}C(CH_4)$ of the seepages was found to be in the range -26.7 to -47.7‰. There is potential fractionation associated with gas transport and microbial oxidation, thus the majority of the CH_4 can be assumed to be thermogenic in origin, but with a bacteriogenic input. Approximately 20% of natural gas fields can be considered of bacteriogenic origin (Stevens, 1988), thus $\delta^{13}C(CH_4)$ = -42‰ for ocean seepages is a reasonable signature.

Consideration of the two North Sea seepages and their geological setting, allowed an estimation of the net methane flux through the seabed of any hydrocarbon rich area of13g CH_4 m^2 yr^{-1} (Hovland and Judd, 1992). The net CH_4 flux through the petroliferous areas of the northern North Sea was estimated to be 2.6 x 10^{12}g yr^{-1} or 2600kt (Hovland and Judd, 1988), which seems an incredible amount, but may be accurate since the Central Graben of the North Sea is documented as only being 2.33% efficient at trapping gas (Cornford, 1993).

The proportion of a CH_4 bubble that survives transit through the water column to enter the atmosphere is dependent principally on water depth, water temperature, bubble size and the bubble boundary film thickness (Hovland et al, 1993). Bacterial CH_4 oxidation is common, efficient and sometimes quite rapid in ocean waters (Hovland et al, 1993), thus most of the bubble CH_4 dissolved will be oxidised by bacteria and will not contribute to the atmospheric CH_4 budget. Due to lack of information on bubble sizes, the proportion of CH_4 in bubbles that survives transit and thus bacterial oxidation, is extremely difficult to

predict. Ocean seepages are another source of atmospheric methane flux that have not been considered by the Department of the Environment. The Watt Committee estimate of 5kt CH_4 yr^{-1} for 1990 (also likely to be applicable to the 1992 budget) suggests that less than 0.2% of natural gas seepage reaches the atmosphere, but this seems incredibly low. For the revised budget, it seems reasonable to consider that 5% of all gas seepage (130kt) reaches the atmosphere.

3.3.6. Revised UK CH_4 budget for 1992.

Table 3.9. compares the revised CH_4 budget with that proposed by the Department of the Environment, and additional Watt Committee estimates for 1990 that are likely to be applicable to 1992. The isotopic signature for each source is given. Where possible $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measured from this research has been used, however for some sources it was necessary to use published data. There is a lack of $\delta D(CH_4)$ measurements in the literature, thus in some cases $\delta D(CH_4)$ has been estimated.

Source	Dept. of Env. (kt) (% of total)	Revised figure (kt)	Proposed isotopic composition $\delta^{13}C/\delta D$
Energy production	1226 (25.32)	2349.79 (47.18% from coal, 52.82% from oil)	coal: -37‰[1,2] / -168‰[3,4] oil assoc.: -40‰[3] / -231‰[3] nat. gas.: -44‰ / -144‰[4] weighted av: -42.1‰ / -165‰
Landfill / incineration	1919 (39.64)	1600-3000 (landfill only)	-57.1‰ / -288‰
Sewage	72 (1.48)	100♣	-51.6‰ / -265‰
Agriculture	1517 (31.33)	774.497♥	-65.4‰ / -310‰
Peat wetlands♠	100 (2.06)	155.79-472.66 *	-75.6‰ / -294‰
Forests / landuse biomass ♠	2 (0.04)	2	-28‰
Termites ♠	-	-	-
Seepages / oceans ♠	5 (0.10)	130	-42‰/-171‰
Total	4841	5112.077-6828.947	

[1] Deines (1980); [2] Whiticar (1989); [3] Schoell (1980); [4] Hitchman et al., (1989)

♠ These are sources considered in previous years budgets by the Watt Committee, but not included in the Department of the Environment 1992 budget; ♣ Watt Committee estimate used; ♥ this budget includes ruminant emissions only; * does not include N.Ireland

Table 3.9. Revised CH_4 budget and source isotopic composition.

The largest difference in CH_4 source flux sizes lies with emissions associated with

energy production, from landfill and from peat and wetlands, and possibly from ocean fluxes, all of which will have quite a significant impact upon $\delta^{13}C(CH_4)$ of all total atmospheric fluxes.

3.4. Calculation of atmospheric $\delta^{13}C(CH_4)$ for the United Kingdom CH_4 budget:

The isotopic signatures and flux sizes given in Table 3.9. can be used to predict the isotopic composition of atmospheric CH_4. The first step is to determine the isotopic composition of the overall CH_4 flux from n sources by mass balance using the following equation:

$$\delta_T m_T = \delta_1 m_1 + \delta_2 m_2 + ... + \delta_{n-1} m_{n-1} + \delta_n m_n \qquad 3.21a$$

which can be represented as:

$$\delta_T m_T = \sum_{i=1}^{n} \delta_i m_i \qquad 3.21b$$

Consider first $\delta^{13}C(CH_4)$ of the United Kingdom CH_4 flux given by the Dept. of the Environment budget, where sources 1-4 are those listed, by order, in Table 3.9. :

$4734\delta_T = 1226\delta_1 + 1919\delta_2 + 72\delta_3 + 1517\delta_4$

$4734\delta_T = 1226(-42.1) + 1919(-57.1) + 72(-51.6) + 1517(-65.4)$

$\delta^{13}C(CH_4)_T = -55.8‰$

Thus, according to the Department of the Environment budget $\delta^{13}C(CH_4)_T$ flux from the United Kingdom in 1992 would have been -55.8‰. Incorporating the additional sources considered by the Watt Committee would give rise to $\delta^{13}C(CH_4)_T$ flux of -56.2‰, which is isotopically lighter due to the input from wetland emissions.

A similar calculation can be carried out for $\delta D(CH_4)$. Although $\delta D(CH_4)$ for forests/landuse biomass is unknown, this flux sources comprises <0.04% of the total United Kingdom flux and can therefore be safely ignored in mass balance calculations. Thus, $\delta D(CH_4)_T$ flux from the United Kingdom in 1992 was -263‰ for both $\delta D(CH_4)_T$ calculated using the Department of the Environment budget, and $\delta D(CH_4)_T$ calculated using the combined Dept. of Env./Watt Committee budget.

$\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ of the atmospheric flux calculated by mass balance for five permutations of the United Kingdom CH_4 flux budgets (Dept. of the Env., combined Dept. of the Env. and Watt Committee, and my revised budgets) are given in Table 3.10.

Flux	Dept.of the Env.	Dept.of Env. & Watt Comm.	Revised Budget (lower level)	Revised Budget (higher level)	Revised budget (middle level)
$\delta^{13}C(CH_4)$	-55.8‰	-56.2‰	-51.5‰	-53.8‰	-52.8‰
$\delta D(CH_4)$	-263‰	-263‰	-232‰	-246‰	-240‰

Table 3.10. Isotopic composition of atmospheric CH_4 flux as a function of different flux budgets.

$\delta^{13}C(CH_4)$ for total atmospheric flux ($(\delta^{13}C(CH_4)_{TF}$) ranges from -56.2‰ to

-51.5‰. $\delta^{13}C(CH_4)$ for all revised budgets is isotopically heavier than that for the Department of the Environment budget and this is due to the larger input from energy production in the revised budgets. The proportionally larger CH_4 flux from energy production in the revised budgets, results in an isotopically heavier $\delta^{13}C(CH_4)$ signature for the lower level budget. This isotopically heavy flux is masked in the higher and middle level revised CH_4 budgets by the increased atmospheric flux of isotopically light bacteriogenic CH_4 (from landfill sites and wetlands) causing $\delta^{13}C(CH_4)_{TF}$ to be isotopically lighter. $\delta D(CH_4)$ for total atmospheric flux ($\delta D(CH_4)_{TF}$) ranges from -232‰ to -263‰. The DoE. budgets, dominated by agriculture and landfill site emissions, give rise to the isotopically lightest $\delta D(CH_4)_{TF}$ signature, while $\delta D(CH_4)_{TF}$ signature for all revised budgets is again isotopically heavier due to the increasing proportion of emissions associated with energy production. The variation in $\delta D(CH_4)_{TF}$ signature for revised budgets is a result of varying flux sizes from the isotopically heavy energy production and the isotopically lighter landfill, which dominate all other sources.

The mean isotopic composition of the United Kingdom atmospheric flux is $\delta^{13}C(CH_4)_{TF}$ = -54.0‰ and $\delta D(CH_4)_{TF}$ = -249‰. The measured isotopic composition of atmospheric CH_4 in 1987 was $\delta^{13}C(CH_4)_A$= -46.8‰ and $\delta D(CH_4)_A$ = -80±8‰ (Wahlen et al., 1987). There does not appear to be more current data for $\delta^{13}C(CH_4)_A$ and $\delta D(CH_4)_A$ in the literature. However, between 1983 and 1987, $\delta^{13}C(CH_4)_A$ increased by 0.09‰ yr^{-1}, attributable to a decrease in the flux of isotopically lighter CH_4 (Stevens, 1988). If this trend had continued, $\delta^{13}C(CH_4)_A$ for 1992 would be -46.4‰, 7‰ heavier than the United Kingdom atmospheric flux.

As a modelling exercise, 1992 $\delta^{13}C(CH_4)_A$ can be calculated from $\delta^{13}C(CH_4)_{TF}$ and compared to the suggested value of -46.4‰. It is unlikely that $\delta^{13}C(CH_4)_{TF}$ represents accurately the total Northern hemispheric flux emission isotopic signature, but it is likely to be similar. The average $\delta^{13}C(CH_4)_{TF}$ of sources in 1980 was calculated from measurements of $\delta^{13}C(CH_4)_A$ to be -55.66‰ (Stevens, 1988). $\delta^{13}C(CH_4)_{TF}$ given in Table 3.10, ranging from -51.5‰ to -56.2‰, are isotopically heavier, but $\delta^{13}C(CH_4)_A$ is also suggested to have increased by 0.09‰ yr^{-1} between 1987 to 1992.

$\delta^{13}C(CH_4)_A$ is more depleted in $^{13}CH_4$ than $\delta^{13}C(CH_4)_{TF}$ due to the kinetic fractionation occurring from the sink reaction of CH_4 with hydroxyl radicals. This can be quantified by expanding equation 3.17, to quantify the fraction of CH_4, lost to the following:

$$\delta_{TF} = \delta_A + F(\alpha - 1)(1 + 10^{-3}\delta_A)10^3 \qquad \text{(Stevens, 1988) 3.22}$$

α is the fractionation factor associated with the removal of CH_4 by oxidation with OH radicals (α =0.9946, from Table 3.1). F is the fractional removal from the atmosphere by reaction with OH radicals vs. total removal from the atmosphere by both this sink and non-fractionating transport to the stratosphere by eddy diffusion. Ehhalt and Schmidt (1978) have calculated F = 0.86-0.96 if the total annual loss rate is ~ 600 Tg yr^{-1} (Khalil and Rasmussen, 1982). Total sink for 1992 is estimated to be between 450-626 Tg yr^{-1} (Figure

3.1), thus the mean value of 0.91 will be used.

For non-steady state conditions:

$$\frac{\Delta(C\delta)}{\Delta(t)} = S\delta_{TF} - C\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] \qquad \text{(Stevens, 1988) 3.23}$$

and

$$\frac{\Delta(C)}{\Delta(t)} = S - C\lambda \qquad \text{(Stevens, 1988) 3.24}$$

where λ is the overall loss rate. Equation 3.23 can be expressed as :

$$\delta_{TF} = \frac{\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] + \frac{\Delta\delta}{\Delta t} + \delta C^{-1}\frac{\Delta C}{\Delta t}}{\lambda + C^{-1}\left(\frac{\Delta C}{\Delta t}\right)} \qquad \text{(Stevens, 1988) 3.25}$$

where the lifetime = $\lambda^{-1}$of ~ 8 years, (λ = 0.1096), the concentration growth, $C^{-1}\left(\frac{\Delta C}{\Delta t}\right)$ = 0.01yr^{-1} and the global average isotopic trend, $\frac{\Delta\delta}{\Delta t}$, is assumed to be 0.09‰ yr^{-1}. The derivation of 3.25 from equation 3.23 is given in Appendix 3.

$\delta^{13}C(CH_4)_A$, calculated for both steady and non-steady state conditions from $\delta^{13}C(CH_4)_{TF}$ signatures, using equations 3.22. and 3.25 respectively are given in Table 3.11.

atmospheric. $\delta^{13}C(CH_4)$	DoE.	DoE. & Watt	Revised Budget (lower)	Revised Budget (higher)	Revised budget (middle	Mean $\delta^{13}C(CH_4)$‰
steady state conditions	-51.1‰	-51.5‰	-46.8‰	-49.1‰	-48.1‰	-49.3±2.0‰ -48±1.2‰*
non-steady state conditions	-52.3‰	-52.7‰	-48.0‰	-50.2‰	-49.3‰	-50.5±2.0‰ -49.2±1.1‰*
$\delta^{13}C(CH_4)_{TF}$	-55.8‰	-56.2‰	-51.5‰	-53.8‰	-52.8‰	-54.0±2.0‰

* mean of revised budgets only

Table 3.11. Isotopic composition of U.K. contribution to atmospheric CH_4, calculated from $\delta^{13}C(CH_4)$ of source fluxes, as a function of different U.K. flux budgets.

It is clear that, as expected due to the kinetic isotope fractionation effect associated with the reaction of CH_4 with hydroxyl radicals, $\delta^{13}C(CH_4)_A$ is isotopically heavier than $\delta^{13}C(CH_4)_{TF}$ by 4.7‰ for steady state conditions, and by 3.5‰ for non-steady state conditions. $\delta^{13}C(CH_4)_A$ suggested for 1992 (based on the assumption $\delta^{13}C(CH_4)_A$ had continued to increase by 0.09‰ yr^{-1}) was -46.4‰. Mean $\delta^{13}C(CH_4)_A$ for steady and non-steady state conditions from the five proposed budgets were -49.3±2.0‰ and -50.5±2.0‰ respectively. $\delta^{13}C(CH_4)_A$ for non-steady state conditions is 1.2‰ isotopically lighter than $\delta^{13}C(CH_4)_A$ for steady state conditions. This may be attributed to the calculation for non-

steady state conditions taking into consideration the lifetime of CH_4 in the atmosphere. If $\delta^{13}C(CH_4)_A$ suggested for 1992 is considered accurate, then the discrepancy between $\delta^{13}C(CH_4)_A$-calculated from $\delta^{13}C(CH_4)_{TF}$ using equations 3.22 and 3.24, and $\delta^{13}C(CH_4)_A$-proposed (when $\delta^{13}C(CH_4)_A$ is presumed to continue increasing by 0.09‰ yr^{-1}), could be attributed to either increasing fluxes of isotopically light sources (from landfills and wetlands), decreasing fluxes of isotopically heavier sources (from energy production), a larger isotopically fractionating sink (most likely by destruction by hydroxyl radicals) or a combination of all three.

The atmospheric reactions that carbon in the CH_4 molecule undergoes are relatively well understood and thus $\delta^{13}C(CH_4)$ measurements can be used to further constrain global CH_4 budgets. Unfortunately, at present, a similar modelling process cannot be executed for $\delta D(CH_4)$ due to both lack of recorded $\delta D(CH_4)$ source flux measurements and uncertainty about the atmospheric chemistry processes.

The complex atmospheric chemistry of methane hydrogen is easily demonstrated in Figure 3.2., which shows all paired isotopic measurements made during this research, $\delta^{13}C$ and δD of atmospheric CH_4 (Wahlen et al., 1987) and outlines the approximate boundaries for CH_4 from the four main source types.

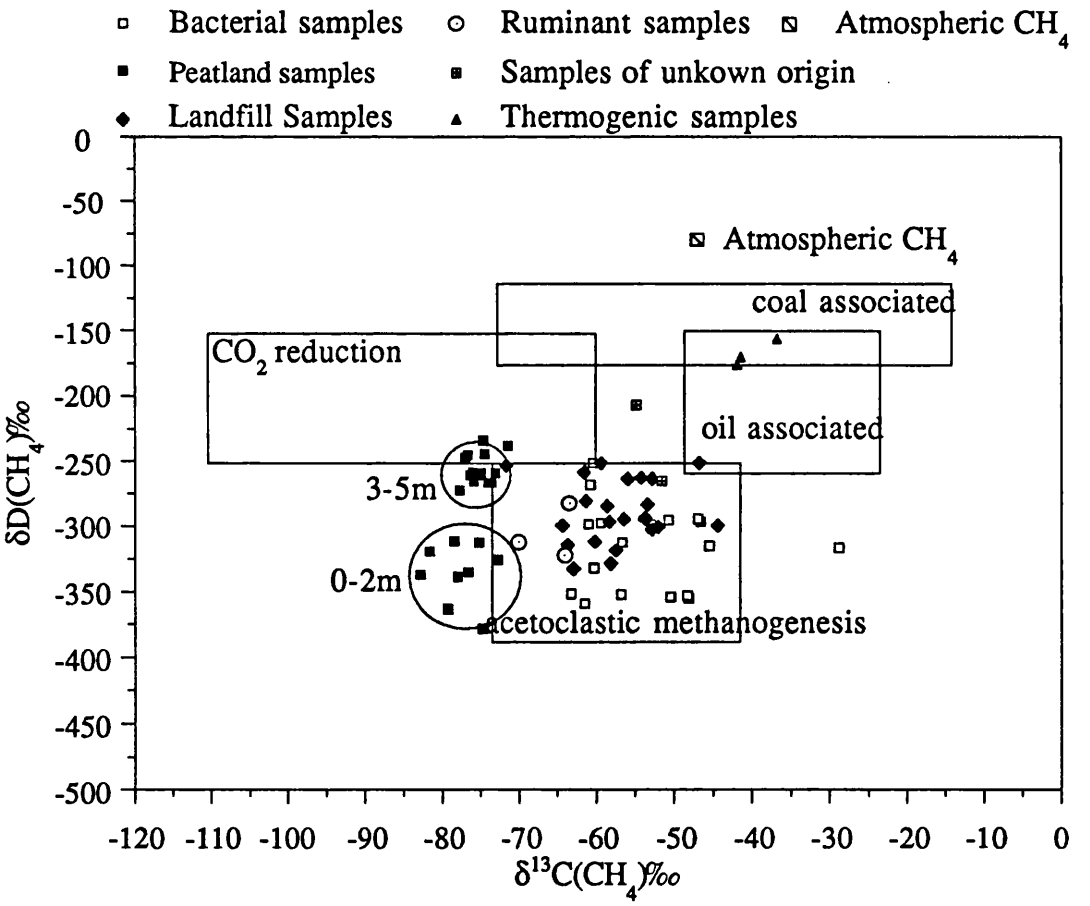


Figure 3.2. $\delta^{13}C(CH_4)$ vs. $\delta D(CH_4)$ for all samples in this study. Adapted from Schoell (1988).

$\delta^{13}C(CH_4)$ ranges from ~ -110 to -15‰, thus it is not surprising that $\delta^{13}C(CH_4)_A$ has a value of ~-47‰, as this is within this range and potentially calculable by mass balance. In comparison, $\delta D(CH_4)$ of source flux ranges from ~-380 to -100‰, and yet $\delta D(CH_4)$ is isotopically heavier than the source range, with a measured value of ~-80‰ (Wahlen et al., 1987). It is clear from figure 3.2., that the source boundaries need to be redefined, however this appears more necessary for $\delta^{13}C$ rather δD measurements, and it is unlikely that the source composition will become significantly D enriched. From the 'Isotopic composition of methane "Guinness Book of Records" ' (Schoell, 1988), the most deuterium enriched methane was thermogenic and from the Rotliegend sandstone, with $\delta D(CH_4)$ = -71‰. This is more D enriched than atmospheric $\delta D(CH_4)$, however this is an extreme example, rather than the norm. By consideration of simple mass balance principles, $\delta D(CH_4)_A \neq$ ~ -80‰, unless a large source of isotopically heavy hydrogen is introduced that can exchange with the methane hydrogen. The likely source is another prolific greenhouse gas.

Water absorbs infra-red radiation even more strongly than CO_2, thus greatly influencing the Earth's heat balance. The water vapour content of the atmosphere varies over a wide range, particularly in the lower atmosphere. The normal range is 1-3% on a volume basis, although air may contain as little as 0.1%, or as much as 5% water vapour (Manahan, 1990). The % water in the atmosphere decreases rapidly with increasing altitude. The cold tropopause serves as a barrier to the movement of water into the stratosphere, with the main source of stratospheric water resulting from the photochemical oxidation of methane (Manahan, 1990). $\delta D(H_2O)$ is isotopically heavier than $\delta D(CH_4)$, with the volumetrically most important meteoric waters ranging from +10 to -70‰ and $\delta D(H_2O)$ of ocean waters relatively uniform, ranging from +5 to -7‰ (Sheppard, 1986).

Gaseous water in the troposphere is involved in the formation of hydroxyl and hydroperoxyl radicals, equations 3.26. and 3.27 respectively:

$$H_2O + h\nu \longrightarrow HO^{\cdot} + H \qquad 3.26$$

$$H + O_2 \longrightarrow HOO^{\cdot} \qquad 3.27$$

85% of the CH_4 that is emitted into the atmosphere is destroyed in the troposphere by reaction with the gas phase hydroxyl radical, OH (Equation 3.2, section 3.2.1.). With OH radicals produced from the photolysis of H_2O, subsequently involved in the destruction of methane (producing the intermediate CH_3 in NO_x rich air columns, possibly in the presence of free hydrogen) it may be that hydrogen exchange and reformation of CH_4 (from CH_3 and free hydrogen) could incorporate isotopically heavy hydrogen.

3.5. Comparison of United Kingdom $\delta^{13}C(CH_4)$ flux with global $\delta^{13}C(CH_4)$ flux.

Large uncertainties in flux size and sink exist (Figure 3.1), $\Delta\delta / \Delta t$, is not known, and it is quite possible that the assumed value of +0.09‰ yr^{-1} may be incorrect. However calculation of global $\delta^{13}C(CH_4)_{TF}$ and $\delta^{13}C(CH_4)_A$ using given flux ranges will be similar in signature to the real $\delta^{13}C(CH_4)_{TF}$ and $\delta^{13}C(CH_4)_A$.

Table 3.12. gives the flux size range and median for global CH_4 fluxes (taken from

Rudolph, 1994). Also given is $\delta^{13}C(CH_4)$ signature for each flux. Where the flux type is the same as those listed for the United Kingdom budget, the same $\delta^{13}C(CH_4)$ signature has been used. Where the flux type differs, e.g. rice cultivation is potentially a major global source, but not one that is present in the United Kingdom, $\delta^{13}C(CH_4)$ has been taken from published data. Where this was not possible $\delta^{13}C(CH_4)$ has been estimated after consideration of the methanogenic environment.

The second part of Table 3.12. lists $\delta^{13}C(CH_4)_{TF}$ calculated by mass balance using equation 3.21 and $\delta^{13}C(CH_4)_A$, calculated for steady state and non-steady state conditions using equations 3.22 and 3.25 respectively.

Flux source	Lowest estimate Tg yr^{-1}	Highest estimate Tg yr^{-1}	Middle estimate Tg yr^{-1}	$\delta^{13}C(CH_4)$
Natural wetlands	58	195	126.5	-75.6‰
Rice cultivation	20	122	71	-67‰[1]
Fermentation	60	100	80	-65.4‰
Landfills	15	65	40	-57.1‰
Biomass	12	45	28.5	-28‰[1]
Domestic sewage	12	35	23.5	-51.6‰
Coal mining	25	45	35	-37‰
Oil and gas usage	35	128	81.5	-42‰
Termites	0	60	30	-65.1‰[2]
Various	7	35	21	-42‰
Total	244	830	537	
$\delta^{13}C(CH_4)_{TF}$	-58.0‰	-58.6‰	-58.5‰	Mean -58.4±0.3‰
$\delta^{13}C(CH_4)_A$ steady state	-53.3‰	-54.0‰	-53.9‰	-53.7±0.4‰
$\delta^{13}C(CH_4)_A$ non-steady state	-54.5‰	-55.1‰	-55.0‰	-54.9±0.3‰

[1] Stevens and Engelkemeir (1988); [2]Tyler (1986)

Table 3.12. Global CH_4 budget and source signature, $\delta^{13}C(CH_4)_{TF}$ and $\delta^{13}C(CH_4)_A$ for steady and non-steady state conditions.

Mean global $\delta^{13}C(CH_4)_{TF}$ is estimated as -58.4±0.3‰, whilst the United Kingdom mean $\delta^{13}C(CH_4)_{TF}$ is estimated as -54.0±2.0‰. Likewise mean global $\delta^{13}C(CH_4)_A$ is estimated as -53.7±0.3‰ and -54.9±0.3‰ for steady state and non-steady state conditions respectively, whilst the United Kingdom $\delta^{13}C(CH_4)_A$ is estimated as -49.3±2.0‰ and

-50.5±2.0‰ for steady state and non-steady state conditions respectively. For all parameters estimated global $\delta^{13}C(CH_4)$ is isotopically lighter than estimated United Kingdom $\delta^{13}C(CH_4)$ by ~5.0‰. This is not surprising for almost 67-70% of the global flux budget is composed of isotopically light sources, whilst only 56-66% of the United Kingdom global flux budget is composed of isotopically light sources. All calculated global and United Kingdom fluxes are isotopically lighter than the suggested value of -46.4‰, but without confirmation of $\Delta\delta/\Delta t$ or improved flux budgets and isotopic source signatures, more accurate $\delta^{13}C(CH_4)$ signatures cannot be ascertained.

Figure 3.3. is a graph illustrating modelled, changes in $\delta^{13}C(CH_4)_A$ as a function of time, for both global and United Kingdom CH_4 fluxes. The chosen starting budget was the middle budget for both global and United Kingdom CH_4 fluxes. All data used are listed in Appendix 4.

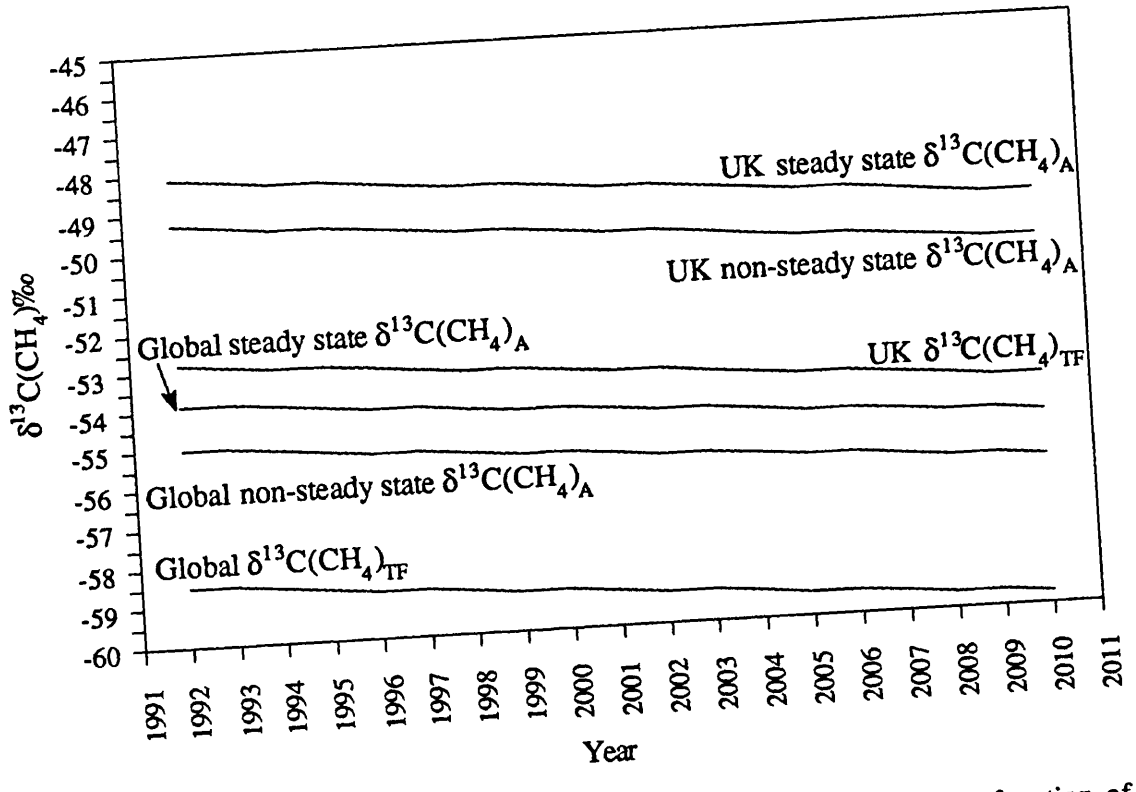


Figure 3.3. Changes in UK and global $\delta^{13}C(CH_4)_{TF}$ and $\delta^{13}C(CH_4)_A$ as a function of changing flux budgets.

The criteria chosen for this model are as follows:

• The global production of oil is peaking at present and from 1998 is expected to decline. A similar pattern may be expected for gas production, although it is possible that the technology will soon be developed to recover CH_4 from gas hydrates. A rate of decline of ~1.25% per year (Fuller, 1993) has been applied to both the global and United Kingdom energy production budgets.

• The vast majority of animals that produce large volumes of CH_4 (cattle, sheep and buffalo) are domesticated and reared for either food production or as draught animals, thus

it would seem reasonable that the number of animals is directly dependent on the human population. With a projected increasing population, CH_4 emissions from animals are projected to increase by between 1.0 and 1.5% yr^{-1} (Anastasi and Simpson, 1993).

• Likewise, domestic landfill is likely to increase as a result of increasing population. However CH_4 emissions to the atmosphere may not increase substantially as the increasing number of small-scale, unplanned landfill sites are replaced by large, planned, multi-cellular sites from which CH_4 produced can be utilised efficiently as an energy source. Thus the net increase in CH_4 emissions from landfill sites will be considered negligible in this model. CH_4 emissions from rice cultivation (an unnatural wetland) and sewage are also likely to be linked to changes in population, thus as with ruminant cattle, CH_4 emissions from both are modelled to increase by 1.0-1.5% yr^{-1}.

• In contrast, natural wetland area is unlikely to increase substantially, and CH_4 flux should stay constant unless there is a change in ambient atmospheric temperature (Hameed and Cess, 1983). Thus it will be assumed that the flux from natural wetland will remain constant during this model.

• Due to lack of information to suggest otherwise CH_4 emissions from termites, ocean seepages, forests and landuse biomass and 'various' are considered to remain constant.

$\delta^{13}C(CH_4)$ for all parameters (flux and global $\delta^{13}C(CH_4)$, steady and non-steady state $\delta^{13}C(CH_4)$) becomes isotopically lighter with the changing budgets described previously. The trends shown reflect the increasing input from isotopically light emissions from rice cultivation, animal fermentation, and sewage and the decreasing input from isotopically heavy CH_4 emissions from oil and gas production. This trend is not directly related to concentration of CH_4 in the atmosphere for total flux (kt) for the UK budget is decreasing through time, while total flux (kt) for the global budgets is increasing with time. Furthermore, similar $\delta^{13}C(CH_4)_A$ trends can be observed under different conditions. For example, steady-state flux to the atmosphere (concentration and $\delta^{13}C(CH_4)$) coupled with an annual reduction in atmospheric OH radical concentration over a given period of time, would result in $\delta^{13}C(CH_4)_A$ becoming isotopically lighter with time as less CH_4 is destroyed by OH radicals. $\delta^{13}C(CH_4)_{TF}$ however would remain constant, which demonstrates the importance in monitoring $\delta^{13}C(CH_4)_{TF}$ contemporaneously with $\delta^{13}C(CH_4)_A$.

For the model illustrated in Figure 3.3., $\frac{\Delta\delta}{\Delta t}$ is ~0.07‰ per year. Larger increases or decreases (than 1.25% yr^{-1}) in the volumetrically significant fluxes (as perhaps would have occurred during the Gulf War) would result in a larger $\frac{\Delta\delta}{\Delta t}$, and changes in budgets would be easier to pinpoint.

3.6. Source characterisation and isotopic databases.

3.6.1. Paired $\delta^{13}C(CH_4)$ and $\delta^{13}D(CH_4)$ measurements.

Figure 3.2., shown again overleaf, is a graph of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ for all paired samples measured during the course of this research. Although few CH_4 samples of

thermogenic origin were analysed during this research, the boundaries delineating CH_4 of thermogenic origin have been constructed based on published isotopic pairs. Most samples plot within the field attributable to CH_4 produced by acetoclastic methanogenesis. Samples from Ellergower Moss are distinguished by the isotopically lighter $\delta^{13}C(CH_4)$ signature.

It is clear that field boundaries are not rigid and probably can be extended, at least for CH_4 produced by acetoclastic methanogenesis and by CO_2 reduction. While there are areas of overlap, stable isotope analysis of CH_4 can be used, in many cases, to determine the origin of an unknown source of CH_4. At present it is not possible to determine by isotopic analysis alone whether a CH_4 sample with a bacteriogenic signature is a ruminant emission or from a landfill site. In cases such as these the geological and industrial setting must also be taken into consideration. Appendix 5 contains a published paper describing the combined application of stable isotope analysis and geological setting in determining the origin of an unknown CH_4 sample.

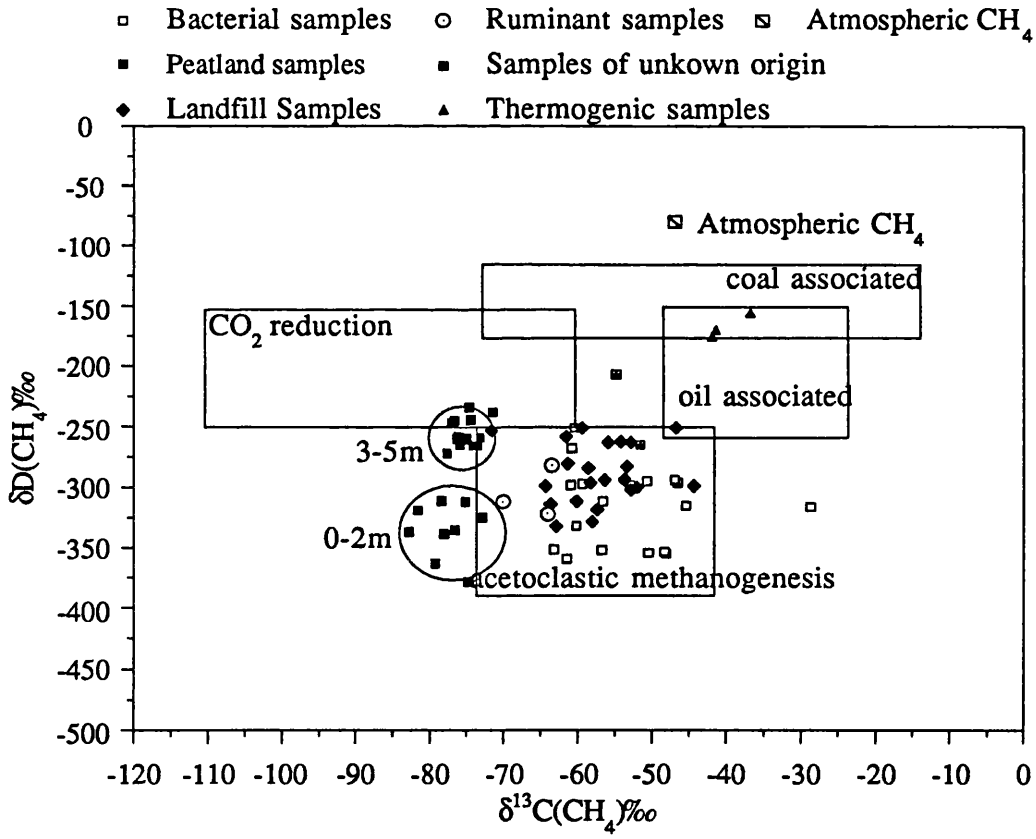


Figure 3.2. $\delta^{13}C(CH_4)$ vs. $\delta D(CH_4)$ for all samples. (Adapted from Schoell, 1988)

3.5.2. Paired $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ measurements.

Figure 3.4. is a graph of $\delta^{13}C(CO_2)$ vs. $\delta^{13}C(CH_4)$ for all samples from which CO_2 was collected. $\delta^{13}C(CO_2)$ ranges from 19.0‰ to -22.5‰, with a mean of 0.2±8.5‰. From this data set as shown on this graph, it does not appear possible to distinguish sources of CH_4 unambiguously by use of $\delta^{13}C(CO_2)$ rather than $\delta D(CH_4)$ measurements - both end

member $\delta^{13}C(CO_2)$ measurements are from landfill sites, thus one source can cover the whole range. While some groups may appear distinguishable, for example peatland samples, this is a function of $\delta^{13}C(CH_4)$ rather than $\delta^{13}C(CO_2)$. The exception is the bacterial samples which form two groups as a function of $\delta^{13}C(CO_2)$. As will be discussed in chapter 4, this is caused by change in anaerobic degradation pathway, from the short-lived aerobic catabolism of organic matter to methanogenesis producing isotopically heavier $\delta^{13}C(CH_4)$. The initial, short period of isotopically light $\delta^{13}C(CO_2)$ production is likely to be masked in a natural 'open' system. $\delta^{13}C(CO_2)$ measurements however, can be useful in suggesting when CH_4 has been subject to bacterial oxidation. This is discussed in Chapter 5, when considering the Ellergower Moss samples and again in the paper in Appendix 5.

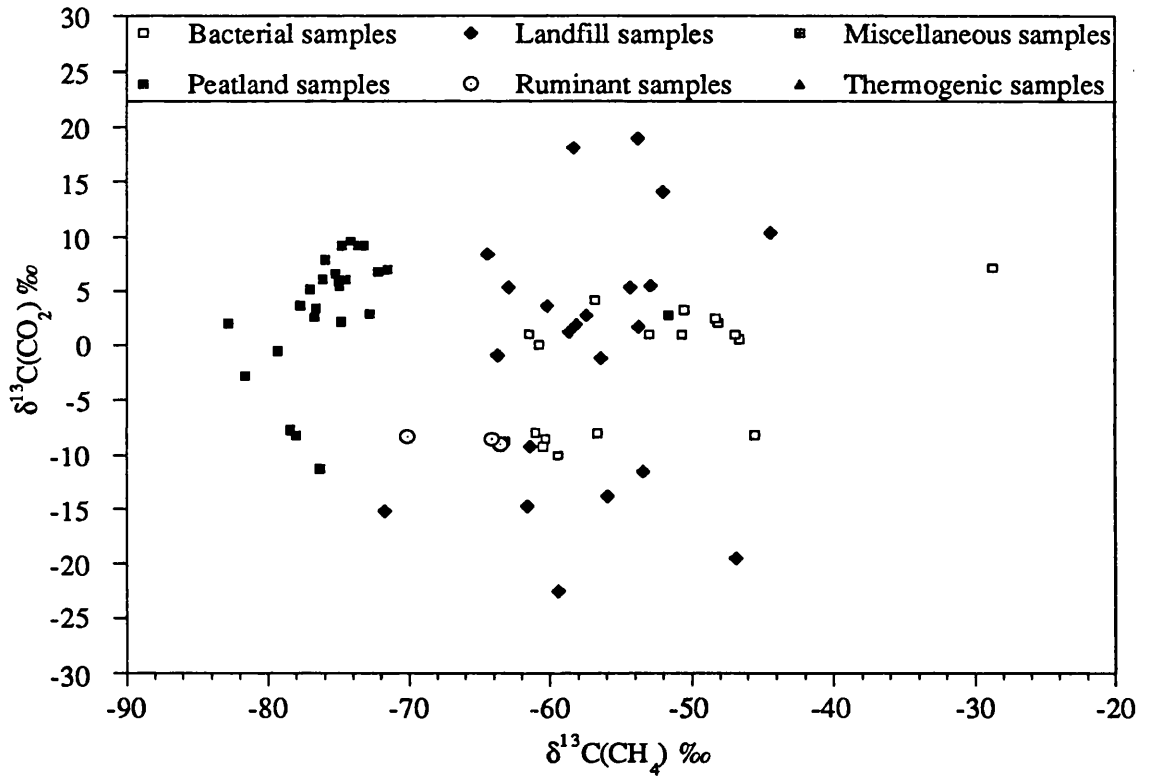


Figure 3.4. $\delta^{13}C(CO_2)$ vs. $\delta^{13}C(CH_4)$ for all samples.

3.7. Conclusions.

In this chapter, data produced from stable isotopic fingerprinting of sources have been utilised to demonstrate the application of stable isotope studies of trace gas emissions to predict atmospheric $\delta^{13}C(CH_4)$ for global and United Kingdom flux budgets. Likewise, from temporal measurement of atmospheric $\delta^{13}C(CH_4)$, changes in flux budget may also be observed and understood. One potential flux emission scenario has been modelled to demonstrate changes in $\delta^{13}C(CH_4)$ with time as a result of changing flux budgets. This modelling also demonstrates the difficulty in accurate interpretation of atmospheric $\delta^{13}C(CH_4)$, for without sufficient data characterising flux size, isotopic composition and

current atmospheric chemical concentrations (for example OH radical concentration), the secular trend of $\delta^{13}C(CH_4)$ may be misinterpreted. Chapter 4 and chapter 5 report research undertaken to elucidate controls of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ at source and thus accurately characterise a bacteriogenic CH_4 flux.

Measurements of atmospheric $\delta D(CH_4)$ have, at present, little application in unravelling the global atmospheric flux of CH_4. However current research to allow $\delta D(CH_4)$ measurements from very small volumes of air, will increase the interest in $\delta D(CH_4)$ and progress in understanding the atmospheric chemistry will follow. Combined $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ of source flux and atmospheric methane should be a very powerful tool by which to understand and monitor global CH_4 budgets.

CHAPTER 4: THE BACTERIA EXPERIMENTS

4.1. Abstract:

Four anaerobic enrichment systems were monitored over a period of three months: Systems 1 and 2, selecting for butyrate degrading bacteria and systems 3 and 4 for hexanoate degrading bacteria. System 1 and 3 $\delta D(H_2O)$ differed from system 2 and 4 $\delta D(H_2O)$ by 118‰. System 1 $\delta^{13}C(CH_4)$ ranged from -63 to -48‰, $\delta^{13}C(CO_2)$ ranged from -8.6 to 2.1‰, $\delta D(CH_4)$ remained constant with a mean of -354±3‰. System 2 $\delta^{13}C(CH_4)$ ranged from -61 to -47‰, $\delta^{13}C(CO_2)$ ranged from -8.0 to 0.6‰, $\delta D(CH_4)$ remained constant with a mean of -296±2‰. Furthermore, the difference in $\delta D(CH_4)$ between systems 1 and 2 was 56±4‰. Systems 3 and 4 failed after an initial sample however $\delta D(CH_4)$ showed the same difference in magnitude as between systems 1 and 2. $\delta D(CH_4)$ is in part, strongly influenced by an isotopically homogenous source i.e. the water, the difference between systems reflects the isotopically distinct water used. The trend in $\delta^{13}C(CH_4)$ is attributed to changing dominance of methanogenic pathway.

Biochemical pathways important during acetoclastic methanogenesis are reviewed. Particular attention is given to the β-oxidation of fatty acids to acetate demonstrating more H_2O than previously considered is incorporated into the resultant CH_4 molecule. As fatty acid chain length increases, the influence of δD(organic) on $\delta D(CH_4)$ is shown to lessen while the influence of $\delta D(H_2O)$ increases.

4.2. Introduction.

At present, $\delta^{13}C$ characterisation of CH_4 sources is well established and aids continued isotopic monitoring of atmospheric CH_4 to determine the dominant fluxes, natural or anthropogenic, and in regard to global carbon budgets, although the atmospheric chemistry may be complex. By comparison, δD characterisation of global sources of CH_4 is poorly established and the atmospheric chemistry remains undocumented. To date global budget estimates have concentrated on $\delta^{13}C(CH_4)$, perhaps due to the complexity of the hydrogen cycle in the atmosphere, however another reason may be due to lack of measurements of $\delta D(CH_4)$.

$\delta^{13}C(CH_4)$ from a series of measurements made from several Scottish landfill sites ranged evenly from -44 to -66‰, mean of -56.5±6‰ (Appendices 1 & 5). $\delta D(CH_4)$ varied from -258 to -352‰, but in contrast 80% of the values lay in the narrow range from -294 to -318‰, mean of 302±8‰. Given that landfill waste is unlikely to be isotopically homogenous for both C and H, and that larger hydrogen than carbon isotope fractionations are observed in nature, at least as large a range in $\delta D(CH_4)$ would have been expected as observed in $\delta^{13}C(CH_4)$. These data suggested that $\delta D(CH_4)$ was influenced by a large, isotopically homogenous hydrogen reservoir. Furthermore stable isotope analysis of CH_4 collected from two closed culture anaerobic enrichment systems inoculated with landfill waste and supplemented with hexanoate or butyrate yielded stable isotopic ratios of $\delta^{13}C$ = -46‰, δD = -315‰ and $\delta^{13}C$= -29‰, δD= -316‰ respectively. The difference in $\delta^{13}C$ of 17‰ is significant, while δD values can be

considered identical. The largest source of isotopically homogenous hydrogen common to both systems was the laboratory distilled water.

Moisture content of refuse at emplacement can be as high as 60% (Schmidell et al., 1986) which can increase as a result of infiltrated moisture and moisture generated by microbial activity. Landfill sites which are kept drained produce insignificant volumes of methane. The isotope measurements from the landfill sites and their analogue, the bacterial cultures, suggest that $\delta D(CH_4)$ is derived from the same source. Common to all landfill sites sampled and to the bacterial cultures was local meteoric water and laboratory distilled water respectively.

A large uncertainty still exists regarding $\delta D(CH_4)$ produced by the acetoclastic methanogenic (commonly known as fermentation) pathway, (Jenden and Kaplan, 1986, Schoell et al., 1988). This chapter will report the results of several experiments set up to further investigate the influence of $\delta D(H_2O)$ on $\delta D(CH_4)$ produced by one important methanogenic pathways, acetoclastic methanogenesis. It is postulated from this research that δD of the local water has a much stronger influence on $\delta D(CH_4)$ produced by the acetoclastic methanogenic pathway than previously acknowledged (by Schoell, 1980 and Whiticar et al., 1986). If $\delta D(CH_4)$ for each source can be more clearly constrained by prediction from δD of the local water, monitoring environmental pollution, on a local or global scale, will be facilitated.

In this chapter, a brief overview of methanogenesis is necessary to demonstrate the complexity of the biochemical pathways and the consortium of bacteria involved. Neither the reactions nor the bacteria have been fully characterised, which makes isotopic interpretation difficult, however the trend exhibited by $\delta^{13}C(CH_4)$ is attributed to changing dominance of methanogenic pathway. New models are proposed to characterise the influence of $\delta D(H_2O)$ on $\delta D(CH_4)$ produced via acetoclastic methanogenesis from a fatty acid substrate. This is contrasted with previous isotope modelling of acetoclastic methanogenesis (Whiticar et al., 1986).

4.3. A biochemical and isotopic review of bacterial methanogenesis :

The biochemistry and physiology of methanogenic bacteria have been extensively studied, with over 49 species currently described (Vogels et al., 1988). Methanogenic bacteria are chemolithotrophic, i.e. they use inorganic substrates for energy production (Senior and Balba, 1983). The species use hydrogen as a sole source of reducing power for methanogenesis and cell carbon synthesis from substrates such as formate (Large 1988), methyl amine (King et al., 1983), methanol and acetate (Senior and Kasali, 1990). Although some species can synthesise all cellular carbon from CO_2 while growing at the expense of hydrogen oxidation, autotrophy has been difficult to detect in other species because of the very slow growth rate of certain organic compounds (Zeikus, 1977).

Despite a more detailed description of bacterial methanogenesis by Cicerone and Oremland (1988), it is still a common misconception that methanogenic bacteria are responsible for the total degradation of complex organic compounds e.g. landfill waste, plant matter, to provide energy for growth while producing the by-products $CH_4 \pm CO_2$. In reality, breakdown of polymers in anaerobic environments requires a minimum of three groups of bacteria, which

in most anaerobic systems co-exist as a complex mixed population, the methanogens being the terminal member. Therefore, only limited information about methanogenesis can be gained by using isolated bacterial cultures. To understand all potential fractionation processes in methanogenesis it is imperative that each stage in the anaerobic degradative process be considered.

Acetoclastic methanogenesis, considered to be the dominant methanogenic biochemical reaction in freshwater sediments, can be described in several stages:

The first stage in the degradative process, microbial hydrolysis, the bio-polymers protein, carbohydrate and lipids are split in the presence of hydrolytic enzymes excreted from the cell (Senior and Balba, 1983) into fragments, forming amino acids, sugars and fatty acids respectively, by the incorporation of water. This is an essential first step since many proteins, fats and carbohydrates are both insoluble and too large to penetrate the cell wall of the bacteria.

In the absence of oxygen, primary fermentation, (the anaerobic catabolism of the hydrolysis products), results in the formation of compounds such as H_2, CO_2, formate and acetate (CH_3COOH) and, in addition, fatty acids such as ethanol, propionate, butyrate, succinate and lactate. The latter five compounds are typical fermentation products of bacterial activity in which reduced end products are generated as a sink for excess electrons in the form of $NADH^+$ derived from the growth substrate. The difference in redox potential of the substrate and the electron acceptor derived from it provides the energy for ATP synthesis (Hamilton, 1988). In fermentation, all components of the reaction schemes are normally soluble and ATP is generated by phosphorylation of the substrate. The sole reason for accumulating the reduced fermentation products is to achieve redox balance. Common examples of electron carriers in fermentation are acetyl-CoA, pyruvate and H^+. Sugars are degraded to pyruvate, an intermediate in the fermentation process, via the Embden-Meyerhof-Parnas (EMP) or glycolytic pathway (Hamilton, 1988) before conversion to acetyl-CoA. Synthesis of fatty acids from acetyl-CoA and the actual proportions of products depends upon the concentrations of H_2 in the ecosystem, the specific growth rates of the bacteria and the bacterial substrate affinity.

Homoacetogenic bacteria are important as they can convert sugars, CO_2 and H_2, and CO or methanol with CO_2 into acetate (CH_3COOH), during acetogenesis. Molecular hydrogen is a major fermentation product (Bryant, 1976), and in order that feedback inhibition is prevented and the anaerobic degradation process can continue, an extremely low H_2 partial pressure must be maintained by continual removal. In mixed populations in anaerobic environments there are many bacteria, which, like homoacetogenic bacteria, can utilise dihydrogen. By the removal of dihydrogen, they make conditions more favourable for secondary fermentation, allowing the development of a group of acetogenic bacteria termed obligate proton reducers. In the secondary fermentative stage these ferment the longer chain fatty acids such as ethanol, butyrate and lactate to acetate and H_2 creating a suitable substrate for the methanogens. Degradation of fatty acids takes place via a process termed the β-oxidation pathway, (Fig. 4.2., section 4.6.5.2.). Complicated by interspecies hydrogen transfer, some of the following equations may not be balanced.

$$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$$

Butyrate Acetate

$$CH_3(CH_2)_4COO^- + 2H_2O \rightarrow CH_3COO^- + CH_3(CH_2)_2COO^- + H^+ + 2H_2$$

Hexanoate

Since these reactions are only energetically favourable when the partial pressure of H_2 is maintained at a very low level (10^{-3} atm. or below), these bacteria can only grow as a component of an obligately syntrophic association, dependent again upon the removal of H_2 by another acetogen, sulphate-reducer or methanogen. The term 'interspecies hydrogen transfer' is used to describe such an association, which has a structural as well as metabolic association in that the bacterial partners are to be found in close physical contact (Hamilton, 1988).

The final stage in the acetoclastic methanogenesis pathway involves the methanogenic bacteria, which obtain energy by the oxidation of dihydrogen under anaerobic conditions.

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$$

Discrete ^{14}C labelling patterns used by Buswell and Sollo (1948) and Stadtman and Barker (1949) found that during acetoclastic methanogenesis the methyl carbon of the substrate is incorporated into the CH_4 molecule and the carbon of the carboxyl group is incorporated into the CO_2 molecule. (The superscripts denote separate ^{14}C labelling.):

$$\overset{*}{C}H_3\overset{\#}{C}OOH \rightarrow \overset{*}{C}H_4 + \overset{\#}{C}O_2$$

Pine and Barker (1956), using deuterium as a tracer determined that the hydrogen attached to the methyl carbon was also transferred intact into the CH_4 molecule during acetoclastic methanogenesis and that the remaining hydrogen was derived from the water:

$$CD_3COOH + H_2O \rightarrow CD_3H + CO_2$$

$$CH_3COOH + D_2O \rightarrow CH_3D + CO_2$$

Whether the methanogens are responsible for cleaving a water molecule, as suggested above, to release the fourth hydrogen or whether it is a hydrogen freed from water and transferred by interspecies hydrogen transfer from earlier in the food chain, remains undemonstrated as not enough information is given with respect to the reducing agent used in this experiment. By supplementing the system with acetate, Pine and Barker, (1956), provided a suitable methanogenic growth substrate, and circumvented the primary and secondary fermenters and acetogens that are usually active in the chain. Thus conclusions cannot be drawn concerning the source of methyl hydrogen in the acetate molecule. During the series of enzyme catalysed reactions necessary for anaerobic degradation of organic matter, hydrogen from the water may be incorporated into the organic methanogenic substrate. Well documented biochemical pathways pertinent to anaerobic degradation of organic compounds and CH_4 production will be utilised, to model with greater accuracy isotopic mass balance equations for $\delta D(CH_4)$.

4.4. Experimental Technique And Methodology

Gas samples were produced by microbial associations grown in closed-culture anaerobic enrichment vessels (Balba, 1978) at 30°C. The apparatus consisted of 2 litre (system 1 and 2) or 1 litre (system 3 and 4) flasks each connected via butyl rubber tubing to the top of a calibrated cylindrical column. The base of each cylindrical column was connected by butyl rubber tubing to a reservoir of acidified (citric acid 0.5%) saturated salt solution (Fig. 4.1). Saturated salt solution was used to minimise the dissolution of methane in the liquid reservoir and therefore reduce possible isotopic fractionation. Initially, the apparatus was set up and sealed, a hydraulic head created between the column and the reservoir and left for 24-72 hours to test for gas and liquid leakage. Once each system was gas tight it was inoculated.

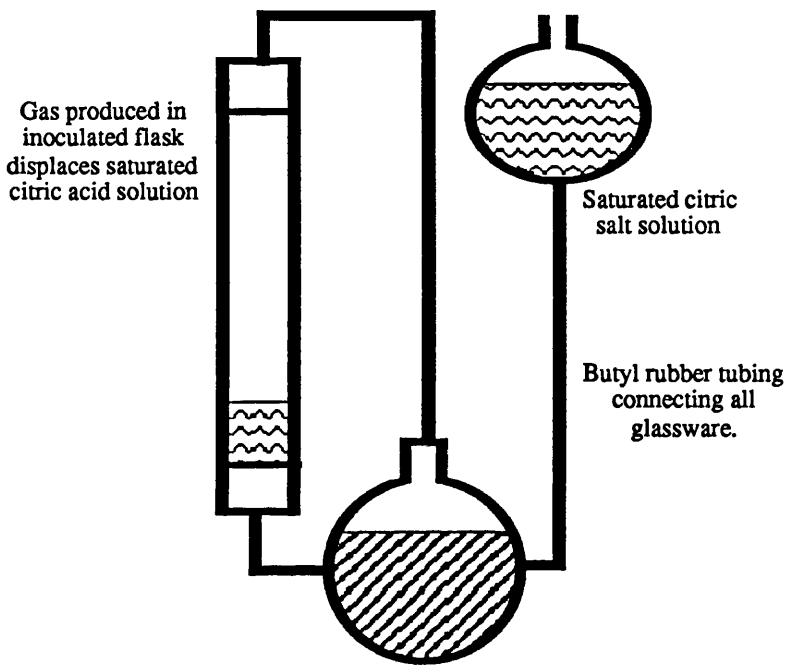


Figure 4.1. Diagrammatic representation of a closed culture enrichment system.

Butyrate-degrading (systems 1 and 2) and hexanoate-degrading associations were each enriched in duplicate systems. The latter associations failed to produce gas after preliminary gas sampling and analysis, but both flasks maintained a hydraulic head for the duration of the research, suggesting the cause was not an air leak inhibiting methanogenesis. The inoculum comprised six-week-old domestic refuse, hand sorted to homogenise size and grade by removing obvious non-biodegradable substances. System 1 and 2 were inoculated with 180g waste in 1200ml 5mM butyric acid. System 3 and 4 were inoculated with 75g waste in 500ml 5mM hexanoic acid solution. Within each pair, δD of the water initially added to each system (as pure water or used in the concentrated acid substrate for resupplementing) differed by 118‰. Isotopic measurements of waste, substrate for resupplementing and water are given in

Table 4.3.

Once inoculated, systems 1 and 2 were monitored for a period of 92 days. 14 days after inoculation, 2ml of supernatant liquid were extracted weekly for fatty acid concentration analysis. Carbon and hydrogen stable isotope analysis of the supernatant liquid commenced on a weekly basis on day 20. A preliminary gas sample was taken on day 22 and then on a weekly basis for 5 consecutive weeks for measurement of $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$. Gas samples were collected in evacuated gas sampling bottles through 6cm narrow bore metal tubing which connected the sample bottle to the culture cells through rubber seals. Due care was taken to allow the sample to equilibriate isotopically as well as hydraulically.

Volatile fatty acid (VFA) concentrations were assayed before resupplementing on a weekly basis by the gas chromatographic method of Watson-Craik and Senior (1989). VFA concentrations are precise (1σ) to 0.1mM.

Methane gas samples were prepared for mass spectrometry in a specially constructed and dedicated vacuum line similar to that designed by Stevens (Stevens and Rust, 1982), which was described more fully in Chapter 2. $\delta^{13}C(CH_4)$ is precise (1σ) to ±2.2‰ and $\delta D(CH_4)$ to ±5.5‰. $\delta^{13}C$ and δD measurements of the waste used as inoculum, the substrate used for resupplementation, and the supernatant liquid in each system were carried out using a closed tube combustion method (Stuermer et al., 1978) for which $\delta^{13}C$ precision is ±0.2‰, while δD is quoted to an accuracy of ±2‰. $\delta^{13}C$ was measured using a VG Sira 10 mass spectrometer, δD was measured using a VG Micromass 602B. All δD measurements are quoted relative to SMOW and $\delta^{13}C$ measurements are quoted relative to PDB (Craig, 1957).

4.5. Results.

4.5.1. Notation:

For interpretation, the following notation has been used. $\delta^{13}C(CH_4)$‰ 'measured' refers to $\delta^{13}C$ measurement of CH_4 produced by the bacterial systems. This value is representative of the mixture of CH_4 produced by acetoclastic methanogenesis and CO_2 reduction.

$\delta^{13}C(CH_4)$‰ 'determined' refers to the value of CH_4 determined from the $\delta^{13}C(CH_4)$‰ measured that is proposed to have been produced solely by acetoclastic methanogenesis. $\delta^{13}C(CH_4)$‰ 'calculated' refers to any hypothetical value calculated from modelling. $\delta D(H_2O)$‰ refers to δD measurement of the liquid in which the bacteria grew. Although dissolved organics were present, water was the dominant compound and therefore this notation has been used. This term is also used in Table 4.3 to refer to the composition of the water that was added to each system at the beginning of the experiment; however as these values are only used in preliminary calculations (equations 4.1-4.6) it was not deemed necessary to devise another term for the supernatant liquid.

Measured $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ for systems 1 and 2 and preliminary values for systems 3 and 4 are given in Table 4.1. The minimum concentrations of CH_4 and CO_2 present in the headspace gas are also given . $\delta^{13}C$ and δD of the supernatant liquid for all four systems is given in Table 4.2. $\delta^{13}C$ and δD of potential substrates is given in Table 4.3.

Sample	D.E.S.I.	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$	$\delta^{13}C(CH_4)$ calculated	min. % CH_4	min. % CO_2
System 1	22	-63	-8.6	-350		14	54
System 2	22	-61	-8.0	-298		10	50
System 3	27	-59	-9.9	-297		11	45
System 4	27	-61	-9.2	-250		2	35
System 1	57	-62	1.0	-359	-60	39	25
System 2	57	-61	0.0	-277	-61		
System 1	64	-58	4.2	-352	-50	51	22
System 2	64	-53	1.0	-298	-37	53	28
System 1	71	-51	3.3	-354	-37	67	25
System 2	71	-51	1.0	-295	-47	47	27
System 1	78	-48	2.5	-353	-42	68	25
System 2	78	-47	1.0	-294	-39	66	31
System 1	85	-48	2.1	-355	-48	63	25
System 2	85	-47	0.6	-296	-47	63	30

DESI : Days elapsed since inoculation.

Table 4.1. Gas sample composition (%) and isotopic composition (‰).

DESI	System 1		System 2		DESI	System 3		System 4	
	$\delta^{13}C$	δD	$\delta^{13}C$	δD		$\delta^{13}C$	δD	$\delta^{13}C$	δD
20	-26.5	-51	-26.9	37	25	-27.9	-52	-26.6	37
27	-27.6	-48	-26.3	27	32	-29.6	-49	-27.1	28
38	-25.7	-38	-24.4	37	43	-26.3	-48	-27.2	12
45	-24.5	-50	-22.5	42	50	-27.2	-52		
52	-19.4	-45	-19.0	37	57	-26.7	-51	-26.6	36
57	-23.3	-50	-24.9	33	62	-26.0	-54	-25.4	33
64	-14.2	-50	-13.4	35	69	-17.4	-50	-28.4	37
71	-15.5	-49	-16.4	40	76	-27.7	-49	-27.6	37
78		-47	-22.0	40	83	-27.7	-48	-26.6	34

DESI : Days elapsed since inoculation.

Table 4.2. Isotopic composition of the supernatant liquid in the closed culture enrichment systems.

System 1	Substrate	$\delta^{13}C$	δD	$\delta D(H_2O)$
1	Butyrate	-28.2	-52	-56
2	Butyrate	-26.7	43	62
3	Hexanoate	-29.6	-81	-56
4	Hexanoate	-29.4	6	62
Waste 1 (paper like)		-25.2	-81	n.a.
Waste 2 (fabric like)		-25.0	-103	n.a.

n.a. not applicable

Table 4.3. $\delta^{13}C$‰ and δD‰ compositions of the acid used to resupplement each system and of two samples of waste typical of the inoculum.

$\delta^{13}C$ of both CH_4 and CO_2 in system 1 and 2 increases with time (Table 4.1). After the first sample systems 3 and 4 failed so no further data was produced. In system 1, $\delta^{13}C(CH_4)$ ranged from -63‰ at the beginning of the experiment to -48‰ on day 78 and $\delta^{13}C(CO_2)$ ranged from -8.6 to 2.0‰. In system 2, $\delta^{13}C(CH_4)$ ranged from -61 to -47‰ and $\delta^{13}C(CO_2)$ from -2.1 to 0.6‰. Between days 57 and 78 (a period of 3 weeks), $\delta^{13}C(CH_4)$ became isotopically heavier by 13‰ for system 1 and 14‰ for system 2, which is a substantial difference. From day 78 onwards, $\delta^{13}C(CH_4)$ for systems 1 and 2 can be considered to be of a constant isotopic composition.

$\delta^{13}C(CO_2)$ rose sharply after the preliminary measurement, only to level off, and, in the case of system 1, fall. In comparison with $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ remained essentially constant with time after day 57 (system 1 : 2.6±1.2‰ (n=5), system 2 : 0.7±0.4‰ (n=5)), (Fig3).

Throughout the duration of the experiment $\delta D(CH_4)$ produced for both system 1 and 2 remained essentially constant. δD of system 1 ranged from -359 to -350‰ with a mean of -354±3‰, n=6. δD ranged from -298 to -277‰ with a mean of -293±8‰, n=6. With the exception of the samples taken on day 57, the difference in $\delta D(CH_4)$ between system 1 and 2 at each sampling interval ranged from 52 to 59‰, mean of 56.6±3.4‰, n=5. $\delta D(CH_4)$ from system 3 and 4 was isotopically heavier with values of -297‰, n=1 and -250‰, n=1, respectively.

CH_4:CO_2 concentrations remained constant in systems 1 from day 71 onwards (mean, 66±2.6% CH_4 : 25±0% CO_2, n=3). System 2 concentrations remained constant from day 78 onwards (mean, 64.5±2.1% CH_4 : 30.5±0.7% CO_2, n=12). The relative concentration of each gas was not substantially different in system 2 from system 1.

4.6. Interpretation :

4.6.1. Assumptions and notable points:

The complete anaerobic degradation of waste, involving methanogenesis, is complex, with some biochemical pathways not yet fully understood. Therefore, based on available

evidence the following assumptions have been made for the purposes of modelling.

Firstly, that by resupplementing with a specific fatty acid, the corresponding degrading association was dominant and active within the system. Systems 1 and 2 were therefore dominated by butyrate degrading associations while in system 3 and 4 hexanoate degrading associations were dominant.

Secondly, although gas was produced by systems 1 and 2 in the same volume as before, once resupplementing ceased, the gas samples taken during this period are assumed to be produced by degradation of the landfill waste by the same pathways as dominant when resupplementing.

System 3 and 4 appeared to fail after the first gas sample was taken. The maintenance of a hydraulic head in each system for the remainder of the experiment suggested air was not inhibiting methanogenesis. Although only one data set for CH_4 and CO_2 were produced from each system in comparison to systems 1 and 2 where n= 6 and 5 respectively, there is no reason to doubt that when first sampled 3 and 4 were not functioning healthily. As such the third assumption will be made that system 3 and 4 would have continued to develop at the same rate and in the same manner as system 1 and 2. $\delta D(CH_4)$ would therefore have remained essentially constant with time and as such the primary measurement will be considered to be representative of the mean value for each system if the further five gas samples could have been taken for the duration of the experiment.

The amount of carbon considered to have gone into cell biomass in comparison to the amount of substrate utilised is small (pers. comm., Senior, 1993). The fourth assumption is that reasonable fractionation factors can be calculated between the substrate and the gases measured without concern that such estimates are inaccurate due to fractionation caused by C fixation by bacteria.

Finally, the design of the apparatus did not allow sampling of all headspace gas. Approximately 1/3 of headspace gas was abstracted at each sampling time. It therefore should be borne in mind that $\delta^{13}C$ of CH_4 and CO_2 produced between sampling might be isotopically lighter / heavier (depending on trend shift) than the composition measured from day 57-78. Probable values for gas isotope composition produced between sampling are given in Table 4.1. and have been calculated from simple mass balance equations, assuming one third was extracted during sampling.

4.6.2. Applying volatile fatty acid analysis (VFA) to determine biochemical pathways:

The dynamics of a methanogenic system may allow the transient dominance of one of the pathways outlined earlier, depending on limiting factors e.g. availability of substrate. As the limiting factors change so may the dominant methanogenic pathway. Resupplementation was stopped on day 78 with the last gas sample taken on day 85. Observation of systems 1 and 2 on day 92 showed that systems 1 and 2 had continued to produce gas to the same extent as previously observed. As it is likely that the added butyric acid would have been utilised within a few days, this indicates that, throughout the experiment, the refuse still provided a pool of

substrates suitable for methanogenesis and these were most probably degraded via the butyric acid pathway. However, once resupplementation ceased, the input by butyric acid-degrading associations may have been less, with methane produced by the production and degradation of other intermediates influencing $\delta D(CH_4)$ to a greater extent. A sample was not taken on day 92.

Volatile fatty acid analysis alone cannot document multiple substrate utilisation because of simultaneous production /utilisation; however this technique can give an indication that methanogenesis is occurring by certain pathways.

From day 39 systems 1 and 2 were supplemented with butyric acid (5mM), which allowed the development and dominance of a bacterial association competent to degrade butyric acid. Supplementation with butyrate did not commence until 39 days after inoculation as until then concentrations increased to a maximum (measured) of 11.5mM in system 1 and 13.9mM in system 2, which suggests that genesis exceeded trophy, i.e. production rates exceeded utilisation rates. Subsequent to day 39, concentrations of residual butyrate, 7 days after resupplementation never exceeded 0.1mM. Net valerate and hexanoate concentrations were comparable. This suggests that either the bacteria producing the latter two were not active or that hexanoate and valerate degrading bacteria were also active.

Volatile fatty acid analysis of system 1 indicated that acetate levels rose to a maximum concentration of 17.5mM on day 22 falling to between 5 and 10mM for the remainder of the experiment. This pattern is characteristic of batch anaerobic fermentation in which, prior to the development of strongly reducing conditions and a methanogenic population, acetogenic activity results in a transient accumulation of acetate. Butyrate levels rose, (without resupplementation with butyric acid) to a maximum of 7.7mM on day 27 but then dropped sharply, averaging less than 0.1mM for the rest of the experiment, despite weekly resupplementing to bring the butyrate concentration back up to 5mM. This indicates that butyrate-degrading bacteria were highly active after this date. This pattern of acetate and butyrate production and degradation was repeated by system 2 and in similar concentrations.

Systems 3 and 4, selecting for hexanoate-degrading bacteria, did not show the same pattern. Hexanoate concentrations decreased to ~1.5mM concentration in system 3 on day 27 and on day 21 in system 4, but then rose to ~2.5 mM concentration, at which level they remained for the rest of the experiment. Acetate concentrations were > 10mM for the duration of the experiment in both systems, which may suggest that it was neither being produced by the acetogens nor utilised by the methanogens. VFA analysis suggests however, that systems 3 and 4 were functioning healthily at the time the sample was taken, and there is therefore no reason to doubt that $\delta D(CH_4)$ would not have remained constant as with system 3 and 4. Due to lack of data from systems 3 and 4 the interpretation will deal mainly with systems 1 and 2.

4.6.3. Interpretation of $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$:

$\delta^{13}C$ of both CH_4 and CO_2 in system 1 and 2 increases with time (Figure 4.3). The trend shown by $\delta^{13}C(CH_4)$ could be similar to that of a closed system, particularly if substrate limited, with progressive isotopic enrichment of the substrate reflected by a progressive $\delta^{13}C$

increase of the CH_4 produced. It has been suggested that bacteria preferentially utilise the lighter isotope, in this case ^{12}C, first (Daniels et al., 1980). However to assume such selectivity may be misleading. It is more likely to be a function of reaction rates, with bonds between lighter isotopes broken marginally faster than bonds involving heavier isotopes. The early reaction products are thus isotopically light, becoming heavier with time. Furthermore isotopic fractionation may be dominated by interaction between the enzyme and the corresponding molecular shape. Either way, the production of ^{12}C enriched CH_4 leaves the remaining substrate enriched in ^{13}C (Rosenfield and Silverman, 1959). However, there are several problems with attributing the trend in $\delta^{13}C(CH_4)$ to a closed system.

When resupplementation of fatty acid ceased, both systems 1 and 2 produced CH_4 in the same proportions as when resupplemented. This would suggest that the waste still contained labile compounds which the bacteria could utilise, and the availability of substrate is not a limiting factor. In contrast to this research, Rosenfield and Silverman (1959) maintained a closed system, with no substrate resupplementation. Details of experimental duration are not given, except to state that 85% of theoretical yield was reached after 25 days. This makes it difficult to draw an accurate comparison for it suggests that the timescale of their experiment was less than this present study.

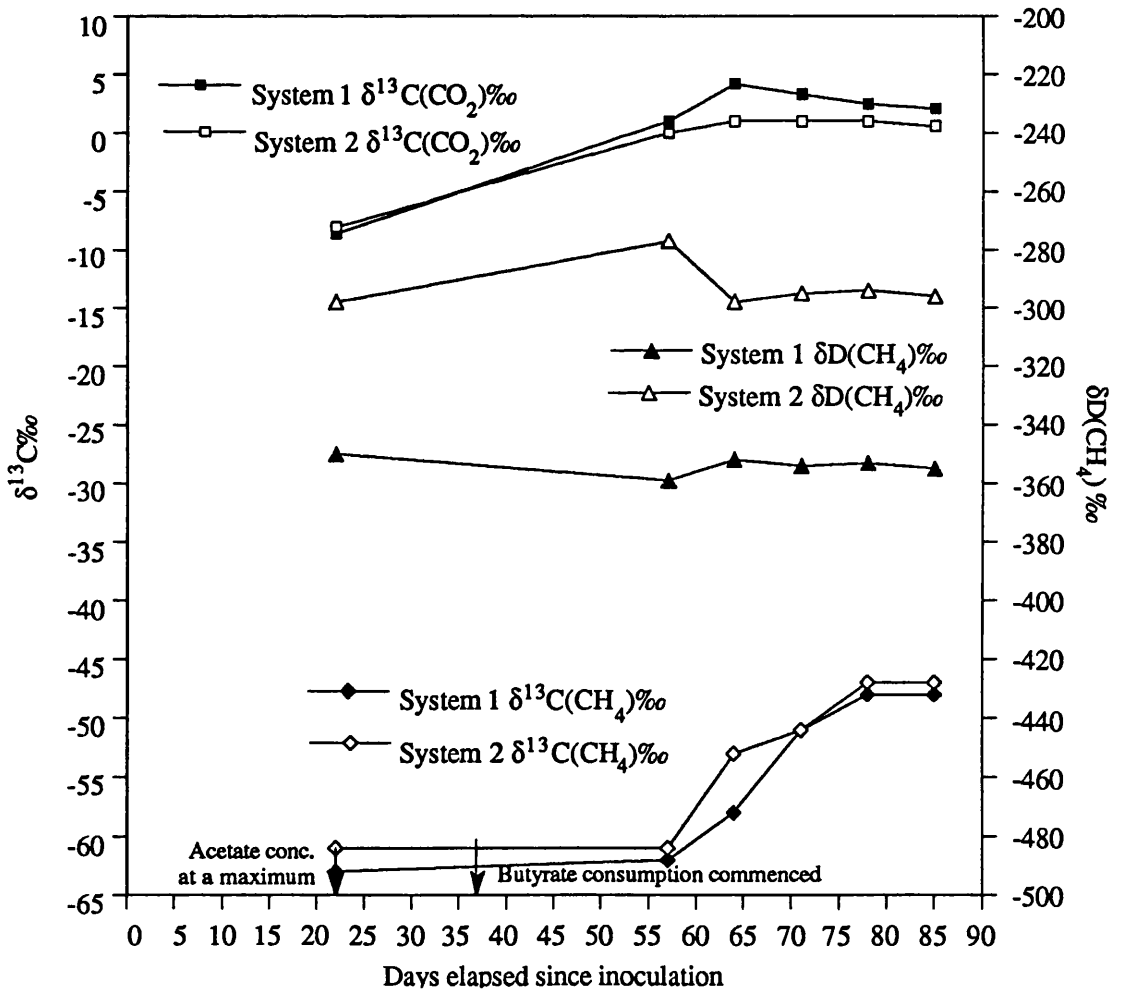


Figure 4.3. $\delta^{13}C(CH_4)$, $^{13}C(CO_2)$ and $\delta D(CH_4)$ for systems 1 and 2 with time.

The trend shown by $\delta^{13}C(CH_4)$ when plotted as a function of time (Fig. 4.3), is similar to that displayed by a bacterial growth curve (Pirt, 1975). Although gas was not sampled between 0-40 days it is unlikely that it varied significantly. This period in a bacterial growth curve (Hobson et al., 1981), the lag phase, is a period of bacterial metabolic adjustment, the duration of which depends on factors such as the time that has elapsed since the inoculation of the parent culture. The rapid change in $\delta^{13}C(CH_4)$ between days 57-78 could be equated to the period of intense growth that occurs, after the lag period, if the environment is suitable. As the cells grow they continuously modify the composition of their sustaining medium (which may be reflected in isotope measurements of the supernatant liquid given in Table 4.2.) by nutrient consumption and waste product excretion. From day 78 onwards, $\delta^{13}C(CH_4)$ for systems 1 and 2 can be considered to be of a constant isotopic composition, with this constancy perhaps reflecting a biological system in dynamic equilibrium, where growth is minimum / equalled by death. If the isotope values reflected a closed system, such dynamic equilibrium would not be observed, but rather continued enrichment of $\delta^{13}C(CH_4)$.

It is not yet known whether CH_4 production (and therefore $\delta^{13}C(CH_4)$) is growth related. As such, absolute conclusions cannot be drawn relating isotope fractionation to bacterial growth and levels of CH_4 production; however the similarity of $\delta^{13}C(CH_4)$ to a bacterial growth curve should not be ignored.

Average $\delta^{13}C$ values of the potential organic substrate for system 1 and 2 were estimated to be -26.1±1.8‰ and -25.6±0.9‰ respectively. These were calculated from the mean of the corresponding system substrate and the two waste values (Table 4.3.). $\Delta^{13}C$ between potential substrate and CH_4 for systems 1 and 2 between days 20-57 was 36.4‰ and 35.4‰ respectively. Once dynamic equilibrium is achieved, (day 78 onwards), and methane production was considered to be dominated by acetoclastic methanogenesis $\Delta^{13}C$ for systems 1 and 2 was 21.9‰ and 21.4‰ respectively. This fractionation for both systems 1 and 2 is 0.6 of that calculated for the initial fractionation.

One complementary interpretation of the trend shown by $\delta^{13}C(CH_4)$ could be as follows. Results obtained by Kryzcki et al., (1987), who incubated *Methanosarcina barkeri* on acetate, CO_2 and methanol, support the contention of Whiticar et al., (1986) that acetoclastic methanogenesis produces less isotopic fractionation than the reduction of CO_2. CH_4 produced from acetate by *Methanosarcina barkeri* was relatively unenriched in ^{13}C, with the average $\Delta^{13}C$ value half of that observed for CO_2 utilisation and one third of that observed for methanol utilisation. It may be possible that the early isotopically light $\delta^{13}C(CH_4)$ sampled from system 1 and 2 from day 20 - 57 is attributable to the dominance of the CO_2 reduction pathway. CO_2 availability would not have been a limiting factor due to its abundance as a product of the early aerobic and subsequent anaerobic catabolism of the waste.

CO_2 produced simultaneously during the degradation process (and with CH_4 during acetoclastic methanogenesis), can be utilised as a substrate by other bacteria e.g. methanogenesis by the reduction of CO_2, or for the production of acetate (Braun et al., 1981); it can become dissolved in the supernatant liquid or it can diffuse into the headspace gas. Under

conditions of isotope equilibrium headspace $\delta^{13}C(CO_2)$ will be isotopically lighter than that of dissolved inorganic carbon (Deuser and Degens, 1967). The reverse is observed when comparing $\delta^{13}C$ of the supernatant liquid with the corresponding headspace $\delta^{13}CO_2$ measurements. Being biologically mediated the system is unlikely to be in isotopic equilibrium and furthermore, $\delta^{13}C$ of the supernatant liquid (Table 4.2.) is a measurement of all dissolved organic carbon in the system, not just CO_2. Two incubations, controlled to produce CH_4 dominantly by the reduction of CO_2, had headspace $\delta^{13}C(CO_2)$ compositions differing by approximately 7‰, yet gave rise to identical $\delta^{13}C(CH_4)$ measurements. This suggested that CO_2, produced in situ by the bacterial consortium, was utilised to produce CH_4 before equilibration could occur with the headspace gas (Sugimoto and Wada, 1993).

It is difficult to ascertain the nature and isotopic composition of the substrate during days 22-57. $\delta^{13}C(CO_2)$ measured on day 57 is substantially isotopically heavier than day 22, yet $\delta^{13}C(CH_4)$ composition for both system 1 and 2 is much closer in composition to day 22. VFA analyses show that acetate levels reached a maximum on day 22, and from day 39 show that butyrate was being consumed (converted to acetate and used as a substrate for acetoclastic methanogenesis), thus the input to $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ of the headspace gas sampled on day 57 from acetoclastic methanogenesis must be considered. Only $\delta^{13}C(CH_4)$ sampled on day 22 is truly representative of CH_4 produced by CO_2 reduction. $\delta^{13}C(CO_2)$ measured on day 22 is the residual of the CO_2 used to produce $\delta^{13}C(CH_4)$ measured on day 22. The bacterial associations will utilise dissolved CO_2 (Sugimoto and Wada, 1993), therefore if the system was in equilibrium $\delta^{13}C(CO_2)$ substrate may have been isotopically lighter than that measured. If CO_2 (dissolved) was not in equilibrium with CO_2 (headspace), but utilised upon production, then it is still likely the early CO_2 consumed was isotopically lighter than that measured in the headspace.

The period of rapidly changing $\delta^{13}C(CH_4)$ could be the result of increasing dominance of CH_4 production from acetate, with a reduced isotopic fractionation between substrate and methane, as suggested by Kryzcki et al.(1987), resulting in high $\delta^{13}C(CH_4)$. Sugimoto and Wada (1993) noted that acetate consumption was accompanied by rapid CH_4 production and rapid increase of $\delta^{13}C(CH_4)$, from -60 to -33‰. The gas sample taken on day 57 is very similar in isotopic composition to the first gas sample, however subsequent sampling until day 78 when acetate concentrations are very low, showed an increase in $\delta^{13}C(CH_4)$ of 14‰, less than that of Sugimoto and Wada (1993), but still substantial.

The constant values after day 80 onwards reflect systems that could be considered to be in dynamic equilibrium, with acetoclastic methanogenesis dominating CO_2 reduction as suggested by the relatively heavy $\delta^{13}C(CH_4)$ measured. As $\delta^{13}C(CH_4)$ measured from day 78 onwards is likely to be representative of CH_4 produced by acetoclastic methanogenesis with minor CO_2 reduction, fractionation of the substrate solely by acetoclastic methanogenesis will be smaller.

A summary of the isotopic composition of the substrate suggested at day 22 (CO_2 reduction dominant) and from day 78 onwards (acetoclastic methanogenesis dominant) and a

comparison of $\Delta^{13}C$ for systems 1 and 2 for each period is given in Table 4.4. $\Delta^{13}C$ for system 1 from day 78 onwards is greater than 0.41 of that for day 22. $\Delta^{13}C$ for system 2 from day 78 onwards is greater than 0.40 of that for day 22. Both values are very similar to the figure 0.5 calculated by Kryzcki et al. (1987) when comparing $\Delta^{13}C$ for CH_4 produced by acetoclastic methanogenesis and CO_2 reduction.

	$\delta^{13}C(CO_2)$	$\delta^{13}C(CH_4)$	$\delta^{13}C$(methyl)	$\Delta^{13}C$
Day 22				
System 1	< ~ -8.6	-62.5		< ~ 53.9
System 2	< ~ -8.0	-61.0		< ~ 53.0
Days 78-85				
System 1		-48	< -26.1	< 21.9
System 2		-47	< -25.6	< 21.4

Table 4.4. $\Delta^{13}C$ calculations for systems 1 and 2. $\delta^{13}C$(methyl) represents $\delta^{13}C$ of the methyl carbons in the acetate molecule substrate. All values are expressed in ‰.

$\delta^{13}C(CO_2)$ rose sharply, only to level off, and, in the case of system 1, fall. The initial isotopically light CO_2 produced can, with certainty, be attributed to aerobic catabolism of the waste during the primary fermentative stages, while the system was evolving from aerobic to anaerobic. A proportion of this will have been consumed to produce the early CH_4 sampled, therefore $\delta^{13}C(CO_2)$ produced by aerobic catabolism of the waste will be isotopically lighter than the initial sample analysed. During this period the methanogens would not be active, but would establish themselves as the conditions became more reducing. As acetate utilising methanogens become dominant, isotopically heavy CO_2 ($\delta^{13}C(CO_2)$ >4.2‰ for system 1 and >1.0‰ for system 2) would be produced, dominating the resultant isotope composition of remaining CO_2 present. In comparison with $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ remained essentially constant with time after day 54 (Fig 4.3).

Fractionation factors between CH_4-CO_2-substrate have not been calculated in this study for the following reasons. As outlined previously, neither $\delta^{13}C(CH_4)$ nor $\delta^{13}C(CO_2)$ are solely representative of acetoclastic methanogenesis, e.g. CH_4 production by CO_2 reduction also occurs and CO_2 may be utilised at or close to source before equilibration with the headspace gas is possible. $\delta^{13}C$ of the substrate cannot be defined, only estimated. While $\delta^{13}C$ of the fatty acid supplement was characterised, two measurements of different types of landfill waste, although giving essentially identical values, are not suffice to characterise the overall $\delta^{13}C$ of the labile compounds. Finally, $\delta^{13}C$ of the supernatant liquid is unlikely to be representative of the substrate from which CH_4 sampled contemporaneously was produced, but rather represents the soluble component (and cell biomass) which may be utilised for future CH_4 production

Although CH_4 and CO_2 are produced in equal amounts as end products of acetoclastic methanogenesis, CO_2 production (and utilisation) in the fermentative stage, acetogenesis and

by possible homoacetogenic activity complicates calculation of input by acetoclastic methanogenesis vs. CO_2 reduction. Gas composition measurements (Table 4.1.) also show that more CO_2 than CH_4 was present in the early stages of the experiment, but that these proportions change with time, before levelling off in system 1 from day 71 and in system 2 from day 78. For a system producing CH_4 solely via acetoclastic methanogenesis the CH_4:CO_2 ratio should be 1:1. In this experiment the volume of CH_4 present was far more than the volume of CO_2, which suggests CO_2 reducing methanogens were also active or that the CO_2 produced was used to produce acetate. There are not sufficient gas measurements from system 3 and 4 to compare with 1 and 2 with regard to interpretation of $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ or concentration.

4.6.4. Estimate of carbon isotope composition of substrate:

$\delta^{13}C$(substrate) is most easily estimated by considering the CH_4 production pathway. The experiments by Buswell and Sollo (1948) and Stadtman and Barker (1949) (outlined earlier in section 4.3. A biochemical and isotopic review of bacterial methanogenesis') suggested that there was no carbon substitution of the substrate during acetoclastic methanogenesis and that significant carbon isotope fractionation does not occur here. The model of carbon and electron flow proposed by Zeikus et al. (1985) for energy conservation of methanogenic bacteria grown on acetate outlined clearly the biochemistry of the reduction of acetate to CH_4 and CO_2. From their diagrammatic representation it does not appear that any other source of carbon is introduced during the process.

Methyl coenzyme M is the last intermediary metabolite of methanogenesis. Its reduction to methane by the enzyme methylreductase is the common step in methanogenesis from acetate, CO_2 and methanol (Zeikus et al., 1985). The different fractionation factors observed by Kryzcki et al. (1987) for the substrates methanol, acetate and CO_2 by *Methanosarcina barkerri* suggest that the reduction of methyl coenzyme M to methane by methylreductase is not the major site of isotopic fractionation during methanogenesis. Any fractionation of the carbon that occurs during the reduction of acetate to CH_4 and CO_2 will be due to incorporation of carbon into the cell biomass, incomplete consumption of the acetate pool and a kinetic fractionation due to the difference in reaction rate of processing an isotopically heavier acetate molecule to a lighter one.

Therefore, if $\delta^{13}C(CH_4)$ for acetoclastic methanogenesis alone could be determined, it will be representative of $\delta^{13}C$(methyl) of the acetate that was utilised. For system 1 and 2 it can be suggested that $\delta^{13}C$(methyl) is heavier than -48‰ for system 1 and -47‰ for system 2 (Table 4.4.). $\delta^{13}C$(methyl) recorded in the literature has been as low as -42.9‰ (Sugimoto and Wada, 1993). It is suggested that $\delta^{13}C(CH_4)$ from day 22-57 was representative of CH_4 production dominated by CO_2 reduction, while $\delta^{13}C(CH_4)$ from day 78 onwards was dominated by acetoclastic methanogenesis with minor CO_2 reduction. $\Delta^{13}C$ for day 22 for system 1 and 2 averaged 36‰. Kryzcki et al. (1987) propose that $\Delta^{13}C$ for acetoclastic methanogenesis is half that for CO_2 reduction. $\Delta^{13}C$ for acetoclastic methanogenesis alone would therefore be 18‰, which would yield $\delta^{13}C(CH_4)$ of approximately -44‰, close to the

measured values. If the estimate of approximately -26‰ for $\delta^{13}C$ substrate for both systems is reasonable then $\Delta^{13}C$ of 18‰ between organic matter and methanogenic substrate acetate is also reasonable. One CO_2 reducing acetogen, *Acetobacter woodii*, produced acetate which was isotopically homogenous at both the methyl and carboxyl site and yet depleted in ^{13}C by as much as 57‰ relative to the total carbonate fraction (Gelwicks et al., 1989; Blair and Carter, 1992). The isotope effect was believed to be associated with the enzyme carbon monoxide dehydrogenase (Gelwicks et al., 1989). The first batch of acetate produced (before day 22, perhaps by the reduction of the early CO_2) may have been isotopically lighter than that produced later from less labile compounds and during butyrate degradation. $\Delta^{13}C$ for acetoclastic methanogenesis is less than that for CO_2 reduction, however the smaller fractionation may have been masked due to the isotopically lighter substrate and thus $\delta^{13}C(CH_4)$ appears similar to that sampled on day 22.

It is therefore suggested that the processes responsible for the synthesis of acetate will incur the largest isotope fractionations from the original organic substrate.

In principle, $\delta^{13}C$(acetate) should be controlled by the isotopic fractionation of its precursors, the isotopic fractionation associated with its synthesis and consumption and the relative rates of all processes which influence its pool size (Blair et al., 1985). Little is known about isotope effects associated with anaerobic synthesis of acetate. In systems 1 and 2, (selecting for butyrate degrading associations), the bulk of the acetate is likely to have been produced by degradation of butyrate via the β-oxidation pathway. The diagrammatic representation of the β-oxidation pathway (Fig. 4.2., overleaf) suggests that the methyl and carboxyl carbons retain the isotope signature of the fatty acid hydrocarbon chain from which they were derived.

I propose that $\delta^{13}C$(methyl) is isotopically light and as such $\delta^{13}C$ of Σ(methyl carbon precursor) on the hydrocarbon is likely to be depleted in ^{13}C. Galimov (1985) initially suggested that isotopic abundances at individual carbon positions should be principally controlled by structural factors, just as equilibrium distributions between CH_4 and CO_2 can be calculated from the reduced partition functions for these species. Approximate calculations suggested that highly reduced positions should be systematically depleted in ^{13}C, and that oxidised (often planar or linear, bonded to O or some other electro-negative element) positions should be enriched in ^{13}C. This would imply that the fatty acid hydrocarbon chain would be isotopically light and, as such, acetate that was produced could have isotopically equal methyl and carboxyl carbons. This is not reflected in measurements of $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$. Monson and Hayes, (1982) suggested that carbon atoms cannot be shuffled among locations within a complex structure in order to establish an equilibrium distribution of isotopes, but rather that they inherit their signature from their precursor. Fig. 4.2. depicting the β-oxidation of fatty acids suggests that acetate produced by this pathway will inherit the carbon isotopic signature of its precursor and thus, from $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$, measurements suggest that methyl carbon positions in the fatty acid molecule will be depleted in ^{13}C relative to the carboxyl carbon positions.

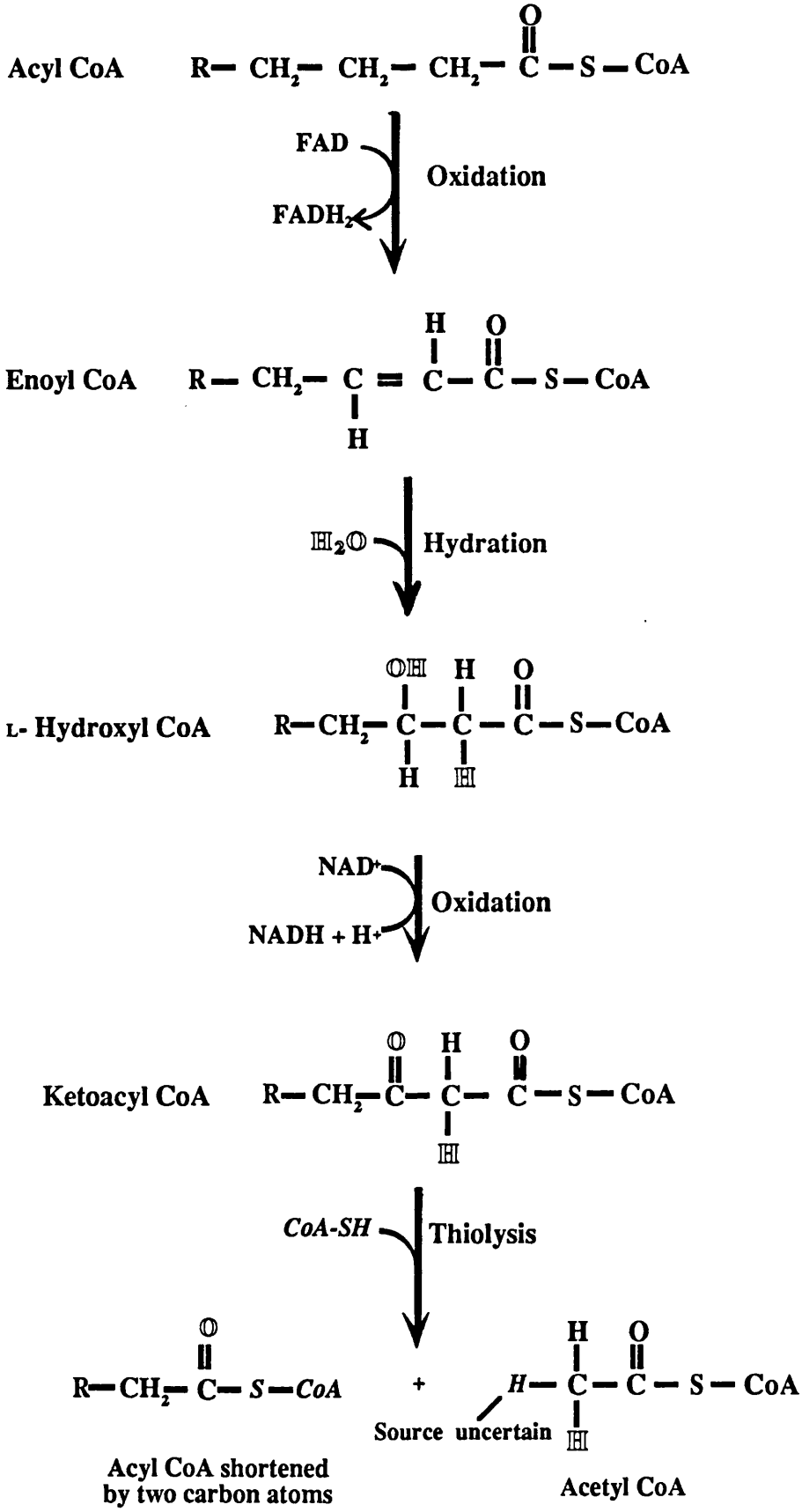


Figure 4.2. β-oxidation degradation of fatty acids (from Stryer, 1981).

4.6.5. Interpretation of $\delta D(CH_4)$:

It is noteworthy that $\delta D(H_2O)$ added to system 2 and 4 had a value of 62‰ and yet the first supernatant fluid $\delta D(H_2O)$ measured for each had a value of 37‰ (Table 4.2.). This has been attributed to mixing with landfill waste with a high moisture content typical of local meteoric water where $\delta D = \sim -55‰$. The same volume of water would have been added via the waste to systems 1 and 3; however as the H_2O added to this system was distilled local meteoric water where $\delta D = -56‰$, δD of the supernatant fluid appeared unchanged from that of the starting H_2O.

Throughout the duration of the experiment $\delta D(CH_4)$ produced for both system 1 and 2 remained essentially constant. If the trends shown by the $\delta^{13}C$ measurements reflect closed system bacterial fractionation, such selectivity would have been expected also to be reflected in $\delta D(CH_4)$, based on the assumption that 3/4 hydrogens on the methane molecule are from the acetate substrate (Whiticar et al, 1986). This is clearly not the case and casts further doubts that the pattern shown by $\delta^{13}C$ measurements reflects a closed system.

Furthermore, the homogeneity of $\delta D(CH_4)$ in comparison to changing $\delta^{13}C(CH_4)$ suggests that more than one hydrogen in the product CH_4 molecule is from an isotopically constant reservoir, large enough to dominate any small scale variation in other substrates present. Analyses of two differing components of landfill site waste, characteristic of that used in the inoculum for both systems (Table 4.3.) show that δD of the waste was isotopically lighter than the local meteoric water, but not homogenous. It is therefore suggested that the water added to each system is strongly influential in the constancy of $\delta D(CH_4)$. This is further strengthened by the fact that the only difference between substrates for the two pairs of systems was δD of the added water with the system with isotopically heavy H_2O in system 2 and 4 producing isotopically heavier CH_4.

H_2O is considered to be the sole source of hydrogen in CH_4 produced by the reduction of CO_2. Nakai et al. (1974) and Schoell (1980) reported the following linear relationship between natural biogenic CH_4 and formation water:

$$\delta D(CH_4) = \delta D(H_2O) - 160\ (\pm 10)‰ \qquad 4.1$$

Methanogenesis in freshwater sediments has been reported to represent a 70:30 mixture of acetoclastic methanogenesis (A.M): CO_2 reduction (R) (Whiticar et al., 1986). By comparing volume for volume CO_2/CH_4, if CO_2 was not being produced or consumed in any other part of the anaerobic degradative processes then the corresponding ratios would be 69%:31% for system 1, 74%:26% for system 2, in agreement with the ratio most commonly quoted in the literature of 70:30.

For system 1, where $\delta D(H_2O)$ mean = -48‰

$$\delta D_T M_T = \delta D_1 M_1\ (\textit{acetoclastic methanogenesis}) + \delta D_2 M_2\ (\textit{CO}_2\ \textit{reduction}) \qquad 4.2$$

$$\delta D(CH_4) = (\delta D(CH_4)M_1) + .(\delta D(CH_4)M_2)$$

$$= 0.69\delta D(CH_4) + 0.31(\delta D(H_2O) - 160)$$

$\delta D(CH_4)$ by acetoclastic methanogenesis = -420‰.

For system 2 where $\delta D(H_2O)$ = 36‰, $\delta D(CH_4)$ by acetoclastic methanogenesis = -351‰.

To summarise, $\delta D(CH_4)$ determined to have been produced by acetoclastic methanogenesis in system 1 and 2 had values of -420‰ and -351‰ respectively. $\delta D(CH_4)$ measured from each system is isotopically heavier, but this is due to approximately 30% input of CH_4 produced by CO_2 reduction, which had values of -208‰ in system 1 and -124‰ in system 2. Although the $\delta D(CH_4)$ acetoclastic methanogenesis input will not be determined for systems 3 and 4 for the reasons outlined previously, $\delta D(CH_4)$ determined via this pathway is likely to be isotopically lighter than that measured.

4.6.5.1. Relationship between $\delta D(CH_4)$ and $\delta D(H_2O)$:

Schoell (1980), found experimentally with sewage sludges that

$$\delta D(CH_4) = \delta D(H_2O) - 321‰ \qquad 4.3$$

Using this equation, $\delta D(CH_4)$ for system 1, 2, 3 and 4 can be calculated as -369‰, -285‰, -373‰ and -284‰ respectively. Measured values were -354‰, -296‰, -297‰ and -250‰ respectively. Values for system 1 and 2 are close to those predicted, however values for system 3 and 4 are isotopically lighter than those measured, which suggests that this equation is not suitable for modelling all methanogenic systems. This is not surprising, for in sewage sludges a complete anaerobic degrading bacterial colony will be present and therefore acetoclastic methanogenesis is only one methanogenic pathway that may be active. As the substrate and environment change so may the dominant pathway by which CH_4 may be produced. More accurate linear relationships relating $\delta D(H_2O)$ to $\delta D(CH_4)$ for this research are as follows:

For systems 1 and 2 : $\delta D(CH_4) = 0.69\delta D(H_2O) - 320‰$ 4.4

For systems 3 and 4 : $\delta D(CH_4) = 0.52\delta D(H_2O) - 269‰$ 4.5

Whiticar et al. (1986), described $\delta D(CH_4)$ formed by the acetoclastic methanogenesis pathway as follows

$$\delta D(CH_4) = 0.25\delta D(H_2O) + b \qquad 4.6$$

where 75% of the hydrogen is derived from the methyl group and the formation water is taken to be the ultimate source of the remaining 25% of hydrogen (Pine and Barker, 1956).

Whiticar et al. (1986) postulate b is dependent on

a) δD of the hydrogens in the transferred CH_3 group of the acetate or other precursor

b) the hydrogen isotope fractionation associated with the transfer

c) the isotopic fractionation associated with the addition of the final hydrogen.

It is therefore not surprising that equation 4.3 cannot be used to relate $\delta D(H_2O)$ to $\delta D(CH_4)$ for all bacterial gas production. The influence of $\delta D(H_2O)$ on the resultant $\delta D(CH_4)$ is less clearly defined for the acetoclastic methanogenesis pathway than for the CO_2 reduction pathway.

With few δD paired measurements of bacterial CH_4 and the coexisting formation water available in the literature (Whiticar et al., 1986) it is necessary to look closer at the

biochemistry of CH_4 production to aid elucidation of hydrogen isotope fractionation.

To understand the significance of $\delta D(CH_4)$ from this experiment, the synthesis and cleavage of acetate and its precursors must be considered. The role of the acetogens in degrading short chain fatty acids to acetate is of particular interest.

4.6.5.2. Applying biochemistry to isotopic modelling:

The addition of one hydrogen from the formation water to a CH_3 group in methanogenesis (Whiticar et al., 1986) is not questioned. However, the uncertainty which lies in the value of b (equation 4.6) may be overcome by consideration of the biochemical processes which form and locate b in acetate.

Acetate molecules can be produced by the degradation of fatty acids (of chain length n) by the β-oxidation pathway (Fig. 4.1.) As the bacterial associations selected for in this research are degrading butyrate (n=4) and hexanoate (n=6), this discussion will concentrate on the degradation of even numbered fatty acids. Degradation of odd chain fatty acids proceeds by the same pathway, except that in the final thiolysis step propionyl CoA (n=3) and acetyl CoA are produced rather than two molecules of acetyl CoA (Stryer, 1981).

The fatty acid is degraded to n/2 acetate molecules, by a recurring sequence of 4 reactions, involving the removal of two carbon atoms (attached to the acid functional group) and a hydrolysis reaction for each complete degradation cycle. The hydrolysis and thiolysis reactions are important when modelling $\delta D(CH_4)$. In the former, one further hydrogen from the formation water (in this research, supernatant H_2O) is incorporated into the organic hydrocarbon chain, while during thiolysis one further hydrogen is added to the acetyl CoA molecule. The most likely source of the hydrogen on the enzyme is also from the water as HS dissociates readily in water. If the hydrogen was not originally derived from the water, subsequent isotopic exchange with the water is likely.

Acetyl CoA must be converted to acetate before expulsion from the cell and this requires one further molecule of water, with a hydroxyl group replacing the CoA bonded to the carboxyl carbon. If this happens contemporaneously with thiolysis it is quite possible that the third hydrogen on the methyl group is again derived from the water

The remaining smaller chain (C'= n-2) acid undergoes the same β-oxidation degradation process until depleted.

The most important conclusions from the biochemistry of fatty acid degradation is that with the exception of the last molecule of acetate to be produced from the fatty acid, the methyl group of all other molecules ((n/2)-1) of acetyl Co-A contains one original hydrogen from the organic matter, 1 (possibly 2), hydrogens from the addition of water and possibly one hydrogen from the CoA-SH enzyme in the methyl group. Further, it is quite likely that the H from the enzyme may also be derived from the water.

The dynamics of methanogenesis make it a very complex situation to model isotopically, complicated by interspecies hydrogen ion transfer, exchange with the surrounding H_2O, and the involvement of enzymes, making isotope mass balance equations difficult to formulate.

However it is clear from Fig. 4.1. that mass balance equations can be constructed based on the biochemical pathways.

For the mass balance equations, it is assumed that $\delta D(H_2O)$ measured from the organic rich supernatant liquid will be dominated by δD of the added water and therefore the mean of all δD supernatant liquid values measured is used for $\delta D(H_2O)$. It is also assumed that although the bacterial association selected for was active, the majority of CH_4 produced by these pathways was using landfill waste substrate and not the added acid. As such δD of the landfill waste is used for δD(organic). Measurement of δD(organic) gave rise to two close values (table 4.3), therefore the mean of these has been used. δD(enzyme CoA-SH) is unknown, but is likely to be the same as $\delta D(H_2O)$ for the hydrogen in a thiol bond exchanges readily with the solvent, in this case water.

The mass balance equation for $\delta D(CH_4)$ produced by acetoclastic methanogenesis, (with β-oxidation of fatty acids providing the acetate substrate), could thus be expanded as follows:

$$\delta D(CH_4) = 0.25\delta D(H_2O) + 0.75\delta D(\text{methyl group}) \quad 4.7a$$
$$= 0.25\delta D(H_2O) + 0.75(\delta D(H_2O) + \delta D(\text{organic}) + \delta D(\text{enzyme CoA-SH})$$

If the third hydrogen on the methyl group is from water and not from enzyme CoA-SH this simplifies to

$$\delta D(CH_4) = 0.25\delta D(H_2O) + 0.75\delta D(\text{methyl group})$$
$$= 0.25\delta D(H_2O) + 0.75(\delta D(H_2O) + \delta D(\text{organic})) \quad 4.7b$$

Each molecule of acetate produced is cleaved to one molecule of CH_4 and one molecule of CO_2. From the diagrammatic representation of the β-oxidation pathway it can be envisaged that as the fatty acid chain length increases, the proportion of hydrogens derived from the water in the acetyl -CoA molecules increases. Conversely, the proportion of hydrogen derived from organic matter as a total of acetate molecules produced during β-oxidation degradation decreases as the length of the fatty acid increases. As such a higher proportion of hydrogen in the final reduced product of n/2 methane molecules is from the formation H_2O and a smaller proportion from organic matter.

4.6.5.3. Application of the above model to this research:

The following two equations model the hydrogen input to a complete CH_4 molecule, describing $\delta D(CH_4)$ in terms of the proportions of hydrogen from each source, as a function of the length of fatty acid being degraded. As before, n refers to the carbon chain length of the fatty acid.

Where n is an even number:

$$\delta D(CH_4) = \frac{n-1}{2n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) + \frac{n-2}{4n}\delta D(enzymeCoA-SH) \quad 4.8$$

If the third hydrogen on the methyl group (added during thiolysis) of (n/2) - 1 acetate molecules is also derived from the water then this simplifies to

$$\delta D(CH_4) = \frac{3n-4}{4n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) \quad 4.8a$$

System 1 and 2 were selecting for an association of butyrate (n=4) degrading bacteria; system 3 and 4 were selecting for hexanoate degrading bacteria (n=6). The appropriate mass balance equations for each pairs of systems would therefore be as follows. Fractions have not been simplified to the lowest common denominator, for the denominator is representative of the total number of hydrogens needed to convert all acetyl-CoA molecules to CH_4. Dividing the denominator by 4, or dividing n by 2 is an indication of the number of molecules of methane will be produced if complete conversion of the fatty acid occurs.

For system 1 and 2, n=4:

$\delta D(CH_4) = 3/8\delta D(H_2O) + 4/8\ \delta D(\text{organic matter}) + 1/8\ \delta D(\text{enzyme CoA-SH})$ 4.9

If the third hydrogen is derived from the H_2O, and not enzyme CoA-SH, this can be simplified to :

$\delta D(CH_4) = 4/8\delta D(H_2O) + 4/8\ \delta D(\text{organic matter})$ 4.9a

For system 3 and 4, n=6:

$\delta D(CH_4) = 5/12\ \delta D(H_2O) + 5/12\ \delta D(\text{organic}) + 2/12\ \delta D(\text{enzyme CoA-SH})$ 4.10

If the third hydrogen is derived from the H2O this can be simplified to :

$\delta D(CH_4) = 7/12\ \delta D(H_2O) + 5/12\ \delta D(\text{organic})$ 4.10a

In system 3 and 4, a minimum of 4.16% more hydrogen from the formation water will be incorporated into the product CH_4 than in system 1 and 2. If the third hydrogen in the methyl group is also derived from the water then in system 3 and 4 8.3% more hydrogen from the formation water will be incorporated into the product CH_4 than in system 1 and 2. Consider the proportions of each input, with increasing chain length where the third hydrogen is not derived from the water.

$\delta D(H_2O)$ can be considered to dominate the decreasing influence of δD(enzyme CoA-SH), but for acetate methyl groups produced from short chain fatty acids the input from $\delta D(H_2O)$ is equalled or dominated by δD(organic). As the chain length increases, $n > 6$, $\delta D(H_2O)$ input will be greater than δD(organic). As such, the longer the chain length, the higher water input and the isotopically heavier / lighter $\delta D(CH_4)$ may become, dependent on the value of $\delta D(H_2O)$. In all cases either $\delta D(H_2O)$ or δD(organic) dominates the composition of δD(enzyme CoA-SH).

However, if the hydrogen in the enzyme CoA-SH is derived from the water, it is likely that δD(enzyme CoA-SH) is the same as $\delta D(H_2O)$. Therefore, when $n = 4$ the input from $\delta D(H_2O)$ equals that of δD(organic). If $n > 4$, then the input from $\delta D(H_2O)$ is greater than that from δD(organic).

Furthermore, as only a fraction of $\delta D(H_2O)$ is incorporated into $\delta D(CH_4)$, then the difference in $\delta D(CH_4)$ between two systems identical, but supplemented with different

$\delta D(H_2O)$, will be less than the difference between the two $\delta D(H_2O)$, as was observed with measured $\delta D(CH_4)$ values.

4.6.5.4. Applying the above modelling to the measured data from Table 4.1.:
Substituting the appropriate values gives calculated $\delta D(CH_4)$ as follows.
For system 1 and 2, butyrate degrading associations where n = 4 using equation 8

$$\delta D(CH_4) = \frac{n-1}{2n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) + \frac{n-2}{4n}\delta D(enzymeCoA-SH)$$

$= 3/8\delta D(H_2O) + 4/8\ \delta D(\text{organic matter}) + 1/8\ \delta D(\text{enzyme CoA-SH})$

$\delta D(CH_4)$ of system 1 = -70‰ 4.11

$$\delta D(CH_4) = \frac{n-1}{2n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) + \frac{n-2}{4n}\delta D(enzymeCoA-SH)$$

$= 3/8\ \delta D(H_2O) + 8/16\ \delta D(\text{organic matter}) + 1/8\ \delta D(\text{enzyme CoA-SH})$

$\delta D(CH_4)$ of system 2 = -28‰ 4.12

$\delta D(CH_4)$ for systems 3 and 4 have been calculated in the same manner using equation 4.8, except that as only one gas sample from each system was taken δD of the supernatant liquid measured three days before the gas sample was taken is used for $\delta D(H_2O)$, instead of the mean of all supernatant liquid measurements.

For system 3 and 4, hexanoate degrading associations where n = 6

$$\delta D(CH_4) = \frac{n-1}{2n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) + \frac{n-2}{4n}\delta D(enzymeCoA-SH)$$

$= 5/12\ \delta D(H_2O) + 5/12\ \delta D(\text{organic}) + 2/12\ \delta D(\text{enzyme CoA-SH})$

$\delta D(CH_4)$ for system 3 = -69‰. 13
Likewise $\delta D(CH_4)$ for system 4 = - 17‰. 4.13

To summarise, $\delta D(CH_4)$ calculated, using unfractionated substrate values, for systems 1 and 2, butyrate degrading associations, is -70‰ and -28‰ respectively. $\delta D(CH_4)$ for systems 3 and 4, hexanoate degrading associations, is -69‰ and -17‰ respectively. System 3 is similar to system 1, likewise system 4 is similar to system 2, in all aspects except the fatty acids used to supplement had a longer chain length by 2 carbon atoms and therefore 4% more hydrogen from the H_2O will be incorporated into the CH_4.

$\delta D(CH_4)$ for system 3 gives essentially the same value as system 1, whilst calculated $\delta D(CH_4)$ for system 4 is, as predicted by the modelling, 11‰ heavier than that of system 2. $\delta D(CH_4)$ is however, a function of chain length, $\delta D(H_2O)$ and $\delta D(\text{organic})$. In system 3, more water hydrogen is incorporated into the CH_4, but $\delta D(H_2O)$ is 4‰ lighter than $\delta D(H_2O)$ for system 1 and as the organic matter is isotopically lighter than the water, the difference between $\delta D(CH_4)$ of system 1 and 3 becomes less. There is a bigger difference between systems 2 and 4 as $\delta D(H_2O)$ used to model each system is different by only 1‰.

During β-oxidation of fatty acids, more isotopically heavy formation water would be incorporated into the acetate, (and hence CH_4) in system 3 and 4 than system 1 and 2. If the pathway modelled is to be validated, then a similar pattern of results from the calculated data should be observed as in the measured data. $\delta D(CH_4)$ measured from system 1 is -354±3‰, with input solely from acetoclastic methanogenesis < -420‰. $\delta D(CH_4)$ measured from system 3, (input from acetoclastic methanogenesis likely to be approximately 70‰ lighter), is heavier as the modelling predicts with a value of -297‰. $\delta D(CH_4)$ measured from system 2 is -296±2‰, with input solely from acetoclastic methanogenesis < -356‰. $\delta D(CH_4)$ measured from system 4 is again isotopically heavier at -250‰, even accounting for determined input from acetoclastic methanogenesis which is likely to be approximately 70‰ lighter.

From this preliminary modelling, it is immediately obvious that calculated $\delta D(CH_4)$ for all systems is substantially isotopically heavier than $\delta D(CH_4)$ measured or $\delta D(CH_4)$ estimated to have been produced by acetoclastic methanogenesis. This is not surprising. δD values substituted in the earlier equations modelled the composition of $\delta D(CH_4)$ if all reactants were converted to CH_4. They did not take into account isotopic fractionation of the reactants that will undoubtedly occur.

4.6.5.5. Hydrogen isotope fractionation within the cell:

Methanogenesis takes place within the cell (Stryer, 1981). In Archaebacteria the cytoplasmic membrane consists of glycerolipids in which L-glycerol is ether-linked to long chain phytl groups (Hamilton, 1988). The lipid layer acts as a hydrophobic barrier which is essentially impermeable to the passage of most ions, including H+ (except via protein based transport systems), but can allow the rapid movement of uncharged species such as H_2O, O_2, CO_2 and NH_4 (Jones, 1988). Although this movement is controlled by homeoviscous adaption i.e. an optimum state is maintained by varying the lipid composition of the cytoplasmic membrane in response to changes in the environment, it is apparent that the water outwith the cell may enter into the cell. It is likely that the cell water will be kinetically fractionated substantially in comparison to the external water (Daniels et al, 1980), as there will be a faster diffusion rate for isotopically light molecules

If only H_2O (an unlimited reservoir) and not D_2O or HDO enters the cell, then $\delta D(CH_4)$ for system 1, 2, 3 and 4 would be identical. The initial $\delta D(H_2O)$ would be irrelevant and intracellular $\delta D(H_2O)$ for all four systems would be -1000‰. As such, $\delta D(CH_4)$ calculated using equations 4.8 would give values isotopically lighter than $\delta D(CH_4)$ measured or determined for acetoclastic methanogenesis. Fractionation of the organic matter would render $\delta D(CH_4)$ lighter still. It is clearly not the case that, for the bacterial cultures studied in this research, $\delta D(H_2O)$ in the cell is -1000‰.

It is plausible that the water may be more fractionated than the organic matter due to the substantial mass difference between deuterium and hydrogen affecting the rate of isotopic cell wall penetration. In contrast to previous manipulations, assigning $\delta D(H_2O)$ a value of -1000‰ gives rise to values for $\delta D(CH_4)$ in systems 3 and 4 isotopically lighter than $\delta D(CH_4)$ for

systems 1 and 2. This is still the outcome suggested by the modelling, for if $\delta D(H_2O)$ is isotopically lighter than δD(organic), as longer chained fatty acids are degraded there is a larger input of isotopically light hydrogen and $\delta D(CH_4)$ becomes lighter.

The difference in $\delta D(CH_4)$ between systems 1 and 3, and 2 and 4, ('measured' and 'calculated' value for acetoclastic methanogenesis input), suggests that $\delta D(H_2O)$ is isotopically heavier than δD(organic), for as the fatty acid chain length increases (systems 3 and 4), $\delta D(CH_4)$ becomes heavier. It is possible that $\delta D(H_2O)$ has been fractionated more than δD(organic), but this is masked as δD(organic) may have been initially isotopically lighter, than the mean value used. Two δD measurements may not be enough to characterise the reservoir of organic hydrogen feed from landfill waste. δD of the acid supplement has been characterised, but this is considered to be dominated by the landfill hydrogen. When the effect of fractionation on each source of hydrogen is unknown, it is clearly difficult to model $\delta D(CH_4)$ produced from a fatty acid substrate.

To summarise, it is suggested that when CH_4 is produced from fatty acids degraded by the β–oxidation pathway, as the chain length of the acid increases more acetate and hence CH_4 is produced that has a hydrogen atom component derived from H_2O. Calculations using the equations derived to model this process suggest all sources of hydrogen in CH_4 have been fractionated during methanogenesis. It is possible that $\delta D(H_2O)$ incorporated into CH_4 produced by these cultures is isotopically heavier than δD(organic). As methanogenesis takes place within the cell, it is likely that δD(enzyme CoA-SH) is also from the water and has a similar value to $\delta D(H_2O)$.

There is however one further conundrum. In the introduction, an example was given of measurements from two closed culture enrichment systems supplemented with butyrate or hexanoate. These yielded stable isotopic ratios of $\delta^{13}C$ = -28.7‰, δD = -316‰ and $\delta^{13}C$= -45.5‰, δD= -315‰ respectively. The difference in $\delta^{13}C$ of 16.8‰ is significant, while δD values can be considered identical. If the respective degrading association was active and dominant in each system, (as is assumed in the modelling) and all CH_4 produced was via β–oxidation of fatty acids, then $\delta D(CH_4)$ for the hexanoate degrading system would be expected to be isotopically heavier than that for the butyrate degrading system.

When sampled both these systems had been active for approximately 500 days, far longer than the duration of this research project. Thus caution must be applied in drawing comparisons between 2 dynamic systems, both at different stages of development. Unfortunately data for $\delta D(H_2O)$ of the supernatant liquid is not available, therefore there is an additional uncertainty as to whether this was the same for both systems. Furthermore it is possible that both systems were strongly influenced by CH_4 production via propiogenesis (propionate is a product of β–oxidation degradation of odd numbered fatty acids), another methanogenic pathway that becomes more dominant with time. The similarity in $\delta D(CH_4)$ of these two cultures does not cast aspersions on the modelling processes outlined previously, but merely serves to indicate the complexity of a methanogenic system.

4.6.6. Potential hydrogen isotope exchange, incorporation and fractionation during methanogenesis:

The same general principles as outlined in the carbon isotope fractionation regarding complete utilisation of substrate, reaction rates etc. are still applicable when considering hydrogen isotope fractionation.

This chapter has considered in more detail hydrogen isotope fractionation during acetoclastic methanogenesis, and looked at the biochemistry of fatty acid degradation to provide a greater understanding of the influence of H_2O on the resultant composition of the CH_4. There are however other stages during the anaerobic degradative process where H_2O could be incorporated into the organic molecule that is the final methanogenic substrate.

Consider a molecule of cellulose $(C_6H_{10}O_5)_n$, with more than 1500 glucose units per molecule. It is the most widespread organic material known (Morrison and Boyd, 1987), found in wood, plant fibres, cotton, paper and therefore could be considered a likely starting material in both landfill sites and natural environments. Carbon bound and oxygen bound hydrogen, both present in cellulose, behave differently in terms of isotope exchange. Carbon bound hydrogens in cellulose are non-exchangeable whereas the hydroxyl hydrogens exchange readily (Epstein et al., 1976). Before any biochemical degradation can occur it is quite possible that 30% of the hydrogen in the cellulose molecule (three out of ten hydrogens on each glucose unit) may be derived from the water.

As outlined earlier, one component of the first stage in the anaerobic degradation of the organic matter is hydrolysis, where complex organic compounds are split using extra-cellular hydrolytic enzymes, by the addition of water. If cellulose is degraded into its component glucose compounds, it is likely to be at the oxygen links between the glucose units (two on each glucose unit), with one water molecule added to each glucose unit in the form of one C-H bond and one C-OH bond. Then n-2 resultant glucose molecules now have 5/12 hydrogens derived from the water. In the preliminary stages there can be a substantial water input into the 'organic' substrate and as such, it is conceivable that in these experiments a proportion of the hydrogen on the fatty acids, before subject to β-oxidation to acetate, was H_2O derived.

The pathway from sugar to fatty acid has not been modelled isotopically, but rather the assumption is made that all hydrogen on the fatty acid is the original hydrogen on the organic matter, although this is unlikely to be the case. For instance, in the biochemical degradation of glucose to acetyl-CoA via the glycolytic pathway (Hamilton, 1988 and Stryer, 1981) there does not appear to be any input of H_2O into the organic matter, although there are additions of H^+ necessary at some stages, notably during the conversion of fructose 1, 6-diphosphate to dihydroxyacetone phosphate, an isomer of glyceraldehyde 3-phosphate, and also in the conversion of phosphophenolpyruvate to pyruvate. The ultimate origin of these hydrogen ions is uncertain, whether taken from the water or transferred during interspecies hydrogen transfer.

Methanogenesis does occur by other pathways e.g. methanol degradation is possible, with a multitude of intermediates produced during the anaerobic degradation of organic compounds before the final methanogenic substrate is produced. The biochemistry of these

processes may provide a greater understanding of isotope fractionation and proportional inputs during methanogenesis.

Pine and Barker (1956) showed that the hydrogen attached from the methyl group was transferred intact into the CH_4 molecule, with the fourth hydrogen derived from the water. Any fractionation during the methyl group transfer will be a kinetic one, dependent on transfer reaction rates, with an insignificant amount of hydrogen incorporated into the cell biomass. However fractionation will likely occur during the addition of the fourth hydrogen from the water.

Further hydrogen isotope fractionation will have occurred during hydrolysis and thiolysis in the synthesis of acetate from long chain fatty acids by β-oxidation, and in the early stages of hydrolysis outlined earlier. A significant fractionation is likely to occur in the transfer of compounds into and out of the cell, whether by diffusion, particularly significant when considering hydrogen isotope fractionation, or by a biological transfer.

4.7. Conclusions.

Four closed culture anaerobic enrichment systems were set up to produce CH_4 and monitored for a period of 92 days. Systems 1 and 2 were selecting for butyrate degrading associations and systems 3 and 4 for hexanoate degrading associations. $\delta D(H_2O)$ added to system 1 and 3 was identical and differed from $\delta D(H_2O)$ added to systems 2 and 4 by 118‰. Headspace gas was analysed for $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$. Supernatant liquid, the inoculum landfill site waste and the acid supplement were analysed for $\delta^{13}C$ and δD. Systems 1 and 2 were active and producing gas for the duration of the experiment, systems 3 and 4 failed after the first gas sample was taken.

Progressive enrichment in $\delta^{13}C$ of CH_4 with time for both systems 1 and 2 is not attributed to a closed system. Gas production continued after resupplementation ceased, suggesting that the waste still contained labile compounds and the systems were not substrate limited. Such enrichment is not observed in $\delta D(CH_4)$ which would be expected if the systems were closed and the source of the hydrogen was from the same substrate as the carbon. Furthermore, for the last 14 days of the experiment $\delta^{13}C(CH_4)$ remained essentially constant.

Rather, the pattern shown by $\delta^{13}C(CH_4)$ may reflect that of a bacterial growth within the system, although it is not yet known if CH_4 production is quantitatively related to bacteria growth. The initial isotopically light CO_2 can be attributed to aerobic catabolism of the waste during the primary fermentative stages. The early stages of CH_4 production may be dominated by the CO_2 reduction pathway, with the following period of rapidly changing $\delta D(CH_4)$ attributed to the increasing dominance of acetoclastic methanogenesis. The final period of dynamic equilibrium may be attributed to methanogenesis dominated by acetoclastic methanogenesis with a minor input from CO_2 reduction.

$\delta D(CH_4)$ measured is of accumulated CH_4 which has been produced by several methanogenic pathways. The relative input of CH_4 produced by acetoclastic methanogenesis in comparison to that produced by the reduction of CO_2 cannot be determined, due to production

and consumption of CO_2 at other stages during the anaerobic degradation of organic matter. $\delta D(CH_4)$ produced by acetoclastic methanogenesis is likely to be lower than the measured composition, while $\delta D(CH_4)$ produced by the reduction of CO_2 is isotopically heavier.

$\delta D(CH_4)$ for system 1 and 2 remained essentially constant with time. The constancy in $\delta D(CH_4)$ suggests that it was very strongly influenced by an 'unlimited' isotopically homogeneous reservoir, most likely water. $\delta D(CH_4)$ for systems 2 and 4 is isotopically heavier by approximately 50‰ than systems 1 and 3 respectively. The difference in $\delta D(CH_4)$ between system 1 and system 2 remained constant for the duration of the experiment, differing by approximately 56‰. This was attributed to the use of H_2O which was 110‰ heavier than used in the counterpart system.

Describing a universal linear relationship between $\delta D(CH_4)$ and $\delta D(H_2O)$ was not possible due to variability in the dominance of methanogenic pathways in different systems. In order to understand the significance of $\delta D(CH_4)$ to $\delta D(H_2O)$, the synthesis and cleavage of acetate and its precursors must be considered in more detail.

Equations have been suggested to model $\delta D(CH_4)$ produced via acetoclastic methanogenesis, when the acetate substrate is derived from fatty acids by the β-oxidation pathway. These quite clearly indicated that as the chain length of the acid increased more H_2O was incorporated into the acetate and hence CH_4. δD(enzyme CoA-SH) is likely to be the same as $\delta D(H_2O)$. Therefore, depending on the composition of $\delta D(H_2O)$ in relation to δD(organic) for two systems of identical acid chain length, $\delta D(CH_4)$ of the system degrading the longer chained fatty acid will be isotopically heavier / lighter than the system degrading the shorter chained fatty acid. As only a fraction of $\delta D(H_2O)$ is incorporated into $\delta D(CH_4)$, then the difference in $\delta D(CH_4)$ between two identical systems, supplemented with different $\delta D(H_2O)$, will be less than the difference between the two $\delta D(H_2O)$. Fractionation of the reactants occurred, and this research suggests that despite fractionation δD(organic) is still isotopically lighter than $\delta D(H_2O)$. $\delta D(H_2O)$ within the cell is unlikely to be -1000‰.

CHAPTER 5: CARBON & HYDROGEN STABLE ISOTOPE RATIOS OF CH_4 AND CO_2 AND THE SURROUNDING ENVIRONMENT, WITHIN ELLERGOWER MOSS, A RAISED PEAT BOG IN SW SCOTLAND.

5.1. Abstract.

The stable isotope ratios, $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$‰ of CH_4 and CO_2 from Ellergower Moss, a raised peat bog in S.W. Scotland were measured from gas samples collected in situ, from two profiles, at 1m intervals, from the surface to a depth of 5m. One of the profiles was sampled underneath a greenhouse, which was erected to consider the impact that global warming might exert on $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$‰ and temperature profiles within the peat. $\delta^{13}C(CH_4)$ had a mean of -75.6±3‰ (n=22); $\delta^{13}C(CO_2)$ had a mean of 3.8±5.3‰ (n=22); $\delta D(CH_4)$ had a mean of -294±39‰ (n=21). In each profile sampled $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ showed a general gradation from isotopically heavy at the 5m depth to isotopically light at the surface. $\delta D(CH_4)$ suggested the presence of a boundary between 2-3m as the mean measured value at 3-5m was substantially heavier than 0-2m by 52±18‰. Such a difference was also observed with $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ measurements. $\delta^{13}C$ and δD of the peat and of the pore water were also measured to examine the influence of δD(porewater) on $\delta D(CH_4)$. However such a boundary was not observed in δD(porewater). $\delta^{13}C$(peat) had a mean isotopic composition of -26.98±5‰. δD(peat) within the profile and at each measured depth was isotopically heterogeneous with a mean of -92.6±11.4‰, generally becoming isotopically heavier with decreasing depth. δD(porewater) had a mean of -37.7±3.1‰ and showed a gradual gradation with depth becoming isotopically heavier by 8‰ in the greenhouse profile and 6‰ in the control profile. In general δD(porewater) in the control profile was isotopically lighter than the greenhouse profile by 4±2.2‰. The effect that simulating global warming by placing a greenhouse of a 7.06m^2 section of the peat from May - Oct. 1992 was monitored by continuous measurements of temperature at depth. The greenhouse profile showed a significant increase in temperature down to a depth of 2m with warming of 2°C, which has implications for increased CH_4 production and hence global warming. It may be possible that the increase in ambient peat temperature caused by the erection of the greenhouse may be responsible for the isotopically heavier greenhouse profile samples collected between 0-2m.

5.2 Introduction

Biogenic CH_4 releases are an ubiquitous feature of recent anoxic environments, with flux measurements and isotopic signatures well documented from both freshwater and marine environments (Whiticar et al., 1986). One methanogenic freshwater environment is that of peatlands. These are unbalanced systems in which the rate of production of organic matter by living organisms exceeds the rate at which these compounds are decomposed (Moore & Bellamy, 1974). Permanent water-logging of the majority of the peat body results in anaerobic

conditions, and with a high organic content peatlands provide a suitable environment for bacteriogenic CH_4 production. Almost 3% (Clymo, 1987) of the Earth's land surface is covered by peat and yet this is one environment for which there are few paired $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measurements.

CH_4 production rates from wetland environments have been shown to increase with increased temperature, which could result in a potential positive feedback for global warming (Hameed and Cess, 1983). However, although peatlands are an important source of CH_4, not all CH_4 produced is actually emitted to the atmosphere due to oxidation within the peat by methylotrophs (Lidstrom and Somers, 1984), postulated to be a major CH_4 sink in peatland environments. This takes place aerobically and possibly anaerobically, although an obligate anaerobic organism living on CH_4 has yet to be isolated (Yavitt et al., 1990). Yavitt et al. (1988) found that on a daily basis during the summer between 11 and 100% of CH_4 produced was consumed within experimental peat columns.

To provide further information about the biogeochemical cycle of CH_4 and more completely understand the isotopic effects associated with bacterial oxidation it would be advantageous to characterise both $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ at source, as bacterial oxidation leaves the remaining CH_4 isotopically heavy (Coleman et al., 1981). Furthermore, not all CH_4 fluxed from a peatland may be subject to bacterial oxidation. Thus, it is important, for both characterisation of CH_4 fluxed that has not been subject to bacterial oxidation and for the purposes of comparison with isotopic composition of the fluxed CH_4, subject to bacterial oxidation that measurements are made before the CH_4 reaches the oxidising microenvironment at the soil / atmosphere interface.

The Dahlem workshop recommended that future environmental research programmes should address several questions including the response of northern ecosystems to expected climatic changes that may be induced by global warming (Schimel et al., 1989). The effectiveness of stable isotope measurements over time as an aid in understanding the biogeochemical cycle of CH_4 was reiterated and it was suggested that such measurements should be included in as many field studies as possible. Establishment of manipulated experiments were recommended in a) greenhouses and b) experimental fields, including sites with the water table lowered by drainage and flooded sites in areas with long meteorological / botanical records. The minimal set of measurements should include soil temperature profiles, water content, soil porosity, and texture measurements, ambient concentrations and flux measurements of CO_2, CH_4, N_2O, CO and O_3, and organic matter decomposition rates.

Ellergower Moss in S.W. Scotland is a peatland mire utilised by two groups within the NERC funded British research programme Terrestrial Initiative into Global and Environmental Research (TIGER). Clymo's team are investigating the measurement of fluxes and concentration profiles of CH_4 (amongst other gases) in monoliths isolated from peatland wet hollows, lawns and hummocks in response to variations in temperature, water level, overlying peat depth and nutrition. These variables will be incorporated into a model predicting CH_4 fluxes from peatlands. Fowler's team have a climatic station on Ellergower Moss as part of their

research project to quantify the effect of temperature, nutrient input and water table on measured CH_4 fluxes (NERC, 1993). One of the aims of this project was to characterise $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ from peat at depth in a site where many of the complementary measurements recommended by the Dahlem Workshop were being made.

A pilot experiment was devised which not only measured $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ in situ, but also, as recommended, incorporated manipulation of the ecosystem by use of a greenhouse in the field. The role of the greenhouse was to simulate global warming while a) monitoring the stable isotope composition of CH_4 produced in-situ and b) monitoring the effect on the ambient temperature within the bog. Monitoring the magnitude of this change is important for, at present, methanogenesis is considered to be slow in most peatlands due to suboptimal temperature and pH (Dinel et al., 1988). In this experiment, gas samples were collected at known depths within the peat 'reservoir'. $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$ were measured for each sample, and the temperature at each depth recorded. To provide a clearer understanding of $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$, $\delta^{13}C$ and δD of the peat and porewater at the corresponding depths were also measured.

5.3. Methodology.

5.3.1. Site description:

Upland, ombrogenous peat is the predominant type to be found in Britain (Claricoates, 1990). The peat is usually 2-3m deep, but may reach depths of 5-6m, or exceptionally 8m. Blanket mires often develop hummocks and pools over 1.5-3m of peat. Permission was given by Scottish Natural Heritage and the Forestry Commission to site the experiment on Ellergower Moss (NX 482796) within the New Galloway Forest Park in Dumfries and Galloway region, SW Scotland. Ellergower Moss is one of the few remaining examples of an intact raised mire left in Wigtown District. It is a site of special scientific interest (SSI) notified under the 1981 Act on 14th October 1988 (Scottish Natural Heritage, 1988). Adjacent to Loch Dee, the 34.6 hectare bog has a maximum thickness of 6m (Ingram, 1987) and forms the most southerly of those remaining mires which make up the Silver Flowe complex. Unusually, Ellergower Moss has formed on almost flat granite at the present loch level.

The mire surface exhibits a hummock-hollow pattern with a few open pools. There are actively growing *Sphagnum* hummocks with *Calluna vulgaris* (heather) and *Erica tetralix* (cross-leaved heath) upon the tops. *Sphagnum imbricatum* and *S. fuscum* have formed hummocks on the centre of the moss. Within the low-lying hollows there are *Sphagnum* carpets consisting mainly of *Sphagnum papillosum* interspersed with *Eriophorum angustifolium* (common cottongrass) and *Rhynchospora alba* (white beak sedge). Some *Sphagnum cuspidatum* filled pools are found on the centre of the moss with bare peat pools at the head of the erosion gullies (Scottish Natural Heritage, 1988).

5.3.2. Sampling techniques:

The gas samplers, known as 'Jane samplers', used for the collection of gas samples

within the peat were based on a design pioneered by Dr. J. Claricoates (presently of Slimbridge Wild Fowl and Water Trust, Slimbridge) and Prof. R.S. Clymo (of Queen Mary & Westfield College, University of London). Emplaced at 1m intervals to a depth of 4.5m (maximum possible) in the control profile and 5m (maximum possible) in the greenhouse profile, the samplers were designed to be permeable in both directions to any likely peat gases and also to the nitrogen used to inflate the sampler, at a rate which would make the sampling period practicable i.e. at a minimum, the same time as the equilibration period of gas transfer into the sampler. The sampler was designed to collect a peat gas sample by gas diffusion rather than mass flow for at depth the gas would be under pressure and might be present in bubbles (Dinel et al., 1988). Furthermore, any such bubbles under the surface might be released ebullitively under extra pressure, such as that exerted on approach for sampling. Gas collection by diffusion meant that the time lag involved in gas exchanging through the membrane ensured that erratic gas concentrations, or an increase in the ambient gas concentrations, did not occur if a sample was collected immediately after the samplers were approached.

It was also important that the samplers were water tight and strong enough to withstand a considerable amount of physical abuse, both during emplacement in the peat and from pressure while underground. Finally the ability to sample and be refilled repeatedly without causing disturbance or damage to the sampler itself or to the surrounding peat body was considered in the design, i.e. the sampler was buried in the peat at the required depth on the first visit and all subsequent sampling and refilling could be done from the surface without disturbance.

The above properties must hold under pressures greater than atmospheric pressures as the samplers were buried in peat. If the peat, which is waterlogged, can be considered as a column of water then the pressure delivered at a point approximately 5m down the peat profile will be 1.5 atm. 1.5 atm. is thus the maximum pressure under which a sampler must operate. Claricoates (1990) carried out successful tests to show that the samplers were fully operational down to the required depth in the peat.

The sampler consisted of a thin rubber inner of standard thickness, plugged at its open end with a rubber bung. One end of a U-shaped brass tube was passed through the centre of the rubber bung and then retracted slightly to prevent it snagging the inner during inflation or sampling. The necessary length of small bore, thick walled, PVC tubing needed for the appropriate depth each sampler was to be placed was connected to the other end of the brass tubing and secured with wire. The tubing was sealed at the other end with a clamp and plugged with a nail. The sampler was protected from physical damage by an external cylinder composed of two layers of expanded aluminium, the inner layer a fine fly mesh to protect the rubber membrane from plant material as much as possible and the outer layer, necessary to provide the sampler with strength, with a more open framework. The cylinder was slightly larger than that of the inner, and being an open framework, fully permeable to gas and water. The cylinder was held in place by crimping the open ends around a rubber bung at each end. The rubber bung attached to the rubber inner was used at one end, another plain rubber bung at the other end. The enclosed volume of the sampler was 130cm^3. The length of PVC tubing

attached to each sampler was sufficient length to reach the surface of the bog from sampling position plus 30cm to facilitate manipulation at sampling time. Care was taken to ensure each connection was as watertight and gastight as possible and each sampler was tested in the laboratory before emplacement in the field. Both control and greenhouse samples were sited in pools.

5.3.3. Operation of gas samplers

The samplers were filled immediately after emplacement from a $60cm^3$ syringe attached to a cylinder of research grade nitrogen by a three way tap. The PVC tubing to the sampler was attached to the third outlet on the tap, creating a closed system, thus reducing the chances of contaminating the nitrogen with air and introducing oxygen into an otherwise anaerobic system during the inflation of the samplers. As the volume of the sampler was 130cm3 this, this was the minimum volume of gas used to inflate all the samplers. Compensation was made for the effect of increasing pressure with depth and also for the additional volume in the length of tubing attached to each sampler. The tubing was considered rigid enough to withstand the pressure exerted by the peat so this volume was not corrected for depth.

5.3.4. Equilibration time and sampling period:

Once inflated, each sampler was left until at least the required time necessary for equilibration of the peat gases across the membrane by diffusion had lapsed. The diffusion of gases is much slower in water than air, therefore gases dissolved in the peat water around the sampler will be less mobile than gases present in the shallower non-waterlogged peat above. Furthermore, mass flow also occurs to a significant extent in shallower peat, in run off water and air currents (Claricoates, 1990). Ingram (1983) suggests that neither of these factors has a significant effect on the transport of gases through waterlogged regions, so assuming a constant rate of production (from the temperature data it can be seen that at depth the temperature is constant for the period of the experiment) and removal by diffusion, the gas sample collected can be assumed to be representative of the gas present within the peat once the equilibration period has passed. Claricoates (1990) showed the equilibration time for the buried samplers to be about fourteen days. After this length of time the concentration of gases within the sampler did not change significantly. The sampling period for peat gases was therefore set to a minimum of fourteen days, although in practice was 28 days. Profile 1 samples were collected on 28.8.92. Profile 2 samples were collected on 26.9.92. The contents of the sampler were collected for analysis in an evacuated glass bottle and analysed as soon as possible after collection.

Peat samples were collected using a Russian corer. A representative sample of each depth was halved and one half dried to constant weight before being ground to a fine powder using an agate mortar and pestle in an attempt to homogenise the sample. The porewater was extracted by centrifuging the other half of the peat sample. All unprocessed peat samples and the water samples were stored in a freezer when not in use. The finely ground peat for $\delta^{13}C$

was stored in a drying oven until use.

The data logger used to record temperatures was a Unidata Starlogger. While the greenhouse was standing ambient soil temperature was recorded on an hourly basis. This interval was increased to six hourly once the greenhouse had been dismantled.

5.3.5. Isotopic analysis:

Methane gas, organic and water samples were prepared for mass spectrometry as outlined in Chapter 2. In cases where δD of the peat did not give two results within the allowed error range the sample was repeated several times to establish the range of δD within the sample.

5.4. Results.

Data collected during this project is outlined in Table 5.1. and Table 5.2. The measurements are first considered below as a single group with a mean isotopic composition for several components of the CH_4 reservoir in Ellergower Moss, followed by some general comments and finally comparison is made between each control (CP1 and CP2) and the corresponding greenhouse profile (GP1 and GP2). Interpretation of the factors controlling and affecting $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ follows in the discussion.

28.8.92	Control Profile 1 CP1			Greenhouse Profile 1 GP1		
Depth	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$
0m	-78	-8.2	-338	-70	2.2	n.m.
1m	-79	-0.5	-363	-77	3.4	-335
2m	-83	2.0	-337	-75	6.6	-313
3m	-75	5.9	-259	-76	7.9	-265
4m	-77	5.2	-247	-73	8.2	-252
4.5m	-74	6.1	-245			
5m				-74	9.2	-266
26.9.92	Control Profile 2 CP2			Greenhouse Profile 1 GP1		
	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$
0m	-76	-10.7	-338	-75	2.2	-348
1m	No sample			-73	4.2	-325
2m	-82	2.8	-319	-72	6.9	-304
3m	-78	3.7	-273	-74	9.6	-267
4m	-76	6.7	-259	No sample		
4.5m	-73	6.3	-261			
5m				-73	9.2	-261

All values are reported in ‰.

Table 5.1. $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$, and $\delta D(CH_4)$ of CH_4 and CO_2 collected in situ at 1m intervals to a depth of 5m.

	Control Profile			Greenhouse Profile		
Depth	$\delta^{13}C$(peat)	δD(peat)	$\delta D(H_2O)$	$\delta^{13}C$(peat)	δD(peat)	$\delta D(H_2O)$
0m	-25.9	-80	-42.3	-26.6	-86	-36.5
1m	-26.8	-80/-117	-40.4	-26.5	-90	-40.1
2m	-27.6	-78/-88	-40.3	-27.0	-85/-90	-37.0
3m	-27.1	-82/-90	-40.8	-27.0	-78/-90/-97	-34.8
4m	-27.8	-84/-93/-105	-38.2	-27.1	-91/-95	-32.9
4.5m	-27.1	-102/-113/-115	-36.6	-	-	-
5m	-	-	-	-27.3	-108	-32.4
Mean	-27.1±0.7	-94±14.3	-39.8±2	-26.9±0.3	-91±8.0	-35.6±3

Table 5.2. $\delta^{13}C$ and δD of peat and porewater profiles. All values are expressed in ‰

5.4.1. Ellergower Moss isotopic reservoir:

For all samples from 0-5m (n=22 except where specified), $\delta^{13}C(CH_4)$ ranges from -83 to -70‰ and has a mean of -76±3‰, while $\delta^{13}C(CO_2)$ ranges from -10.7 to 9.6‰ and has a mean of 4±5‰. $\delta D(CH_4)$ (n=21) ranges from -363 to -245‰ and has a mean of -294±39‰. These measurements are similar to the bubble samples from Kings Lake Bog, USA, collected by disturbing the peat with a long rod . $\delta^{13}C(CH_4)$ (n=23) from Kings Lake Bog ranged from -64.2 to -80.3‰ and had a mean of -73±4‰, while $\delta^{13}C(CO_2)$ (n=23) ranged from -8.6 to 2.5‰ and had a mean of -2±3‰. $\delta D(CH_4)$ (n=10) from Kings Lake Bog ranged from -399 to -276‰ and had a mean of -308±35‰. (Lansdown et al., 1992). Unfortunately the length of the rod used to disturb the peat is not stated and therefore no comparison with mean isotopic reservoir composition to a given depth can be drawn.

The mean isotopic composition of CH_4 and CO_2 fluxing to the atmosphere is likely to be very similar to the mean of all 0m measurements. For Ellergower Moss $\delta^{13}C(CH_4)$ fluxed is likely to be -75±3‰, $\delta^{13}C(CO_2)$ fluxed is likely to be -4±7‰ and $\delta D(CH_4)$ fluxed is likely to be -341±6‰. Lansdown et al. (1992) found $\delta^{13}C(CH_4)$ fluxed to be -74±5‰. However $\delta^{13}C(CO_2)$ fluxed had a mean composition of -24.5±0.8‰, which is substantially lighter than the value suggested for Ellergower Moss. It is likely that $\delta^{13}C(CO_2)$ measured in sealed surface chambers from Kings Lake Bog can be attributed to recycled plant respiration, which is recorded as having a $\delta^{13}C(CO_2)$ value of approximately -25‰ (Ehleringer, 1991), although it may indicate partial bacterial oxidation of CH_4.

For truly representative isotopic compositions of the Ellergower Moss CH_4 and CO_2 reservoir within the bog, the samples just below the surface of the bog at 0m are best excluded because the samples from this depth may have been subject to oxidation and/or fractionation as a result of fluctuations of the water table and atmospheric fluxing. The isotopic compositions for samples only from 1-5m (n=18) are $\delta^{13}C(CH_4)$ ranging from -83 to -72‰, with a mean of -76±3‰, $\delta^{13}C(CO_2)$ ranging from -0.5 to 9.6 with a mean of 5.±3.‰ and $\delta D(CH_4)$, (n=21) ranging from -363 to -245 with a mean of -286±37‰.

5.4.2. General:

Excluding the samples taken at 0m for the reasons outlined above, for both the greenhouse and control profiles there appears to be a relationship between isotopic composition and depth, (Figure 5.1.a, b, c). In general for each profile, $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ become isotopically heavier with increasing depth. With the exception of GP2, CH_4 present at 1m below the surface is isotopically lighter than that present at the 5m profile base. In all four profiles it is interesting to note that this trend is paralleled in $\delta^{13}C(CO_2)$ measurements and $\delta D(CH_4)$ values.

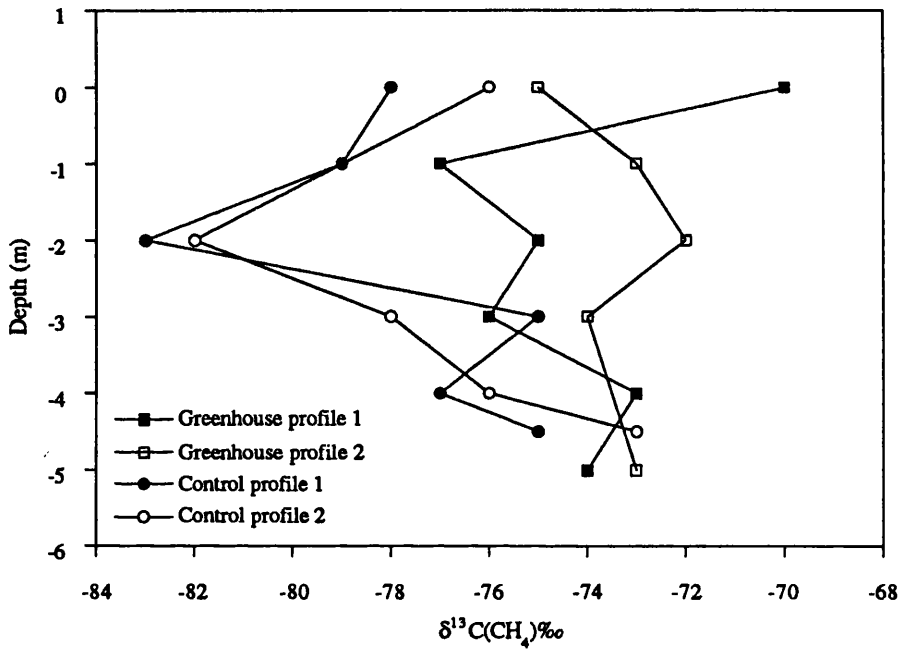


Figure 5.1a. $\delta^{13}C(CH_4)$ of Ellergower Moss methane reservoir with depth.

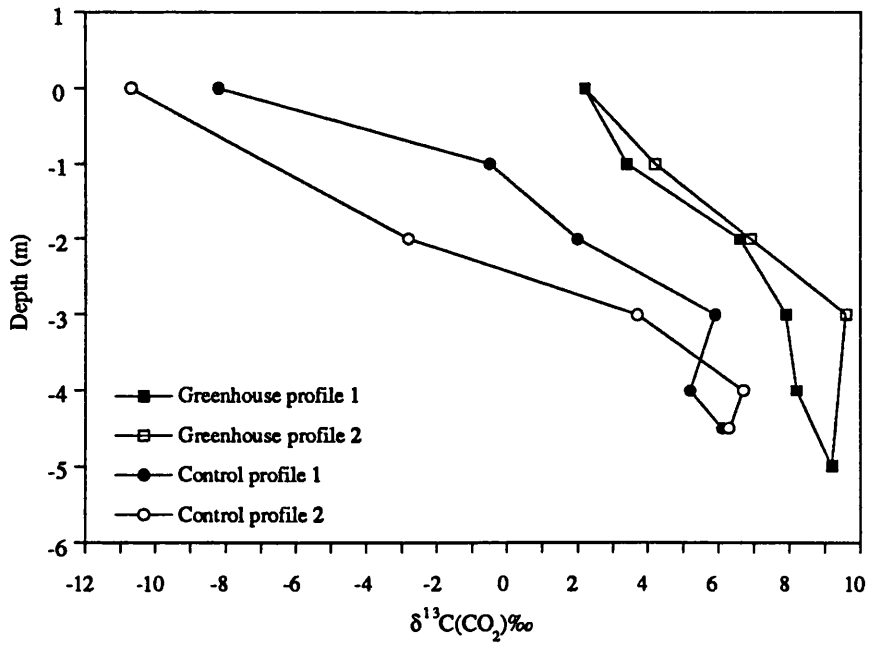


Figure 5.1b. $\delta^{13}C(CO_2)$ of Ellergower Moss carbon dioxide reservoir with depth.

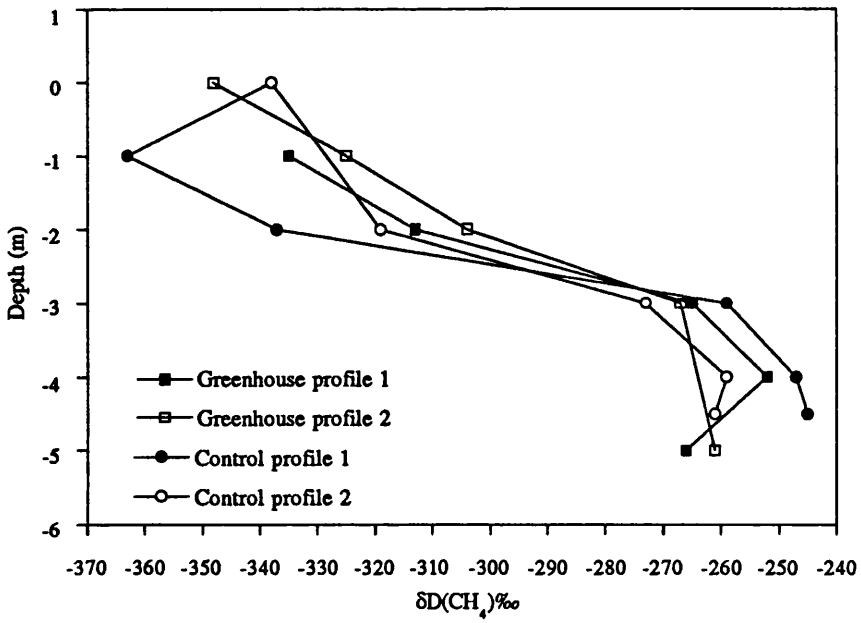


Figure 5.1c. $\delta D(CH_4)$ of Ellergower Moss methane reservoir with depth.

Immediately noticeable from Figure 5.1c. is the dramatic change in $\delta D(CH_4)$ between 2 and 3m, with measurements in all four profiles becoming isotopically heavier by approximately 52±18‰ (n=4), substantially more than the change between other adjacent depths which has a mean of approximately 18±12‰. If the samples above 2m (inclusive) and below 3m (inclusive) are considered as two distinct groups, the $\delta D(CH_4)$ means can be calculated to be -332±17‰ (n=10) and -260±9‰ (n=11) respectively. The spread in $\delta D(CH_4)$ measured in the lower section of the bog is almost half that calculated for samples from the upper half of the bog. The 2/3m 'boundary' also appears to be significant when considering $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$, with a larger spread in measured values above 2m, than the more tightly constrained samples below. From Figure 5.1a. and 5.1b. this is especially evident in the control profiles. Greenhouse and control profiles between 0-2m (n=11) have a mean $\delta^{13}C(CH_4)$ of -76±4‰ while below 3m, although similar in value, the range is again halved with a mean of -75±2‰. Between 0-2m, $\delta^{13}C(CO_2)$ has a mean of 0.5±6‰, $\delta^{13}C(CO_2)$ between 3-5m is substantially isotopically heavier and more tightly defined with a mean of 7±2‰.

5.4.3. Comparison of Greenhouse profiles (GP) with Control profiles (CP):

For the purposes of comparison, greenhouse 5m will be considered closest to control 4.5m measurements.

$\delta^{13}C(CH_4)$ values for the greenhouse profiles tend to be heavier than $\delta^{13}C(CH_4)$ values for the control profiles. This is more pronounced in the 0-2m zone with a difference of 6±4‰ (n=5), although $\delta^{13}C(CH_4)$ measured at 3m and below is more homogenous. $\delta^{13}C(CH_4)$ at 3m and 4.5/5m in GP1 and CP1 is essentially constant. GP2 is isotopically lighter than CP2 by a mean of 5±5‰ (n=3), although $\delta^{13}C(CH_4)$ at 4.5/5m may be considered constant with time.

$\delta^{13}C(CO_2)$ values in greenhouse profiles are isotopically heavier by 6±4‰ (n=10)

(calculated using the mean of similar profiles) than the control profiles. Unlike $\delta^{13}C(CH_4)$ this occurs throughout the 1-5m range.

$\delta D(CH_4)$ measurements in all four profiles become isotopically lighter with decreasing depth, with a substantial jump in $\delta D(CH_4)$ between 2-3m. From 2m upwards $\delta D(CH_4)$ becomes lighter more rapidly than from 5 to 3m. $\delta D(CH_4)$ values in the 3-5m zone are reasonably similar for all four profiles, however, yet again, above 3m $\delta D(CH_4)$ values for the greenhouse profile are isotopically heavier than the control profiles (with the exception of GP2 0m) by a mean of 22±7‰ (n=3).

In summary, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ become substantially heavier with depth for both the greenhouse and control profiles. $\delta^{13}C(CH_4)$ also becomes heavier, but to a lesser extent. Gas samples collected 3m and below have a more tightly constrained isotopic signature than those collected at 2m and above. $\delta^{13}C(CH_4)$ can be considered essentially constant for both control profiles, but GP2 is isotopically heavier. $\delta^{13}C(CH_4)$ for the greenhouse profiles is isotopically heavier than the control profiles, and this is also observed for $\delta^{13}C(CO_2)$ (0-5m) and $\delta D(CH_4)$ (0-2m). $\delta^{13}C(CO_2)$ for the greenhouse profiles can be considered essentially constant, but $\delta^{13}C(CO_2)$ for CP1 is isotopically heavier than $\delta^{13}C(CO_2)$ for CP2. $\delta^{13}C(CO_2)$ however is lighter in the control profiles than the greenhouse profiles for all depths. $\delta^{13}C(CO_2)$ measured at 5m for each pair of profiles can be considered to have remained essentially constant with time. The trend shown by $\delta D(CH_4)$ is very similar for all profiles, however samples collected at 2m and above during the second sampling period (profiles 2) are isotopically heavier than those collected during the first sampling period.

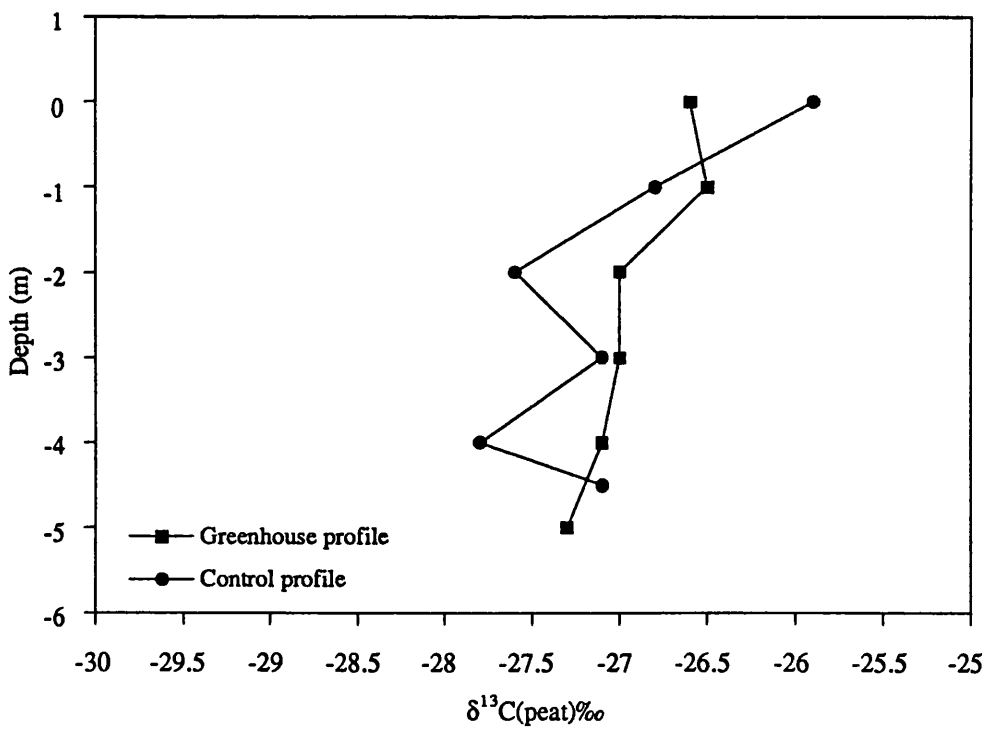


Figure 5.2a. $\delta^{13}C(peat)‰$ of Ellergower Moss peat samples.

$\delta^{13}C$(peat) has a very narrow range from -25.9 to -27.8‰ (Table 5.2. and Figure 5.2a.), mean of -27.0±0.5‰ (n=12). From 1 to 4m depth $\delta^{13}C$(peat) for the control profile can be considered to be slightly isotopically lighter than the greenhouse profile. With increasing depth $\delta^{13}C$(peat) for both profiles shows a general trend of becoming isotopically lighter.

A larger variation is observed with measurements of δD(peat), although the same general trend of becoming isotopically lighter with increasing depth is exhibited (Figure 5.2b.). δD(peat) ranges from -78 to -113‰ (Table 5.2.), with a mean of -93±12‰ (n=12). No accurate judgement can be made of whether the control profile is isotopically heavier or lighter than the greenhouse profile due to the variation measured at each depth.

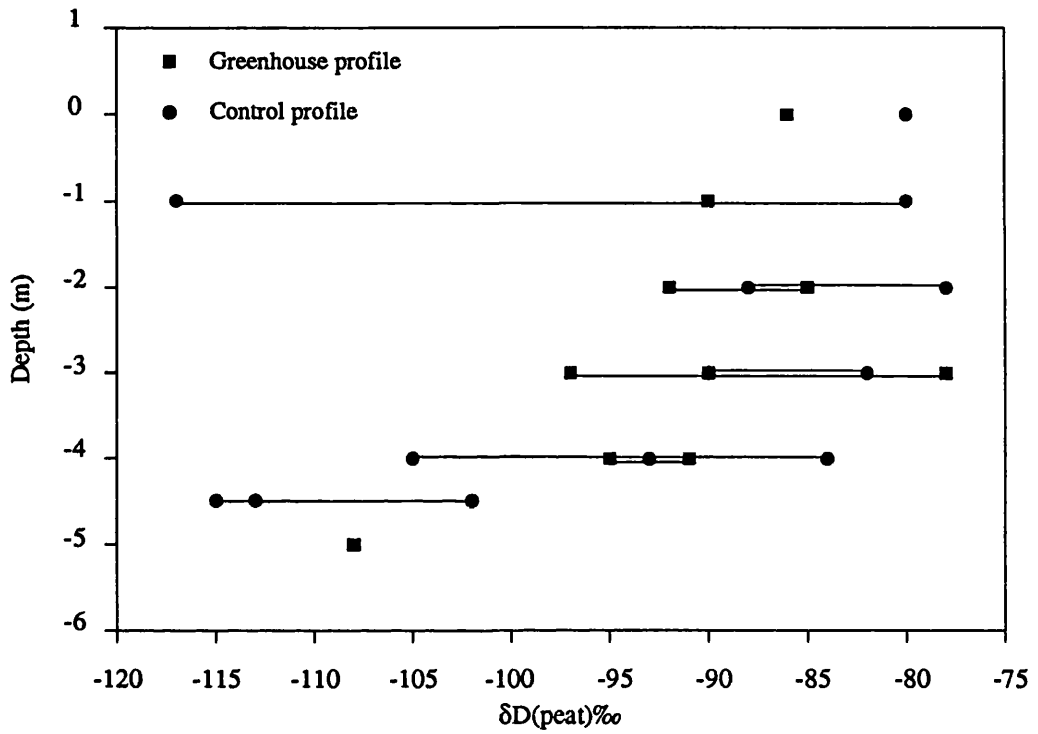


Figure 5.2b. δD(peat) of Ellergower Moss peat samples.

5.5. Discussion & Interpretation:

$\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ all have isotopic values typical of CH_4 and CO_2 of bacteriogenic origin. In Figure 5.3. the data plot close to, but mainly outwith the boxes delineating CH_4 produced by acetoclastic methanogenesis and CO_2 reduction (Schoell, 1988), suggesting that these boundaries are not as rigid as shown. There appears to be both temporal (compare GP1 and GP2 $\delta^{13}C(CH_4)$; CP1 and CP2 $\delta^{13}C(CO_2)$; and both greenhouse and control $\delta D(CH_4)$ - Table 5.1) and spatial (compare corresponding greenhouse and control profiles for all depths - Table 5.1) variation within the peat bog. Potential controls on this variation are numerous, therefore rigorous conclusions explaining the observed variation cannot be drawn at present, only some suggestions offered and some eliminated.

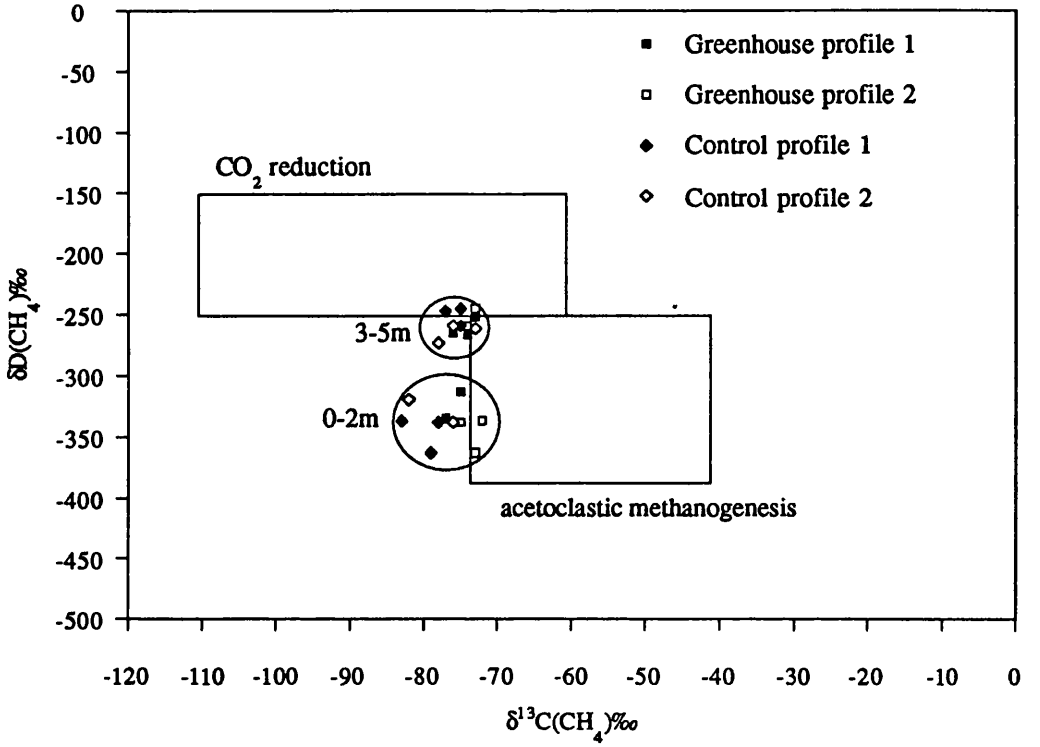


Figure 5.3. $\delta^{13}C(CH_4)‰$ and $\delta D(CH_4)‰$ of samples collected from within Ellergower Moss. Diagram adapted from Schoell (1988).

An explanation of the variation in isotopic composition of CH_4 and CO_2 with time and depth necessitates an understanding of the main features of Ellergower Moss. Most peat profiles show zonation. At the surface there is a relatively shallow layer, in Britain usually 5-20 cm, with a rather open structure containing mostly living plant material. Largely structurally intact dead material, although decomposing, is also present. This layer, the acrotelm (Ingram, 1987), is largely oxygenated, and has a higher hydraulic conductivity than the lower, waterlogged, anoxic zone, the catotelm. The catotelm extends to the base of the peat, is much more highly decayed and has a higher bulk density than the acrotelm. The transition between these two zones is not well defined, as fluctuation of the water table is inevitable and therefore the zone which is oxygenated will also fluctuate. Small anoxic areas may be found in the acrotelm as a result of local water-logging, and conversely oxygen supplied from the roots of bog plants, such as *Eriophorum angustifolium* may result in areas of the catotelm being oxic (Armstrong 1964). Generally however, micro-organisms living in the top of the catotelm utilise oxygen present in the water faster than it can be replaced by diffusion from above (the rate of diffusion of oxygen in water is about 10^{-4} that of oxygen in air) and so the bulk of the catotelm remains anaerobic (Clymo 1987).

Despite seasonal variations in the water table, it is likely that Ellergower Moss was anaerobic from at least 20cm downwards. For the duration of the experiment the water table appeared to be very close to the surface of the bog. Both control and greenhouse profiles were sited in pools. During June, the first month of the experiment, the water table could be seen to

have dropped in the area of the experiment by approximately 10cm, so allowing the atmosphere around the upper sampler to be oxic. Before the samples were collected for profiles 1 and 2, the water table had risen to the level observed at the beginning of the experiment. The level of the water table did not appear to be any different inside the greenhouse to outwith.

5.5.1. Bacterial oxidation : aerobic and anaerobic CH_4 recycling?

Aerobic methylotrophs have been shown to be present in peat samples to a depth of 40cm (Yavitt et al., 1990). It is difficult to relate the presence of methylotrophic populations to samples from Ellergower Moss due to the difference in depth to which Ellergower Moss was sampled. It is possible that aerobic methylotrophs are present throughout the peat column to a depth of 5m in Ellergower Moss, however are not active except as suggested by Yavitt et al. (1990) above 30cm depth i.e. in the zone where oxygen penetrates occasionally or is present, such as the zone affected by fluctuations of the water table. Bacterial oxidation has been shown to leave the resultant CH_4 enriched in ^{13}C with the CO_2 produced depleted in ^{13}C (Coleman et al., 1981). The presence of active methylotrophs is thus important, for by their action, the isotopic composition of CH_4 fluxed to the atmosphere may be heavier than that at source, and more similar to CH_4 produced thermogenically.

In Ellergower Moss $\delta^{13}C(CO_2)$ becomes isotopically lighter with decreasing depth for both greenhouse and control profiles with this trend more marked above 3m (Figure 5.1b.). In Figure 5.1a. $\delta^{13}C(CH_4)$ for the control profiles becomes isotopically lighter from 5m to 2m depth and then becomes isotopically heavier from 2m to the surface. The same trend is shown with $\delta^{13}C(CH_4)$ for GP1, although only becoming isotopically heavier from 1m depth to the surface. Such a trend is not seen with the later set of samples, GP2. It could be suggested that the trends in $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ could be attributed to bacterial oxidation of a small proportion of CH_4 in the upper layers of the peat. Substantial bacterial CH_4 oxidation would result in $\delta^{13}C(CO_2)$ measurements as isotopically light as the $\delta^{13}C(CH_4)$ measured and resultant isotopically heavy CH_4. This is clearly not the case.

Most conclusively, $\delta D(CH_4)$ does not become isotopically heavier with decreasing depth as would be expected if the trend shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ could be attributed to bacterial oxidation. With the exception of CP1, all other profiles become isotopically lighter upwards. It may therefore be suggested that the lighter $\delta^{13}C(CO_2)$ values observed close to the surface may be attributed to mixing with atmospheric $\delta^{13}C(CO_2)$, which in rural areas has a value of approximately -7.8‰ (Boutton, 1991).

Unless fast flowing oxygenated water is moving through the bog at these depths (and this is not suggested by the temperature data) the bog from virtually the surface down can be considered to be anaerobic. Oxygen supplied from the roots of bog plants can result in areas of the catotelm being oxic (Armstrong, 1964). *Eriophorum angustifolium*, which is present in Ellergower Moss, may grow down to 70cm deep or more (Clymo, 1987) and due to the presence of large intercellular gas spaces, the plants probably exist, functioning aerobically in predominantly anoxic peat. It is unlikely that the roots of these plants will reach depths of 2m

(the zone above which $\delta^{13}C(CH_4)$ may be suggestive of bacterial oxidation), but air pockets trapped within dead roots may release O_2 when the plant decays, creating a temporary aerobic microclimate in which aerobic methylotrophs may become active.

Aerobic methylotrophs may be microaerophilic, surviving at concentrations as low as $0.1 mg\ l^{-1}$ (Rudd et al., 1976). An aerobic micro-organism in mostly anaerobic peat is possible. Williams and Crawford, (1984), reported the occurrence of many aerobic heterotrophs in anaerobic *Sphagnum* derived peats in Minnesota bogs. If CH_4 oxidation is occurring to the extent that isotopic signature of the gas within the bog is affected, it may be caused by organisms that can withstand microaerophilic conditions.

Although suspected, the presence of an anaerobic obligate methylotroph has not yet been proven, with one yet to be isolated from peat samples. Zehnder and Brock (1979) have shown that nine strains of methanogenic bacteria are able to oxidise CH_4, but only a small fraction (<1%) of the CH_4 produced is oxidised. They suggest that net CH_4 oxidation could result from a bacterial consortium consisting of a methanogen and another bacterium. Sulphate reducing bacteria are unable to oxidise CH_4 when it is the sole carbon source (Postgate, 1984) but can oxidise CH_4 when an additional electron donor is present (Davis and Yarborough, 1966). Alperin and Reesburgh, (1985), found during inhibition experiments on anaerobic CH_4 oxidation with samples from anoxic marine sediments that anaerobic CH_4 oxidation was either mediated by an unknown organism or a consortium involving an unknown methane oxidiser and sulphate-reducing bacteria. There is difficulty in comparing this research using marine sediments with Ellergower Moss, for in the latter low sulphate concentration is likely to be inhibitory in anaerobic methylotrophy. Furthermore, the volume of CH_4 oxidised to cause the differences observed in $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ is greater than 1%.

However bacterial oxidation of CH_4 oxidation within the anoxic catotelm at Ellergower Moss cannot be ruled out. Whether by aerobic (and microaerophilic) or anaerobic methylotrophs is unknown. The situation is likely to be further complicated by inhomogenous distribution of bacterial colonies throughout the peat, most likely present in niches, and perhaps closely associated with methanogenic associations. This may result in spatial variation of the isotopic signature of similar gases measured throughout Ellergower Moss. The existence of an active anaerobic methylotroph has implications for further carbon cycling within the bog and therefore isotopic fractionation, for the less reduced products of anaerobic oxidation of CH_4 such as methanol, (not CO_2), also provide suitable substrates for methanogenesis. The influence of CH_4 consumption on the isotopic signature of gases measured is unquantifiable without determining if such associations are present, and, if so, use of laboratory based incubations of soil samples using CH_4 of known concentration and isotopic signature which can then be applied to the field situation.

5.5.2 Hydrology, peat and water control

Ellergower Moss is an unusual site in Scotland, for it is a raised bog rather than a blanket bog. In raised bogs the acrotelm and catotelm have fundamentally different hydrological

characteristics (Ingram, 1983), therefore it has quite a distinctive hydrology.

The acrotelm has a relatively high permeability (k>1m/d), but is much more highly conductive at the surface than below. Ingram & Bragg (1984) showed that the hydraulic conductivity of two *Sphagnum* species and their remains (which undergo compaction and humification with depth) is some three orders of magnitude higher towards the top of the acrotelm than near the base. The limit of air entry is generally the surface of the capillary fringe (Ingram, 1987), defined as the layer within which the saturation is maintained above the water table at hydraulic potentials that are negative to atmospheric pressure. The acrotelm provides a route by which the excessive fluxes generated by storms are dispersed without harm to the surface vegetation

The catotelm in contrast has a permeability several orders of magnitude lower than the acrotelm, $10^{-3} > k > 10^{-6}$ metres/day. Stability within the bog depends upon permanent water logging of the catotelm. In raised mires that stability demands the maintenance of a ground water mound (Marino, 1974), coextensive with the peat deposit, most of whose surface lies well above the altitude to which the system drains (Ingram, 1987). The catotelm holds a great quantity of water in storage as a result of dynamic equilibrium between recharge and discharge by seepage. The same equilibrium sustains a ground water mound which governs the geometry of the system and in raised mires at least imposes order upon their development and regularity on their shape (Ingram, 1987).

The overall impression of Ellergower Moss is of a shallow surface water layer with a relatively high permeability, although decreasing to the bottom of the layer. The water in this layer is derived from precipitation and feeds an underlying extremely slow moving ground water body. The surface layer copes with drought and floods, to allow sheet-like runoff, rather than displace the bottom layer. The ground water mound can be considered to be moving very slowly.

All hydrogen in CH_4 produced by the reduction of CO_2 is considered to have come from water (Whiticar et al., 1986). In some instances, at least 50%, possibly closer to 100% of hydrogen in CH_4 produced by acetoclastic methanogenesis is initially derived from the water (Chapter 4). With the ground water mound considered to be slowly moving, then, depending on the rates of methanogenesis, the trend shown by $\delta D(CH_4)$ may be influenced by δD(porewater), which in turn may be isotopically altered as a result of bacterial utilisation. δD(porewater) may therefore become isotopically heavier with depth, with a significant boundary between 2-3m. Likewise the trend shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ may be attributable to a change in $\delta^{13}C$ of the organic substrate with depth.

From Table 5.2. it can be seen that $\delta D(H_2O)$ does become isotopically heavier with depth. The control profile ranges from -42.3‰ at the surface to -36.6‰ at 4.5m, the greenhouse profile is isotopically heavier and ranges from -36.5‰ at the surface to -32.4‰ at 5m depth. The greenhouse profile is isotopically heavier than the control profile, with the exception of 1m depth. There is no significant change between 2-3m in either the control or the greenhouse profile, and in the control profile measurements of porewater at these depths give identical

values. This suggests that the 2-3m boundary present in $\delta D(CH_4)$ measurements is not caused by a difference in δD(porewater) with respective depths, although it still may be possible that the increasing D content with depth is a result of bacterial utilisation, leaving the H_2O more protium depleted .

$\delta^{13}C$(peat) only changes slightly through the profiles, becoming isotopically lighter with depth (Fig. 5.2a.). The change is so small that it could be considered negligible in relation to the difference shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ for each profile. Both greenhouse and control profiles become isotopically lighter with depth, which is the opposite trend to that shown from $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ measurements. This therefore suggests that the trends shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ cannot be attributed to a change in $\delta^{13}C$of the organic substrate.

δD(peat) also becomes isotopically lighter with increasing depth (Fig. 5.2b.), although the pattern is rather erratic. No attempt was made to remove the hydroxyl hydrogens from the cellulose before isotopic analysis, although these are known to exchange readily with hydrogen in water (Epstein et al., 1976). The peat trend is opposite to that shown by δD(porewater). Therefore δD(porewater) would be expected to be independent of δD(peat) except at surface where living material is present. The repetition of a range of values for δD(peat) at a given depth suggests that there will be spatial variation in δD(peat) within the whole of the bog. Spatial variation in the substrate is likely to result in spatial variation of the gas composition produced, as was observed from samples measured.

5.5.3. CO_2 reduction vs. acetoclastic methanogenesis ?

There is a distinct isotopic $\delta D(CH_4)$ boundary between 2m and 3m for the greenhouse and control profiles, with a shift of around 40‰ (Fig. 5.1c.). It is therefore possible that the samples 3m and below, and 2m and above should be considered as separate groups. Table 5.3 gives the range and mean of $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ for each group, 0-2m and 3-5m.

Depth	$\delta^{13}C(CH_4)$	$\delta^{13}C(CO_2)$	$\delta D(CH_4)$
0-2m	-83 to -70	-8.2 to 6.9	-363 to -304
	-76±4 (n=11)	0.5±6 (n=11)	-332±17 (n=10)
3-5m	-78 to -73	3.7 to 9.6	-304 to -245
	-75±2 (n=11)	7±2 (n=11)	-260±9 (n=11)

All values are expressed in ‰.

Table 5.3. Range and mean of $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ measurements from Ellergower Moss when considered as two groups : 0-2m and 3-5m.

Figure 5.3 (p 99) is a plot of $\delta^{13}C(CH_4)$ against $\delta D(CH_4)$ based upon Schoell (1988). The majority of each group lie outwith the boxes delineating the isotopic signature of CH_4 produced by each pathway, suggesting that these boundaries need to be extended. None of the 0-2m samples plot close to the zone attributed to methanogenesis by CO_2 reduction; some have

a signature typical of CH_4 produced by acetoclastic methanogenesis and it is likely that the other samples have been produced by the same pathway. Samples taken from 3-5m plot within and adjacent to the boxes attributed to methanogenesis by CO_2 reduction and acetoclastic methanogenesis which suggests that both methanogenic pathways are operating at this depth. The shift from the area typical of 0-2m samples is large, which suggests that CO_2 reduction may be the dominant pathway at 3-5m depth.

Results obtained by Kryzcki et al., (1987) who incubated *Methanosarcina barkeri* on acetate, CO_2 and ethanol, support the contention of Whiticar et al., (1986) that acetoclastic methanogenesis produces less isotopic fractionation than does the reduction of CO_2. CH_4 produced from acetate by *Methanosarcina barkeri* was relatively unenriched in ^{12}C, with the average $\Delta^{13}C$ value half that observed for CO_2 utilisation and one third that observed for methanol utilisation. Table 5.4 shows the difference between $\delta^{13}C(CO_2)$ (substrate for CO_2 reducing bacteria, suggested for 3-5m zone in both greenhouse and control profiles), $\delta^{13}C$(organic) (substrate for acetoclastic methanogenesis, suggested to occur between 0-2m for both profiles), $\delta^{13}C(CH_4)$ measured and suggested fractionation between substrate and CH_4 produced, $\Delta^{13}C_{substrate-CH_4}$.

Profile / Depth	$\delta^{13}C(CH_4)$ mean	$\delta^{13}C(CO_2)$mean	$\delta^{13}C$(organic) mean	$\Delta^{13}C_{substrate-CH_4}$
CP1:0-2m	-80±3 (n=3)		-26.7±0.3 (n=3)	<53.3
CP1:3-5m	-76±1 (n=3)	5.7±0.5 (n=3)		81.7
CP2:0-2m	-79±4 (n=2)		-26.7±0.3 (n=3)	<52.3
CP2:3-5m	-76±3 (n=3)	5.6±1.6 (n=3)		81.6
GP1:0-2m	-74±4 (n=3)		-26.8±0.8 (n=3)	<47.2
GP1:3-5m	-74±2 (n=3)	8.4±0.7 (n=3)		82.4
GP2:0-2m	-73±2 (n=3)		-26.8±0.8 (n=3)	<46.2
GP2:3-5m	-74±1 (n=2)	9.4±0.3 (n=2)		83.4
			0-2m mean	50±4
All values are expressed in ‰.			3-5m mean	82±1

Table 5.4. Fractionation between substrate and CH_4 produced if the pathway in the 0-2m zone is dominated by acetoclastic methanogenesis and by CO_2 reduction in the 3-5m zone.

Kryzcki et al. supplemented their cultures with acetate and were therefore able to measure a direct fractionation between the acetate substrate and the CH_4 produced. The presence and isotopic composition of acetate was not tested for at Ellergower Moss. If present, it is likely to be isotopically lighter (due to fractionation during formation) than $\delta^{13}C$(organic) measured from peat samples. As such $\Delta^{13}C_{substrate-CH_4}$ calculated for acetoclastic methanogenesis will be less than the value in Table 5.4. $\delta^{13}C(CO_2)$ measured is for gaseous CO_2. Occluded bubbles of CO_2 can be considered to behave in a similar manner to headspace gas. Deuser & Degens, (1967) found that under conditions of isotope equilibrium $\delta^{13}C(CO_2)$ of headspace gas will be isotopically lighter than that of the dissolved inorganic carbon. The

methanogens are more likely to utilise dissolved CO_2, which may be isotopically heavier, making $\Delta^{13}C$ bigger. As Ellergower Moss is likely to be dominated by biological activity and therefore not in thermodynamic equilibrium, the application of this statement is questionable. $\Delta^{13}C$ is therefore no more than a close estimate and it is unlikely that each zone will produce CH_4 solely by the pathway suggested, but rather there may be minor methanogenesis by acetate utilisation in the 3-5m zone and likewise minor methanogenesis by CO_2 reduction in the 0-2m zone.

Despite this, $\Delta^{13}C$ calculated for each 0-2m is close to half of that calculated for the corresponding 3-5m zone, with this estimate a maximum, decreasing if $\delta^{13}C$(acetate) was used in the calculations rather than $\delta^{13}C$(organic). These crude calculations strengthen the suggestion that methanogenic associations in 0-2m utilise acetate as a substrate, while at 3-5m depth CO_2 is the dominant substrate.

Older (and therefore deeper) sediments are more likely to play host to methanogenesis by CO_2 reduction and younger (and therefore shallower) sediments are more likely to be host to methanogens utilising acetate (Jenden and Kaplan, 1986). CO_2 reduction could continue to operate even after the acetate substrate pool is exhausted and the acetoclastic methanogenic pathway ceases (Whiticar et al., 1986) It is therefore reasonable that with increasing depth CO_2 reduction may become the more dominant pathway by which CH_4 production occurs in Ellergower Moss. If so, it would be expected that the CO_2 'reservoir' within the bog will be enriched in ^{13}C caused by fractionation resulting from the utilisation of CO_2 as a methanogenic substrate. This is the case, with $\delta^{13}C(CO_2)$ being significantly heavier in the 3-5m zone than in the 0-2m zone for all four profiles.

Svensson (1984) showed from incubation of bulk peat and enrichment cultures at different temperatures the presence of low temperature adapted methanogens with an optimum of about 20°C and another population with a higher optimum between 24 and 28°C. The two seemed to occupy two nutritional niches: the low temperature population used acetate for CH_4 formation and the one with the higher optimum oxidised hydrogen. It is not necessarily the methanogenic bacteria in these samples that may show the low temperature optimum; any of the micro-organisms involved in the decomposition of peat to acetate, CO_2 and H_2 could cause the observed increase in CH_4 production. The population using hydrogen is more likely to be enriched compared with the population using acetate, since the former substrate is energetically more favourable (McInerney and Bryant, 1981).

This finding contrasts with that of Schoell (1988) who suggested that methanogenic processes are seasonally controlled. In summertime and at warmer sediment temperatures, acetoclastic methanogenesis is the dominant process, whereas in wintertime with lower sediment temperatures CO_2 reduction prevails (Burke et al., 1988). Svensson (1984) suggests that *Methanosarcina* species take part in CH_4 production at low temperatures. The interpretation, that in the warmer (at the time of sampling) 0-2m of Ellergower Moss, CH_4 production is dominated by acetoclastic methanogenesis, while in the lower cooler 3-5m zone, CO_2 reduction is the dominant process, is more sympathetic to Burke and Schoell's hypotheses.

5.5.4. The influence of temperature and the greenhouse:

The greenhouse was erected on 23rd April 1992 and had been standing for a period of 127 days when the first profiles of samples were collected. The temperature (°C) of each profile at the time of sampling is shown in Table 5.-5. All four profiles show a decrease in temperature with depth. The greenhouse profiles suggest that from 4-5m the temperature can be considered to be constant. The logger did not record any temperatures within the bog before the 15th May 1992, therefore the extent of normal temperature fluctuations between sites at the same depth interval within the bog is unknown. The greenhouse clearly has a strong influence down to a depth of 2m in both profiles 1 and 2. The difference between greenhouse and control samples at 0m depth is the same for the first and second sampling period. The influence of the greenhouse upon the ambient temperature within the bog appears to decrease by approximately 50% for each metre of increasing depth.

Depth	CP1	GP1	ΔT°C	CP2	GP2	ΔT°C
0m	13.4	16.6	3.2	10.3	13.5	3.2
1m	11.4	12.8	1.4	10.9	12.2	1.3
2m	9.1	9.9	0.8	9.5	10.2	0.7
3m	8.3	8.5	0.2	8.6	8.8	0.2
4m	8.2	8.4	0.2	8.2	8.3	0.1
5m		8.4			8.4	

All values are in °C

Table 5.5. Temperature of profiles 1 (collected 28.8.92) and profiles 2 (26.9.92) in Ellergower Moss.

The temperature between 0-1m has decreased in CP2 when compared to CP1 due to decreasing atmospheric temperature i.e. the bog is losing heat, and this is most dramatic at the surface. There has been an increase in the ambient peat temperature between 2-3m attributable to a lag period for the sediment at this depth to warm up. Temperature at 4m depth remained constant.

The greenhouse profiles show a very similar pattern with time to the control profiles. Between 0-2m heat has been lost from the bog, most dramatically at the surface. Between 2-3m there has been a rise in the bog temperature, while from 4-5m the temperature can be considered to have remained constant.

Within each profile set, the greenhouse profile is warmer than the control profile. Profiles 2 between 0-1m are cooler than profiles 1, however the opposite trend is shown between 2-3m with an increase in temperature with time.

There is the possibility that with increased temperature, isotopic fractionation between substrate and gas produced may become less. $\alpha(CO_2/CH_4)$, calculated from the data of Krzycki et al. for a culture of *Methanosarcina barkeri* growing on CO_2 at 37°C had a value of 1.048. At 40°C, $\alpha(CO_2/CH_4)$ by *Methanosarcina barkeri* is 1.045±0.002 (Games et al., 1978). The

temperature difference is so small that the α values are within error. A larger difference in temperature would be reflected in a larger difference in α values. The greenhouse profiles may therefore be expected to be isotopically heavier than the control profiles, although as the difference in temperature is small, the differences in isotopic composition may also be small. The influence of temperature should decrease with depth, observed most clearly by comparison of the 0-2m zone with the 3-5m zone. Measurements show $\delta^{13}C(CH_4)$, $\delta^{13}CCO_2)$ and $\delta D(CH_4)$, sampled under the greenhouse to be isotopically heavier than in the corresponding control profile, with this difference largest in the 0-2m zone. $\delta^{13}C(CH_4)$ in GP2 is isotopically heavier than GP1. CP2 is very similar to CP1, but it is uncertain as to whether this is a function of prolonged higher temperatures or not.

Fractionation factors, α, will not be calculated for Ellergower Moss due to the complexity of the methanogenic environment (Chapter 4). A laboratory experiment can be manipulated to ensure that all methanogenic pathways are known. In Ellergower Moss, it is likely that methanogenesis is occurring by more than one pathway, with added error in calculating $\alpha(CO_2/CH_4)$ or α(acetate/CH_4) if indeed there is CH_4 and CO_2 cycling within the bog.

$\alpha(CO_2/CH_4)$ depends not only upon temperature, but on bacterium. For *Methanobacterium* strain M.o.H. (40°C), $\alpha(CO_2/CH_4)$ is 1.061± 0.002, (Games et al., 1978). At 40°C, $\alpha(CO_2/CH_4)$ by *Methanosarcina barkeri* is 1.045±0.002 (Games et al., 1978). From the data of Balabane et al., (1987) a fractionation factor of 1.048 (NATO advanced study institute, 1992) can be calculated for *Methanobacterium formicium* grown for 3 days at 34°C, although I note that the authors preferred not to perform this calculation. There are few paired $\delta^{13}C$ measurements of CH_4 produced and substrate utilised, particularly for acetoclastic methanogenesis. As such, α(acetate/CH_4) cannot be calculated for different temperatures.

If applied carefully, laboratory measured data may be used in very simplistic terms, as α calculated may be close to the field measurement. *Methanosarcina barkeri* is one methanogen that can utilise both acetate and CO_2 for methanogenesis, and it is therefore reasonable to consider α for this bacterium at a given temperature. It is capable of switching from one pathway to another, as is suggested may happen with age and depth in Ellergower Moss.

Using $\alpha(CO_2/CH_4)$ measured by Krzycki et al., *Methanosarcina barkeri* utilising peat from Ellergower Moss as a substrate with $\delta^{13}C(CO_2)$ of -27‰, would produce CH_4 with a $\delta^{13}C(CH_4)$ signature of -71.6‰ (37°C). Lansdown et al., (1992) used ^{14}C labelling to show that CO_2 reduction could account for all CH_4 produced in Kings Lake Bog. $\alpha(CO_2/CH_4)$ was estimated to be 1.072. This is larger than laboratory based measurements, but the temperature of Kings Lake Bog (14-19°C) was lower than with the experiments. The ambient temperature in Ellergower Moss, even at maximum is much cooler than that at which the lab-based fractionations were measured. If CH_4 was being produced within Ellergower Moss by *Methanosarcina barkeri* via CO_2 reduction, $\alpha(CO_2/CH_4)$ is likely to be larger than 1.048 as the temperature is lower and $\delta^{13}C(CH_4)$ lighter than -71.6‰. $\delta^{13}C(CO_2)$ substrate is unknown, although is likely to be very close to $\delta^{13}C(CO_2)$ measured from each profile. With the

exception of GP1(0m), $\delta^{13}C(CH_4)$ is lighter than -71.6‰ and all temperatures measured at the time of sampling are less than 14°C measured at Kings Lake Bog. A bigger fractionation between substrate and CH_4 produced might therefore be expected in Ellergower Moss than King's Lake Bog.

One of the original aims of the experiment was to measure the influence of increased temperature on $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ of CH_4 and CO_2 produced. With only two measurements of greenhouse and control profiles, and the added uncertainty that both spatial and temporal variation in measurements may be an artefact of bacterial methanogenesis, the influence of increased temperature cannot be quantified at present except to say that the fractionation between substrate and gas produced will decrease and $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ may become significantly isotopically heavier with prolonged higher temperatures.

5.5.5. Gas entrapment, diffusion and gas expulsion from Ellergower Moss:

Dinel et al., (1988) suggest that peatland CH_4 release may be episodic and local, influenced by hydrostatic and atmospheric pressure, and temperatures of the surface and subsurface waters, with diffusive flux playing only a minor role. Field observations show that the concentration of CH_4 dissolved and/or occluded in peatland waters increases with depth (Dinel et al., 1988, Yavitt et al., 1988). Laboratory observations also showed a statistically significant increase in CH_4 concentration of the peat with increasing depth (Yavitt, 1990).

The influence that diffusion of CH_4 and CO_2 from the area of production, upwards through the water column, will have on the observed isotopic profiles cannot be ignored. The isotopically lighter gas species will diffuse upwards faster than those isotopically heavier. If all gas production was occurring solely at the base of Ellergower Moss, profiles becoming isotopically heavier with increasing depth would be expected as the concentration of ^{13}C and D increased closer to source. Such trends are observed in Ellergower Moss; however it is highly unlikely that methanogenesis is only occurring at the base of the bog and not throughout the whole of the anaerobic zone. A purely diffusive hypothesis does not explain the 2 to 3m boundary. Undoubtedly, diffusion is influential, but further information is needed about the gas release mechanism from Ellergower Moss to provide a more accurate assessment of its influence. If release is local and episodic dependent on hydrostatic and atmospheric pressure (Mattson and Likens, 1990), it may be that diffusion is insignificant within the bog.

CH_4 supersaturation within the bog and high atmospheric pressure may cause gas produced to be 'trapped' close to its origin. CH_4 supersaturation of pore waters can inhibit further methanogenesis (Williams and Crawford, 1984). Thus, it may be possible that CH_4 produced at depth (below 3m) will not migrate far from source, but stay trapped and have an inhibiting influence upon methanogenesis. CH_4 sampled between 3-5m may therefore represent an accumulation of longterm production. With a decrease in the availability of labile compounds with age, the 3-5m reservoir is likely to be isotopically heavier. Above 3m gas may be released suddenly by eructation, which will allow diffusion of CH_4 upwards from the 3-5m zone (with a resultant kinetic isotope fractionation), until equilibrium with atmospheric pressure

is attained. The decrease in the concentration of CH_4 at depth could trigger methanogenesis with CH_4 production continuing until inhibitory levels are reached once again. Suggested by isotopic profiling, such a gas release mechanism may be further evidenced by increased CH_4 concentration with depth.

Unpublished data supplied by Clymo et al., (Queen Mary and Westfield College, London, 1993) shows that the partial pressure/concentration of CH_4 increases with depth in Ellergower Moss. CH_4 partial pressure stays essentially constant between 0.5-1.5m ranging from 5.5 to 7.0% with a mean of 6.3±0.6% (n=3). The partial pressure increases sharply from 1.5 to 2m and remains unchanged between 2-2.5m with a mean of 11±0‰ (n=2). There is another sharp increase in partial pressure in 2.5-3m zone, with CH_4 partial pressure at 3m and below essentially constant, ranging from 13 to 20%, mean of 17±1.5%. Significantly, these measurements show a very similar pattern to the isotope profiling. From 3-5m measurements of partial pressure show little variation. Measurements above 3m show greater variation, although divisible into two further subsets, 0.5-1.5m and 2.2.5m. The CH_4 partial pressure measurements suggest that there is at least one boundary within Ellergower Moss between 1.5 and 3m depth. The isotope measurements suggest the presence of a boundary between 2-3m depth. The difference between partial pressure profiling (measured at a different site from this work) and isotopic profiling, is further suggestive of spatial variation within Ellergower Moss.

5.5.6. General conclusions of factors influencing isotopic composition:

$\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ exhibit both spatial (with depth and latitude) and temporal variation. This complexity cannot be explained simply, but some potential controls can be eliminated, while others suggested as being dominant. The trends shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ cannot be attributed to a change in $\delta^{13}C$ of the organic substrate. Likewise the pattern shown by $\delta D(CH_4)$ measurements with depth cannot be attributed to variation in δD of the organic matter or porewater, although the latter has been shown to be influential (Whiticar et al., 1986; Chapter 4) and may be influenced by the rate of water flow through the catotelm. It is suggested that the data can be grouped into two sets, 0-2m and 3-5m as a function of methanogenic pathway dominant at each zone, acetoclastic methanogenesis and CO_2 reduction respectively. The dominance of each pathway may be controlled by substrate limitation. Entrapment of gas close to source below 3m, with free rapid diffusion upwards, only after episodic and localised gas release in the 0-3m zone, may also provide isotopic profiles similar to those observed in Ellergower Moss. All measurements at 0m are likely to be affected isotopically by aerobic bacterial oxidation, although the isotopically heavy $\delta^{13}C(CO_2)$ and isotopically light $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ measurements suggest that very little CO_2 is being converted to CH_4 by bacterial oxidation. Rather, the $\delta^{13}C(CO_2)$ signature is attributed to mixing with atmospheric CO_2. It is possible that bacterial oxidation is occurring at greater depths, whether anaerobically or by microaerophilic methylotrophs is uncertain. The potential recycling of carbon and hydrogen that may occur as a result of bacterial oxidation at depth has implications for further isotopic fractionation. Undoubtedly kinetic fractionation during

implications for further isotopic fractionation. Undoubtedly kinetic fractionation during diffusion of CH_4 and CO_2 from source, upwards, through the profile is influencing the pattern shown by isotopic measurements with depth, however this is not considered to be the dominant control and does not account for the 2-3m boundary. Without further information about zones of methane production and factors influencing gas release mechanisms from the bog, this cannot be quantified. It is possible that an increase in ambient soil temperature caused by erection of the greenhouse may be responsible for istopically heavier greenhouse profile samples between 0-2m, but there is insufficient data to draw a firm conclusion.

5.6. Temperature Profiles Within Ellergower Moss.

The greenhouse was erected on 23rd April 1992 and dismantled on 27th September 1992. Figures 5.4 are constructed from the temperature measurements at 12:00 and 00:00. These were not always the maximum and minimum temperature on the given day respectively (although close), rather were chosen to provide a time control for comparison of data. The first temperature profile was logged during the latter half of May (Figures 5.4.a-t.).

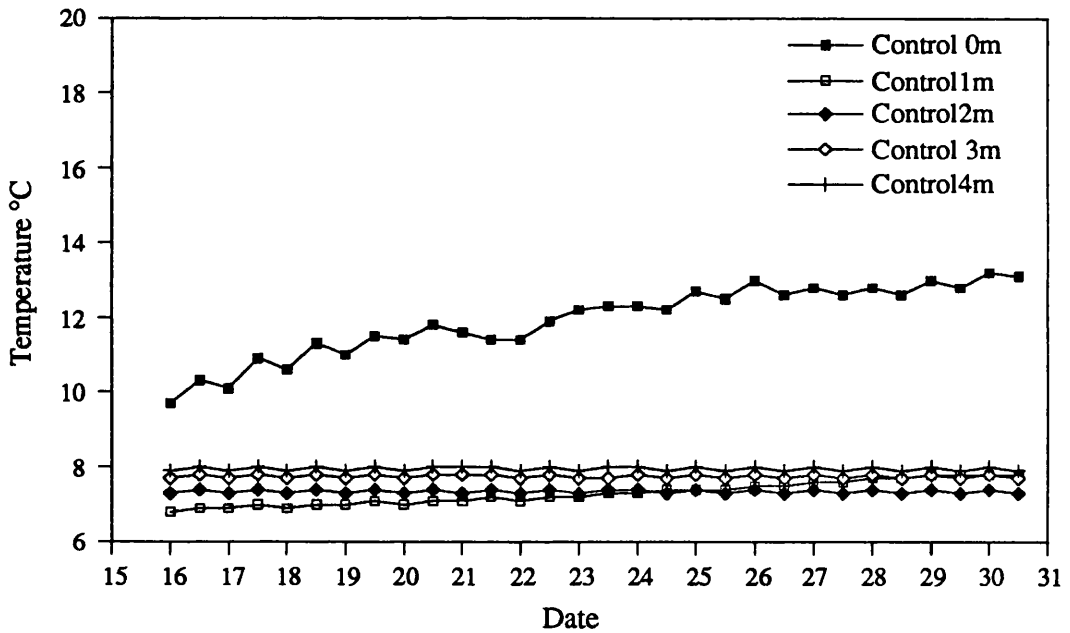


Fig. 5.4a. Temperatures in the control profile from 00:00 hours, 16th May to 12:00 hours, 30th May 1992.

It is apparent from comparison with the control profile (CP) (Figure 5.4b) that at this early stage, the greenhouse is having an effect on the surface temperature of the peat, and towards the end of the month is beginning to raise the temperature at 1m depth (Figure 5.4a).

Due to battery failure, temperature profiles through Ellergower Moss were not recorded in June.

The surface temperature of the greenhouse profile (GP) at 0m was still raised by a mean of 3.9 ± 0.7°C (n=52) in July (Figure 5.4d) in comparison to the control profile (Figure 5.4c.). Significant warming at 1m depth occurred in both profiles, with the GP warmer at 1m depth

than the CP. Warming also occurred at 2m depth although more significant in the GP than CP. The temperature from 3-5m depths for all profiles can be considered to have remained essentially constant at around 8°C.

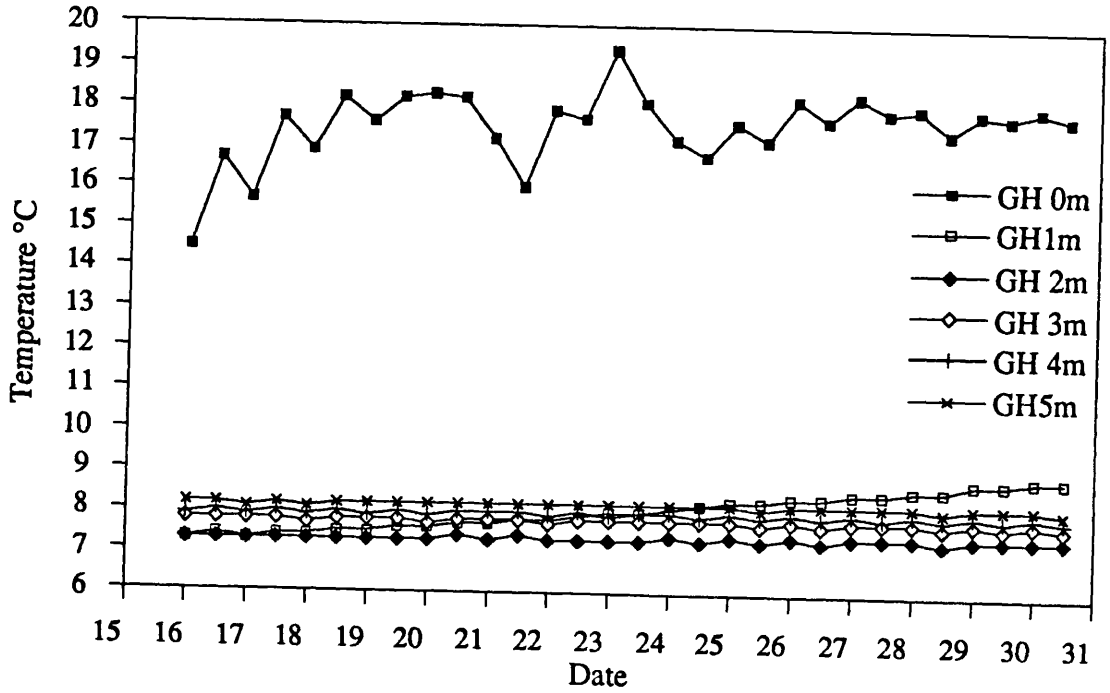


Fig. 5.4b. Temperature in the greenhouse profile from 00:00 hours, 16th May to 12:00 hours, 30th May 1992.

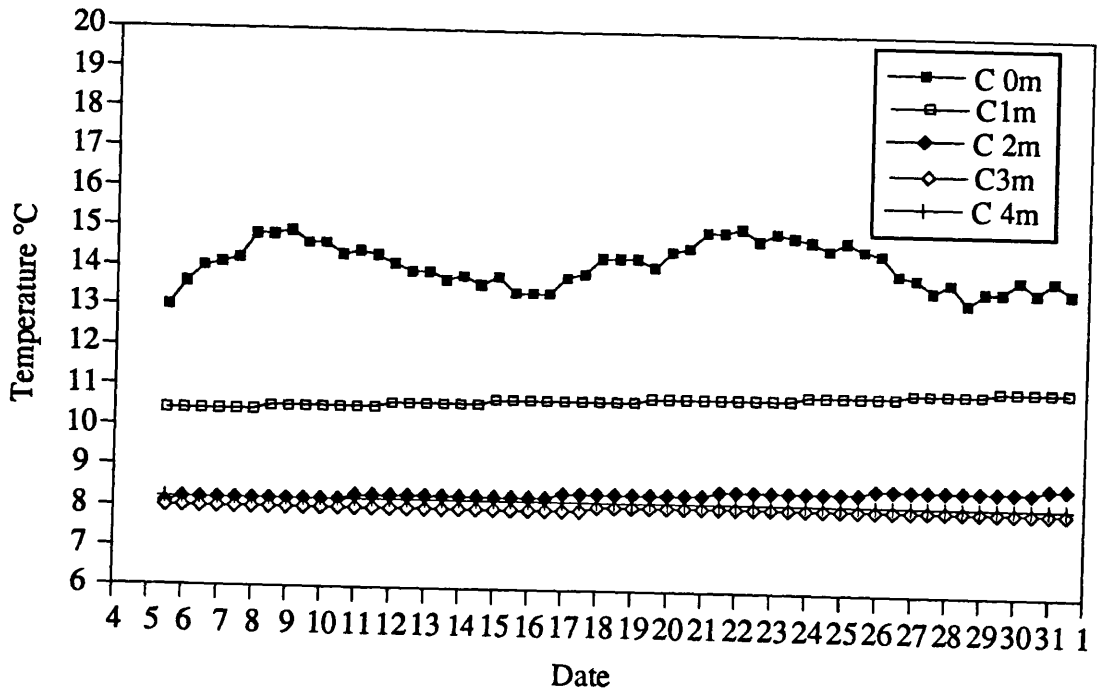


Fig. 5.4c. Temperature in the control profile from 12:00 hours, 5th July to 12:00 hours, 31st July 1992.

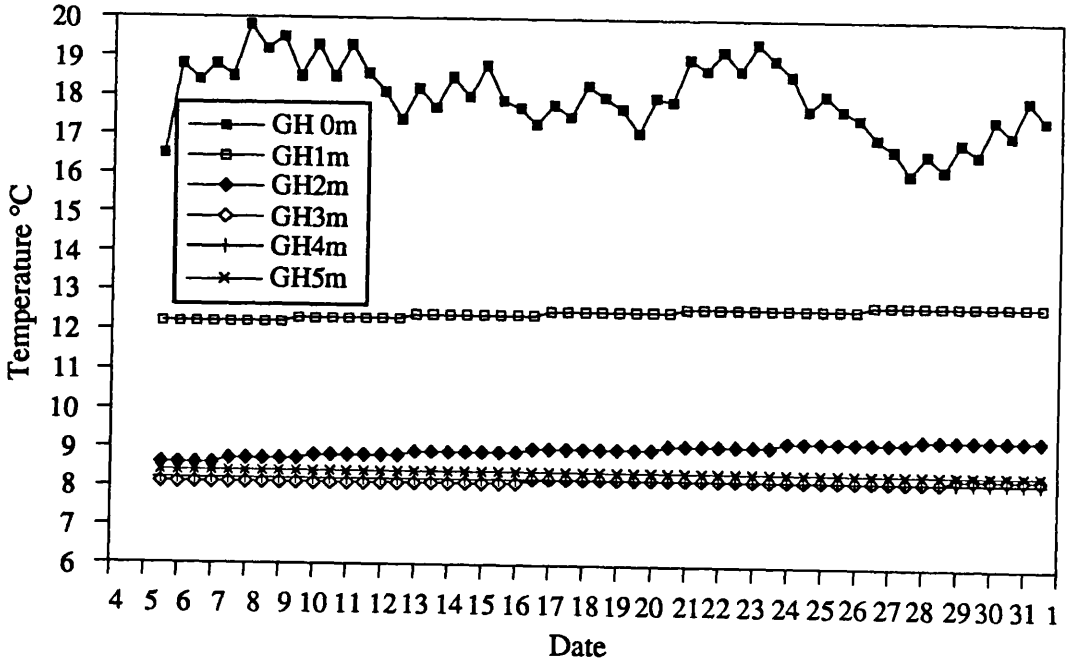


Fig. 5.4d. Temperature in the Greenhouse Profile from 12:00 hours, 5th July to 12:00 hours, 31st July 1992.

During August the temperature at 0m was still elevated in the GP (Figure 5.4e.) with the temperature at 1m and 2m depths higher by a mean of 1°C than the CP (Figure 5.4f.). In both GP and CP the temperature at 3m depth began to rise, however the temperature difference at this depth between the two profiles was too small to assess if the greenhouse was causing an increased effect in the GP.

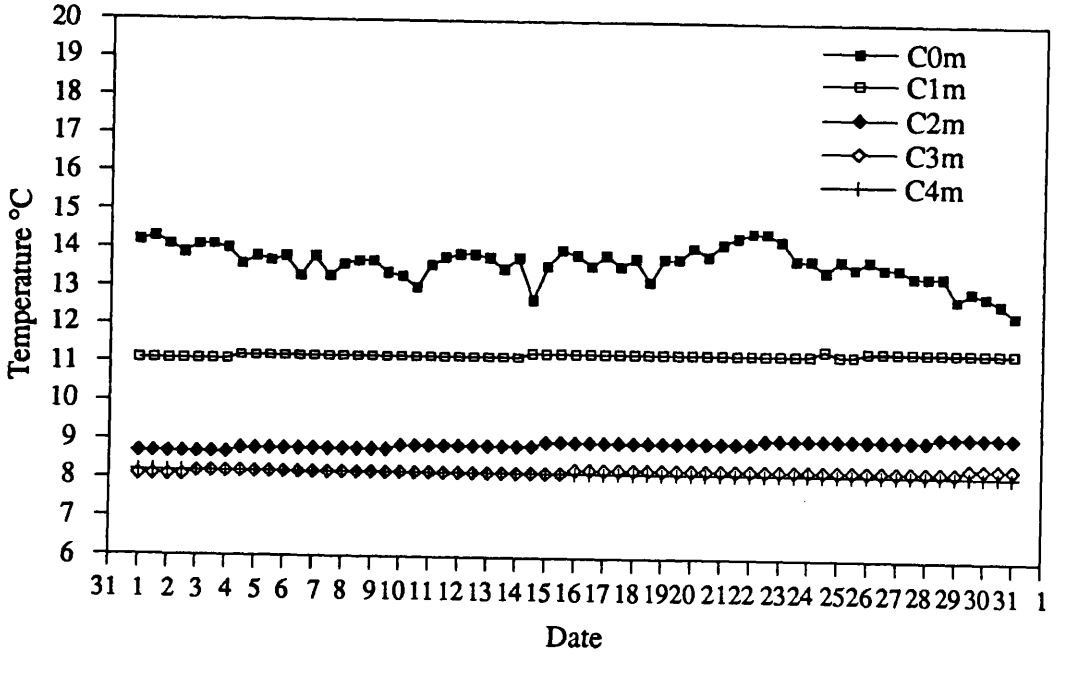


Fig. 5.4e. Temperature in the Control Profile from 00:00 hours, 1st August to 00:00 hours, 31st August 1992.

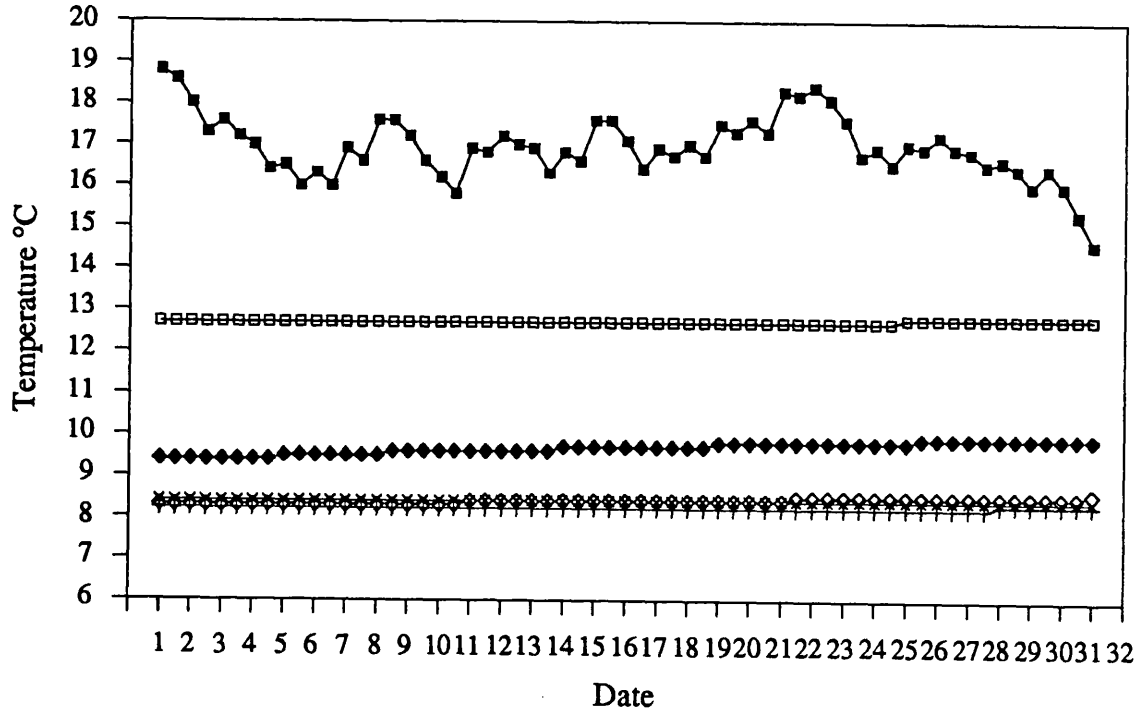


Fig. 5.4f. Temperature in the greenhouse profile from 00:00 hours, 1st August to 00:00 hours, 31st August 1992. For key refer to Fig. 5.4e.

In September, the surface temperature of the bog dropped in both profiles, although cooler in the CP (Fig. 5.4g) than the GP (Fig. 5.4h)

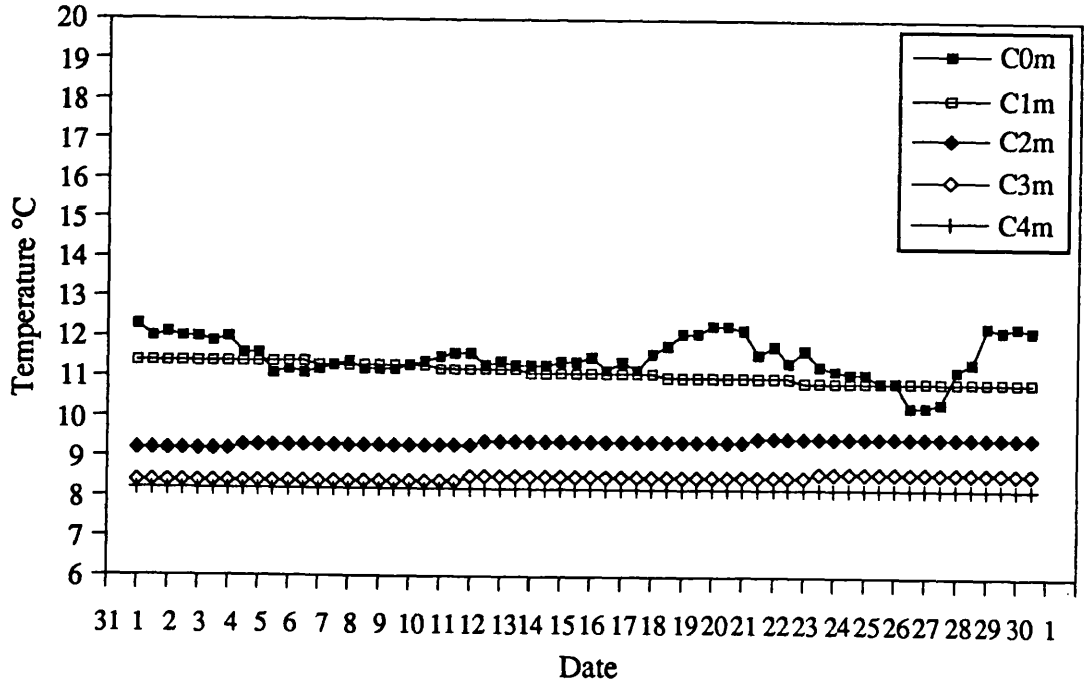


Fig. 5.4g. Temperature in the Control profile from 00:00 hours, 1st September to 12:00 hours, 30th September 1992.

The temperature at 1m, although still elevated in the GP in comparison to the CP, also dropped during September. The temperature at 2m and 3m continued to rise gently with GP2m warmer than CP2m. 4m and 5m depths maintained a constant temperature of approximately 8.5°C. The greenhouse was removed on 27th September 1992 but no immediate effect on temperature was apparent.

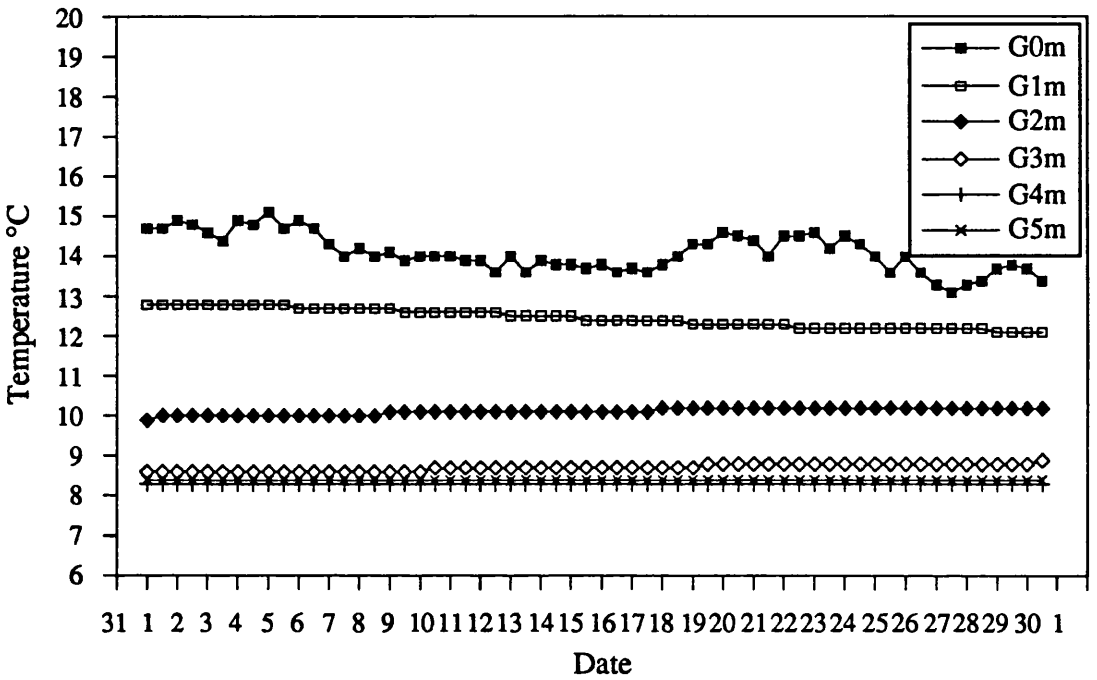


Fig. 5.4h. Temperature in the Greenhouse profile from 00:00 hours, 1st September to 12:00 hours, 30th September 1992.

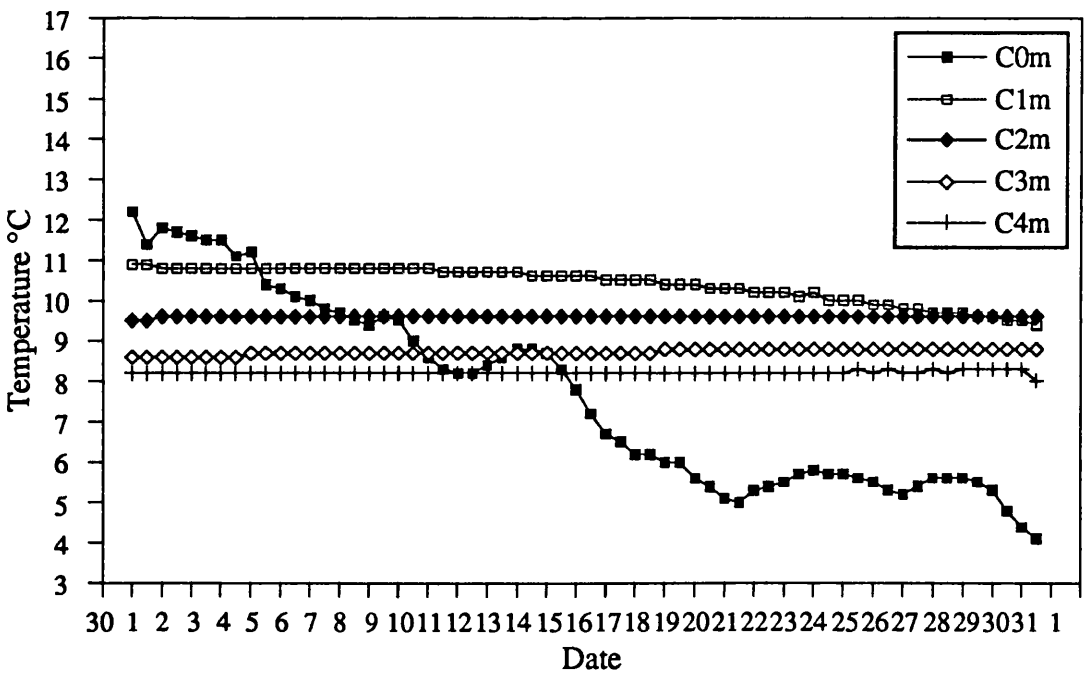


Fig. 5.4i. Temperature in the Control profile from 00:00hours, 1st October to 12:00 hours, 31st October 1992.

With the influence of the greenhouse removed, during October both profiles exhibited very similar temperature profiles (Figure 5.4i/5.4j). The temperature at the surface of the bog dropped substantially from approximately 13°C to 4°C, with the final temperature colder than the temperature at all other depths below the surface of the bog. Heat was also lost at 1m depth in both profiles. At the end of October, the temperature at 1m depth was essentially constant between the GP and the CP, suggesting that more heat was lost from GP1m depth than CP1m depth during this month. 3m depth in both profiles was significantly warmer than the underlying 4m and 5m depths which exhibited little variation in temperature.

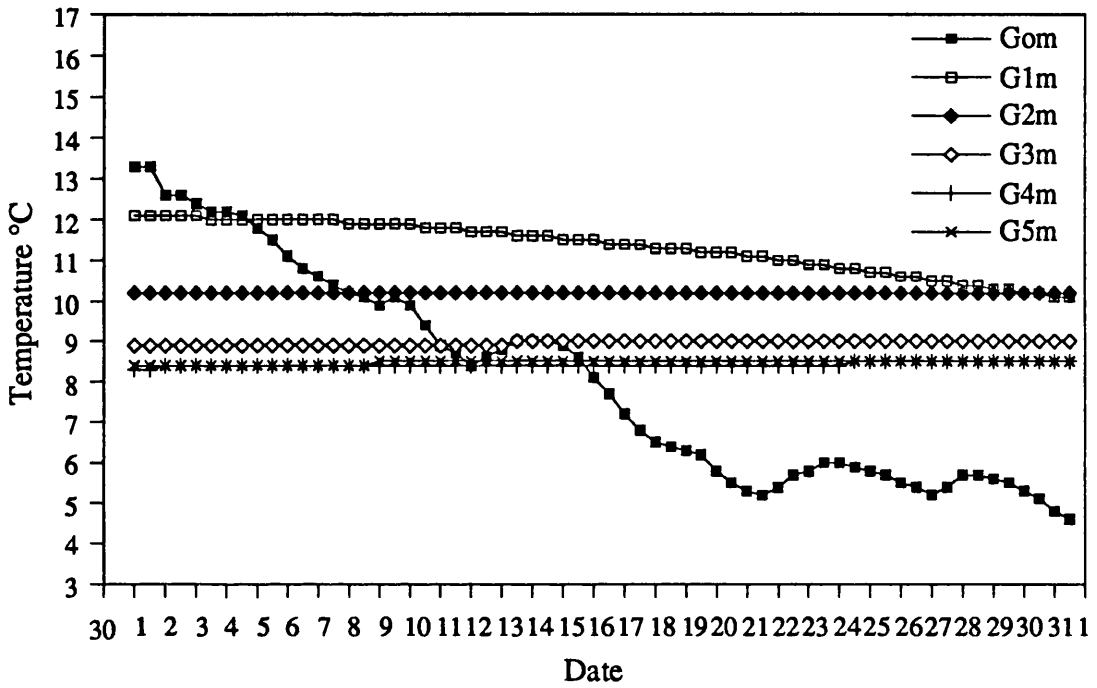


Fig. 5.4j. Temperature in the Greenhouse profile from 00:00hours, 1st October to 12:00 hours, 31st October 1992.

In both profiles during November (Figure 5.4k/5.4l), the surface temperature was lower than the temperature at any other depth. With the removal of the greenhouse more than a month ago both profiles exhibited very similar temperature regimes. Any remaining influence elevated atmospheric temperature had on the GP could be considered to have dissipated. The temperature at 1m and 2m continued to drop and by the end of November, the temperature at 1m was cooler than at 4m and 5m depth. The temperature at 3m rose in both profiles, albeit very slightly.

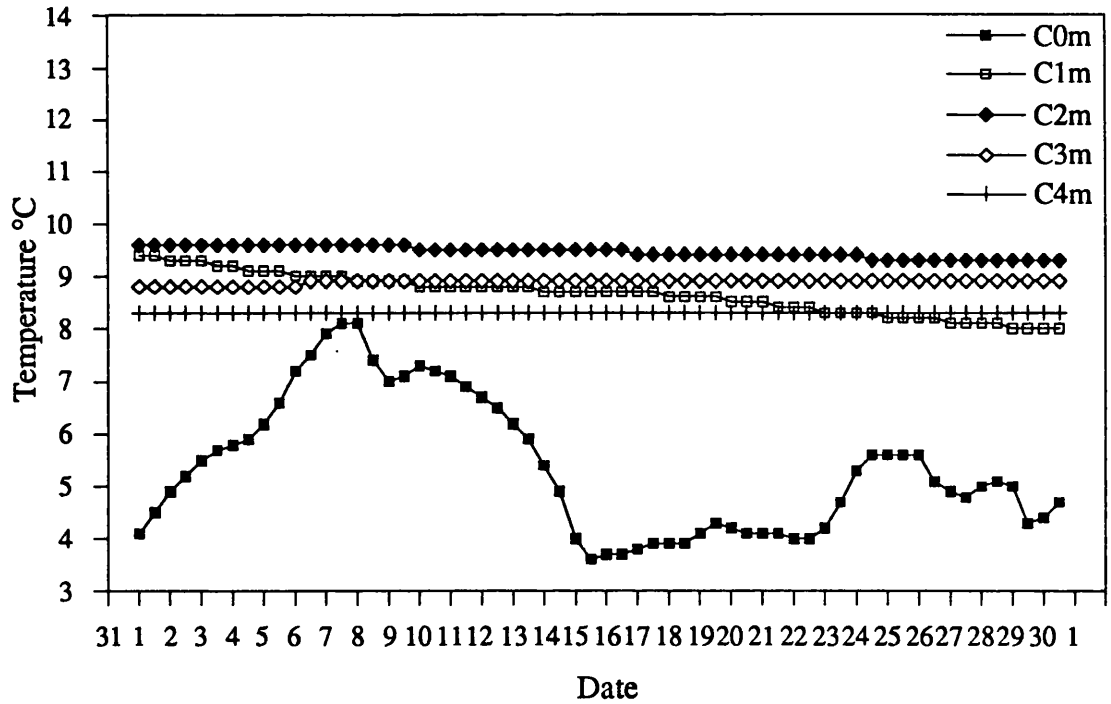


Fig. 5.4k. Temperature in the Control Profile from 00:00hours, 1st November to 12:00 hours, 30th November 1992. Note change in y-axis scaling.

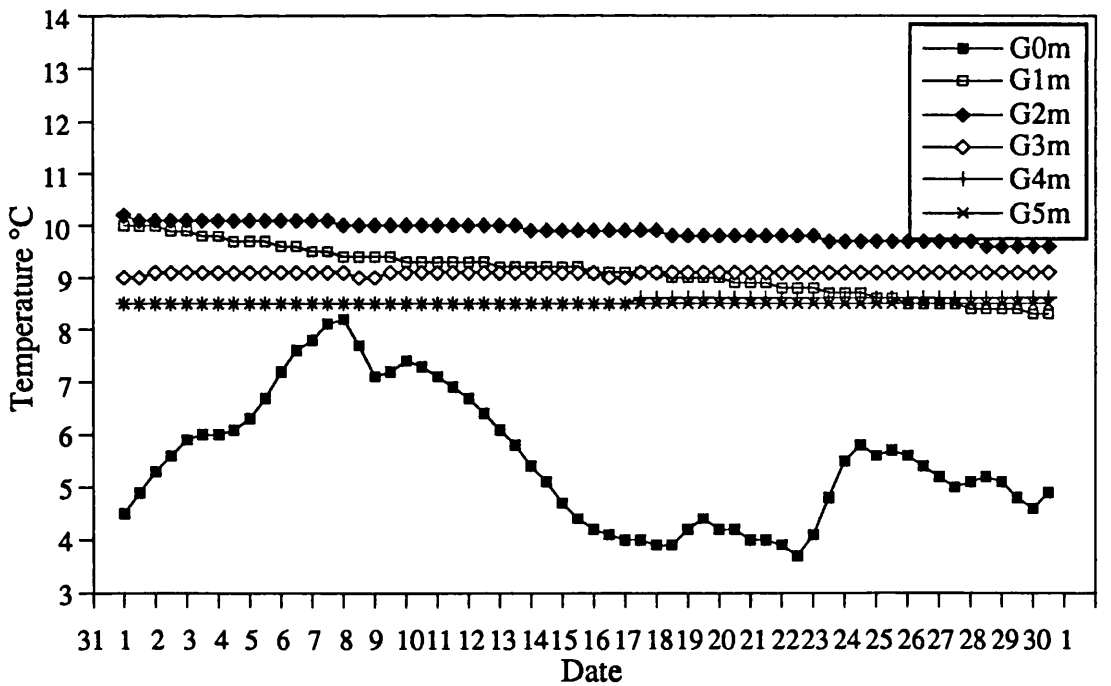


Fig. 5.4l. Temperature in Greenhouse profile from 00:00 hours, 1st November to 12:00 hours, 30th November 1992.

A comparable pattern was observed in December (Fig. 5.4m/5.4n). Both profiles exhibited very similar characteristics. The surface temperature fell to a minimum of 2°C, cooler

than any other depth in the bog. Likewise, the temperature at 1m for both profiles was lower than the underlying peat body. The temperature at 2m continued to fall and towards the end of the month the temperature at 3m depth started to fall. The temperature at 4m depth in the control profile remained constant for the duration of the month. In the GP, the temperature at 4m and 5m depth rose slightly during the month.

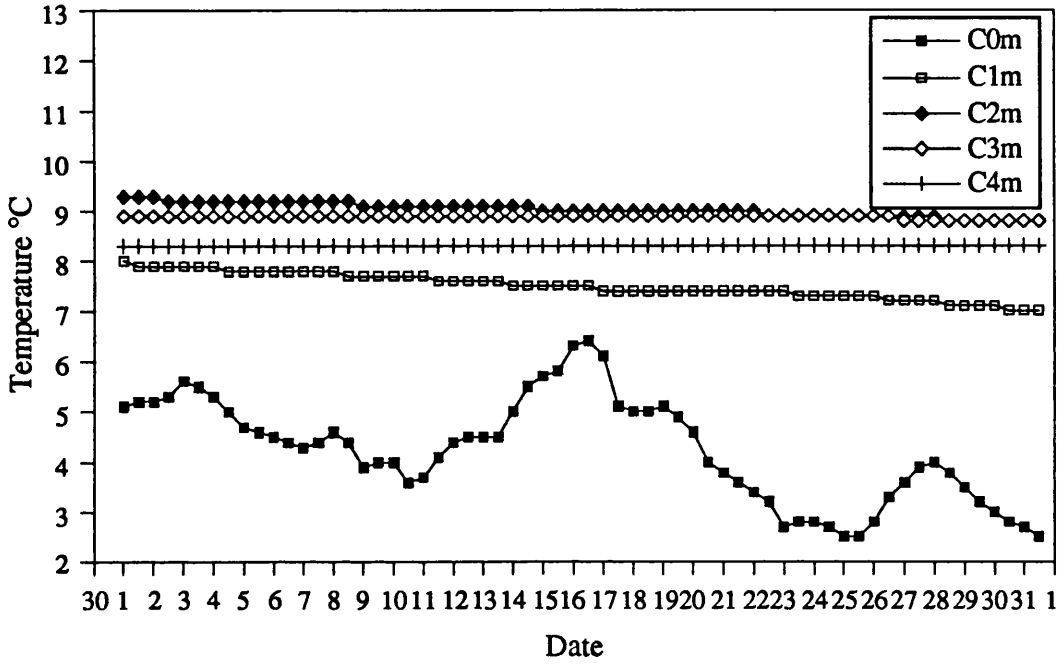


Fig. 5.4m. Temperature in the Control profile from 00:00 hours, 1st December to 12:00 hours, 31st December 1992.

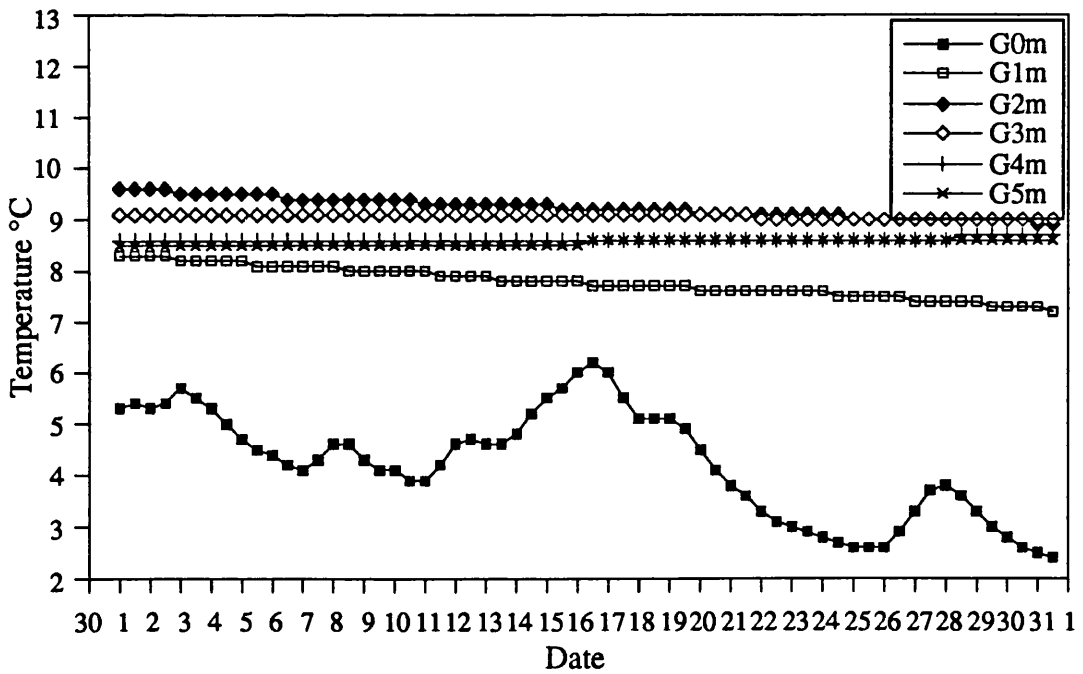


Fig. 5.4n. Temperature in the Greenhouse profile from 00:00 hours, 1st December to 12:00 hours, 31st December 1992.

There was a general trend for the bog surface temperature to rise during January, while the temperature at 1m and 2m continued to drop (Figure 5.4o/5.4p). Towards the end of January, the temperatures at the surface, 1m and 2m depths were lower than the temperature in the deeper peat body. The temperature from 3m down remained relatively constant during January.

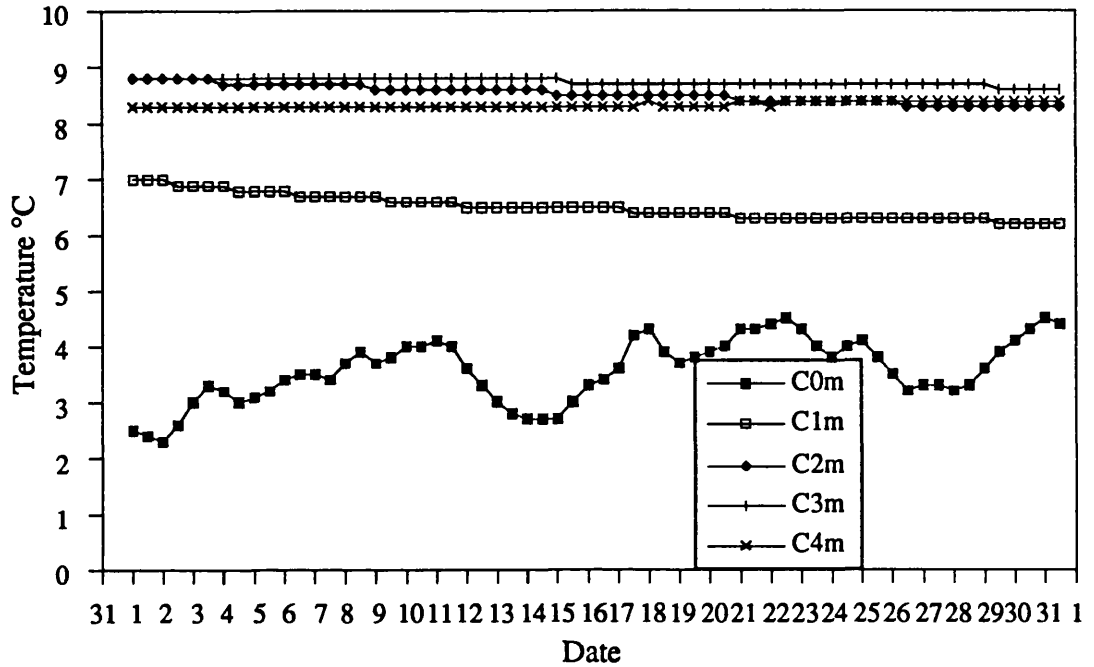


Fig. 5.4o. Temperature in the Control profile from 00:00 hours, 1st January to 12:00 hours, 31st January, 1993.

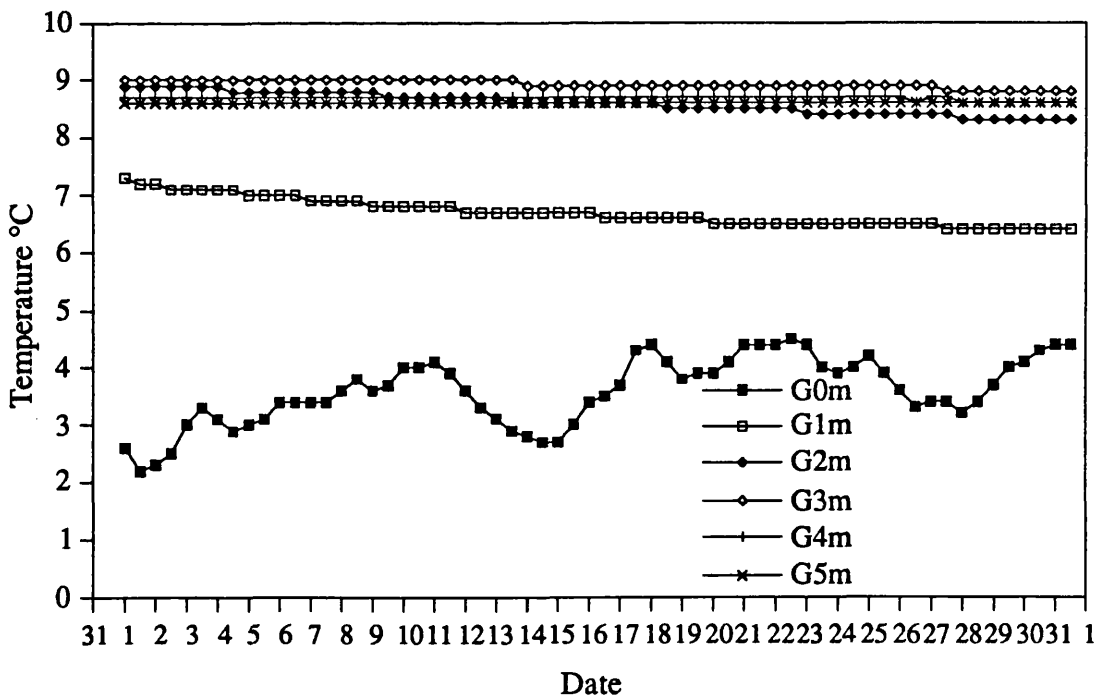


Fig. 5.4p. Temperature in the Greenhouse profile from 00:00 hours, 1st January to 12:00 hours, 31st January, 1993.

During February (Figure 5.4q/5.4r) the ambient temperature at 0m was higher than in January and at one point in the CP0m temperature was greater than CP1m. The temperature at 1m also started to rise. In contrast the temperature at 2m and 3m for both profiles started to fall, more substantially at 2m than 3m.. The temperature at 4m and 5m within the bog remained constant during February.

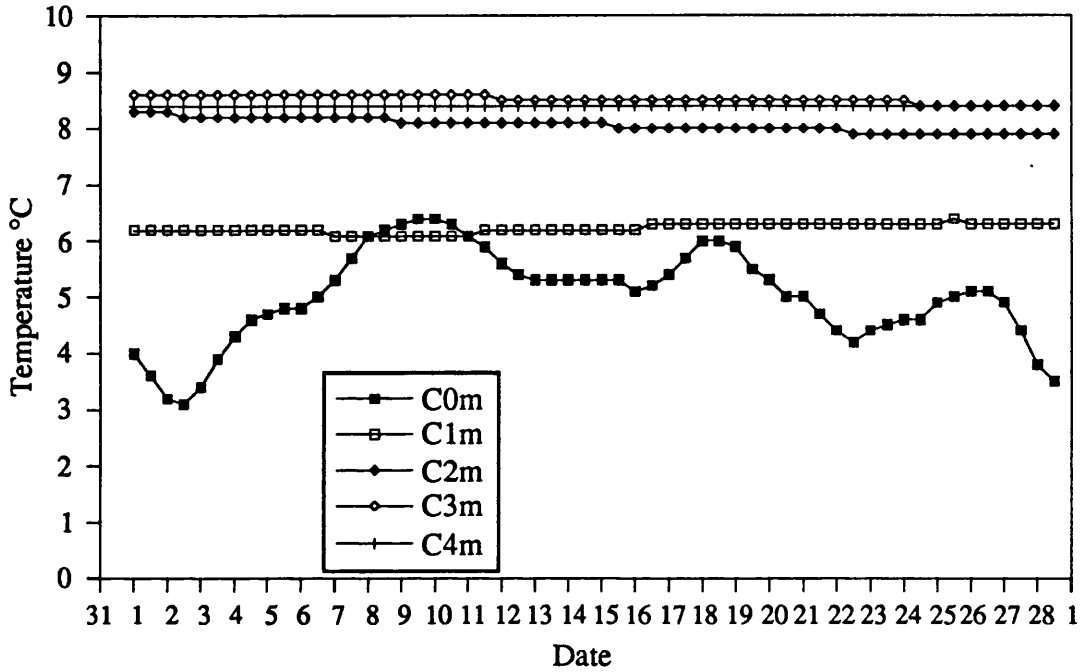


Fig. 5.4q. Temperature in the Control profile from 00:00 hours 1st February to 12:00 hours, 28th February 1993.

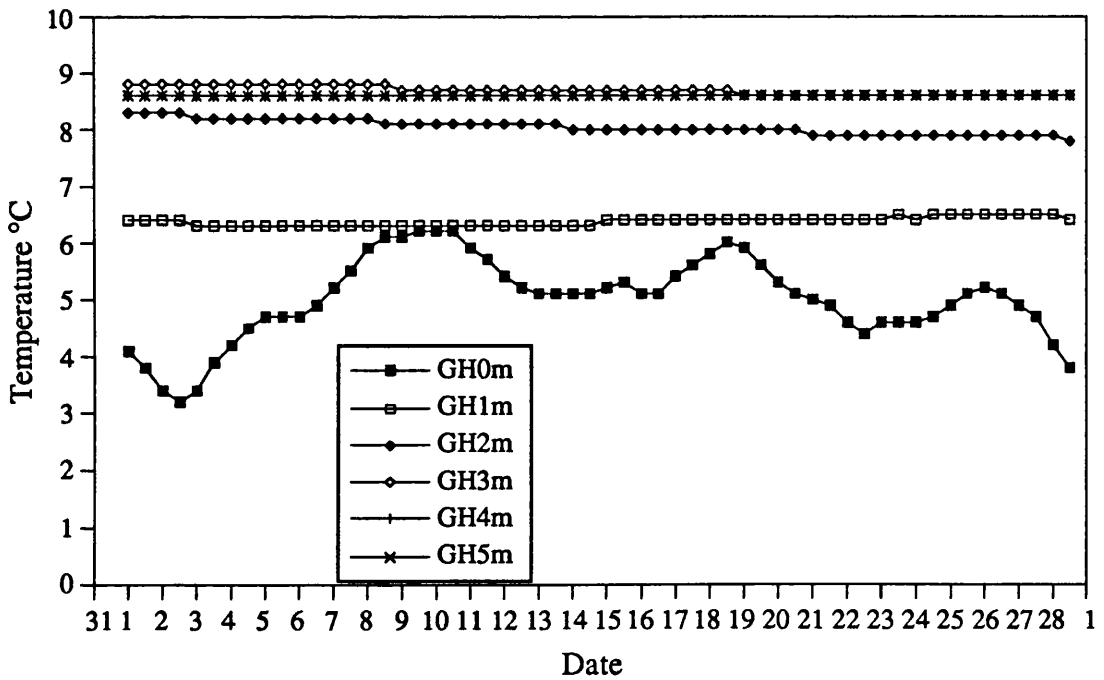


Fig. 5.4r. Temperature in the Greenhouse profile from 00:00 hours, 1st February to 12:00 hours, 28th February 1993.

In March (Figure 5.4s/5.4t) the temperature at the surface of the bog fluctuated quite substantially, peaking on the 19th when the surface temperature became greater than the temperature at 1m depth. The temperature at 1m depth also fluctuated, dropping to a monthly minimum when the surface temperature exhibited a maximum. The temperature at 2m depth continued to drop, as did the temperature at 3m in both profiles and 4m in the GP. GP5m depth stayed constant during March. Both profiles now exhibited a complete temperature inversion becoming warmer with increasing depth for the majority of the month.

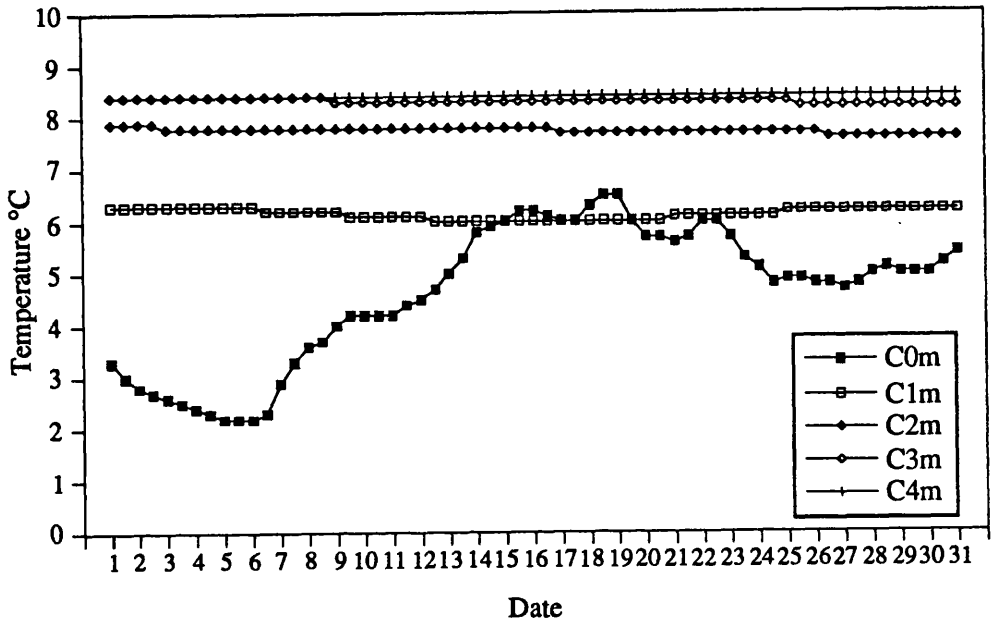


Figure 5.4s. Temperature in Control profile from 00:00 hours, 1st March to 12:00 hours, 31st March 1993.

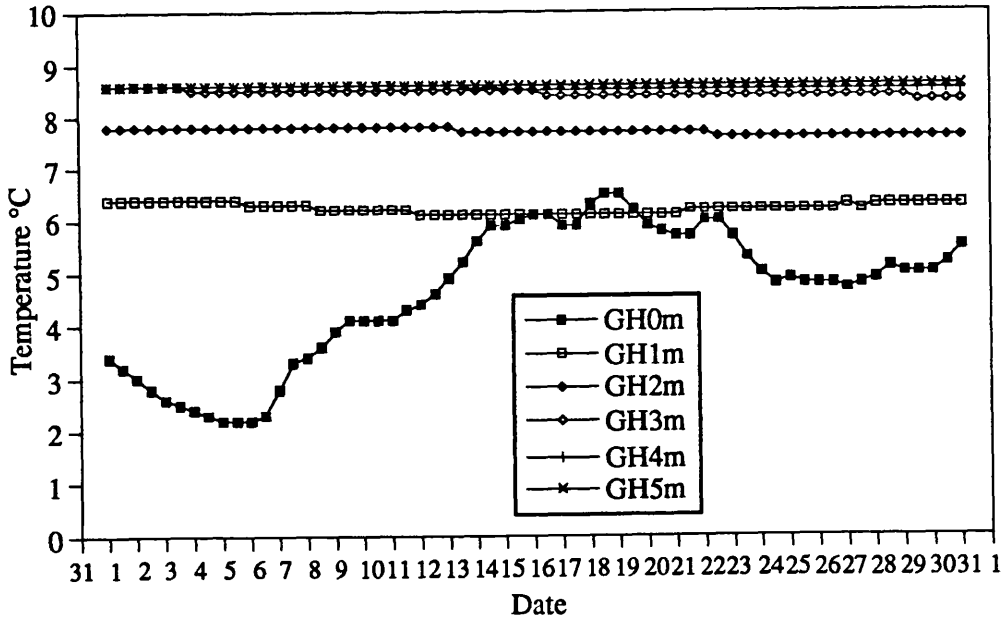


Figure 5.4t Temperature in Greenhouse profile from 00:00 hours, 1st March to 12:00 hours, 31st March 1993.

Atmospheric temperature profiles within the greenhouse (May to Sept. 1992) and outwith (May to September 1992; October 1992- March 1993) are shown in Figures 4u, 4v and 4w respectively. Atmospheric temperature data outside the greenhouse was supplied by the Institute of Terrestrial Ecology, Edinburgh. Figures 5.4u, 5.4v and 5.4w are complex due to the multitude of data points plotted. The data has been plotted in a manner that will not emphasis minor variation within monthly profiles, but rather give an impression of the overall variation during the season shown. The salient features from Figure 5.4u and 5.4w are the highest and lowest temperatures, and the seasonal mean temperature. These figures are only provided to give an indication of the air temperature affecting the peat. Figure 5.4u is also as a control for the temperature inside the greenhouse. All profiles exhibit diurnal variation in temperature.

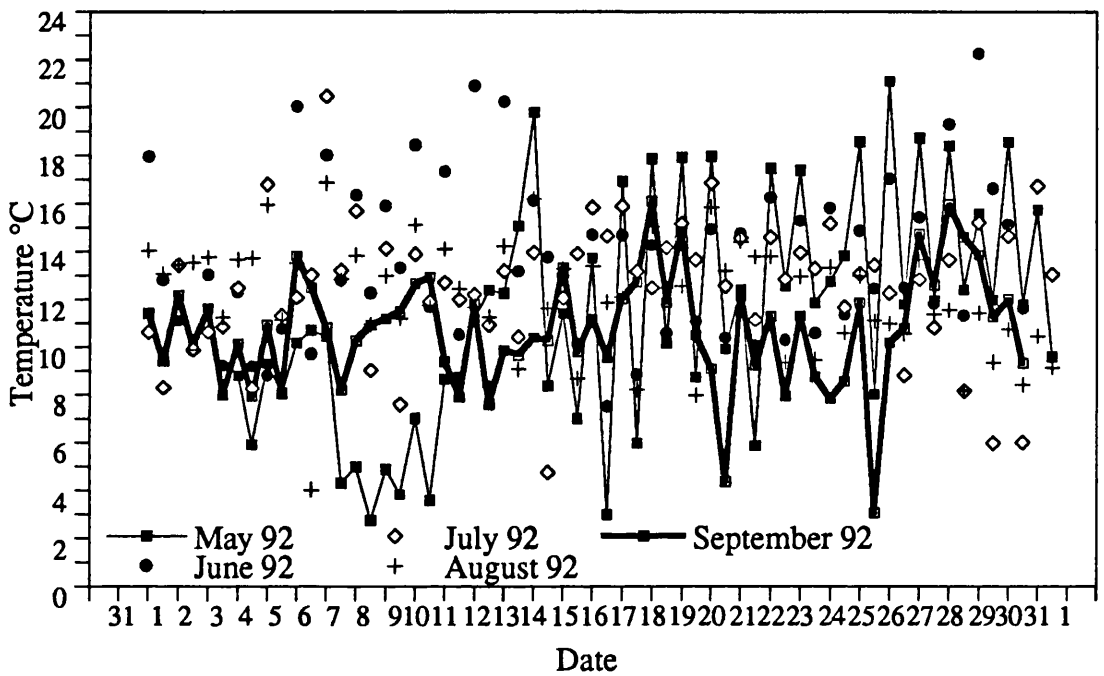


Fig. 5.4u. Atmospheric temperature recorded at Ellergower Moss from May to September 1992.

The greenhouse clearly had a significant effect while standing, to a depth of three metres. The effect was less marked with increasing depth. Barriers were not placed into the peat around the greenhouse to arrest water movement within the bog. The significant increase in temperature observed in the greenhouse profile therefore suggests that the movement of water is very slow within the bog. This therefore suggests that the hydraulic conductivity of Ellergower Moss is so low that the water body can almost be considered stationary. As such it can be concluded that if Ellergower Moss was subject to atmospheric temperatures identical to those recorded within the greenhouse, then a similar pattern of warming within the whole bog would be observed within most of the bog as that under the greenhouse. With increased atmospheric temperatures the potential for warming such an environment exists.

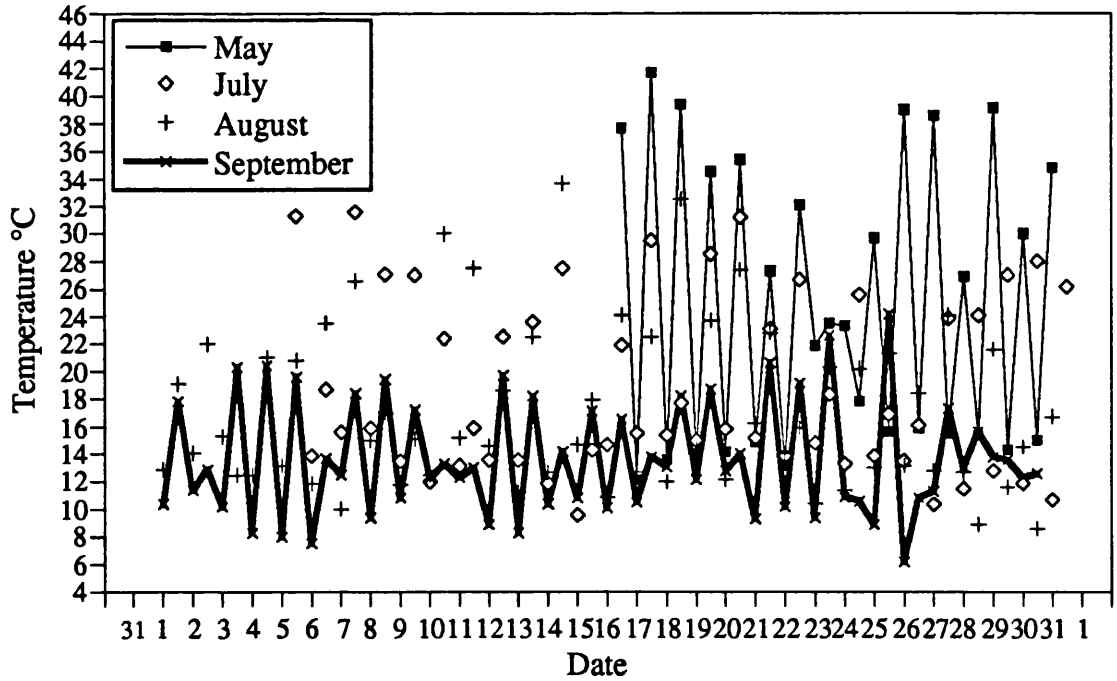


Fig. 5.4v. Atmospheric temperature inside greenhouse sited on Ellergower Moss from May to September 1992.

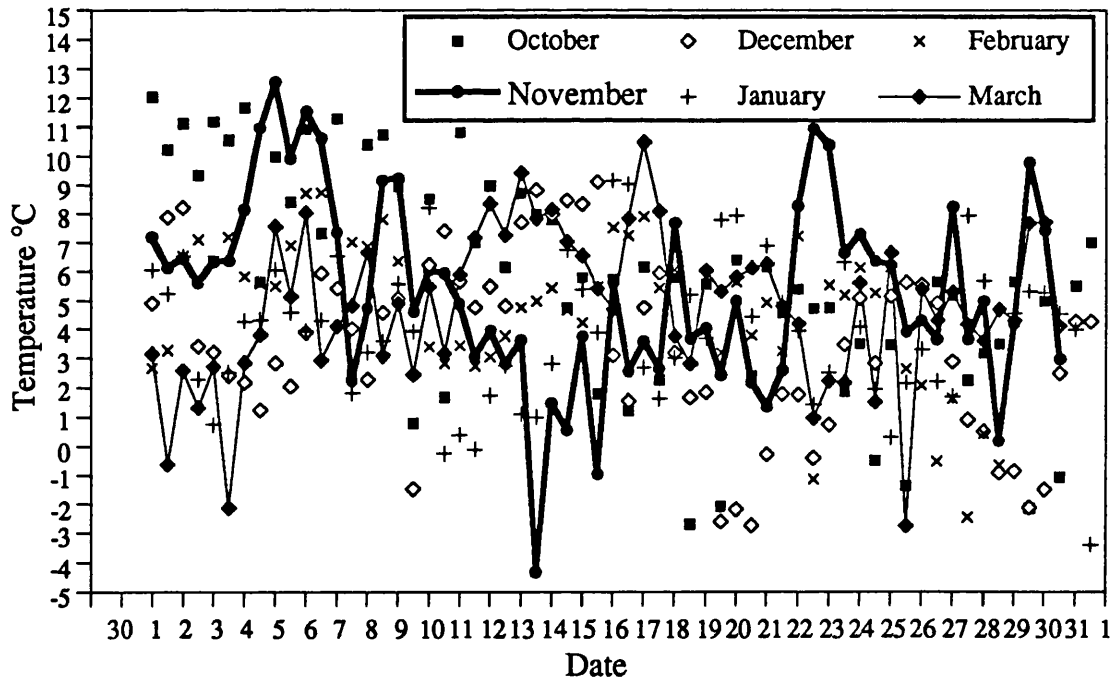


Fig. 5.4w. Atmospheric temperature at Ellergower Moss from October 1992 to March 1993. Data was kindly supplied by ITE, Edinburgh.

The consequences of such warming are most important when considering Ellergower Moss as both a potential source and sink of CH_4 due to bacteriogenic gas production and oxidation.

The greenhouse caused a significant increase in temperature to a depth of 2m while the

peat below remained unaffected. The largest temperature changes were shown closest to the surface, the zone where aerobic methane oxidising bacteria are likely to be present and active. Elevated ambient temperatures (but below optimum) will increase the activity of the methylotrophs (Williams and Crawford, 1984). If solely temperature limited, methanogenic associations at depth (3-5m) in the bog will continue to produce CH_4 at the same rate. With increased oxidation at the surface less CH_4 is likely to be emitted from the bog to the atmosphere. Thus an increase in atmospheric temperature affecting only the upper 2m of the peat may result in increased methane oxidation, which could be considered as a negative feedback to global warming.

Temperature measurements of Ellergower Moss throughout the year are also significant as they provide further information about the methanogenic environment. It is likely that CH_4 is being produced throughout the anaerobic zone of Ellergower Moss. The temperature data shows that while the surface temperature fluctuated substantially throughout the year, and that the temperature at 1m depth ranged from 11.5°C to 6°C (data from control profile only as considering natural environment) the range in temperature shown by all other depths was small, approximately 8.5±1.5°C. This is close to optimum methanogenic temperatures of 12°C measured for Minnesota peatland samples collected below 1.2m (Williams and Crawford, 1984). The methanogenic environment from 2m downwards thus remains relatively stable throughout the year, with little change in temperature despite fluctuations in the overlying peat temperature. CH_4 production is not likely to be temperature limited in the 2-5m zone of Ellergower Moss and may occur throughout the year. The 0-1m zone however is subject to quite large fluctuations in temperature, although more substantial at the surface than at 1m depth. Such changes are significant, as they could affect the activity of the methylotrophs, ultimately increasing the volume of CH_4 that is being fluxed to the atmosphere. In Ellergower Moss CH_4 production may be seasonally controlled, with any decrease in the volume of CH_4 produced attributable to the cessation or decrease in the rate of methanogenesis in the upper 2m, rather than throughout the bog. The proposal that CH_4 is still being produced within the bog, but that there are no active methylotrophs at the surface, resulting in increased fluxes of CH_4 during the winter months appears a possibility and warrants further investigation.

5.7. Conclusions :

Interpretation of the factors controlling the isotopic composition of CH_4 and CO_2 within Ellergower Moss is complex for $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ exhibit both spatial (with depth and latitude) and temporal variation. Interpretation does not appear to be complicated by aerobic bacterial oxidation of CH_4 at 0m depth, however microaerobic / anaerobic oxidation at depth within the peat body cannot be disregarded. The potential recycling of carbon and hydrogen that may occur as a result of bacterial oxidation at depth has implications for further isotopic fractionation. The trends shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ cannot be attributed to a change in $\delta^{13}C$ of the organic substrate. Likewise the pattern shown by $\delta D(CH_4)$ measurements with depth cannot be attributed to variation in δD of the organic matter or porewater, although

the latter has been shown to be influential (Whiticar et al., 1986; this work, Chapter 4) and may be dependent upon the rate of water flow through the catotelm.

The presence of an isotopic boundary between 2-3m is observed in $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and most clearly in $\delta D(CH_4)$ measurements. Undoubtedly kinetic fractionation during diffusion of CH_4 and CO_2 from source, upwards, through the profile, is influencing the pattern shown by isotopic measurements with depth, however this is not considered to be the dominant control and does not account for the 2-3m boundary. Entrapment of gas close to source in the 3-5m zone, with rapid diffusion upwards (causing a kinetic fractionation), only after episodic and localised gas release in the 0-3m zone (triggered perhaps by low atmospheric pressure), may also provide isotopic profiles similar to those observed in Ellergower Moss. Without further information about zones of methane production and factors influencing gas release mechanisms from the bog, this cannot be quantified. It is suggested that the data can be grouped into two sets, 0-2m and 3-5m, as a function of methanogenic pathway dominant at each zone (indirectly age), acetoclastic methanogenesis and CO_2 reduction respectively. The dominance of each pathway may be controlled by substrate limitation.

The greenhouse clearly had a significant effect while standing, causing an increase in ambient temperature to a depth of two metres. This effect was less marked with increasing depth with the largest temperature changes closest to the surface in the zone where aerobic methane oxidising bacteria are likely to be present and active. It is possible that an increase in ambient soil temperature caused by erection of the greenhouse may be responsible for istopically heavier greenhouse profile samples between 0-2m. Unfortunately there is insufficient data to draw a firm conclusion. Although a pilot experiment, the results show that Ellergower Moss (and other similar wetland environments) are susceptible to the influences of atmospheric warming. The consequences of such warming are most important when considering these environments as both a potential source and sink of CH_4 due to bacteriogenic gas production and oxidation.

Measurements of natural temperature profiles within Ellergower Moss throughout the year are also significant as they provide further information about the methanogenic environment. It is quite clear that during the winter months a temperature inversion occurs, with the upper 0-1m zone colder than the lower peat body, which remains at an essentially constant temperature throughout the year. Bacterial activity, both methanogenic and methylotrophic is likely to be adversely affected in this upper zone due to decrease in temperature, while bacterial activity in the lower peat zone will be seasonally unaffected by temperature. The proposal that CH_4 is still being produced within the bog, but that there are no active methylotrophs at the surface, resulting in increased fluxes of CH_4 during the winter months thus appears possible and warrants further investigation.

CHAPTER 6: CONCLUDING REMARKS AND FUTURE RESEARCH

Analytical procedure:

The analytical procedure developed during this research allows accurate measurement of $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$ of CH_4 and CO_2 in an air sample. The precision to which $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ can be measured is less than was desired, but considered sufficient to undertake research projects whose objective was to elucidate controls of $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ at source and thus accurately characterise a bacteriogenic CH_4 flux. Development of the analytical method took one year and future funds were limited, thus a decision was made to accept the limitations of the analysis procedure and instead use the technique to investigate several aspects of isotopic biogeochemical cycle of CH_4. One future research aim would be to improve the technique and increase the precision with which $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ can be measured. This would probably be done effectively by increasing the temperature of the CuO furnace where the CH_4 oxidation takes place, and by using an inert carrier gas to transport all the sample into the CuO furnace, ensuring 100% oxidation of the introduced sample and avoiding potential fractionation.

Fingerprinting of sources:

It is clear from isotopic characterisation of bacteriogenic CH_4 in both natural and laboratory environments during this research, that the field boundaries previously used to depict the isotopic signature of CH_4 produced by acetoclatic methanogenesis and CO_2 reduction are not accurate and should be extended. While there are areas of overlap, measurement of $\delta^{13}C(CH_4)$ and complementary $\delta D(CH_4)$ can still be applied, in many cases, to determine the origin of an unknown source of CH_4. At present it is not possible to determine by isotopic analysis alone whether a CH_4 sample with a bacteriogenic signature is a ruminant emission or from a landfill site. In cases such as these the geological and industrial setting must also be taken into consideration.

From this data collected during this research, it does not appear possible to distinguish sources of CH_4 unambiguously by use of $\delta^{13}C(CO_2)$ rather than $\delta D(CH_4)$ as a complementary measurement to $\delta^{13}C(CH_4)$. $\delta^{13}C(CO_2)$ ranges from 19.0‰ to -22.5‰, with a mean of 0.2±8.5‰. Both end member $\delta^{13}C(CO_2)$ measurements are from landfill sites, thus one source can cover the whole range. While some groups may appear distinguishable, for example peatland samples, they are differentiated by $\delta^{13}C(CH_4)$ rather than $\delta^{13}C(CO_2)$. The exception is the bacterial culture samples which form two groups as a function of $\delta^{13}C(CO_2)$. This is attributed to a change in anaerobic degradation pathway, from the short-lived aerobic catabolism of organic matter producing isotopically light CO_2, to methanogenesis producing isotopically heavier $\delta^{13}C(CH_4)$. The initial, short period of isotopically light $\delta^{13}C(CO_2)$ production observed with the bacterial cultures is likely to be masked in a natural 'open' system. Combined $\delta^{13}C(CO_2)$ and $\delta^{13}C(CH_4)$ measurements however, can be useful in suggesting when CH_4 has been subject to bacterial oxidation, producing CO_2. The reaction rate for the oxidation of $^{12}CH_4$ is faster than that for $^{13}CH_4$, thus $\delta^{13}C(CO_2)$ becomes isotopically lighter due to the input of isotopically light

CO_2 and the resultant $\delta^{13}C(CH_4)$ isotopically heavier due to the proportionally greater loss of ^{12}C.

Application of stable isotope analysis to trace gas budgets:

Data produced from stable isotopic fingerprinting of sources have been utilised in Chapter 3 to demonstrate the application of stable isotope studies of trace gas emissions to predict atmospheric $\delta^{13}C(CH_4)$ for global and United Kingdom flux budgets. Due to complex atmospheric chemistry, measurements of atmospheric $\delta D(CH_4)$ have, at present, little application in unravelling the global atmospheric flux of CH_4. However current research elsewhere within the United Kingdom to allow $\delta D(CH_4)$ measurements from very small volumes of air, will increase the interest in $\delta D(CH_4)$ and progress in understanding the atmospheric chemistry will follow.

From temporal measurement of atmospheric $\delta^{13}C(CH_4)$, changes in flux budget may be observed and understood. One potential flux emission scenario modelled in Chapter 3 demonstrates changes in $\delta^{13}C(CH_4)$ with time as a result of changing flux budgets. The difficulty in accurate interpretation of atmospheric $\delta^{13}C(CH_4)$ is demonstrated through this modelling, for without sufficient data characterising isotopic composition, flux size and current atmospheric chemical concentrations (for example OH radical concentration), the secular trend of $\delta^{13}C(CH_4)$ may be misinterpreted. In order to elucidate the factors controlling $\delta^{13}C(CH_4)$, $\delta D(CH_4)$ and $\delta^{13}C(CO_2)$, both laboratory based closed culture enrichment systems (Chapter 4) and a field site within a CH_4 rich peatland (Chapter 5) were utilised.

Understanding the controls on isotopic signature of CH_4 at source:

Four closed culture anaerobic enrichment systems set up to produce CH_4 were monitored for a period of 92 days. Systems 1 and 2 were active and producing gas for the duration of the experiment, systems 3 and 4 failed after the first gas sample was taken. The pattern shown by $\delta^{13}C(CH_4)$, in systems 1 and 2 may have reflected bacterial growth within the system, although it is not yet known if CH_4 production is quantitatively related to bacteria growth. Progressive enrichment in ^{13}C of CH_4 observed with time for both systems 1 and 2 was not attributed to a closed system. Initial isotopically light CO_2 produced was attributed to aerobic catabolism of the waste during the primary fermentative stages. The early stages of CH_4 production may have been dominated by the CO_2 reduction pathway, with the following period of rapidly changing $\delta D(CH_4)$ attributed to the increasing dominance of acetoclastic methanogenesis. The final period of dynamic equilibrium was attributed to methanogenesis dominated by acetoclastic methanogenesis with a minor input from CO_2 reduction.

$\delta D(CH_4)$ measured was of accumulated CH_4 which has been produced by several methanogenic pathways. The relative input of CH_4 produced by acetoclastic methanogenesis in comparison to that produced by the reduction of CO_2 could not be determined, due to production and consumption of CO_2 at other stages during the anaerobic degradation of organic matter. $\delta D(CH_4)$ produced by acetoclastic methanogenesis was likely to be lower than the measured composition, while $\delta D(CH_4)$ produced by the reduction of CO_2 was isotopically heavier.

$\delta D(CH_4)$ for system 1 and 2 remained essentially constant with time. The constancy in $\delta D(CH_4)$ suggested that it was very strongly influenced by an 'unlimited' isotopically homogeneous reservoir, most likely water. $\delta D(CH_4)$ for systems 2 and 4 was isotopically heavier by approximately 50‰ than systems 1 and 3 respectively. The difference in $\delta D(CH_4)$ between system 1 and system 2 remained constant for the duration of the experiment, differing by approximately 56‰. The heavier $\delta D(CH_4)$ for systems 2 and 4 was attributed to the use of H_2O which was 110‰ heavier than used in the counterpart system.

A universal linear relationship between $\delta D(CH_4)$ and $\delta D(H_2O)$ was not evident due to variability in the dominance of methanogenic pathways in different systems. In order to understand the significance of the relationship of $\delta D(CH_4)$ to $\delta D(H_2O)$, the synthesis and cleavage of acetate and its precursors had to be considered in more detail.

The following equation was derived to model $\delta D(CH_4)$ produced via acetoclastic methanogenesis, when the acetate substrate was derived from a fatty acid of an even numbered chain length by the β-oxidation pathway (Chapter 4):

Where n is an even number:

$$\delta D(CH_4) = \frac{n-1}{2n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic) + \frac{n-2}{4n}\delta D(enzymeCoA-SH)$$

This quite clearly indicates that as the chain length of the acid increased, more H_2O was incorporated into the acetate and hence CH_4. δD of the enzyme CoA-SH involved in acetoclastic methanogenesis was likely to be the same as $\delta D(H_2O)$, and the above equation can thus be simplified to:

$$\delta D(CH_4) = \frac{3n-4}{4n}\delta D(H_2O) + \frac{n+4}{4n}\delta D(organic)$$

Therefore, depending on the composition of $\delta D(H_2O)$ in relation to δD(organic) for two systems of identical acid chain length, $\delta D(CH_4)$ of the system degrading the longer chained fatty acid will be isotopically heavier / lighter than the system degrading the shorter chained fatty acid. As only a fraction of $\delta D(H_2O)$ is incorporated into $\delta D(CH_4)$, then the difference in $\delta D(CH_4)$ between two identical systems, supplemented with different $\delta D(H_2O)$, will be less than the difference between the two $\delta D(H_2O)$. Fractionation of the reactants occurred, and this research suggests that despite fractionation, δD(organic) is still isotopically lighter than $\delta D(H_2O)$.

This project would be worth extending to to allow quantification of the fractionation that will occur between $\delta D(H_2O)$ and $\delta D(CH_4)$ by using two sets of four closed culture enrichment systems, each set selecting for the same degradation pathway, but with isotopically distinct water. The relation ship between $\delta D(H_2O)$ and $\delta D(CH_4)$ when an odd-numbered fatty acid is being degraded requires consideration.

The dependence of $\delta^{13}C(CH_4)$ upon methanogenic pathway may also have been observed in samples collected from Ellergower Moss (Chapter 5), although interpretation of the factors controlling the isotopic composition of CH_4 and CO_2 within the peatland is complex for $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and $\delta D(CH_4)$ exhibit both spatial (with depth and latitude) and temporal variation. Interpretation does not appear to be complicated by aerobic bacterial oxidation of CH_4 at 0m depth, however microaerobic/anaerobic oxidation at depth within the peat body cannot be

disregarded. The potential recycling of carbon and hydrogen that may occur as a result of bacterial oxidation at depth has implications for further isotopic fractionation. The trends shown by $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ could not be attributed to a change in $\delta^{13}C$ of the organic substrate. Likewise the pattern shown by $\delta D(CH_4)$ measurements with depth could not be attributed to variation in δD of the organic matter or porewater, although the latter has been shown to be influential and may be dependent upon the rate of water flow through the catotelm.

The presence of an isotopic boundary between 2-3m was observed in $\delta^{13}C(CH_4)$, $\delta^{13}C(CO_2)$ and most clearly in $\delta D(CH_4)$ measurements. Undoubtedly kinetic fractionation during diffusion of CH_4 and CO_2 from source, upwards, through the profile, influenced the pattern shown by isotopic measurements with depth. However this was not considered to be the dominant control and does not account for the 2-3m boundary. Entrapment of gas close to source in the 3-5m zone, with rapid diffusion upwards (causing a kinetic fractionation), only after episodic and localised gas release in the 0-3m zone (triggered perhaps by low atmospheric pressure), may also have produced isotopic profiles similar to those observed in Ellergower Moss. Without further information about zones of methane production and factors influencing gas release mechanisms from the bog, this cannot be quantified. It is suggested that the data can be grouped into two sets, 0-2m and 3-5m, as a function of methanogenic pathway dominant at each zone (indirectly age), acetoclastic methanogenesis and CO_2 reduction respectively. The dominance of each pathway may be controlled by substrate limitation. Future research may undertake quantification of pathway dominance by a) measurement of acetate and CO_2 substrate concentrations at 1m intervals through Ellergower Moss and b) by measurement of the CH_4 production rate from laboratory incubated peat cores, taken from 1m depth intervals from Ellergower Moss, and supplemented with ^{14}C labelled acetate and/or bicarbonate.

The greenhouse clearly had a significant effect while standing, causing an increase in ambient temperature to a depth of two metres. This effect was less marked with increasing depth with the largest temperature changes closest to the surface in the zone where aerobic methane oxidising bacteria are likely to be present and active. It is possible that an increase in ambient soil temperature caused by erection of the greenhouse may be responsible for istopically heavier greenhouse profile samples between 0-2m. Unfortunately there is insufficient data to draw a firm conclusion. Although a pilot experiment, the results show that Ellergower Moss (and other similar wetland environments) are susceptible to the influences of atmospheric warming. The consequences of such warming are most important when considering these environments as both a potential source and sink of CH_4 due to bacteriogenic gas production and oxidation.

Measurements of natural temperature profiles within Ellergower Moss throughout the year are also significant as they provide further information about the methanogenic environment. It is quite clear that during the winter months a temperature inversion occurs, with the upper 0-1m zone colder than the lower peat body, which remains at an essentially constant temperature throughout the year. Bacterial activity, both methanogenic and methylotrophic is likely to be adversely affected in this upper zone due to decrease in temperature, while bacterial activity in the lower peat zone will be seasonally unaffected by temperature. The proposal that CH_4 is still being

Measurements of natural temperature profiles within Ellergower Moss throughout the year are also significant as they provide further information about the methanogenic environment. It is quite clear that during the winter months a temperature inversion occurs, with the upper 0-1m zone colder than the lower peat body, which remains at an essentially constant temperature throughout the year. Bacterial activity, both methanogenic and methylotrophic is likely to be adversely affected in this upper zone due to decrease in temperature, while bacterial activity in the lower peat zone will be seasonally unaffected by temperature. The proposal that CH_4 is still being produced within the bog, but that there are no active methylotrophs at the surface, resulting in increased fluxes of CH_4 during the winter months thus appears possible and warrants further investigation.

Concluding remarks:

This research has demonstrated the application of stable isotope analysis in constraining and understanding atmospheric CH_4 flux budgets. Isotopic fingerprinting of bacteriogenic terrestrial CH_4 sources has supplied data which may now be used to aid interpretation of the secular trend of atmospheric CH_4. Two unique pilot projects, one using laboratory based methanogenic associations, and one in a CH_4 rich field environment, have demonstrated that comprehensive stable isotope analyses can contribute significantly to understanding methanogenic processes and the environmental controls on the resultant isotopic signature.

APPENDIX 1 : Non-standard sample data.

This appendix lists the data collected from all non-standard samples analysed during the course of this research. Data collected from the analysis of CH_4 standards, NGS1, NGS2 and NGS3, is given in Chapter 2 in Tables 2.5., 2.6. and 2.4. respectively.

Line N°	Sample name	$\delta^{13}C(CH_4)$	$\delta D(CH_4)$	$\delta^{13}C(CO_2)$	%yield carbon	%yield hydrogen	C:H ratio
CHA 654	Spex	-41.5	-170		31	32	1:4.10
CHA 491	S2: 5/5/93	-46.6	-296	0.6	90	90	1:4.02
CHA 486	S1:5/5/93	-48.1	-355	2.1	85	85	1:4.02
CHA 480	S2:28/4/93	-46.9	-294	1.0	96	96	1:4.01
CHA 479	S1:28/4/93	-48.3	-353	2.5	90	91	1:4.01
CHA 471	S2:21/4/93	-50.7	-295	1.0	64	65	1:4.09
CHA 469	S1:21/4/93	-50.5	-354	3.3	90	90	1:4.04
CHA 462	S1:14/4/93	-56.8	-352	4.2	65	66	1:4.05
CHA 455	S2:14/4/93	-53.0	-298	1.0	73	74	1:4.04
CHA 424	S2:7/4/93	-60.8	-268	0.0	4.0	5.0	1:4.48
CHA 418	S1:7/4/93	-61.5	-359	1.0	50	53	1:4.26
CHA 414	S1:3/3/93	-63.2	-351	-8.7	19	21	1:4.35
CHA 411	S3:3/393	-59.4	-297	-10.0	3	3	1:4.66
CHA 408	S4:3/393	-60.5	-251	-9.2	4	4	1:4.02
CHA 401	S2:3/393	-61.0	-298	-7.9	19	19	1:4.15
CHA 388	S2:1/3/93	-56.6	-312	-8.0	17	18	1:4.30
CHA 386	S1:1/3/93	-60.3	-332	-8.5	31	32	1:4.25
CHA 385	BOC 3		-176	N.P.	62	68	1:4.38
CHA 167	BOC2	-36.8	-156	N.P.	105	106	1:4.04
CHA 156	BOC1	-42.0	-176	N.P.	72	74	1:4.13
CHA 305	C2M:9	-81.6	-319	-2.8	-	-	1:4.82
CHA 300	C3M:9	-77.7	-272	3.7	-	-	1:4.39
CHA 299	C4.5M:9	-74.9	-261	5.5	-	-	1:4.05
CHA 298	C0M:9	-76.3	-260	-11.3	-	-	1:4.19
CHA 297	GH2M:9	-72.8	-325	2.9	-	-	1:4.23
CHA 294	GH0M:9	-72,2		6.8	-	-	1:4.33
CHA 291	GH1M:9	-74.8	-378	2.2	-	-	1:4.11
CHA 286	GH3M:9	-74.1	-266	9.6	-	-	1:4.11
CHA 283	C4M:9	-76.1	-258	6.2	-	-	1:4.07
CHA 279	GH5M:9	-73.2	-259	9.2	-	-	1:4.11

Line N°	Sample name	$\delta^{13}C(CH_4)$	$\delta D(CH_4)$	$\delta^{13}C(CO_2)$	%yield carbon	%yield hydrogen	C:H ratio
CHA 261	C3M:8	-75.0	-259	5.9	-	-	1:4.30
CHA 259	C4.5M:8	-74.5	-244	6.1	-	-	1:4.34
CHA 257	C4M:8	-77.0	-247	5.2	-	-	1:4.26
CHA 255	GH2M:8	-75.2	-312	6.6	-	-	1:4.56
CHA 254	GH1M:8	-76.6	-335	3.4	-	-	1:4.34
CHA 253	C0M:8	-78.0	-338	-8.2	-	-	1:4.28
CHA 251	C1M:8	-79.3	-363	-0.5	-	-	1:4.60
CHA 247	C2M:8	-82.8	-337	2.0	-	-	1:4.70
CHA 246	GH3M:8	-75.9	-265	7.9	-	-	1:4.36
CHA 244	GH5M:8	-73.6	-266	9.2	-	-	1:4.28
CHA 241	C0M:9/8	-78.4	-311	-7.7	-	-	1:4.21
CHA 240	C4M:9/8	-76.7	-245	2.6	-	-	1:5.04
CHA 239	GH4M:9/8	-71.5	-238	7.0	-	-	1:6.57
CHA 208	GH5M:5/7	-74.7	-234	9.2	-	-	1:4.46
CHA 190	SB:BUT	-28.7	-316	7.2	56	N.M.	-
CHA 189	SB:HEX	-45.5	-315	-8.2	81	98	1:4.40
CHA 243	EKLS	-63.7	-314	-0.9	85	95	1:4.30
CHA 242	EKLS	-62.9	-332	5.4	75	81	1:4.27
CHA 187	EKLS:B19	-71.7	-253	-15.2	40	45	1:4.46
CHA 153	EKLS:B13	-52.9	-263	N.C.	47	50	1:4.23
CHA 152	EKLS:BH2	-59.4	-251	-22.5	44	50	1:4.56
CHA 166	EKLS:B10	-54.3	-262	5.4	75	74	1:3.96
CHA 165	EKLS:BH9	-61.4	-280.1	-9.2	38	43	1:4.51
CHA 185	GMLS:ME	-53.4	-283	-11.5	76	85	1:4.37
CHA 184	GMLS:GM	-56.4	-294	-1.2	106	106	1:3.70
CHA 154	GMLS	-52.9	-302	5.5	45	51	1:4.49
CHA 183	COW	-63.5	-282	-9.0	14	14	1:3.98
CHA 162	COW	-70.1	-312	-8.3	49	50	1:4.13
CHA 160	SHEEP	-64.1	-322	-8.5	81	95	1:4.74
CHA 181	DLS:BH6	-55.9	-263	-13.8	59	64	1:4.33
CHA 180	DLS:BH7	-53.7	-293	1.7	100	106	1:4.26
CHA 179	SEW. DIG.	-51.6	-265	2.8	93	97	1:4.22
CHA 178	MILL.	-54.9	-207	N.C.	78	81	1:4.14
CHA 148	ECLS	-64.4	-299	8.4	76	83	1:4.35
CHA 146	ECBH2	-61.6	-258	-14.7	39	43	1:4.40

Line N°	Sample name	$\delta^{13}C(CH_4)$	$\delta D(CH_4)$	$\delta^{13}C(CO_2)$	%yield carbon	%yield hydrogen	C:H ratio
CHA 145	GOLS4	-60.2	-311	3.6	56	60	1:4.28
CHA 143	GOLS:B8	-58.6	-284	1.2	34	31	1:3.65
CHA 142	GOLS:CF	-58.1	-328	1.9	85	92	1:4.28
CHA 141	GOLS	-57.4	-318	2.7	89	95	1:4.28
CHA 135	SLSBH21	-46.8	-251	-19.5	8	9	1:4.48
CHA 134	SLSS4	-44.4	-299	10.4	51	51	1:3.84
CHA 132	SLSBH28	-58.3	-296	18.1	125	137	1:4.36
CHA 130	SLSBH27	-52.0	-300	14.1	85	88	1:4.17
CHA 128	SLSBH29	-53.7	-294	19.0	66	67	1:4.05

N.C. not collected

N.M. not measured

- not determinable

C:H ratio

1:4.26±0.2 (n-=65)

1:4.30±0.4 (n=68)

APPENDIX 2: Conversion of Gw/hrs to kt of CH_4

This appendix shows the conversion of Gw/hrs to kt of CH_4, used in Chapter 3 to calculate the United Kingdom atmospheric methane flux budget.

- During 1992 the amount of natural gas recovered in United Kingdom was measured as 597854 Gw/hrs.
- 1million therms = 29.3071 Gw/hrs.
- $\Rightarrow$ the number of therms produced in the United Kingdom was 20399.63.

- There are 1×10^6 therms in 100×10^6 cu.ft. CH_4.
- Thus gas production during 1992 in terms of cu.ft. $CH_4 = 20399.63 \times 100 \times 10^6$,
  $= 2.04 \times 10^{12}$ cu.ft. CH_4.

- 1 cu.ft. $CH_4 = 0.0283 m^3$, $\Rightarrow 2.04 \times 10^{12}$ cu.ft. $CH_4 = 5.77 \times 10^{10} m^3$ CH_4.
- $1 m^3$ contains 1000 litres of gas, $\Rightarrow$ the number of litres of gas in $5.77 \times 10^{10} m^3$ CH_4 is 5.77×10^{13} litres
- 1 mole of gas at S.T.P. = 22.4litres, $\Rightarrow$ the number of moles of CH_4 in 5.77×10^{13} litres is 2.58×10^{12} moles.
- 1 mole of CH_4 has an atomic weight of 16.0426g, $\Rightarrow$ the number of grammes of CH_4 in 2.58×10^{12} moles is 4.132402×10^{13}g.
- This can be converted to kt by dividing by 1×10^{9}, which gives total natural gas production in the United Kingdom during 1992 of 41324.02 kt.

- 3% of total production is considered to be lost, thus the volume of gas that fluxes to the atmosphere each year from natural gas production is 1241.11kt.

Appendix 3: Algebraic manipulation of equations from Stevens (1988), refer to Chapter 3, pp 51-52.

$$\frac{\Delta(C\delta)}{\Delta(t)} = S\bar{\delta}_{TF} - C\lambda\left[\delta_A + F(\alpha-1)\left(1+10^{-3}\delta_A\right)10^3\right] \qquad \text{Eq. 1}$$

and

$$\frac{\Delta(C)}{\Delta(t)} = S - C\lambda, \text{ therefore } S = \frac{\Delta(C)}{\Delta(t)} + C\lambda \qquad \text{Eqs. 2a \& 2b}$$

According to Stevens (1988), Equation 1 can be rearranged using Equation 2b to the following:

$$\bar{\delta}_{TF} = \frac{\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] + \dfrac{\Delta(\delta)}{\Delta(t)} + \delta C^{-1}\dfrac{\Delta(C)}{\Delta(t)}}{C^{-1}\left[\dfrac{\Delta(C)}{\Delta(t)}\right] + \lambda} \qquad \text{Eq. 3}$$

Equation 3 is derived as follows.

1. Use equation 2b to substitute for S in equation 1:

$$\frac{\Delta(C\delta)}{\Delta(t)} = \bar{\delta}_{TF}\left[\frac{\Delta(C)}{\Delta(t)} + C\lambda\right] - C\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] \qquad \text{Eq.4.}$$

2. Rearrange equation 4 to select for $\bar{\delta}_{TF}$:

$$\bar{\delta}_{TF} = \frac{C\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] + \dfrac{\Delta(C\delta)}{\Delta(t)}}{\dfrac{\Delta(C)}{\Delta(t)} + C\lambda} \qquad \text{Eq.5.}$$

3. Multiply all terms in the right hand side of Eq.5 by C^{-1}:

$$\bar{\delta}_{TF} = \frac{\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] + C^{-1}\dfrac{\Delta(C\delta)}{\Delta(t)}}{C^{-1}\left[\dfrac{\Delta(C)}{\Delta(t)}\right] + \lambda} \qquad \text{Eq.6.}$$

4.Using the product rule, $C^{-1}\dfrac{\Delta(C\delta)}{\Delta(t)}$ can be expanded to :

$$C^{-1}\frac{\Delta(C\delta)}{\Delta(t)} = C^{-1}C\frac{(\Delta\delta)}{(\Delta t)} + C^{-1}\delta\frac{(\Delta C)}{(\Delta t)} \qquad \text{Eq.7.}$$

which can be simplified to:

$$C^{-1}\frac{\Delta(C\delta)}{\Delta(t)} = \frac{(\Delta\delta)}{(\Delta t)} + C^{-1}\delta\frac{(\Delta C)}{(\Delta t)} \qquad \text{Eq.8.}$$

5. Substituting Eq.6 with Eq.8 gives Steven's original equation, Eq.3:

$$\bar{\delta}_{TF} = \frac{\lambda\left[\delta_A + F(\alpha-1)(1+10^{-3}\delta_A)10^3\right] + \dfrac{\Delta(\delta)}{\Delta(t)} + \delta C^{-1}\dfrac{\Delta(C)}{\Delta(t)}}{C^{-1}\left[\dfrac{\Delta(C)}{\Delta(t)}\right] + \lambda} \qquad \text{Eq. 3}$$

Appendix 4:

This appendix contains the data points generated for Figure 3.3 in Chapter 3: Changes in UK and global $\delta^{13}C(CH_4)_{TF}$ and $\delta^{13}C(CH_4)_A$ as a function of changing flux budgets.

Year	1	2	3	4	5	6
1992	-52.800	-48.122	-49.271	-58.500	-53.851	-54.997
1993	-52.900	-48.223	-49.371	-58.500	-53.851	-54.997
1994	-53.000	-48.323	-49.472	-58.600	-53.951	-55.097
1995	-53.000	-48.323	-49.472	-58.700	-54.052	-55.198
1996	-53.100	-48.424	-49.572	-58.800	-54.152	-55.298
1997	-53.200	-48.524	-49.673	-58.800	-54.152	-55.298
1998	-53.300	-48.625	-49.773	-58.900	-54.253	-55.399
1999	-53.300	-48.625	-49.773	-59.000	-54.353	-55.499
2000	-53.400	-48.725	-49.874	-59.000	-54.353	-55.499
2001	-53.500	-48.826	-49.974	-59.100	-54.454	-55.599
2002	-53.500	-48.826	-49.974	-59.200	-54.554	-55.700
2003	-53.600	-48.926	-50.075	-59.200	-54.554	-55.700
2004	-53.700	-49.027	-50.175	-59.300	-54.655	-55.800
2005	-53.800	-49.127	-50.275	-59.400	-54.755	-55.901
2006	-53.800	-49.127	-50.275	-59.400	-54.755	-55.901
2007	-53.900	-49.228	-50.376	-59.500	-54.856	-56.001
2008	-54.000	-49.328	-50.476	-59.600	-54.956	-56.102
2009	-54.100	-49.429	-50.577	-59.600	-54.956	-56.102
2010	-54.100	-49.429	-50.577	-59.700	-55.057	-56.202

1 UK $\delta^{13}C(CH_4)_{TF}$.

2 UK $\delta^{13}C(CH_4)_A$ for steady state conditions.

3 UK $\delta^{13}C(CH_4)_A$ for non-steady state conditions.

4 Global $\delta^{13}C(CH_4)_{TF}$.

5 Global $\delta^{13}C(CH_4)_A$ for steady state conditions.

6 Global $\delta^{13}C(CH_4)_A$ for non-steady state conditions.

APPENDIX 5:

This appendix contains a paper published in GREEN '93 (Geotechnics relating to the environment) conference proceedings.

Stable isotope analysis as a means of identifying the source of methane.

S. Waldron, A.J. Hall
Glasgow University, Glasgow, Scotland

A.E. Fallick
Scottish Universities Research & Reactor Centre, East Kilbride, Scotland

R.A. Gilmour, M. MacDonald.
University of Strathclyde, Glasgow, Scotland

ABSTRACT: The application of stable isotope analysis in the identification of methane is demonstrated with five case studies whose combined $\delta^{13}C$ and δD values ranged from -66 to -44‰ and -352 to -263‰ respectively. This is characteristic of CH_4 produced by bacteria and so suggested the source of the gas was landfill. In this paper the theoretical background behind stable isotope analysis will be explained, the cryogenic separation method and CH_4 analysis method will be outlined and the advantages of using stable isotope analysis and problems that may occur when interpreting the results will be discussed.

INTRODUCTION

At 6.30am on 24 March 1986 a methane gas explosion completely destroyed a bungalow in Loscoe Derbyshire, badly injuring the three occupants (Williams & Aitkenhead, 1991). Derbyshire County Council initiated a non-statutory inquiry to identify the source of the gas and its migration pathway, the outcome of which showed the gas to have migrated from a filled and capped landfill site, situated 70m from the site of the explosion.

As early as 1983, there was evidence of gas migration from the landfill site, which had not been in use for a year, into the residential area. A resident, experiencing problems, dug into his lawn to discover why it had died. A 0.5m deep hole exposed muddy water bubbling with gas, a percentage of the ebullitive flux composing of methane. Despite the absence of carbon monoxide, the occupier assumed that the flux was from underlying coal seams and contacted British Coal who installed a stand-pipe with flame trap to allow the soil gas to vent harmlessly to the atmosphere. Analysis of the gas by British Coal (although by what method is not stated in the relevant literature, Williams & Aitkenhead, 1991) suggest that the gas was not associated with coal seams, but rather had a composition similar to that produced from rotting material. Natural gas was supplied to the houses, but investigations several months later by East Midlands Gas Board suggested that the leaking gas was not mains gas.

In the case of Loscoe, if the full significance of the distressed vegetation and 'hot spots' had been realised when first observed some two years prior to the explosion, it is possible that the authorities may have been able to prevent continued gas seepage and so the explosion. Early sampling with appropriate analysis could have revealed quite clearly the origin of gas and a proper investigation subsequently undertaken to trace this source.

Where there is more than one potential source of origin a method is needed that can distinguish between the different sources. Stable isotopic analysis of methane is one such tool that can be used for this differentiation. In this paper, using case studies, this use of stable isotopes in identifying the source of unknown methane will be outlined. It can be definitive by itself but there are however occasions when this technique may need to be complemented by other forms of geochemical analyses. The drawbacks to the technique and a comparison of other techniques is also discussed.

PRINCIPLES OF STABLE ISOTOPE ANALYSIS

Hydrogen and carbon, elements intimately associated with the biosphere, hydrosphere and lithosphere, are amongst those especially susceptible to natural isotope fractionation i.e. the selective partitioning of one isotope into a compound. Approximately 98.89% of all carbon in nature is ^{12}C (carbon of mass number 12), and 1.11% of all carbon is ^{13}C. Hydrogen has two stable isotopes whose abundances are ^{1}H= 99.985% and ^{2}H = 0.015%. The ratio of these two isotopes for each element in natural materials varies slightly around these average values as a result of isotopic fractionation during physical, chemical and biological processes. However differences between materials in the range of several parts per thousand can be significant.

Isotope fractionation is a consequence of the fact that certain thermodynamic properties of molecules depend on the masses of the atoms of which they are composed. Bonds formed by the lighter of two isotopes are weaker and therefore more easily broken, making the molecule with the lighter isotope more reactive than a similar molecule containing the heavier isotope. In methane formation, bacterially controlled fractionation processes such as bacteriogenic production from landfill waste or from fermentation in an animal's rumen, result in methane richer in light isotopes than methane formed from an organic substrate as a more direct result of elevated temperatures and pressures, such as occurs in the formation of fossil fuels. Different sources therefore have different isotopic signatures so measurements of the ratios of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ in the methane molecule allow the characterisation of sources.

A relative difference function, the δ value, is used for reporting stable isotope abundances and variations and is the quantity actually measured on isotope ratio mass spectrometers. If the absolute ratios of the standards employed are known, the absolute ratio of any sample is readily calculated from its δ value. The δ value is defined as follows:

$$\delta_X = \left(\frac{R_X - R_{STANDARD}}{R_{STANDARD}} \right) 10^3 \qquad \text{Eq.1}$$

where, R is an atomic ratio and by convention is always written as the ratio of the heavy to light isotope. Rstandard is the corresponding ratio in a standard and in the case of methane Rx = $(^{2}H/^{1}H)x$ or $(^{13}C/^{12}C)x$. The reference standard for carbon is the Pee Dee Belemnite (PDB) (Craig, 1957), the standard for hydrogen is Standard Mean Oceanic Water (SMOW). The δ value, then, is the difference in isotopic ratio between a sample and a standard, expressed in parts per thousand, or per mil (‰). Negative values indicate that the sample is depleted by that amount relative to the standard, while positive values indicate the standard is enriched in that amount relative to the standard. Fig.1, a plot of $\delta^{13}C$ (‰) against δD (‰), illustrates the suggested boundaries for bacteriogenic methane and thermogenic methane. Further information about the theory behind stable isotope analysis can be gained from O' Neil in Valley et al., 1986.

EXPERIMENTAL TECHNIQUE

Gas samples were collected in one litre Tedlar™ gas sample bags using a vacuum method designed by Mr. L. Thomson of Strathclyde University. Unfortunately it was later discovered that such bags are not suitable for long term storage of methane samples due to diffusion of the gas through the Tedlar™ membrane. However in terms of reference to these results methane stored in a bag for fifteen days became 5.5‰ isotopically heavier in $\delta^{13}C$ and 23‰ in δD. Samples were analysed soon after collecting and for these particular sites such change in isotopic composition would make little difference to the origin of gas attributed for each site. Gas samples are now collected in evacuated glass bottles.

Gas samples are prepared for mass spectrometry at the Scottish Universities Research & Reactor Centre, East Kilbride in a specially constructed and dedicated vacuum line similar to that designed by Stevens of the Argonne National Laboratory, Illinois (Stevens & Rust, 1982).

Before analysis begins the diffusion pumped vacuum line is degassed while open to high vacuum by flaming with a gas torch. The sample gas is then introduced by a syringe from the sampling vessel via a septum into an evacuated area of known volume. This is connected to a fixed volume capacitance manometer so that the amount of gas introduced can be measured quantitatively.

Liquid nitrogen is then placed over the first trap. This will cryogenically remove any atmospheric carbon dioxide, water, hydrogen sulphide and higher weight hydrocarbons while allowing the methane

and other non-condensable gases (non-condensable at -196°C i.e. nitrogen, hydrogen, helium, argon, carbon monoxide) to pass through. The sample is then allowed to expand into the vacuum line passing through another liquid nitrogen trap as it expands. This is a safety precaution in the unlikely event that there are traces of condensable gases still present.

The methane is then combusted by passing through an electrically heated quartz furnace containing copper oxide at approximately 750°C. Oxygen produced at this temperature by the copper oxide is the oxidant, and carbon dioxide, water and oxides of nitrogen are formed. Carbon monoxide may also be present in the reaction by-products. With the exception of carbon monoxide all are frozen into a liquid nitrogen trap placed on the line after the furnace. Products forming as a result of incomplete oxidation if substantial in volume e.g. carbon monoxide, OH, nitrogen oxide can be converted by passing through a trap containing Schutze reagent (iodine pentoxide on silica wool), a powerful oxidising agent, and similarly collected at liquid nitrogen temperature. However Stevens (pers. comm.) has shown that if not done this has minimal influence on the isotopic value and so is probably unnecessary. With all the desired products of the oxidation of methane collected in a liquid nitrogen trap the line can then be opened to the vacuum pump and any undesired non-condensable gases pumped away.

After the oxidation of the methane sample to water and carbon dioxide the traps containing the gases are warmed to -78°C with an acetone-dry ice mixture, allowing the carbon dioxide and any oxides of nitrogen to sublimate. These then pass over a copper furnace at a temperature of 650°C. Nitrous oxides are unstable at this temperature and will become reduced to leave free nitrogen, whereas the carbon dioxide remains stable and can be trapped at liquid nitrogen temperature. The carbon dioxide can then be measured quantitatively and collected for analysis on the VG Sira 10 triple collector mass spectrometer.

Hydrogen is extracted from the gas samples as follows. The purified water is allowed to warm to room temperature and the line heated so that the water evaporates. It is then passed through uranium turnings at ~750°C. This reduces the water to hydrogen which is pumped by a Toepler pump into a chamber of known volume.

Once most of the hydrogen has been pumped away the trap and surrounding piping are flamed to release adsorbed water. Any water that passes through the furnace (usually only a fraction of a mM) is condensed into a second liquid nitrogen trap and then recycled through the uranium furnace to ensure quantitative conversion. The total yield of hydrogen is then measured with a fixed volume capacitance manometer. Using the Toepler the sample is transferred to an evacuated sample tube for transfer to the mass spectrometer.

The hydrogen is analysed using a VG Micromass 602B with a modified inlet system. This inlet system makes use of mercury pistons which enables very small samples of gas (at the present moment $<4\mu M$) to be introduced into the mass spectrometer. The $\delta^{13}C$ permil value for carbon dioxide is measured in a similar manner using a VG Sira 10 Mass Spectrometer. The condensable nature of carbon dioxide allows the use of an inbuilt cold finger to make the isotopic ratio of very small samples possible. As with hydrogen the reference gas is calibrated by running against standards.

At present analysis are quoted to an accuracy for $\delta^{13}C$ of ±2‰, for δD of ±5‰.

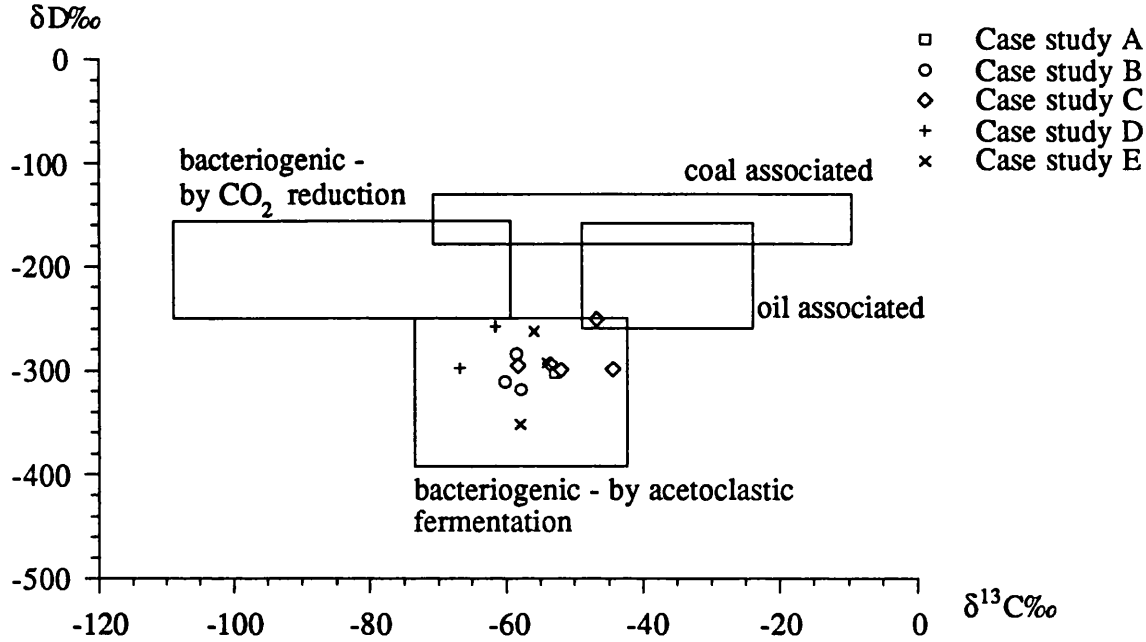
CASE STUDIES

The isotopic composition of methane measured from each case study is given in Table 1 while their composition in relation to other sources of methane can be seen from Figure 1.

Case A : This is probably the simplest of the case studies. The site was documented as containing 'inert' industrial and commercial waste since opening in 1981, but has periodically been giving methane concentrations of 30%. It is situated in Carboniferous age Westphalian Middle and Lower Coal Measures, with the cyclic sequence of mudstones, siltstones, sandstones, coals and seatclays overlain by thin drift deposits of pebbly silts and clays. From Fig. 1 (symbol A), it can be seen that the carbon and hydrogen isotope ratios indicate that the methane that is present has the signature characteristic of methane produced from landfill. Further investigation revealed that in one area of the site where methane concentrations in excess of 50% have been recorded, domestic waste was dumped during the first year of the site being operational.

Table 1: Analysis results
* signifies data from sample collected outwith landfill site.

Figure 1. Plot outlining boundaries of δ^{13} C‰ and δD‰ in naturally occurring methanes. Adapted from Schoell, 1988.



Case study	δ^{13}C ‰	δD ‰
A	-53	-302
B	-58	-318
	-60	-310
	*-59	*-284
C	-54	-294
	-52	-300
	-58	-296
	-44	-299

Case study	δ^{13}C ‰	δD ‰
C	*-47	*-251
D	-62	-258
	-66	-298
E	-58	-352
	*-54	*-293
	*-56	*-263
Mains gas	-45	-197

Case B : This landfill site utilises a disused sand and gravel quarry. Infill started in 1952, and the bottom is neither graded nor lined. The waste, mainly loose with a small percentage baled is composed of 90% industrial, 7% commercial /household and 3% from demolition. 15,000 tonnes is estimated to be received weekly. A cell system is employed and the intermediate cover material is discarded sand/silt from a neighbouring quarry.

Site B is situated adjacent to a residential area, with the nearest house being only a distance of 15m from the site boundary. Despite having boreholes within the site to vent the gas build-up, boreholes placed adjacent to the edge of a completed and capped part of the site were found to beventing methane up to concentrations of 41%. The proximity of the houses and uncertainty to the extent and pathway of methane migration meant that an analysis technique was needed that could help in defining the source of the gas as quickly as possible.

Samples were taken from within the site and from the external borehole for comparison. The results are plotted on Fig.1 (symbol B). The composition of the gas found outwith the site is virtually identical to the gas found within the site. It can therefore be assumed that the landfill site is indeed the source for the gas found in the external boreholes and remedial action be taken identify the extent of the migration pathway and/or curtail the leakage.

Case C : This landfill site utilises a disused opencast coal mine. Infill started in 1984 and the bottom is lined with high density polyethylene. The waste is baled with to high density capacity of 1.15 tonnes/m^3 and in composition is 45%

household waste, 13% commercial waste, 7% street litter, 29% subsoil and 6% demolition waste. A cell system is employed and the intermediate cover material is subsoil. The site is drained with the runoff pumped to a treatment plant for purification. There are plans underway to utilise the gas to power this purification plant, but at present the gas is flared when the pressure builds up.

However, part of this site is a result of unsupervised dumping pre-1986 and therefore is not so well planned. At the moment methane vents at concentrations of up to 70%. As with case B, monitoring boreholes positioned just outside the site, next to the edge of a river and by a public walkway, were found to have concentrations of up to 29%. Stable isotope analysis of methane was employed in this case to ensure that the source of the methane was from the landfill site and not from thermogenic methane associated with the coal measures from the old colliery. Samples from gas vented from boreholes within the old site and from those adjacent to the periphery of the site were analysed and the results are shown on Fig1.

Methane found outwith the site has an isotopic signature attributable to production by bacterial fermentation (i.e. normal landfill gas) and is similar to the signature of the methane produced within the site. The sample outwith the site is isotopically heavier, but this may be attributed to partial oxidation of the gas by bacteria during migration, a phenomenon which is documented as leaving the resultant methane isotopically heavier (Coleman, Risatti & Schoell, 1981). The samples analysed however are quite distinct from the isotopic signature of methane associate with coal and so the coal measures can be disregarded as the likely source of methane found in the monitoring boreholes.

Case D : Explorative boreholes put down during drilling to estimate the extent of a landfill site believed to be the source of migratory methane intersected underground mine workings. As with case C, stable isotope analysis of the gas was undertaken to identify the source of the gas, eliminating either the coal measures or the landfill site. From Fig.1 it can be seen that the isotopic composition of the sample collected is representative of methane produced by bacteria by fermentation, such as the process that happens in a landfill site. The underlying coal measures can therefore be disregarded as the source of methane.

Case E : This landfill partially utilises the space left by quarrying of a quartz-dolerite sill for roadstone but also extends into coal measures. There is, or was, a disused mine entrance within the site (it may now be covered) and in coal seams can still be seen to outcrop in one cell. In fact, during sampling, people have been seen pushing coal filled wheelbarrows from the site.

Three boreholes, placed just outside the edge of this landfill site, have consistently shown methane to be present up to concentrations of 70%, with an isotopic signature representative of bacterially produced methane. The positioning of these boreholes is of interest for, from the geological map, they appear to be situated directly up dip of the outcropping coal seams. No methane has been found present at any of the other boreholes outwith the site which may imply that discontinuity surfaces e.g. bedding planes, within the sedimentary sequence are acting as a conduits along which methane, if produced by the coal, may migrate.

Isotope analyses again suggests that the source of the methane is landfill, although the isotopically heavier values of the gas in the boreholes suggests that during migration it has undergone bacterial oxidation.

DISCUSSION :

Schoell, (1988) has compiled data from recorded variations in the isotopic composition of bacteriogenic methane to outline the zones shown on Fig. 1. which represent methane produced by acetoclastic methanogenesis or by the reduction of carbon dioxide. $\delta^{13}C$ for the former ranges from -73 to -42‰, δD ranges from -390 to -250‰, while for methane produced by the reduction of carbon dioxide $\delta^{13}C$ ranges from -110 to -60‰ and δD from -250 to -150‰.

Unfortunately there is very little data available that combines both typical $\delta^{13}C$ and δD values for methane associated with oil and gas, however $\delta^{13}C$ composition alone is well documented. Thermogenic methane (including deposits associated with crude oil) shows $\delta^{13}C$ ranges from -50 to -25‰ (Stevens, 1988). δD typically has a range from -260 to -150‰ (Schoell, 1980). $\delta^{13}C$ values of methane absorbed in coals show a wide range, -12 to -71‰ and are independent of the degree of coalification of the coal from which the gas was released (Deines, 1980). The lower values of this range overlap with the

attributed ranges for methane produced by bacteria, however less than 10% of all coal methane samples measured fall in the range -55 to -70‰ while 50% of measured $\delta^{13}C$ for coal methane samples fall between -25 and -40‰. Furthermore δD of methane associated with coal is isotopically heavier than δD of bacteriogenic samples with typical values ranging from -180 to -130‰ and is therefore quite isotopically distinct.

In all the aforementioned case studies the isotopic signature of each sample analysed was distinctive of methane of bacteriogenic origin and therefore attributed to the landfill. These results were used to eliminate the sources of methane that would be likely to give another signature, and combined with further information about each site to pin-point the likeliest source of gas, which for each of these cases this was the landfill. Although some samples did plot in the area of overlap between bacterially produced methane and thermogenic produced methane associated with crude oil, the proximity of the gas sampled to landfill and the characteristic bacteriogenic signature suggested that the origin of the gas was from landfill.

Stable isotope analysis of methane can give a signature which is indicative of the process by which the methane formed, i.e. thermogenic or biogenic, however there is one drawback in that within each range there are several sources which may give similar signatures. Methane produced by a ruminant, from a sewerage digester or from anaerobic, organic rich sediment will all have an isotopic signature that is similar to methane produced in a landfill from decomposing rubbish. Likewise, the two main thermogenic sources, coal gas and natural gas, will have a similar signature.

Bacteriogenic methane that has undergone partial oxidation by bacteria during migration can have a resultant isotopic signature that is similar to methane of thermogenic origin, as the bacteria responsible for the oxidation preferentially consume the lighter isotopes leaving the gas isotopically heavier (Coleman et al, 1980). The resultant isotopic composition of a landfill gas that may has been fractionated as a result of oxidation is shown on Fig. 1, Case C. However research carried out by King et al, 1989 which is consistent with Coleman et al., 1980, show that the change in δD value of methane which has been partially oxidised by bacteria is 8-14 times greater than the change in $\delta^{13}C$ value. It should therefore be possible to recognise methane which has been subjected to partial oxidation by bacteria during migration by using the combined carbon and hydrogen isotopic analysis technique, for although the $\delta^{13}C$ value for the gas may be consistent with a thermogenic signature, the δD value may be outside the field normally accepted for thermogenic gas. If bacterial oxidation of landfill gas is suspected then characterisation of samples from mains supply gas or coal related methane in the specific area for comparison would aid identification of the source of the unknown methane.

Another problem that may lead to confusion in identifying the source of methane is where the mixing of two isotopically distinct gases occurs e.g. the mixing of a landfill gas and gas from a coal seam. For example, if the landfill gas started with a composition of $\delta^{13}C$ = -65‰, δD = -300‰, and the coal-derived methane started with an isotopic composition of $\delta^{13}C$ = -29‰, δD = -150‰, with both gases present in equal volumes, then by simple mass balance calculations the resultant isotopic composition of the methane would be $\delta^{13}C$ = -37‰, δD = -225‰. It can be noted that this resulting composition may be similar to the isotopic composition of methane that has been fractionated as a result of oxidation during migration.

In situations such as these, most other conventional analysis techniques such as determination of the chemical composition of the problem gas and age dating by ^{14}C determinations (refer to Williams & Aitkenhead for a further explanation) may also give rise to ambiguous results. If used in conjunction with stable isotope analysis they should be capable of defining the source. In comparison to these techniques, particularly age dating by ^{14}C determinations, stable isotopic analysis of methane is a quick and inexpensive way of determining the likely source of an unknown methane sample. If there is confusion from the initial analysis the results will serve to eliminate certain sources and will be useful in suggesting which alternative, complementary technique should be used. Furthermore, the volume of gas that is required for stable isotope analysis (1cc of 100% CH_4, or the proportionally appropriate volume of a diluted sample e.g. 10cc at 10% CH_4 concentration) is much smaller than that required for ^{14}C age dating. There may be instances where the volume of gas available is not sufficient for ^{14}C determinations, whereas in the majority of cases, unless the sample size is very small and at very low, in the order of L.E.L. (lower explosive limit), concentrations, there should be sufficient

gas for stable isotope analysis.

CONCLUSIONS:

The use of stable isotope analysis of methane to identify the source of unknown methane is viable because different processes of methane generation give rise to large differences in isotopic signatures. It is a relatively quick and inexpensive technique, particularly useful because it utilises small volumes of gas. Alone, it can often give a definitive answer but in more complex environmental / geological situations can be complemented by other available geochemical analysis techniques or information to provide the solution.

REFERENCES:

Coleman, D.D., Risatti, J.B. and Schoell, M. 1981. Fractionation of carbon and hydrogen isotopes by methane-oxidising bacteria. *Geochim. Cosmochim. Acta* 45: 1033-1037.

Craig, H. (1957). Isotopic standards for mass-spectrometric analysis for carbon dioxide. *Geochim. Cosmochim. Acta* 12: 133-149.

Craig, H. (1961). Standard for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science* 133: 1833-1834.

Deines, P. (1980). The isotopic composition of reduced organic matter. *Handbook of Environmental Geochemistry*, Vol 1, The Terrestrial Environment, A. Published by Elsevier.

King, S.L., Quay, P.D. and Lansdown, J.M. (1989). The $^{13}C/^{12}C$ kinetic isotope effect for soil oxidation of methane at ambient atmospheric concentrations. *Journ. Geophys. Res.* 94: D15, 18273-18277

O'Neil, J.R. (1986) Theoretical and experimental aspects of isotope fractionation, in Stable Isotopes in High Temperature Geological Processes, *Reviews in Mineralogy*, 16: 1-40. Published by BookCrafters Inc.

Schoell, M. (1980). The hydrogen and carbon isotopic composition of methane from natural gases of various origins. *Geochimica et Cosmochimica Acta*, 44, 649-661.

Stevens, C.M. and Rust, F.E. (1982). The carbon isotopic composition of atmospheric methane. *Journ. Geophys. Res.* 87, C7: 4879-4882.

Williams, G.M. & Aitkenhead, N. (1991). Lessons from Loscoe: the uncontrolled migration of landfill gas. *Quarterly Journal of Engineering Geology*, 24, 191-207.

REFERENCES:

AFRC (1988) Basic study of landfill microbiology and biochemistry, Agricultural and food research council, ETSU B1159.

Aitchison E. (1993) Methane emissions from UK landfill sites, in *Methane Emissions*, The Watt Committee on Energy, 92-98, London.

Alperin M.J. and Reeburgh W.S. (1985) Inhibition experiments on anaerobic methane oxidation (1985) *Appl. Env. Microb.*, **50**, 4, 940-945.

Anastasi C. and Simpson V.J. (1993) Future methane emissions from animals. *J. Geophys. Res.*, **98**, D4, 7181-7186.

Armstrong W. (1964) Oxygen diffusion from the roots of some British bog plants. *Nature, Lond.* **204**, 801-2.

Balabane M., Galimov E., Hermann M. and Letolle R. (1987) Hydrogen and carbon isotope fractionation during experimental production of bacterial methane. *Org. Geochem.*, **11**, 115-119.

Balba M.T.M. (1978) Part 1-The methanogenic fermentation of aromatic substrates; Part 2-The origin of hexydrohippurate in the urine of herbivores. *Ph.D. Thesis*, University Of Wales.

Blair N.E., Leu A., Munoz E., Olsen J., Kwong E. and DesMarais D. (1985) Carbon isotopic fractionation in heterotrophic microbial metabolism. *App. Env. Microb.*, **50** : 4, 996-1001.

Blair, N.E. and Carter W.D. Jnr. (1992) The carbon isotope biogeochemistry of acetate from a methanogenic sediment. *Geochim. Cosmochim. Acta*, **456**, 1247-1258.

Boutton T.W. (1991) Stable carbon isotope ratios of natural materials : II. Atmospheric, terrestrial, marine and freshwater environments. In *Carbon Isotope Techniques* (ed. D. Coleman and B. Fry), Chap. 11, pp173-185. Academic Press.

Braun M., Mayer F. and Gottschalk G. (1981) *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Arch. Microbiol.*, **128**, 288-293.

Bryant M.P. (1976) The microbiology of anaerobic degradation and methanogenesis with special reference to sewage. *Seminar on Microbial Energy Conservation.* (Edited by Schlegel H.G.), Gottingen, Coltze K.G.

Buchanan D.L. and Corcoran B.J. (1957) Sealed tube combustions for the determination of carbon-14 and total carbon. *Anal. Chem.*, **31**, 1635-1638.

Burke Jr. R.A., Martens C.S. and Sackett W.M. (1988) Seasonal variation of D/H and $^{13}C/^{12}C$ ratios of biogenic methane in surface sediments of Cape Lookout Bight, U.S.A. *Nature*, **332**, 829-831.

Buswell A.M. and Sollo, F.W. (1948) The mechanism of methane formation. *J. Am.. Chem.. Soc.*, **70**, 1778-1780.

Cantrell C.A., Shetter R.E., McDaniel A.H., Galvert J.G., Davidson J.A., Lowe D.C., Tyler

S.C., Cicerone R.J. and Greenberg J.P. (1990) Carbon kinetic isotope effect in the oxidation of methane by the hydroxyl radical. *J. Geophys. Res.*, **95**, D13, 22,455-22,462.

Cicerone R.J. (1988) How has the atmospheric concentration of CO changed? In *The Changing Atmosphere*, Ed. Rowland F.S. and Isaken I.S.A., pp46-91, John Wiley, New York.

Cicerone R.J. and Oremland R.S. (1988) Biogeochemical aspects of atmospheric methane. *Glob. Biogeochem. Cycles*, **2**, 4, 299-327.

Claricoates J. (1990) Gas production during peat decay. Ph.D. Dissertation, University of London.

Clymo R.S. (1987) The ecology of peatlands. *Sci. Prog., Oxf.* , **71**, 593-614.

Coleman D.D., Risatti J.B. and Schoell M. (1981) Fractionation of carbon and hydrogen isotopes by methane oxidising bacteria. *Geochim. Cosmochim. Acta*, **45**, 1033-1037.

Cornford C. (1993) Hydrocarbon flux inefficiencies in the Central Graben of the North Sea. In *Geofluids '93*, Ed. Parnell J., Ruffel A.H. and Moles N.R., pp 76-81.

Craig H. (1957) Isotopic standards for mass spectrometric analysis for carbon dioxide. *Geochim. Cosmochim. Acta.*, **12**, 133-149.

Craig H. (1961) Standard for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science*, **133**, 1833-1834.

Crutzen P.J., Aselmann I. and Seiler W. (1986) Methane production by domesticated animals, wild ruminants, other herbivorous fauna and humans, *Tellus Ser. B.*, **38**, 271-284.

Daniels L., Fulton G., Spencer R.W. and Orme-Johnson, W.H. (1980) Origin of hydrogen in methane produced by *Methanobacterium thermoautotrophicum. J.Bact.*, **141**, 694-698.

Davidson J.A., Cantrell C.A., Tyler S.C., Shetter R.E., Cicerone R.J. and Calvert J.G. Carbon kinetic isotope effect in the reaction of CH_4 with HO *J. Geophys. Res.*, **92**, 2195.

Davis J.B. and Yarborough H.E. (1966) Anaerobic oxidation of hydrocarbons by *Desulfovibrio desulfuricans. Chem. Geol.*, **1**, 137-144.

Deines P. (1980) The isotopic composition of reduced organic carbon. In *Handbook of Enivronmental Isotope Geochemistry, Vol. 1, The Terrestrial Environment, A*, (Ed. by Fritz P. and Fontes J.Ch.), Ch.9, Elsevier, Amsterdam, pp 380-406.

Deuser W.G. and Degens E.T. (1967) Carbon isotope fractionation in the system CO_2 (gas)-CO_2 (aqueous)- HCO_3-(aqueous).*Nature*, **215**, 1033-1035.

Dinel H., Mathur S.P., Brown A. and Levesque M. (1988) A field study of the effect of depth on methane production in peatlands waters: equipment and preliminary results. *Journ. Ecol.*, **76**, 1083-1091.

Dlugokencky E.J., Masaire K.A., Lang P.M., Tans P.P., Steele L.P. and Nisbet E.G. (1994) A dramatic decrease in the growth rate of atmospheric methane in the northern hemisphere during 1992. *Geophys. Res. Lett.*, **21**, 1, 45-48.

Donner L. and Ramanathan V. (1980) Methane and nitrous oxide: Their effects on the terrestrial climate, *J. Atmos. Sci.*, **37**, 119-124.

Dutton E.G. and Christy J.R. (1992) Solar radiation forcing at selected locations and evidence for global lower tropospheric cooling following eruptions of El Chicon and Pinatubo. *Geophys. Res. Lett.*, **19**, 2313-2316.

Ehhalt D.H. and Schmidt A. (1978) Sources and sinks of atmospheric methane. *Pure. Appl. Geophys.*, **116**, 452-464.

Ehleringer J.R. (1991) $^{13}C/^{12}C$ fractionation and its utility in terrestrial plant studies. In *Carbon Isotope Techniques* (ed. D. Coleman and B. Fry), Chap. 12, pp187-200. Academic Press.

ENDS (1993) Uncertainty over landfills crucial to methane emission plan. Briefing in *ENDS Report*, **220**, 23-24.

Engel M.H. and Maynard R.J. (1989) Preparation of organic matter for stable carbon isotope analysis by sealed tube combustion : A cautionary note. *Anal. Chem.*, **61**, 1996-1998.

Epstein S., Yapp C.J. and Hall J.H. (1976) The determination of D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth. Plan. Sci. Lett.*, **30**, 241-251.

Frazer J.W. and Crawford R. (1963) Modifications in the simultaneous determination of carbon, hydrogen and nitrogen. *Mikrochim. Acta*, **1963/3**, 561-566.

Friedman I. and Smith R.L. (1958) The deuterium content of water in some volcanic glasses. *Geochim. Cosmochim. Acta.*, **15**, 218-228.

Fuller J. (1993) The oil industry today. *British Association lectures.*

Fung I., John J., Lerner J., Matthews E., Prather M., Steele L.P. and Fraser P.J. (1991) Three dimensional model synthesis of the global methane cycle. *J. Geophys. Res.*, **96**, 13,033-13,065.

Galimov E.M. (1985) Experimental evidence of thermodynamically ordered distribution of isotopes in biological systems. In *The Biological Fractionation of Isotopes* (Edited by Meinschein W.G.), Chap4, pp. 94-124.

Gallagher M. (1994) On the trail of the Scottish Plume: Methane measurements in Northern Scotland. *TIGER Eye*, **7**, 2-3.

Games L.M., Hayes J.M. and Gunsalus R. (1978) Methane producing bacteria : natural fractionations of the stable carbon isotopes. *Geochim. Cosmochim. Acta*, **42**, 1295-1297.

Gelwicks J.T., Risatti J.B. and Hayes J.M., (1989) Carbon isotope effects associated with autotrophic acetogenesis. *Org. Geochem.*, **14**, 441-446.

Gleason J.F., Bhartia P.K., Hermann J.R., McPeters R., Newman P., Stolarski R.S., Flynn L., Labow G., Larko D., Seftor C., Wellermeyer C., Kmohyr W.D., Miller A.J. and Planet W. (1993) Record low global ozone in 1992. *Science*, **260**, 523-526.

Gordon S. and Mulac W. (1975) Proceedings of the symposium on chemical kinetics, Data for the upper and lower atmosphere . *Int. J. of Chem. Kinetics*, p289.

Grace J.D. and Hart G.F. (1986) Giant gas fields of northern West Siberia. *Amer. Assoc. Petrol. Geol. Bull.*, **70**, 830-852.

Hamilton W.A. (1988) Energy transduction in anaerobic bacteria. In *Energy transduction in anaerobic bacteria* (Edited by Anthony C.), Academic Press, London.

Hameed S. and Cess R.D. (1983) Impact of global warming on biospheric sources of methane and its climatic consequences. *Tellus*, **35B**, 1-7.

Harris N.S. (1989) Modern vacuum practice. *McGraw-Hill Book Company.*

HMSO (1993) Digest of United Kingdom Energy Statistics. Published by *The Department of Trade and Industry.*

Hitchman S.P., Darling W.G. and Williams G.M. (1989) Stable isotope ratios of methane containing gases in the United Kingdom. *British Geological Survey Technical Report* WE/89/30.

Hobson P.N., Bonsefield S. and Summers R. (1981) The microbiology and biochemistry of anaerobic digestion. In *Methane Production From Agricultural and Domestic Wastes, Energy from Waste Series.* Chap. 3, pp. 10-51. App. Sci. Pub. Ltd.

Hovland M. and Judd A.G. (1988) Seabed pockmarks and seepages. Impact on geology, biology and the marine environment. Graham and Trottman London.

Hovland M. and Judd A.G. (1992) The global production of methane from shallow marine sources. In Methane in Marine Sediments. *Continental Shelf Research*, **12**, 10, 1231-1238.

Hovland M., Judd A.G. and Burke R.A. Jnr. (1993) The global flux of methane from shallow marine sediments, *Chemosphere*: Atmospheric methane: Sources, sinks and role in global change, **26**, 1-4, 559-578.

Ingram H.A.P. (1983) Hydrology. In *Ecosystems of the world* (ed. A.J.P. GORE), Vol. 4 (Mires: swamp, bog, fen and moor), pp 67-158. Elsevier, Amsterdam.

Ingram H.A.P. (1987) Ecohydrology of Scottish Peatlands. *Trans. Roy. Soc. Edin. : Earth Sci.* **78**, 287-296.

Ingram H.A.P. and Bragg O.M. (1984) The diplotelmic mire: some hydrological consequences reviewed. *Proc. 7th Int. Peat Congr.*, **1**, 220-234.

James C.G. (1990) Natural gas and the greenhouse. *Nature*, **347**, p720.

Jenden P.D. and Kaplan I.R. (1986) Comparison of microbial gases from the Middle American Trench and Scripps Submarine Canyon : Implications for the origin of natural gas. *Appl. Geochem.*, **1**, 631-646.

Jenkin G.R.T. (1988) Stable isotope studies in the Caledonides of S.W. Connemara, Ireland. Ph.D. Dissertation, University of Glasgow.

Jones C.W. (1988) Membrane associated energy conservation in bacteria : a general introduction. In *Bacterial Energy Transduction* (Edited by Anthony C.), Chap.1, pp. 1-82. Academic Press.

Khalil M.A.K. and Rasmussen R.A. (1982) Secular trends of atmospheric methane (CH_4). *Chemosphere*, **11**, 877-883.

Khalil M.A.K. and Rasmussen R.A. (1983) Sources, sinks and seasonal cycles of atmospheric methane. *J. Geophy. Res.*, **88**, 5131-5144.

Khalil M.A.K. and Rasmussen R.A. (1990) Atmospheric methane: Recent global trends. *Environ. Sci. Technol.*, **24**, 549-553.

Khalil M.A.K. and Rasmussen R.A. (1993) Decreasing trends of methane: unpredictability of future concentrations. *Chemosphere*, **26**, 803-814.

Khalil M.A.K., Rasmussen R.A. and Moraes F. (1993) Atmospheric methane at Cape Meares: Analysis of a high-resolution data base and its environmental implications. *J. Geophy. Res.*, **86**, D1, 9826-9832.

King G.M., Klug J.M. and Lovley D.R., (1983) Metabolism of acetate, methanol and methylated amines in intertidal sediments of Lowes Cove, Maine. *Appl. Environ. Microbiol.*, **45**, 1848.

Krzycki J.A., Kenealy W.R., Deniro M.J. and Zeikus J.G. (1987) Stable carbon isotope fractionation by *Methanosarcina barkeri* during methanogenesis from acetate, methanol of carbon dioxide-hydrogen. *App. Env. Microb.*, **53**, 10, 2597-2599.

Lansdown J.M., Quay P.D. and King S.L. (1992) CH_4 production via CO_2 reduction: a source of ^{13}C depleted CH_4. *Geochim. Cosmochim. Acta*, **56**, 3493-3503.

Large P.L. (1988) *Methylotrophy and Methanogenesis* (Edited by Cole, J.A., Knowles C.J. and Schlessinger D.) John Wiley and Sons, New York.

Lidstrom M.E. and Somers L. (1984) Seasonal study of methane oxidation in Lake Washington. *Appl. Env. Microbiol.* **47**, 1255-1260.

M^{c}Inerney M.J. and Bryant M.P. (1981) Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effects of H_2 on acetate degradation. *Appl. Env. Microbiol.*, **41**, 346-354.

M^{c}Kinney C.R., M^{c}Crea J.M., Epstein S., Allen H.A. and Urey H.C. (1950) Improvement in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Rev. Sci. Instrum.*, **21**, 724-730.

Maclean D. (1993) *House of commons written answers*, Cols. 366-7, 11th May.

Manahan S.E. Photochemical Smog in *Environmental Chemistry*. Lewis Publishers.

Marino M.A. (1974) Growth and decay of groundwater mounds induced by percolation. *J. Hydrol.*, Amst., **22**, 295-301.

Mattson M.D. and Likens G. (1990) Air pressure and methane fluxes. *Nature*, **347**, 718-719.

Milk Marketing Board (1993) United Kingdom Dairy Fact and Figures, publ. by the Federation of United Kingdom Milk Marketing, Milk Marketing Board Statistics Dept.

Ministry of Agriculture, Fisheries and Food (1994) Agricultural and horticultural census: 1 June 1993, United Kingdom and England. Final results. *MAFF Statistics Notice 21/94.*

Monson K.D. and Hayes J.M. (1982) Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochim. Cosmochim. Acta*, **46**, 139-149.

Moore P.D. and Bellamy D.J. (1974) Peatlands. *London Elek Science.*

Morrison R.T. and Boyd R.N. (1987) Structure of cellulose. In *Organic Chemistry*, Allyn and Bacon, Inc, U.S.A.

Nakai M., Yoshida Y. and Ando N. (1974) Isotopic studies on oil and natural gas fields in Japan. *Journ. Earth Sci.*, **8**, 174-180.

NERC (1993) Terrestrial Initiative in Global Environmental Research : *Programme Report & Science Highlights 1990-1992.* NERC Publishing Services.

NATO Advanced Study Institute (1992) Quantitative diagenesis : recent developments and applications to reservoir geology, University of reading, 6-19th Sept.

O'Neil J.R. (1986) Appendix : Terminology and standards in Reviews in Mineralogy : *Stable Isotopes in High Temperature Geological Processes.* (Ed. by Valley J.W., Taylor Jnr. H.P. and O'Neil J.R.). Publ. Min. Soc. Am.

Pine M.J. and Barker H.A. (1956) Studies on methane fermentation XII. The pathway of hydrogen in the acetate fermentation. *Journ. Bacteriol.*, **71**, 644-648.

Pirt J.S. (1975) *Principles of microbe and cell cultivation*, Chap.4, pp. 22-28. Blackwell Scientific Publications, Oxford.

Postgate J.R. (1984) *The sulphate reducing bacteria*, 2nd ed. Cambridge University Press, Cambridge.

Prinn R., Cunnold D., Simmond P., Alyea F., Boldi R., Crawford A., Fraser P., Gutzler D., Hartley D., Rosen R. and Rasmussen R. (1992) Global average concentration and trend for hydroxyl radicals deduced from ALE/GAGE trichloroethane (methyl chloroform) data for 1978-1990. *J. Geophy. Res.*, **97**, 2445-2461.

Ramanathan V., Cicerone R.J., Singh H.B. and Kiehl J.T. (1985) Trace gas trends and their potential role in climate change, *J. Geophys. Res.*, **90**, 5547-5566.

Rasmussen R.A. and Khalil M.A.K. (1981) Atmospheric methane: Trends and seasonal cycles. *J. Geophy. Res.*, **98**, D8, 14,753-14,770.

Rosenfield W.D. and Silverman S.R. (1959) Carbon isotope fractionation in bacterial production of methane. *Science*, **130**, 1658-1659.

Rudd J.W.M., Furutani A., Flett R.J. and Hamilton R.D. (1976) Factors controlling methane oxidation in shield lakes: the role of nitrogen fixation and oxygen concentration. *Limnol. Ocean.*, **21**, 357-364.

Rudolph J. (1994) Anomalous methane. *Nature*, **368**, 19-20.

Rust F.E. and Stevens C.M. (1980) *Int. J. of Chem. Kinetics*, **12**, 371.

Schimel D.S., Andreae M.O., Fowler D., Galbally I.E., Harriss R.C., Ojima D., Rodhe H., Rosswall T., Svensson B.H. and Zavarzin G.A. (1989) Priorities for an international research programme on trace gas exchange. In Dahlem Workshop Reports, Life Sciences Research Report 47, *Exchange of trace gases between terrestrial ecosystems and the atmosphere.* (ed. M.O. Andreae and D.S. Schimel), Chap. 21, pp321-331.

Schmidell W., Craveiro A., Peres C.S., Hirata Y.S. and Varella R.F. (1986) Anaerobic digestion of solid municipal wastes, *Water Sci. Technol.*, **18** (12), 163.

Schoell M. (1980) The hydrogen and carbon isotopic composition of methane from natural gases of various origins. *Geochim. Cosmochim. Acta*, **44**, 649-661.

Schoell M. (1988) Multiple origins of methane in the Earth. *Chem. Geol.*, **71**, 1-10.

Schoell M., Tietze K. and Schoberth, S.M. (1988) Origin of methane in Lake Kivu (East-Central Africa). *Chem. Geol.*, **71**, 257-265.

Scottish Natural Heritage (1988) Ellergower Moss site of special scientific interest citation. File reference 1755.

Scottish Natural Heritage (1993) The national peatland resource inventory: Lowland raised bogs in Great Britain.

Senior E. and Balba M.T.M. (1983) The microbiology and biochemistry of landfill biotechnology. *Sci. Conf.*

Senior E.(Ed.) and Kasali G.B., (1990) Landfill gas in *Microbiology of landfill sites*, Chap. 5., CRC Press.

Sheppard S.M.F. (1986) Characterisation and isotopic variation in natural waters, in Stable Isotopes in High Temperature Geological Processes. *Reviews in Mineralogy*, **16**, Chap. 6, 165-184.

Stadtman T.C. and Barker, H.A. (1949) Studies on methane fermentation XII. Tracer experiments on the mechanism of methane formation. *Arch. Biochem.*, **21**, 256-264.

Stevens C.M. (1988) Atmospheric methane. *Chem. Geol. (Isotope Geosciences)*, **71**, 11-21.

Stevens C.M. and Engelkemeir A. (1988) Stable carbon isotopic composition of methane from some natural and anthropogenic sources. *J. Geophys. Res.*, **93**, D1, 725-733.

Stevens C.M. and Rust F.E. (1982) The carbon isotope composition of atmospheric methane. *Journ. Geophys. Res.*, **87**, C7, 4879-4882.

Stevens C.M. and Wagner A.F. (1989) The role of isotope fractionation effects in atmospheric chemistry. *Z. Naturforsch* **44a**, 376-384.

Stryer L. (1981) Biochemistry. W.H. Freeman and Company, San Francisco.

Stuermer D.H., Peters K.E. and Kaplan I.R. (1978) Source indicators of humic substances and proto-kerogen. Stable isotope ratios, elemental compositions and electron spin resonance spectra. *Geochim. Cosmochim. Acta*, **42**, 989-997.

Sugimoto A. and Wada E. (1993) Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contributions of acetate and CO_2/H_2. *Geochim. Cosmochim. Acta*, **57**, 4015-4027.

Svensson B.H. (1984) Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate of hydrogen. *Appl. Env. Microbiol.*, **48**, 2, 389-394.

Tiger Eye (1992) UK Methane Budget, **4**, p7.

Tyler S.C. (1986) Stable carbon isotope ratios in atmospheric methane and some of its sources. *J. Geophys. Res.*, **91**, D12, 13,232-13238.

Vaghjiana G.L. and Ravishankara A.R. (1991) New measurement of the rate coefficient for the reaction of OH with methane, *Nature*, **350**, 406-409.

Valley J.W., Taylor H.P. Jnr. and O'Neil J.R. (1986) Stable isotopes in high temperature geological processes. **16**, *Reviews in Mineralogy*, publ. BookCrafters Inc.

Vogels G.D., Keltjens J.T. and Van Der Drift C. (1988) Biochemistry of methane production. In *Biology of anaerobic micro-organisms* (ed. Zehnder, A.J.B.), pp. 707-770, Wiley-Liss, New York, .

Wahlen M., Tanaka N., Henry R., Yoshimara T., Faorbanks R.G., Sheruesh A. and Broecker W.S. (1987) ^{13}C, D and ^{14}C in methane. *Eos, (Trans. Am. Geophys. Union)*, **68**, 1220 (abstract).

Waldron S., Hall A.J., Fallick A.E., Gilmour R.A. and Macdonald M. (1993) Stable isotope analysis as a means of identifying the source of methane. Waste Disposal By Landfill : Geotechnics related to the environment, **2**, *Proc. GREEN '93. Sci. Conf.* , pp. 209-215.

Wallis M.K. (1990) Leaky answer to greenhouse gas? *Nature*, **344**, 25-26.

Wallis M.K. (1993) Reassessing methane from UK landfills. *Waste disposal by landfill* : Green '93 Conference syposium proceedings.

Watson-Craik I.A. and Senior E. (1989) Effects of phenol wastewater co-disposal on the attenuation of the refuse leachate molecule hexanoic acid. *Lett. Appl. Microbiol.*, **9**, 227-232.

Whiticar M.J. (1989) A geochemical perspective of natural gas and atmospheric methane. *Org. Geochem.*, **16**, 531-547.

Whiticar M.J., Faber E. and Schoell M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation - Isotope evidence. *Geochim. Cosmochim. Acta*, **50**, 693-709.

Williams R.T. and Crawford R.L. (1984) Methane production in Minnesota peatlands. *Appl. Env. Microbiol.* , **47**, 1266-1271.

Wolin M.J. (1981) Fermentation in the rumen and the human large intestine, *Science*, **213**, 1463-1468.

Yavitt J.B., Lang G.E. and Downey D.M. (1988) Potential methane production and methane oxidation rates in peatland ecosystems of the Appalachian Mountains, Unites States. *Glob. Biogeochem. Cycl.*, **2**, 253-268.

Yavitt J.B., Downey D.M., Lancaster E. and Lang G.E. (1990) Methane consumption in decomposing *Sphagnum* derived peat. *Soil Biol. Biochem.*, **22** : 4, 441-447.

Zeikus J.G. (1977) The biology of methanogenic bacteria. *A. Rev. Microbiol.*, **31**, 309.

Zeikus J.G., Kerby R. and Krzycki J.A. (1985) Single-carbon chemistry of acetogenic and methanogenic bacteria. *Science*, **227**, 1167-1173.

Zehnder A.J.B. and Brock T.D. (1979) Methane formation and methane oxidation by methanogenic bacteria. *J. Bacteriol.*, **137**, 420-432.