Chemical Geology, 12 (1973) 161–169

GROWTH PATTERN AND $^{13}C/^{12}C$ ISOTOPE FRACTIONATION OF CYANIDIUM CALDARIUM AND HOT SPRING ALGAL MATS*

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(Accepted for publication August 29, 1973)

ABSTRACT

Seckbach, J. and Kaplan, I.R., 1973. Growth pattern and $^{13}C/^{12}C$ isotope fractionation of *Cyanidium caldarium* and hot spring algal mats. *Chem. Geol.*, 12: 161–169.

A study was undertaken with the thermophilic green alga *Cyanidium caldarium* which grows optimally at low pH and high concentrations of CO_2. Carbon-isotope fractionation was not found to be a simple linear function of temperature. Maximum enrichment of ^{12}C in cellular material occurred under optimum growth conditions (at approximately pH 2 and at temperatures between 40–50°C in a CO_2 atmosphere). A maximum measured fractionation of $-24^{0}/_{00}$ may account for low values ($\delta^{13}C < -30^{0}/_{00}$ PDB) in Precambrian kerogen presumably derived from algal mats.

INTRODUCTION

Photosynthetic activity of *Cyanidium caldarium* grown under pure CO_2 was recently reported (Seckbach et al., 1970; Seckbach et al., 1971). We now provide additional data for this thermophilic-acidophilic alga concerning its growth and $^{13}C/^{12}C$ isotope fractionation characteristics. This hot-spring alga was grown in a CO_2 atmosphere at different temperatures for various periods of time while monitoring the pH. Controls cultured in laboratory air were grown in parallel.

Data for the C-isotope compositions were determined by combustion of the cells in a vacuum system using the modified technique described by Craig (1953). The CO_2 collected was transferred to a 6-inch radius, 60° deflection, dual-collecting mass spectrometer manufactured by Nuclide, Inc. (Pennsylvania, U.S.A.), and measured against the U.C.L.A. calcite standard. All results are quoted relative to PDB. The δ value indicates the difference in per mil of the $^{13}C/^{12}C$ ratio of the sample relative to a standard, and is defined as:

$$\delta\ (^{0}/_{00}) = \frac{^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ standard}}{^{13}C/^{12}C \text{ standard}} \times 1000$$

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The $\delta^{13}C$ for air CO_2 was taken as $-7‰$, whereas for tank CO_2 it was measured as $-34‰$ in experiments carried out at U.C.L.A. and $-30‰$ in CO_2 used at the Hebrew University.

Measurements of $^{13}C/^{12}C$ isotope ratios in photosynthetic systems, indicate that most higher plants (including all lower vascular plants and all gymnosperms except *Welwitchia*) yield $\delta^{13}C$ values of -24 to $-34‰$ (Smith and Epstein, 1971). Algae, lichens and nanoplankton generally yield values of -12 to $-20‰$ (Park and Epstein, 1960). One possible explanation suggested for the difference, is that the algae are metabolizing bicarbonate, which is about 7 or $8‰$ more enriched in ^{13}C than atmospheric or dissolved CO_2. The increase in solubility of CO_2 with decrease in temperature was used to explain high enrichment in ^{12}C ($\delta^{13}C \approx -26‰$) in algae grown at low temperatures (10°C) in the laboratory (Deuser et al., 1968; Degens et al., 1968a) and in ocean plankton hauls obtained in high latitudes (Degens et al., 1968b). This effect is of potential ecological significance, as a means of determining environment of growth by studying organic matter in sediment. In a study recently reported, Calder and Parker (1973) were unable to reproduce the temperature effect reported above, but did find increased fractionation factors at increased carbon-dioxide concentration. However, the maximum enrichment in ^{12}C in the cellular carbon of blue-green algae relative to the starting CO_2 measured by Calder and Parker was $-18‰$ for cultures grown at or near neutral pH. *Cyanidium caldarium* was especially chosen for this study as it grows at low pH and, therefore, must only fix dissolved CO_2.

RESULTS AND DISCUSSION

The cultures were grown in agitated mineral media and aspirated with CO_2 or air as previously described (Seckbach et al., 1971). Typical growth curves of *Cyanidium caldarium* cells grown at room temperature (26°C) and at elevated temperature (45°C) are presented in Fig.1A and B, respectively. Growth value is expressed as the optical density of the suspension at 580 mμ which also gives an estimate of the volume (ml/l) of packed cells. It is clear from the growth of *Cyanidium* at room temperature (Fig.1A) that cultures grown on pure CO_2 have a lag period of adaptation of nearly two weeks and then show a higher rate of cellular increase, whereas, aerated cultures show a growth response within one or two days. When *Cyanidium* is incubated under CO_2 at 45°C (Fig.1B), visible growth is observed within 24–48 hours and the growth rate is higher than in the aerated control, in contrast to results obtained with CO_2 at room temperature (Fig.1A). The growth curves shown in Fig.1A and 1B were compiled from three different experiments. An additional characteristic of this organism, is the decrease in pH (Fig.2) in the liquid growth media to ≈ 2 or lower.

Table I illustrates the temperature influence on carbon-isotope composition in *Cyanidium* grown either in CO_2 or in air. An increase of temperature from 25° or 26°C to 45° or 50°C in the air-grown cells does not result in any significant change in the cellular isotopic ratio of the cells ($\Delta\delta^{13}C = -11.7‰$ at 25°C and $-13.5‰$ at 40–50°C).

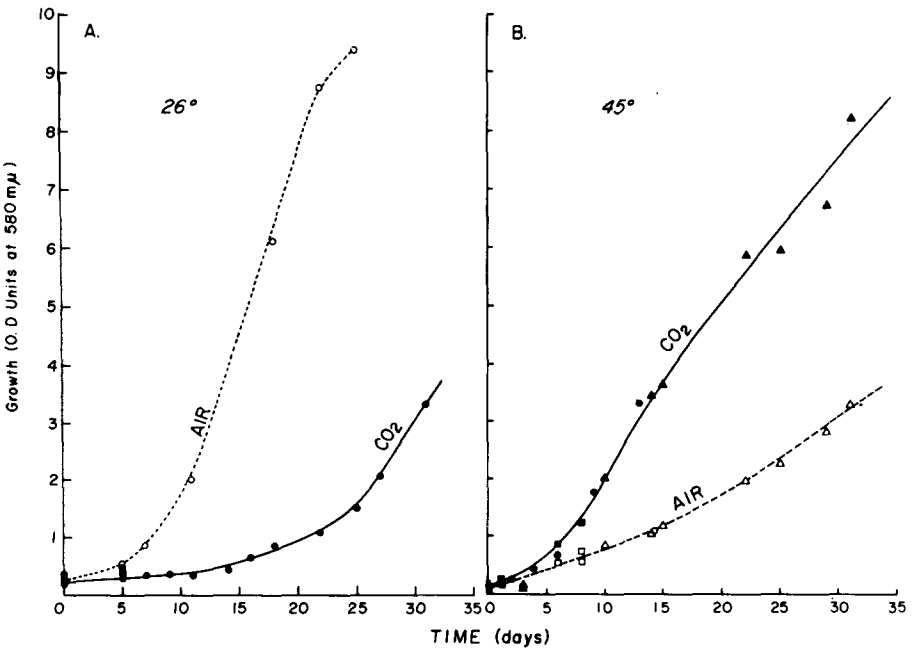

Fig.1. Growth of *Cyanidium caldarium* as a function of CO_2 (solid circles) or air (open circles) at room temperature (A) and at 45° (B). Growth values are expressed in units of O.D. at 580 mμ (which gives a relatively accurate picture of the cell mass at this wavelength).

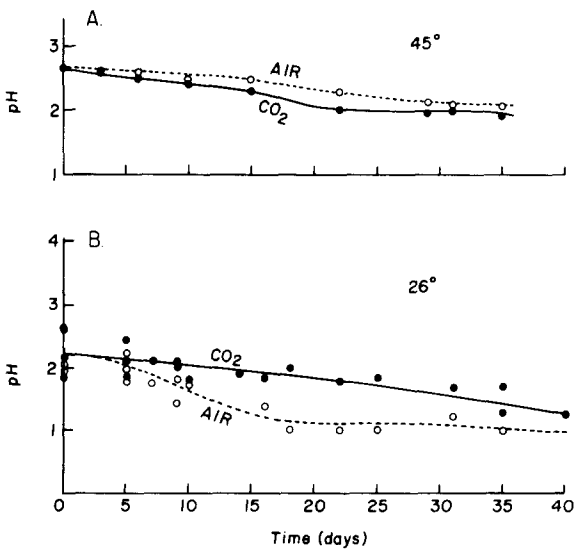

Fig.2. The pH modification of the nutrient media during growth of *Cyanidium caldarium* cells under CO_2 (solid line) or air (dash line) at 45° (A) and 26° (B).

TABLE I

Carbon-isotope fractionation, $\Delta\delta^{13}C^{*1}$, by laboratory grown *C. cyanidium* in the presence of air or CO_2 at various temperatures

Temp. of growth (°C):	25–26		40	45			50	
Age of culture (days):	14	41	11	14	17	18	14	26
Substrate:								
CO_2	−5.1[1] *[2] −11.0[3]	−19.0[3]	−17.0[4]	−16.5[1]	–	−24.0[3]	−19.3[1]	−16.6[4]
Air	−12.3[1] −12.0[2]	−11.0[3]	−14.9[4]	–	−13.0[2]	−11.0[3]	−13.8[1]	−14.8[4]

*[1] $\Delta\delta^{13}C = \delta^{13}C$ sample − $\delta^{13}C$ substrate.

*[2] Experiment number.

CO_2-grown cultures consistently show a greater enrichment in the light isotope (decrease in $\delta^{13}C$) at the higher temperature than air-grown cells (average $\Delta\delta^{13}C \approx -18.4‰$ for range 40°–50°). Two cultures grown on CO_2 at 25° and 26° have $\Delta\delta^{13}C$ values of $-5‰$ and $-11‰$, respectively.

These results are in contrast to the normally-accepted biogenic isotope effect, which predicts that the kinetically-controlled process should show greater fractionation at lower metabolic rates (lower temperatures). From data presented in Table I, attention should be focused on two values for CO_2-grown cells at 26°C in experiment 1. When cells were still in their early growth stage (14 days old) the analyzed value was $-11‰$, whereas after 41 days of growth, the culture yielded a measured $\Delta\delta^{13}C$ value of $-19‰$.

Additional information on the stable C-isotope distribution was obtained from lipid fractions extracted from anaerobically (CO_2) and aerobically (grown *Cyanidium* cells). Table II demonstrates that $^{13}C/^{12}C$ ratios in the lipid fraction of CO_2-grown cells are similar to the ratio of unextracted cell components, whereas in air-grown cells, the light isotope is enriched in the lipid carbon as previously observed in plankton (Degens et al., 1968a). The lipid fractions were obtained from ca. 100 mg lyophilized algae powder which was extracted by refluxing over a boiling water bath with 50 ml of methanol:chloroform: benzene (1:2:2) for 2.5 hours. The extract was centrifuged for 30 min and the lipid supernatant was removed from the residue, both fractions were dried prior to the ^{13}C determination.

TABLE II

$^{13}C/^{12}C$ ratios of *Cyanidium* lipid extracted from cells grown under pure CO_2 and air (0.03% CO_2) at different temperatures

	$\delta^{13}C$ (‰); 25°C, 41 days		$\delta^{13}C$ (‰); 45°C, 18 days	
Fraction:	lipid	residue	lipid	residue
Condition:				
CO_2	−18.9	−17.0	−24.5	−23.3
air	−14.1	−11.2	−19.6	−10.7

Increase in temperature and decrease in the nutrient pH lower the solubility of CO_2, which is 1.7 times higher for water at 25° than at 50° (Dodds et al., 1956). Thus, increase in isotope fractionation with increase in temperature cannot be a function of the availability of carbon. We believe the effect is probably due to the involvement of different temperature-controlled rate-limiting processes, either in activation or transfer of carbon dioxide by enzyme-bound moieties. In nature, different algal species could have optimum enzyme-substrate reactions at a variety of temperatures, pH and substrate levels, hence yielding different isotope effects even under identical conditions of growth.

For comparison, samples of hot-spring algae were measured from environments of

known pH and temperature. A mat of *C. calderium* (collected by J. Oehler, June 1971) from Nymph Creek in Yellowstone Park actively growing in water at 44°C and pH 2.7, was analyzed some weeks after collection and yielded $\delta^{13}C = -12.3‰$ or $\Delta\delta^{13}C = -5.3‰$ (assuming air $CO_2 = -7‰$). This value is less than that measured for air-grown cells in the laboratory and $\Delta\delta^{13}C$ may in fact be larger if the surface-growing cells also metabolize dissolved CO_2 with a $\delta^{13}C$ value considerably different from air. However, data on $\delta^{13}C$ in gases and carbonates from Yellowstone Park indicate that $^{13}C/^{12}C$ of dissolved CO_2 should not be very different from atmospheric values (Craig, 1953; Friedman, 1970).

Algal mats from the Orakei Korako geothermal area of New Zealand (see Kaplan, 1956) were collected by E. Lloyd of D.S.I.R. around springs, ponds and geysers during 1960 and 1961 at different seasons. The water temperature at the source and that in contact with the algal mats was measured. The pH was measured after permitting the water to cool at atmospheric temperature (Table III). The algal mats were then air-dried by spreading them out in a warm room. All samples were first treated with 1*N* HCl to dissolve carbonate, before combustion for isotope measurement.

TABLE III

Algal mats from Orakei Korako (New Zealand) hot springs

Sample No.	Date collected	Site	Spring temp. (°C)	Temp. sampling site (°C)	pH	$\delta^{13}C$ (‰)
1	3-21-60	I	99.8	44	9.75	−19.61
3	3-21-60	I	99.8	44	9.75	−18.26
34	5-25-60	I	99	30.5	9.6	−20.00
74	8- 2-60	I	104	35	9.56	−20.81
139	1-18-61	I	–	–	–	−19.89
7	3-21-60	III	98	63	9.3	−22.81
38	5-25-60	III	97.5	–	9.32	−14.6
76	8- 2-60	III	98	60.5	9.22	−19.47
146	10-11-60	III	–	63.7	–	−19.89
13	3-21-60	VI	75.5	63.7	8.46	−23.80
14	3-21-60	VI	–	43	–	−11.40
42	5-25-60	VI	73	56.5	8.38	−18.38
79	8- 2-60	VI	74	–	–	−15.97
105	10-11-60	VI	75	–	8.5	−15.41
149	1-18-61	VI	–	40–30	–	−11.09

It is apparent that at the high pH in this environment (pH 8.4–9.8), bicarbonate will predominate as the dissolved inorganic carbon species. The spread in $\delta^{13}C$ is from −11.1 ‰ to −23.8‰ over a temperature of growth range of 30°–63°C. However, a glance at Table III indicates that there is no direct relationship between temperature of growth and the $\delta^{13}C$ of the algae. Paradoxically, the greatest depletion in ^{13}C occurred at the highest temperature of growth. Unfortunately, the $\delta^{13}C$ of the dissolved HCO_3^- is not

known, but one may assume that it is reasonably constant for all the pools, as the environment of growth is localized and the source of water is probably the Waikato River (Lloyd, 1972).

The preliminary results suggest that caution must be exercised in interpreting $^{13}C/^{12}C$ data measured on algal populations in natural environments, and their degraded products in soil or sediment. It is apparent from these studies and those recently presented by Calder and Parker (1973), that $\Delta\delta^{13}C$ of algal cells cannot be simply equated to temperature of growth of the organism. For example, harsh wind conditions (stirring) or rapid mixing of the upper water column may be responsible for lowering pH in surface seawater by introducing atmospheric or respired CO_2 into the water. Furthermore, experiments with bacteria indicate that an organism may show different $^{34}S/^{32}S$ fractionation responses under different rate-controlling conditions (Kaplan and Rittenberg, 1964). Hence, temperature is not the only control on concentration of dissolved CO_2, and fractionation of carbon isotopes in algal cellular material resulting from photosynthetic fixation may be influenced by several rate-controlling steps, of which temperature is only one (Brock, 1970).

However, the results of the study may be used for interpreting the origin of organic matter in sediments. Reduced carbon (as kerogen) in Precambrian rocks is characteristically enriched in ^{12}C, with $\delta^{13}C$ values in the range -25 to -35‰ (Hoering, 1967; Smith et al., 1970; Oehler et al., 1972). It is widely believed that during the Early Proterozoic, life was restricted to procaryotic organisms (blue-green algae and bacteria?) and later to primitive eucaryotes (probably resembling green algae) at about 800–1000 m.y. ago (Schopf, 1970). *C. caldarium* is unique because it has a pigment suite (chlorophyll and phycobilins) resembling blue-green algae, but is classified as a chlorophyte because of its reproductive habit (Allen, 1959). More recent studies on cell structure indicate it may be a primitive rhodophyte (Seckbach and Ikan, 1972). Hence, *C. caldarium* may represent an early primitive form of eucaryotic organism. Its present ecological distribution is in acid hot-spring water where amorphous or hydrated siliceous sinter is depositing. During burial, at elevated temperature and pressure, this sinter may be transformed to chert (Oehler, 1972). Most preserved Precambrian microfossils are found in chert sediments (Schopf, 1970).

Present-day algal mats have $\delta^{13}C$ values in the range -8 to -15‰ (Calder and Parker, 1973), therefore, to explain the isotopically light values in the Precambrian, four alternatives may be contemplated: (1) the $\delta^{13}C$ values for Precambrian kerogen represent alteration during preservation; (2) isotope fractionation effects were much larger in Precambrian organisms because of different metabolic pathways; (3) the carbon-isotopic composition of the atmospheric CO_2 was substantially lighter in Precambrian times (perhaps $\delta^{13}C = -15$ to -25‰); and (4) the organic matter found preserved represents algal mats of organisms (similar to *C. caldarium*) growing under high P_{CO_2}, possibly in thermal areas.

The first proposition may be true for kerogen which has undergone mild metamorphism and loss of ^{13}C-rich CO_2, but probably this has not occurred in all cases. In fact, comparison of kerogen $\delta^{13}C$ values between metamorphosed and less or unmetamorphosed sedimentary equivalents indicates a shift toward heavier, rather than lighter values (Baker and

Claypool, 1970; Barker and Friedman, 1969). There is no evidence for the second proposition. Measurements of $\delta^{13}C$ on Precambrian limestone and dolomite indicate that the ratios fall within $\pm 5‰$ of PDB (Galimov et al., 1968; Perry and Tan, 1972). This range is not significantly different from that in Phanerozoic marine carbonates, and it has therefore been suggested that $\delta^{13}C$ of atmospheric CO_2 must have been about the same as in the present atmosphere. Furthermore, Galimov et al. (1968) suggest that in order for $\delta^{13}C$ of Precambrian atmospheric CO_2 to be approximately equal to that of present-day CO_2, it must have been a major atmospheric component during the Proterozoic when carbonates began to form. In view of the above arguments, we wish to propose (speculate) that low $\delta^{13}C$ values measured in Precambrian kerogen, may in part represent organic matter deposited in algal mats growing at elevated temperatures and under atmospheric conditions where P_{CO_2} was substantially greater than at present.

ACKNOWLEDGEMENT

This work was supported by grants from NASA No.237-62; NGR 05-007-221) to U.C.L.A. and by a special grant from the Hebrew University to J. Seckbach. We wish to thank Professor W.F. Libby for his interest in this study and Ed Ruth and Chari Petrowski for making the mass-spectroscopy measurements.

REFERENCES

Allen, M.B., 1959. Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. *Arch. Microbiol.*, 32: 270–277.

Baker, D.R. and Claypool, G.E., 1970. Effects of incipient metamorphism on organic matter in mudrock. *Am. Assoc. Petrol. Geol. Bull.*, 54: 456–458.

Barker, F. and Friedman, I., 1969. Carbon isotopes in pelites of the Precambrian Uncompahgre Formation, Needle Mountains, Colorado. *Geol. Soc. Am. Bull.*, 80: 1403–1408.

Brock, T.D., 1970. High temperature systems. *Ann. Rev. Ecol. Systems*, 1: 191–220.

Calder, J.A. and Parker, P.L., 1973. Geochemical implications of the induced changes in ^{13}C fractionation by blue-green algae. *Geochim. Cosmochim. Acta*, 37: 133–140.

Craig, H., 1953. The geochemistry of the stable carbon isotopes. *Geochim. Cosmochim. Acta*, 3: 53–92.

Degens, E.T., Guillard, R.R.L., Sackett, W.M. and Hellebust, J.A., 1968a. Metabolic fractionation of carbon isotopes in marine plankton, 1. Temperature and respiration experiments. *Deep-Sea Res.*, 15: 1–9.

Degens, E.T., Behrendt, J., Gottardt, B. and Reppmann, E., 1968b. Metabolic fractionation of carbon isotopes in marine plankton, 2. Data on samples collected off the coast of Peru and Ecuador. *Deep-Sea Res.*, 15: 11–20.

Deuser, W.G., Degens, E.T. and Guillard, R.R.L., 1968. Carbon-isotope relationships between plankton and seawater. *Geochim. Cosmochim. Acta*, 22: 657–660.

Dodds, W.S., Stutzman, L.F. and Sollami, B.J., 1956. Carbon dioxide solubility in water. *Ind. Eng. Chem., Chem. Eng. Data Ser.*, 1: 92.

Friedman, I., 1970. Some investigations of the deposition of travertine from hot springs, 1. The isotopic chemistry of a travertine-depositing spring. *Geochim. Cosmochim. Acta*, 34: 1303–1315.

Galimov, E.M., Kuznetsova, N.G. and Prokhotov, V.S., 1968. The composition of the former atmosphere of the earth as indicated by isotopic analysis of Precambrian carbonates. *Geochim. Int.*, 5: 1126–1131 (translated from *Geokyimya*, pp. 1376–1381, 1968);

Hoering, T.C., 1967. Organic geochemistry of Precambrian rocks. In: P.H. Abelson (Editor), *Researches in Geochemistry*, Wiley, New York, N.Y., Vol. 2, pp. 87–111.

Kaplan, I.R., 1956. Evidence of microbiological activity in some of the geothermal regions of New Zealand. *N.Z.J. Sci. Technol.*, 37: 637–663.

Kaplan, I.R. and Rittenberg, S.C., 1964. Microbiological fractionation of sulfur isotopes. *J. Gen. Microbiol.*, 34: 195–212.

Lloyd, E.F., 1972. Geology and hot springs of Orakei Korako. *N.Z. Geol. Surv. Bull.*, 85: 164.

Oehler, D.Z., Schopf, J.W. and Kvenvolden, K.A., 1972. Carbon isotopic studies of organic matter in Precambrian rocks. *Science*, 1246–1248.

Oehler, J.H., 1972. "Stromatoloids" from Yellowstone Park, Wyoming. *Geol. Soc. Am. (Cordilleran Soc. 68th Ann. Meeting) Abstr.*, 2: 212–213.

Park, R. and Epstein, S., 1960. Carbon-isotope fractionation during photosynthesis. *Geochim. Cosmochim. Acta*, 21: 110–126.

Perry, E.C. and Tan, F.C., 1972. Significance of oxygen and carbon-isotope variations in Early Precambrian cherts and carbonate rocks of southern Africa. *Geol. Soc. Am. Bull.*, 83: 647–664.

Seckbach, J., Shugarman, P.M. and Baker, F.A., 1970. Algae thrive under pure CO_2. *Nature*, 227: 744–745.

Seckbach, J., Gross, H. and Nathan, M.B., 1971. Growth and photosynthesis of *Cyanidium caldarium* cultured under pure CO_2. *Isr. J. Bot.*, 20: 84–90.

Seckbach, J. and Ikan, R., 1972. Sterols and chloroplast structure of *Cyanidium caldarium*. *Plant Physiol.*, 49: 457–459.

Schopf, J.W., 1970. Precambrian micro-organisms and evolutionary events prior to the origin of vascular plants. *Biol. Rev. Cambr. Phil. Soc.*, 45: 319–352.

Smith, B.N. and Epstein, S., 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol.*, 47: 380–384.

Smith, J.W., Schopf, J.W. and Kaplan, I.R., 1970. Extractable organic matter in Precambrian cherts. *Geochim. Cosmochim. Acta*, 34: 659–675.