APPENDIX D

Experimental Procedures For $\delta^{13}C$ And C/N Analyses

Details of experimental techniques involved in the $\delta^{13}C$ and C/N analyses of organic matter present in the sediments are described in this appendix. These are dealt with under the following sections:

D.1 Calibration of the gas extraction assembly line.

D.2 Sample pre-treatment and gas extraction.

D.3 Mass spectrometric measurements.

D.1 Calibration of the assembly line:

A schematic diagram of the glass gas extraction line, especially constructed for this work, is shown (Fig. D.1).

Experiments were undertaken to calibrate the gas extraction line by constructing a plot of P (pressure as measured in the manometer tube) vs micromoles of gas (nitrogen or CO_2) in a fixed volume (manometer and glass/quartz tube). For this purpose a gas bottle of known volume was taken. After evacuation to 10^{-3} torr, it was filled with purified N_2 gas at a known pressure from a N_2 gas cylinder. The purification step involved passing the N_2 gas from the cylinder through liquid nitrogen (LN_2) cooled tubes, U1 and U2, to remove any impurity of CO_2 or water that may be present. Using the gas law, the number of micromoles of purified gas in the bottle was calculated. All the gas in the N_2 bottle was then released into the evacuated assembly line. The N_2 was absorbed onto the charcoal finger (labelled N in Fig. D.1) at LN_2 temperature. Once all the gas had been so collected, the manometer tube was closed and the charcoal finger torched to released the N_2. Upon cooling to room temperature, the pressure in the manometer tube was measured and the gas pumped away. The charcoal finger was torched again and kept ready for the next lot. A series of such measurements gave a plot of $P(N_2)$ vs Micromoles of N_2 (Fig. D.2).

A similar procedure was followed for obtaining the calibration curve for CO_2 gas with the difference that the gas was collected in the glass finger

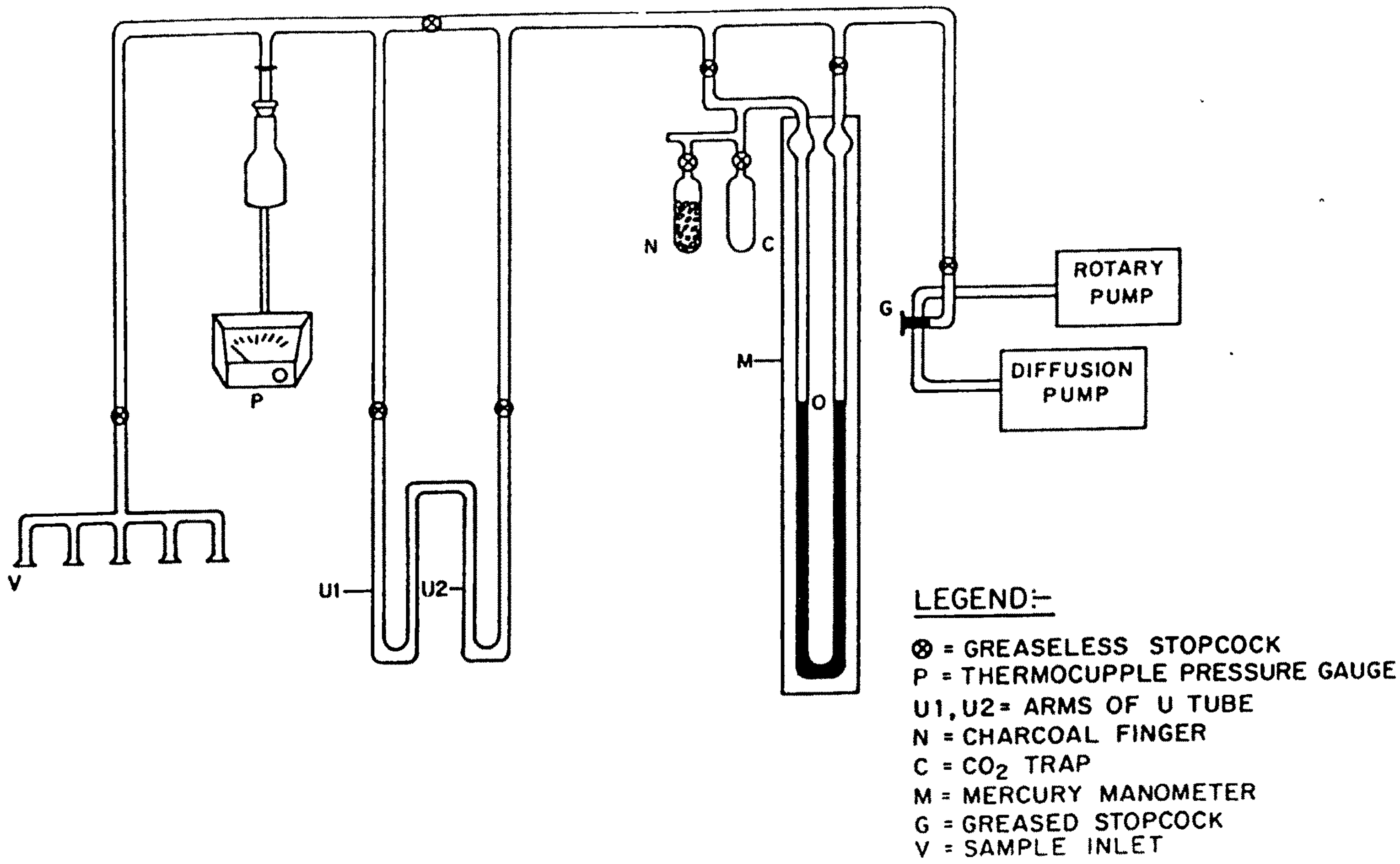

Fig D.1 Assembly line for extraction of Carbon dioxide and Nitrogen.

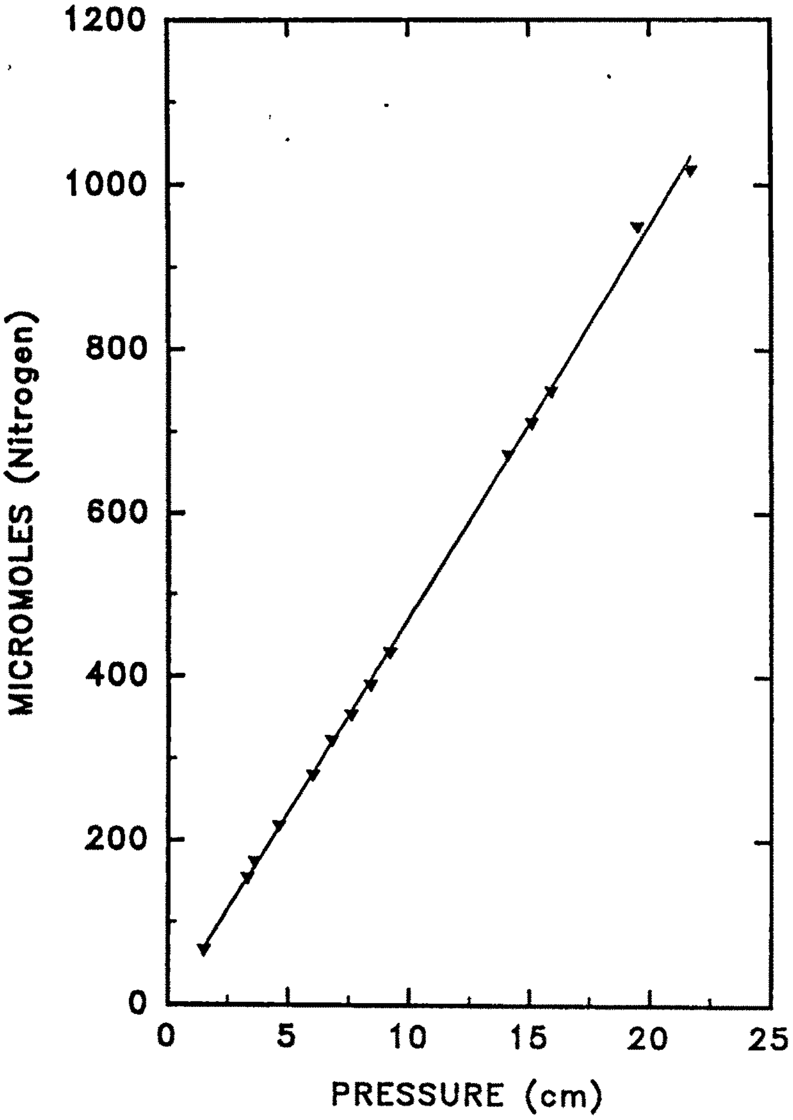

Fig. D.2 Calibration curve for determination of nitrogen.

(labelled C in Fig. D.1). The calibration curve for carbon dioxide is shown in Fig. D.3.

In both the cases, the efficiency of gas absorption/freezing was monitored using a thermocouple pressure gauge.

D.2 Sample pre-treatment and gas extraction:

B1. *Sample pre-treatment*: Since the aim of this study was to analyse the organic fraction of the sediments for $\delta^{13}C$ and C/N values, the first step was to remove inorganic carbon from each sample. The carbonate minerals constitute the main source of inorganic carbon in the sample. To remove them, a 10% Hydrochloric acid treatment is repeatedly given to 1g of dried and powdered sample. Dilute HCl was added till the reaction was over. A fresh batch of acid was then added and the samples left overnight to ensure complete removal of all the carbonates. Once the acid reaction was over the samples were repeatedly washed with double distilled water and dried at 70°C. Dried samples were powdered and stored in clean dry vials.

B2. *Extraction of CO_2 and N_2 from organic matter:* For the extraction of CO_2 and N_2 the following procedure was used.

Quartz tubes with break seals were used for combustion of samples. Before the sample was loaded, the tubes were left overnight in a furnace at 600°C to remove any possible source of contamination. About 400mg of pre-treated, accurately weighed, dry sample was loaded into a quartz tube (10mm OD, 8mm ID, 10cm long) with break seal at one end. Alongwith the sample, about 1g of pure E-Merck copper oxide was added which acted as a oxygen donor for the oxidation of the sample. This copper oxide, in wire form, had been baked previously at 400°C for 10 hours to remove any traces of organic matter which might have been present. The quartz tube with the sample and CuO was then evacuated to 10^{-3} Torr for at least 3 hours and then sealed under vacuum. The sealed quartz tube containing the sample and CuO was then inserted into stainless steel jackets, to ensure uniform heating, and heated to 800°C for 6 hours in a furnace.

With each batch of 7 sample tubes a Urea ($NH_2.CO.NH_2$) and a UCLA glucose standard was also processed to monitor the combustion

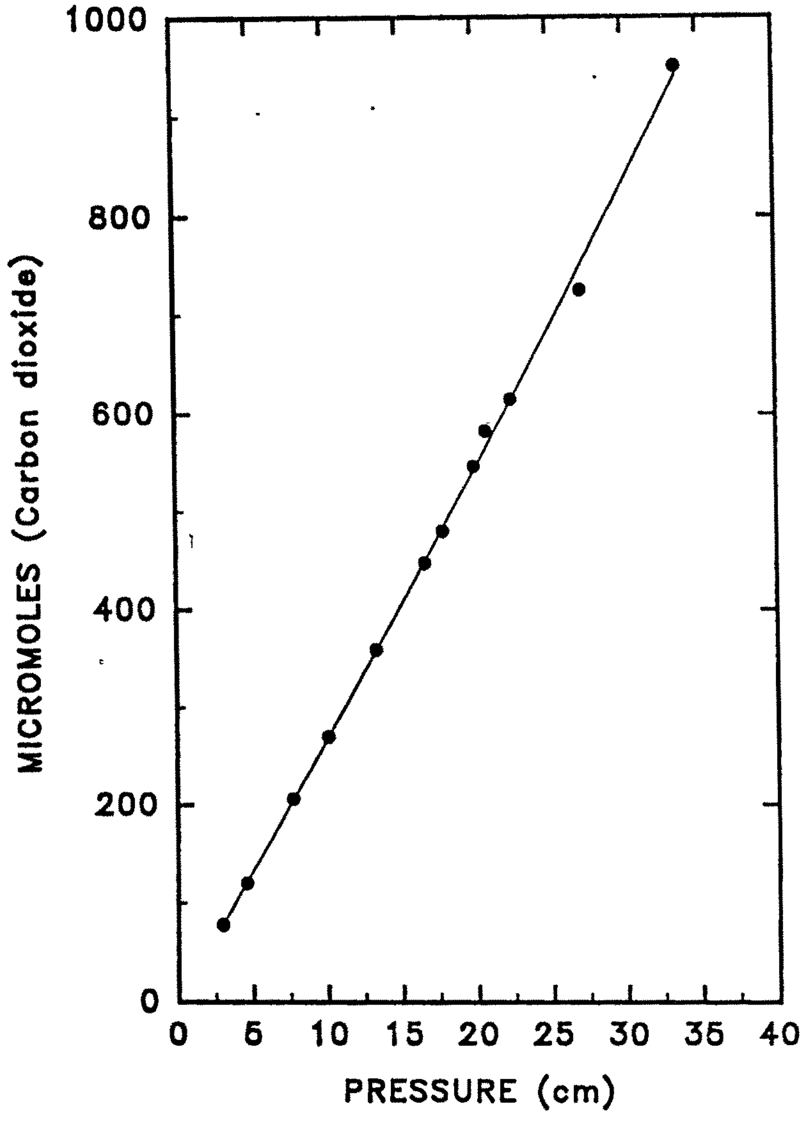

Fig. D.3 Calibration curve for determination of carbon dioxide.

efficiency. The yield for UCLA glucose was over 90%. Urea was used as a standard to monitor the reproducibility of %C and %N whereas UCLA glucose was used to monitor the reproducibility of $\delta^{13}C$. Blanks were periodically measured and found to be less than 3 micromoles as compared to the total gas yield of around 300 micromoles.

The cooled quartz tube, with a stainless steel ball resting on its break seal, was contained in a glass tube and introduced into the vacuum line through valve 'V'. After evacuation the break seal was cracked open by moving the steel ball using an external magnet. The released gases comprising water vapour, CO_2 and N_2 were made to pass through the U tubes, U1 and U2, which had been cooled to liquid nitrogen temperature to trap water vapour and CO_2 The N_2 gas was trapped on the charcoal contained in a quartz finger 'N' at liquid nitrogen temperature. The manometer valve was closed and the N_2 released by torching. After cooling, the yield was measured by reading the manometer. Knowing the weight 'w' of the sample and number of micromoles 'n' of nitrogen the yield was calculated as,

$$\% \text{ Nitrogen} = 2.8 * n / w$$

The nitrogen was pumped away and the charcoal finger torched again, evacuated and kept ready for the next sample.

CO_2 was separated from water vapour by replacing the liquid nitrogen trap around U1 and U2 by a alcohol - liquid nitrogen slurry maintained at -90°C. The CO_2 was collected in the glass finger and the yield measured. If 'c' is the number of micromoles of carbon dioxide produced, the carbon content of the sample was calculated using the formula,

$$\% \text{ Carbon} = 1.2 * c / w$$

D.3 Mass spectrometric measurements

After extraction, the CO_2 gas samples were taken to the mass spectrometer laboratory and again purified using a alcohol-LN_2 slurry trap. In principle, a mass spectrometer may be divided into four different parts: (1) double inlet system, which is used to introduce the sample and the reference gas; (2) An ion source where ions are formed (thermally or by electron impact), accelerated and focused into a narrow beam; (3) mass analyser, where the beams emerging from the ion source are subjected to a magnetic field due to which the ions are deflected into circular paths, the radius of which is proportional to square root of Mass/charge, and (4) ion collector, where the separated ions are collected in the ion detector and converted into an electrical pulse which is then fed into an amplifier.

The relative variations of the isotopic composition of different samples can be measured with high accuracy, with respect to a reference sample which is introduced into the mass spectrometer alternately with the sample under investigation, using a double inlet system. While analysing CO_2 by mass spectrometry, different ionic species with the same molecular weight will interfere with each other and corrections were applied for the same using the Craig (1957), equations. $\delta^{13}C$ was measured using a VG micromass 602D mass spectrometer, relative to tank CO_2 which served as an internal standard. Knowing the value of tank CO_2 with respect to PDB, the $\delta^{13}C$ value of sample wrt PDB was calculated using the equation,

$$\delta^{13}C^{X}_{PDB} = \delta^{13}C^{X}_{TC} + \delta^{13}C^{TC}_{PDB} + 10^{-3}\delta^{13}C^{X}_{TC}\delta^{13}C^{TC}_{PDB}$$

where X=sample, TC=tank CO_2.

In this case, $\delta^{13}C^{TC}_{PDB} = -22.17‰$, a value close to the $\delta^{13}C$ value of the sample being measured.

Results of standards

Results of $\delta^{13}C$ (‰) measurements on UCLA glucose:

- Mean = -10.12±0.19, n=14
- Value obtained at UCLA = -9.78±0.14

Results of %C and %N measurements on Urea ($NH_2.CO.NH_2$)

% Nitrogen:	measured = 47±2 (n=11)	expected = 46.62
% Carbon:	measured 19.7±0.6 (n=8)	expected = 19.98