ISOTOPE FRACTIONATION IN THE BIOSPHERE

INTRODUCTION

As we noted, biological processes often involve large isotopic fractionations. Indeed, biological processes are the most important cause of variations in the isotope composition of carbon, nitrogen, and sulfur. For the most part, the largest fractionations occur during the initial production of organic matter by the so-called primary producers, or *autotrophs*. These include all plants and many kinds of bacteria. The most important means of production of organic matter is photosynthesis, but organic matter may also be produced by chemosynthesis, for example at mid-ocean ridge hydrothermal vents. Large fractions of both carbon and nitrogen occur during primary production. Additional fractionations also occur in subsequent reactions and up through the food chain as *hetrotrophs* consume primary producers, but these are generally smaller.

CARBON ISOTOPE FRACTIONATION DURING PHOTOSYNTHESIS

The most important of process producing isotopic fractionation of carbon is photosynthesis. As we earlier noted, photosynthetic fractionation of carbon isotopes is primarily kinetic. The early work of Park and Epstein (1960) suggested fractionation occurred in several steps. Subsequent work has elucidated the fractionations involved in these steps, which we will consider in more detail here.

For terrestrial plants (those utilizing atmospheric CO_2), the first step is diffusion of CO_2 into the boundary layer surrounding the leaf, through the stomata, and internally in the leaf. The average $\delta^{13}C$ of various species of plants has been correlated with the stomatal conductance (Delucia et al., 1988), indicating that diffusion into the plant is indeed important in fractionating carbon isotopes. On theoretical grounds, a 4.4‰ difference in the diffusion coefficients is predicted ($^{12}CO_2$ will diffuse more rapidly; see Lecture 26) so a fractionation of –4.4‰ is expected. Marine algae and aquatic plants can utilize either dissolved CO_2 or HCO_3^- for photosynthesis:

$$CO_{2(g)} \rightarrow CO_{2(aq)} + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

An equilibrium fractionation of +0.9 per mil is associated with dissolution ($^{13}CO_2$ will dissolve more readily), and an equilibrium +7 to +12‰ fractionation (depending on temperature) occurs during hydration and dissociation of CO_2.

At this point, there is a divergence in the chemical pathways. Most plants use an enzyme called *ribulose bisphosphate carboxylase oxygenase* (RUBISCO) to catalyze a reaction in which *ribulose bisphosphate carboxylase* reacts with one molecule of CO_2 to produce 3 molecules of 3-phosphoglyceric acid, a compound containing 3 carbon atoms, in a process called *carboxylation* (Figure 27.1). Energy to drive this reaction is provided by another reaction, called *photophosphorylation*, in which electromagnetic energy is used to dissociate water, producing oxygen. The carbon is subsequently reduced, carbohydrate formed, and the ribulose bisphosphate regenerated. Such plants are called C_3 plants, and this process is called the *Benson-Calvin*, or *Calvin*, cycle. C_3 plants constitute about 90% of all plants and include algae and autotrophic bacteria and comprise the majority of cultivated plants, including wheat, rice, and nuts. There is a kinetic fractionation associated with carboxylation of ribulose bisphosphate that has been determined by several methods to be -29.4‰ in higher terrestrial plants. Bacterial

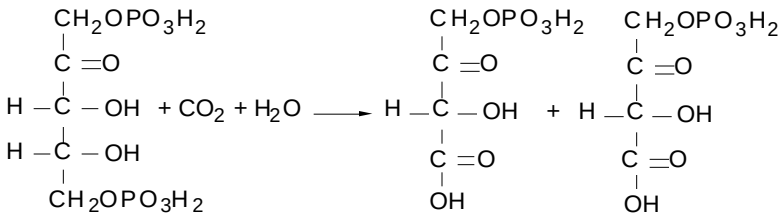

Figure 27.1. Ribulose bisphosphate (RuBP) carboxylation, the reaction by which C_3 plants fix carbon during photosynthesis.

carbolaxylation has different reaction mechanisms and a smaller fractionation of about –20‰. Thus for terrestrial plants a fractionation of about –34‰ is expected from the sum of the fraction. The actual observed total fractionation is in the range of –20 to –30‰.

The disparity between the observed total fractionation and that expected from the sum of the steps presented something of a conundrum. The solution appears to be a model that assumes the amount of carbon isotope fractionation expressed in the tissues of plants depends on ratio the concentration of CO_2 inside plants to that in the external environment. The model may be described by the equation:

$$\Delta = a + (c_i/c_a)\,(b - a) \qquad 27.1$$

where a is the isotopic fractionation due to diffusion into the plant, c_i is the interior CO_2 concentration, c_a is the ambient or exterior CO_2 concentration, and b is the fractionation occurring during carboxylation. According to this model, where an unlimited amount of CO_2 is available (i.e., when $c_i/c_a \approx 1$), carboxylation alone causes fractionation. At the other extreme, if the concentration of CO_2 in the cell is limiting (i.e., when $c_i/c_a \approx 0$), essentially all carbon in the cell will be fixed and therefore there will be little fractionation during this step and the total fractionation is essentially just that due to diffusion alone. Both laboratory experiments and field observations provide strong support for this model.

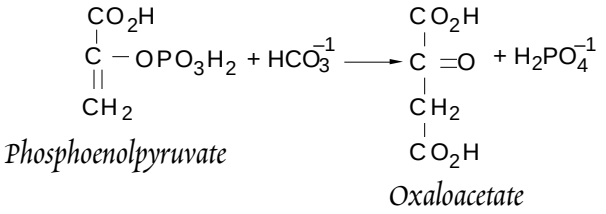

Figure 27.2. Phosphoenolpyruvate carboxylation, the reaction by which C_4 plants fix CO_2 during photosynthesis.

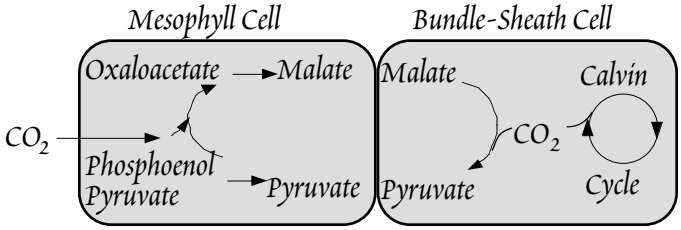

Figure 27.3. Chemical pathways in C_4 photosynthesis.

The other photosynthetic pathway is the Hatch-Slack cycle, used by the C_4 plants which include hot-region grasses and related crops such as maize and sugarcane. These plants use *phosphoenol pyruvate carboxylase* (PEP) to initially fix the carbon and form oxaloacetate, a compound that contains 4 carbons (Fig. 27.2). A much smaller fractionation, about -2.0 to -2.5‰ occurs during this step. In phosphophoenol pyruvate carboxylation, the CO_2 is fixed in outer mesophyll cells as oxaloacetate and carried as part of a C_4 acid, either malate or asparatate, to inner bundle sheath cells where it is decarboxylated and refixed by RuBP (Fig. 27.3). The environment in the bundle sheath cells is almost a closed system, so that virtually all the carbon carried there is refixed by RuBP, so there is little fractionation during this step. C_4 plants have average $\delta^{13}C$ of -13‰. As in the case of RuBP photosynthesis, the fractionation appears to depend on the ambient concentration of CO_2. This dependence can be modeled as:

$$\Delta = a + (b_4 + b_3\phi - a)(c_i/c_a) \qquad 27.2$$

where a is the fractionation due to diffusion of CO_2 into the plant as above, b_4 is the fractionation during diffusion into bundle-sheath cells, b_3 is the fractionation during carboxylation (~ –3‰), ϕ is the fraction CO_2 leaked from the plant.

A third group of plants, the CAM plants, have a unique metabolism called the 'Crassulacean acid metabolism'. These plates generally use the C_4 pathway, but can use the C_3 pathway under certain conditions. These plants are generally adapted to arid environments and include pineapple and many cacti, they have $\delta_{13}C$ intermediate between C_3 and C_4 plants.

Terrestrial plants, which utilize CO_2 from the atmosphere, generally produce greater fractionations than marine and aquatic plants, which utilize dissolved CO_2 and HCO_3^-, together referred to as *dissolved inorganic carbon* or DIC. As we noted above, there is about an +8‰ equilibrium fractionation between dissolved CO_2 and HCO_3^-. Since HCO_3^- is about 2 orders of magnitude more abundant in seawater than dissolved CO_2, many marine algae utilize this species, and hence tend to show a lower net fractionation during photosynthesis. Diffusion is slower in water than in air, so diffusion

is often the rate limiting step. Most aquatic plants have some membrane-bound mechanism to pump DIC, which can be turned on when DIC is low. When DIC concentrations are high, fractionation in aquatic and marine plants is generally similar to that in terrestrial plants. When it is low and the plants are actively pumping DIC, the fractionation is less because most of the carbon pumped into cells is fixed. Thus carbon isotope fractionations can be as low as 5‰ in algae. The model describing this fractionation is:

$$\Delta = d + b_3 + (F_3/F_1) \qquad 27.3$$

where d is the equilibrium effect between CO_2 and HCO_3^-, b_3 is the fractionation associated with carboxylation, and (F_3/F_1) is the fraction of CO_2 leaked out of the cell. Though the net fractionation varies between species and depends on factors such as light intensity and moisture stress, higher C_3 plants have average bulk $\delta^{13}C$ values of -27‰; algae and lichens are typically -12 to 23‰.

In aquatic systems where the pH is lower, CO_2 becomes a more important species and algae can in some cases utilize this rather than HCO_3^-. In those cases, the total fractionation will be greater. An interesting illustration of this, and the effect of the CO_2 concentration on net fractionation is shown in Figure 27.4, which shows data on the isotopic composition of algae and bacteria in Yellowstone hot springs.

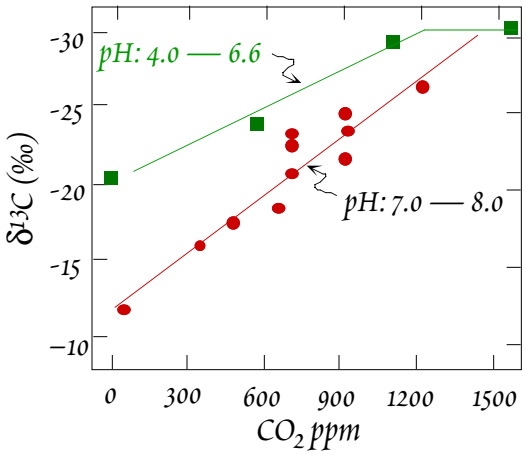

Figure 27.4. Dependence of $\delta^{13}C$ of algae and bacterial on CO_2 concentration from hydrothermal springs in Yellowstone National Park. Carbon isotope fractionation also depends on the pH of the water, because this determines the species of carbon used in photosynthesis.

Some fractionation is also associated with respiration (the oxidation of carbohydrate to CO_2), but the net effect is uncertain.

Not surprisingly, the carbon isotope fractionation in C fixation is also temperature dependent. Thus higher fractionations are observed in cold water phytoplankton than in warm water species. However, this observation also reflects a kinetic effect: there is generally less dissolved CO_2 available in warm waters because of the decreasing solubility at higher temperature. As a result, a larger fraction of the CO_2 is utilized and there is consequently less fractionation. Surface waters of the ocean are generally enriched in ^{13}C because of uptake of ^{12}C during photosynthesis (Figure 27.5). The degree of enrichment depends on the productivity: biologically productive areas show greater enrichment. Deep water, on the other hand, is depleted in ^{13}C (perhaps it would be more accurate to say it is enriched in ^{12}C). Organic matter falls through the water column and is decomposed and "remineralized", i.e., converted in inorganic carbon, by the action of bacteria, enriching deep water in ^{12}C. Thus biological activity acts to "pump" carbon, and particularly ^{12}C from surface to deep waters.

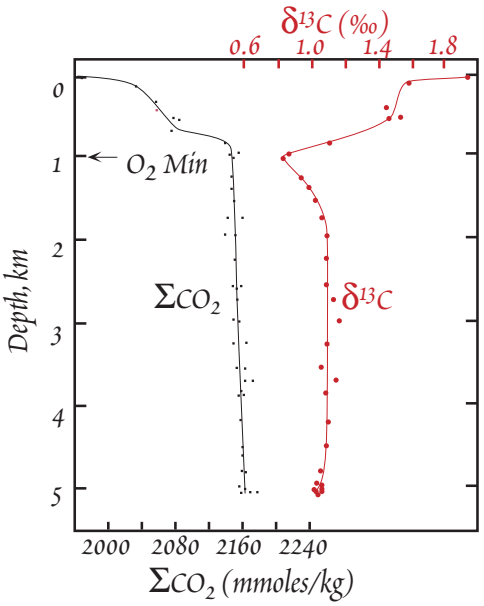

Figure 27.5. Depth profile of total dissolved inorganic carbon and $\delta^{13}C$ in the North Atlantic.

Essentially all organic matter originates through photosynthesis. Subsequent reactions convert the photosynthetically produced carbohydrates to the variety of other organic compounds utilized by organisms. Further fractionations occur in these reactions. These fractionations are thought to be kinetic in origin and may partly arise from organic C-H bonds being enriched in ^{12}C and organic C-O

bonds are enriched in ^{13}C. ^{12}C is preferentially consumed in respiration (again, because bonds are weaker and it reacts faster), which would tend to enrich residual organic matter in ^{13}C. Thus the carbon isotopic composition of organisms becomes more positive moving up the food chain.

NITROGEN ISOTOPE FRACTIONATION IN BIOLOGICAL PROCESSES

Nitrogen is another important element in biological processes, being an essential component of all amino acids and proteins. The understanding of isotopic fractionations of nitrogen is much less advanced than for carbon. There are five important forms of inorganic nitrogen (N_2, NO_3^-, NO_2^-, NH_3 and NH_4^+). Equilibrium isotope fractionations occur between these five forms, and kinetic fractionations occur during biological assimilation of nitrogen. Ammonia is the form of nitrogen that is ultimately incorporated into organic matter by growing plants. Most terrestrial plants depend on bacteria for *fixation* (i.e., reduction) of N_2 and other forms of nitrogen to ammonia. Many plants, including many marine algae, can utilize oxidized nitrogen, NO_3^- and NO_2^-, and others (legumes, for example) are able to utilize N_2 directly. In these cases, nitrogen must first be reduced by the action of reductase enzymes. $\delta^{15}N$ fractionations of 0 to –24‰ have been measured for the assimilation of NO_3^-. Fractionation of 0 to -20‰ has been measured for assimilation of NH_4^+. Fractionations of –3 to +1‰ have been measured for the fixation of N_2. There is a –19‰ equilibrium fractionation in the conversion of NH_3 to NH_4^+. While NH_3 is generally the form ultimately used, in most natural waters NH_4^+ will be the most abundance species.

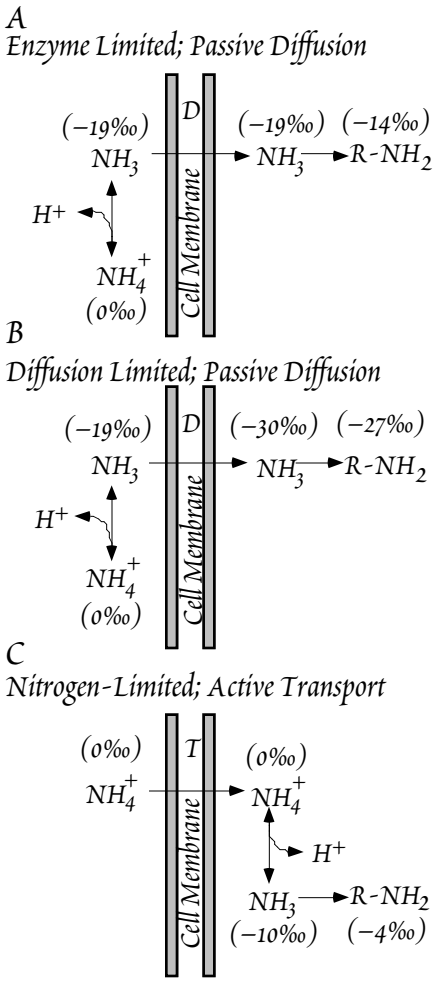

Figure 27.6. Fractionation of Nitrogen during assimilation of autotrophs. After Fogel and Cifuentes (1993).

There are two principle reactions by which ammonia is incorporated into organic matter: formation of glutamate from α-ketoglutarate via the glutamate dedhydrogenase reaction, and formation of glutamine from gluatmate via the enzyme glutamine synthetase. A positive fractionation (i.e., the product is enriched in ^{15}N) of +2 to +4 has been measured for the glutamate dedhydrogenase reaction, and the fractionation for the glutamine synthetase reaction is also expected to be positive, because N is bound more strongly in the product than in ammonia.

While isotope fractionations during assimilation of ammonium are still poorly understood, it appears there is a strong dependence on the concentration of the ammonium ion. As with carbon, fractionation may occur in each of several steps that occurs in the nitrogen assimilation process. A model proposed by Fogel and Cifuentes (1993) describes this dependence as:

$$\Delta = E_q + D + (C_i/C_o)(E_{enz} + D) \qquad 27.4$$

where Δ is the difference in $\delta^{15}N$ between ambient NH_4^+ and nitrogen in tissues, E_q is the equilibrium fractionation between NH_3 and NH_4^+, D is the fractionation associated with diffusion of NH_3 in and out of the cell, C_i/C_o is the ratio of concentration of ammonia inside to ammonia outside the cell, and E_{enz} is the fractionation associated with enzymatic fixation of NH^-. This model is illustrated in Figure 27.6a for the case where the abundance of the enzyme is limiting, and 28.6b for the case where diffusion is limiting.

As we saw for CO_2, when ammonium concentrations are low, which they generally are in natural waters, plants and bacteria have the capacity to actively transport it across cell membranes. Where active transport occurs, Fogel and Cifuentes (1993) propose the following model for fractionation:

$$\Delta = (F_3/F_1)(E_q + E_{enz}) \qquad 27.5$$

where (F_3/F_1) is the ratio of NH_4^+ leaked from the cell to the ratio of NH_3 and NH_4^+ inside the cell. This model is illustrated in Figure 27.6b.

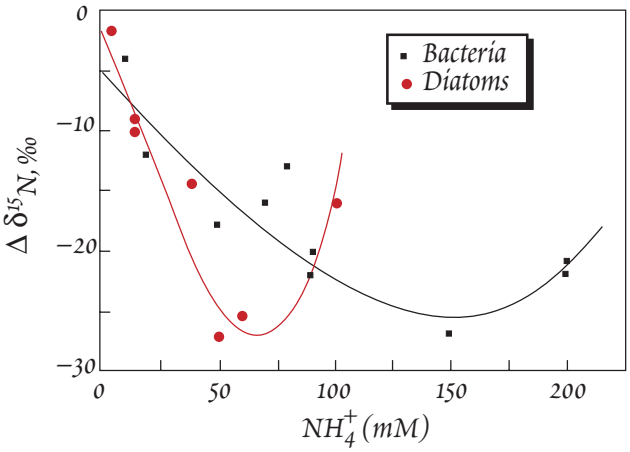

Figure 27.7. Dependence of nitrogen isotope fractionation by bacteria and diatoms on dissolved ammonium concentration.

These models imply, as for carbon, a dependence of fractionation on the abundance of ammonium. Such dependence has been observed, as for example in Figure 27.7. The complex dependence in Figure 27.7 is interpreted as follows. The increase in fractionation from highest to moderate concentrations of ammonium reflects the switching on of active ammonium transport by cells. At the lowest concentrations, essentially all available nitrogen is transported into the cell and assimilated, so there is little fractionation observed.

The isotopic compositions of marine particulate nitrogen and non-nitrogen-fixing plankton are typically –3‰ to +18‰ $\delta^{15}N$. Terrestrial plants have a similar range, –5‰ to +18 per mil, but are isotopically lighter on average. Marine blue-green algae range from –2 to +4‰ and nitrogen-fixing terrestrial plants range from –6 to +6‰. The reason for this difference may in part reflect the large fractionations that occur during nitrification:

$$NH_4^+ \rightleftarrows NO_2^- \qquad \alpha = -1.02 \text{ to } -1.035$$

and denitrification:

$$NO_3^- \rightleftarrows N_2 \qquad \alpha = -1.02$$

This leaves residual dissolved ammonia and nitrate, which is utilized by non-nitrogen-fixing plants, that is isotopically heavy. Plants capable of fixing nitrogen utilize atmospheric or dissolved N_2 (only a very small fractionation occurs when N_2 dissolves in water), which has $\delta^{15}N \approx 0$.

Oxygen and Hydrogen Isotope Fractionation by Plants

Oxygen is incorporated into biological material from CO_2, H_2O, and O_2. However, both CO_2 and O_2 are in oxygen isotopic equilibrium with water during photosynthesis, and water is the dominant form. Therefore, the isotopic composition of plant water determines the oxygen isotopic composition of plant material. The oxygen isotopic composition of plant material seems to be controlled by exchange reactions between water and carbonyl oxygens (oxygens doubly bound to carbon):

$$\diagdown\!\!\diagup C = {}^{16}O + H_2{}^{18}O \longleftrightarrow \diagdown\!\!\diagup C = {}^{18}O + H_2{}^{16}O$$

Fractionations of +16 to +27‰ (i.e., the organically bound oxygen is heavier) have been measured for these reactions. Consistent with this, cellulose from most plants has $\delta^{18}O$ of +27±3‰. Other factors, however, play a role in the oxygen isotopic composition of plant material. First, the isotopic composition of water varies from $\delta^{18}O \approx -55$‰ in Arctic regions to $\delta^{18}O \approx 0$‰ in the oceans. Second, less than complete equilibrium may be achieved if photosynthesis is occurring at a rapid pace, resulting in less fractionation. Finally, some fractionation of water may occur during transpiration, with residual water in the plant becoming heavier.

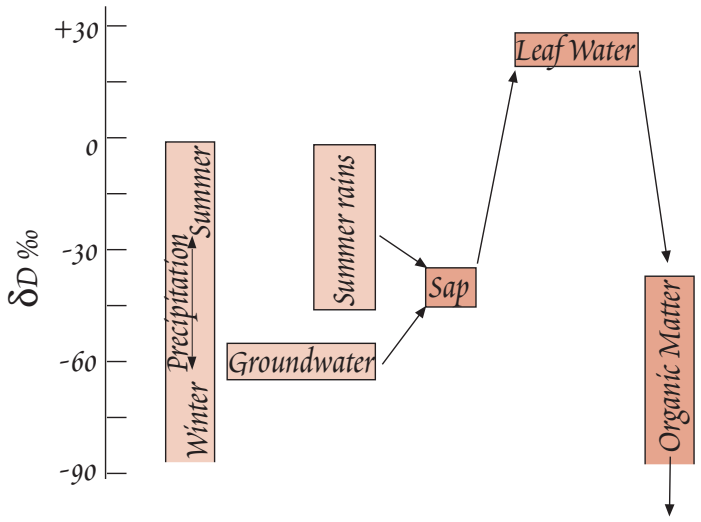

Figure 27.8. Isotopic Fractionations of hydrogen during primary production in terrestrial plants. After Fogel and Cifuentes (1993).

Figure 27.8.

Hydrogen isotope fractionation during photosynthesis occurs such that the light isotope is enriched in organic material. In marine algae, isotope fractionations of –100 to –150‰ have been observed, which is similar to that observed in terrestrial plants: –86 to -120‰. Among terrestrial plants, there appears to be a difference between C_3 and C_4 plants. The former fractionations of –117 to –121‰, while fractionations -86 to –109‰ have been observed in C_4 plants. However, little is known in detail about the exact mechanisms of fractionation.

As is the case for oxygen, variations in the isotopic composition of available water and fractionation during transpiration are important in controlling the hydrogen isotopic composition of plants. This is illustrated in

BIOLOGICAL FRACTIONATION OF SULFUR ISOTOPES

Though essential to life, sulfur is a minor component is living tissue (C:S atomic ratio is about 200). Plants take up sulfur as sulfate and subsequently reduce it to sulfide and incorporate into cysteine. There is apparently no fractionation of sulfur isotopes in transport across cell membranes and incorporation, but there is a fractionation of +0.5 to –4.5‰ in reduction process, referred to as *assimilatory sulfate reduction*. This is substantially less than the expected fractionation of about -20‰, suggestingthat nearly all the sulfur taken up by primary producers is reduced and incorporated into tissue.

Sulfur, however, plays two other important roles in biological processes. First, sulfur in the form of sulfate can act as an electron acceptor or oxidant, and is utilized as such by sulfur-reducing bacteria. This process, in which H_2S is liberated, is called *dissimilatory sulfate reduction* and plays an important role in biogeochemical cycles, both as a sink for sulfur and source for atmospheric oxygen. A large fractionation of +5 to –46‰ is associated with this process. This process produces by far the most significant fractionation of sulfur isotopes, and thus governs the isotopic composition of sulfur in the exogene. Sedimentary sulfate typically has $\delta^{34}S$ of about +17, which is similar to the isotopic composition of sulfate in the oceans (+20), while sedimentary sulfide has a $\delta^{34}S$ of –18. The living biomass has a $\delta^{34}S$ of ≈ 0.

The final important role of sulfur is a reductant. Sulfide is an electron acceptor used by some types of photosynthetic bacteria as well as other bacteria in the reduction of CO_2 to organic carbon. Most unique among these perhaps are the chemosynthetic bacteria of submarine hydrothermal vents. They utilize H_2S emanating from the vents as an energy source and form the base of the food chain in these unique ecosystems. A fractionation of +2 to – 18‰ is associated with this process.

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