Invited Paper

Extremophiles and Chemotrophs as Contributors to Astrobiological Signatures on Europa: A Review of Biomarkers of Sulfate-Reducers and Other Microorganisms

Joseph Seckbach* and Julian Chela-Flores**

*The Hebrew University of Jerusalem, Home: P.O.B. 1132, Efrat 90435 Israel. e-mail: seckbach@huji.ac.il, URL: **www.springer.com/series/5775**
**The Abdus Salam International Centre for Theoretical Physics, Strada Costiera 11; 34014 Trieste, Italy, and Instituto de Estudios Avanzados, Apartado Postal 17606 Parque Central, Caracas 1015A, R. B. Venezuela. e-mail: chelaf@ictp.it, URL: http://www.ictp.it/~chelaf/index.html

ABSTRACT

Microorganisms occupy almost every habitable niche on Earth. Some of them may use inorganic or organic substances rather than light as energy sources. We refer to this group as chemotrophs in order to distinguish them from those organisms that use light as an energy source (phototrophs). Both chemotrophs and phototrophs are abundant in environments where one or more physical, or chemical parameters show values far from the lower or upper limits known for life. These ambient habitats are referred to as normal environments. When microorganisms succeed in adapting themselves to harsh niches they are referred to collectively as extremophiles (Seckbach 2004, 2006).

We review arguments that militate in favor of microorganisms that in the past and present may have occupied other niches in the Solar System. Among the extremophiles we find representatives of the three domains of life (*Bacteria*, *Archaea*, and *Eukarya).* Some of the possible candidates for life in the Solar System are the extremophiles including chemotrophs, especially sulfur-reducing bacteria (SRB). Another example of chemotrophs are the methanogens. Those microbes are capable of producing methane as a metabolic byproduct of the reduction of carbon dioxide, a process that is called methanogenesis. Searching for new forms of life (within the extraterrestrial regions) is the object of planning within the Cosmic Vision Program of ESA, in collaboration with NASA, and other space agencies. Indeed, sulfur traces on Jupiter's moon Europa detected by the Galileo mission have been conjectured to be endogenic, most likely of cryovolcanic origin, due to their non-uniform distribution in patches. The Galileo space probe first detected the sulfur compounds, as well as revealing that this moon almost certainly has a volcanically heated and potentially habitable ocean hiding beneath Europa surface layer of icy water. In this paper we restrict our attention to possible biomarkers that could signal on Europa the presence extremophiles in general and chemotrophs, especially the presence of sulfur reducers.

Keywords: Extremophiles, Europa, SRB, microorganisms, TSR, Sulfur metabolism.

1. CAN WE DISTINGUISH LIFE FROM NON-LIFE ELSEWHERE IN THE SOLAR SYSTEM?

Life has existed on Earth for at least for 3.5 to 3.8 billion years. Sulfur-reducing microorganisms including bacteria and archeans (SRB) have some groups occupying deeply rooted branches on the

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phylogenetic tree of life. These microorganisms represent some of the earliest-arising lineages that show fractionations like those of contemporary SRBs on Earth (Dertmers *et al,* 2001). In order to investigate the early history of SRBs, there are three possible lines of approach, given current knowledge and technology (Shen and Buick, 2004). Firstly, one could search for the preserved remains of the organisms themselves, but they are difficult to distinguish from other microfossils (Knoll, 1992). Secondly, specific molecules that may have been preserved in the geologic record have also a difficulty with respect to the SRBs, since only young sediments can be trusted to preserve such molecules (Taylor and Parkes, 1985). Finally, isotopic fractionation has been considered for a long time one of the most reliable methods (Kaplan, 1975, Chela-Flores, 2006). We shall dwell on this avenue for selecting out the most reliable biomarkers that would be possible for Europa. But we hasten to point out that for the study of early life an answer to the following requires special attention:

- *What characteristics of life (structural and biogeochemical) also are produced by abiogenic processes and, consequently, how can we distinguish between signatures of past life and signatures of nonlife?* (Westall, 2005).

Fortunately, the question of distinguishing between signatures of past life and signatures of nonlife in the sulfur cycle of the Earth has been carefully discussed (Machel *et al,* 1995, Machel, 2001). Distinguishing criteria of bacterial and **T**hermochemical **S**ulfate **R**eduction (TSR) have been considered. Redox-reactions occur, whereby sulfate is reduced by hydrocarbons with concomitant oxidation of the organic compounds, either bacterially or abiotic inorganically. These two processes form similar products and by-products. The mere presence of any of reaction products does not permit discrimination of biogenic processes due to SRB from nonlife processes (i.e., TSR). Relationships arising from the study of rocks including their mode of origin, chemical and mineral composition (petrological studies) may help towards the necessary distinction, but additional stable isotope and gas-chromatographic data are often needed. In particular, to differentiate products of life from nonlife we can use the isotopic data through the "delta functions" of sulfur and carbon (to be defined in Sections. 3 and 5 respectively). Alternatively, elemental, and chromatographic analysis can be used. We shall return to this topic in Sec. 6.

Extremophilic SRBs may serve as models for microbes that could perhaps live under the harsh conditions that exist on extraterrestrial environments. Life represented by terrestrial extremophiles may have existed in the past elsewhere in the Solar System. A discussion on the possibility of ancient life elsewhere in the Solar System includes Venus in its early cytherean oceans (Seckbach and Libby, 1970). Terrestrial microorganisms can live in similar conditions as presumably primordial biota autochthonous to ancient Venus under high CO_2, elevated temperature, and even tolerate sulfuric acid. One such microorganism is *Cyanidium caldarium* (Seckbach, 1992; 1994a, 1999, Pinto, 2007). At present the absence of liquid water on the planetary surface excludes Venus from the high priority searches from the point of view of astrobiology. Mars is a well-known case that has been extensively pursued by the space agencies and is prominent in the ESA Cosmic Vision Program (2005), as well as the European Exploration Program (2007). NASA has been involved in this respect since the early Mariner missions. (Other possibilities for the presence of microorganisms are on the moons of Saturn: Enceladus and Titan.)

However, the focus of our attention is guided by the remark that planets forming at a few astronomical units from the Sun, such as Jupiter, are likely to have retained significant quantities of materials as volatile as water, rather than ammonia, and methane. Sulfur is clearly an important element in the geophysics, oceanography and evolution of this Galilean satellite. Models of Europa suggest that a type of chondrite carry sufficient amount of water (13.35%), carbon compounds (2.46%) and sulfur (3.25%) to stand as good models of the planetesimals that gave rise to the proto-Europa (Oro *et al.,* 1992). The meteorite in question is petrographic type-2 carbonaceous chondrite of chemical class CM, i.e., similar to the prototypical Mighei meteorite (Cronin and Chang, 1993). Other models have been discussed during the last decade independently (Kargel *et al.,* 1999). There would have been also sufficient carbon input for eventually inducing a substantial biota capable of deriving its energy from redox reactions (cf., Sec. 5). This shows that in this model of Europa, collisions with the proto-satellite planetesimals of this composition would have carried with them sufficient amounts of water to account for an ocean.

2. ON THE NATURE OF THE ICY SURFICIAL PATCHES OF EUROPA

The relatively young surface of Europa is significant for two aspects: firstly, its lack of numerous craters, and secondly its patches of sulfur that were detected by the Galileo mission (for references, cf., Chela-Flores, 2006). The first remarkable feature has been gradually understood in terms of resurfacing and cryovolcanism ever since the low-resolution images of the Voyagers became available (Fagens, 2003). Cryovolcanism has been defined as the eruption of liquid or vapor phases (with or without entrained solids) of water or other volatiles that would be frozen solid at the normal temperature of the icy satellite's surface (Geissler, 2000).). At the time of the Voyager Mission, cryovolcanic resurfacing is assumed to be the most likely explanation for the relatively young surface. At resolutions much better than those of Voyager images, Galileo data acquired between 1996 and 2003 show that some of the features previously thought to be cryovolcanic are best explained by other formative mechanisms, including tectonism and diapirism (diapers are intrusions of relatively light material into pre-existing ice, doming the overlying icy surface).

The second remarkable feature of the icy surface, namely its icy patches, remain a significant aspect of our knowledge on Europa, being potentially rich of information on the nature of its ocean. If there was life on Europa, *in-situ* studies of ocean water chemistry, or of minerals deposited at the ocean floor (and subsequently raised to the surface) may carry a fingerprint of the biota itself. The chemistry of the Europan Ocean should give some indication of how the sulfur reservoir has been processed between sulfide and sulfate. As we discussed above, models of proto-Europa imply that there can be sulfur in its core that could be in contact with the bottom of the ocean (unlike the cases of the probable oceans of Ganymede and Callisto). In that case rock-water interactions are relevant, as we could conceive of an Europan hydrothermal system associated with volcanic eruptions at the seafloor, in analogy with the multiple examples known in the Earth oceans, where hydrothermal systems occur along mid-ocean ridges, or in isolated volcanic locations, such as Hawaii and the Solfatara volcanic area near Naples, Italy. Such rock-water interactions arise from the heat of therocks that induces convective fluid circulation, drawing seawater down into the crust. Duringcirculation and heating, the fluid reacts with the rocks in the subsurface, altering the composition of the fluid, as well as the chemical composition of the rocks (McCollom, 1999). Theheated fluid then rises buoyantly back to the ocean floor where it mixes with seawater.During mixing, chemotrophs can use energy liberated from chemical reactions among dissolvedcompounds in seawater as energy sources. Future missions now being planned for returning to Europa should search for such contact of the ocean and the satellite core that would contribute to the redox state of the ocean and be the source of an autochthonous biota. These remarks suggest the need for a detailed discussion of geochemistry, as we shall do in the next few sections.

3. A WIDELY ACCEPTED HYPOTHESIS

Sulfur consists largely of two stable isotopes, ^{32}S and ^{34}S, which have natural abundance of 95.02% and 4.21%, respectively (Ryu, et al. 2006). Bacterial sulfate reduction results in the depletion of ^{32}S in the remaining dissolved sulfate and the enrichment of ^{32}S in sulfide. The redistribution of the primordial isotopic mixtures can be followed up in terms of the appropriate parameter, namely:

$$\mathbf{delta\ {}^{34}S = [({}^{34}S/{}^{32}S)_{sa} / ({}^{34}S/{}^{32}S)_{st} - 1] \times 10^3\ [{}^{0}/_{00}, CDT]}$$

For simplicity this function will be referred to as the delta ^{34}S parameter, or simply as the delta parameter. Its value is close to zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite that is a triolite (FeS), abbreviated as CDT. This parameter allows a comparison of a sample (sa) with the standard (st) CDT. The relevant terms are the dominant sulfur isotope (^{32}S) and the next in abundance (^{34}S). In fact, ($^{34}S/^{32}S$)st coincides with the average terrestrial fraction of the two most abundant isotopes of sulfur. We obtain positive values of the delta-parameter when by comparison we have a larger quantity of the less abundant isotope ^{34}S. Once the primordial planetary mantle material (for example, on the Earth), or satellite internal silicate nucleus (for example, Europa) had entered their corresponding geochemical cycles, their initial isotope mixtures began to be redistributed. The Earth upper mantle and crust are believed to reflect broadly the isotopic distribution patterns of chondritic meteorites (Libby, 1971). An analogous case should apply to Europa.

If the CDT meteorite was typical of the materials from which the Earth was formed, this similarity of the isotopic abundances tells us that the process of the formation of the Earth had little influence on the observed isotopic fractionation of sulfur between sulfide and sulfate in the present environment. This has led to the widely accepted hypothesis that the global delta parameter for sulfur has always been near to zero. There is a considerable body of evidence that supports the view that at earth-surface temperatures remarkable fractionation is associated with biologically catalyzed reactions. At higher temperatures ($> 100^0$ C) generally smaller fractionations may be induced inorganically (Machel *et al,* 1995). Indeed, it should be kept in mind that the total reservoir of isotopic-biased sulfur will reveal isotope fractionation of sulfur that takes place during hydrothermal reduction of seawater sulfate. These fractionations vary as a function of temperature, giving rise to isotopic-heavy sulfates that are easily distinguished from marine sulfate in the geologic record. Isotopic depletions can also take place during magmatic reduction of gaseous SO_2 to H_2S, giving rise to delta ^{34}S depletions in the range $15\text{-}20^{0}/_{00}$. Therefore, we should be cautious with the interpretation of S-signatures, especially due to a possible hydrothermal source of whichever sample is being considered.

4. MICROORGANISMS ABLE TO WITHSTAND EXTREME SOLAR SYSTEM CONDITIONS

PSYCHROPHILES. Organisms living in cold habitats are called psychrophiles. They are distributed in places such as the Arctic and the Antarctic, where sea-ice organisms thrive in the ice (Thomas and Dieckmann, 2002; Junge *et al.,* 2004). Other psychrophiles live in the permafrost of Siberia. Some microbes can resist a frozen period and may survive subzero temperatures, and then germinate under warmer conditions. Furthermore, bacterial spores are almost immortal and can retain their viability for millions of years under harsh conditions and finally be revived. In the Antarctic there are icy environments that contain many planktonic organisms including bacteria, algae (most conspicuous are the pinnate diatoms) protists, flatworms and small crustaceans. Bacterial activity has been documented at -20^0 C—making the limits of life on Earth wider. Psychrophilic SRBs are known in sediments of permanently cold areas around Svalbard and northern Norway (Sagemann *et al.,* 1998). Other relevant examples of psychrophilic SRB are: *Desulfofrigus oceanense, Desulfotalea psychrophila* (Dertmers et al, 2001; Gunde-Cimerman, 2006).

MESOPHILES. In contrast with all of the methanogens cultured from submarine hydrothermal vents to date, which are thermophiles (cf., below), numerous aerobic chemotrophs living at hydrothermal vents areonly capable of growth at lower temperatures: They are called mesophiles. The isotope fractionation of 26 mesophilic SRBs has been recently characterized (Dertmers *et al*, 2001).

THERMOPHILES. At the other end of the thermal scale, thermophiles, or the heat loving microorganisms (Bonch-Osmolovskaya, 2006), are ubiquitously found in hot springs and hot locations, such as the Solfatara volcanic area. Thermophilic cyanobacteria inhabit an even higher temperature range, living in neutral, or alkaline hot springs at temperatures of up to ~70^0 C. *Bacteria* and *Archaea* are abundant in high temperatures and inhabit most hot environments. The extremophiles have adapted to active hydrothermal vents (Jørgensen *et al.,* 1992). Relevant examples of a thermophilic SRB that have been recently characterized as: *Desulfotomaculum geothermicum, Desulfotomaculum thermocisternum, Thermodesulfobacterium commune, Thermodesulfovibrio yellowstonii* (Dertmers *et al*, 2001).

HYPERTHERMOPHILES. Most phylogenetic models predict that the first microorganisms may have been hyperthermophiles (Rossi *et al.,* 2003; Seckbach, 1994b). Following the Hadean era, Earth's subsurface remained hot due to the meteorite bombardments; the oceans might have been heated up to just over 100^0 C (Rossi *et al.,* 2003). Hyperthermophiles could have been the first living pioneers within such hot environments, or the only survivors following such sterilizing hot events. It has been proposed that such hyperthermophiles may also serve as candidates for microorganisms in Solar System bodies that may have similar physical conditions as occur in extremely hot environments on Earth. Among the hyperthermophiles are the prokaryotes, *Bacteria* and *Archaea* that have been observed distributed from 80 ^{0} C to temperatures higher than 100 ^{o} C. Stetter and his coworkers have determined the uppermost temperature of life in *Pyrolobus fumarii* at 113^0 C (Blochl *et al.,* 1997), and this organism can survive incubation in an autoclave at 121^0 C for over 1 hour. Such hyperthermophiles may have been the initial microbes that evolved in the depth of the hot oceans, or the only survivors in the primeval environment (Rossi *et al.,* 2003). In the depth of the subsurface and deep in the oceans these microbes could also have been protected from harmful UV radiation during the primeval era of Earth, as well as shielded from the meteorites' impact during the early states of the Earth. A relevant recent characterization of a hyperthermophilic SRB is: *Archeaoglobus fulgidus* (Dertmers *et al,* 2001).

ACIDOPHILES, ALKALIPHILES AND THERMOACIDOPHILES. The acidophiles thrive in lower ranges of pH (Seckbach, 2000), such as in sulfur hot springs. On the other side of the pH scale are the alkaliphiles that live in alkaline environments, such as in the African soda lakes. Some microorganisms combine diverse adaptations to extreme environments, for example the red unicellular thermoacidophilic *Cyanidium caldarium* is an enigmatic alga (Seckbach, 1992, 1994a, Pinto, 2007). This rhodophytan grows in very low pH areas (cf., 'halophiles below), at temperatures up to 56–57^0C. It thrives in media very well bubbled with pure CO_2 (Seckbach *et al.,* 1970). Its cohorts in the family Cyanidiaceae cohabit the same elevated temperature and low pH environments (Seckbach, 1992). Measurements from the Galileo Mission imply that light reflected from the moon's icy surface bears the spectral fingerprints of hydrogen peroxide and strong acids, perhaps close to pH 0, if liquid (Carlson *et al.,* 1999b).

HALOPHILES. The halophiles are organisms living in very salty environments, such as in salt lakes, salt mines or saline ponds sometimes containing saturated salt solutions. In the seas and oceans the concentration of the salts is 34 g per liter, while the Dead Sea may reach 10 times as much dissolved salts. Some halophiles (e.g., the green alga *Dunaliella salina*) accumulate organic compounds, such as glycogen or β carotene. Its internal content of glycerol balances the external high osmotic pressure.

High brine salinities may cause major dehydration stress for ice-trapped organisms. Square and triangular halophilic *Archaea* have been reported in salt media (Oren, 1999). A review on the halophilic environments has been published (Oren, 2002, 2006). Significantly, evidence for a conducting layer near the surface of Europa has been obtained by the Galileo Mission: Indeed, the Galileo magnetometer measured changes in the magnetic field predicted if a current-carrying outer shell, such as a planet-scale liquid ocean, is present beneath the icy surface. The evidence that Europa's field varies temporally strengthens the argument that a liquid ocean exists beneath the present-day surface. The most likely explanation is that Europa has a salty, global water ocean beneath its ice shell (Kivelson *et al,* 2000).

5. THE REDOX STATE OF THE EUROPAN OCEAN AND HINTS OF BIOGENIC ACTIVITY

Learning about the operation of the sulfur cycle on Europa would be expected to yield analogous information as we can expect from the sulfur cycle on the Earth. In this context, three clear and significant properties of the Earth SRBs are relevant:

- Sulfate-reducers have some groups occupying deeply rooted branches on the phylogenetic tree representing some of the earliest-arising lineages that show fractionations like those of contemporary SRBs (Dertmers *et al,* 2001). Hence, it is likely that ancient sulfate-reducing organisms fractionated isotopes in a similar manner to contemporary SRBs.
- A comprehensive study of the relation between sulfate reduction rates and temperature demonstrate that rates of sulfate reduction for a wide variety of SRBs are not coupled to temperature (Canfield *et al.,* 2000).
- Data on isotopic fractionation by both pure cultures and natural populations of sulfate-reducing microbes show that isotopic fractionations during sulfate reduction by SRBs from psychrophiles to thermophiles have manifestly proved to be independent of specific reduction rates (Brüchert *et al.,* 2001).

These three properties together suggest that the minimally fractionated early Archean sulfides are due to either biological sulfate reduction at low sulfate concentrations, implying poorly oxygenated oceans, or a non-biological volcanogenic origin, hence sulfate-reducers had not yet evolved (Shen and Buick, 2004). Hints of the sulfur cycle on Europa are promising targets for the most reliable biomarkers that are possible within the feasible technological possibilities that are now being considered for the next stages of the exploration of Europa. The spectroscopic signature of this Galilean satellite indicates water-ice composition, with minor amounts of sulfur compounds (Carlson *et al.,* 1999a, 2002), and hydrated salt minerals such as magnesium sulfates or sodium carbonates (McCord *et al.,* 1998, 1999) likely acting as contaminants to produce briny (cryomagmatic) fluids.

What sort of information could we draw on the putative Europan biota from the Galileo Mission discovery of surficial sodium carbonates? The abundances of ^{13}C in ocean water carbonate and in fresh organic material have taught us that these elements are determined by (Strauss *et al.*, 1992):

- Relative rates of immobilization of oxidized and reduced carbon in sediments and,
- Isotopic fractionations imposed by the biological carbon cycle.

Knowledge of variations of these abundances that could be retrieved from our return to Europa is, therefore, of great interest. Much would be learnt about the Europa global environment and the evolution of the autochthonous Europan biota if we could take the first steps in reconstructing the cycles of the biogenic elements, especially carbon and sulfur, and to a lesser extent iron, as there are ways to use the available isotopic systems of Fe to examine interactions between the biosphere and the abiotic environment (Archer and Vance, 2006). A review of recent work on biologically mediated iron redox reactions has been published (cf., Konhauser, 2007, Box 7.10). In the context of the carbonates detected by the Galileo Mission a definite possibility is that they may carry some significant biosignatures carry (together with organic carbon). The delta ^{13}C [$^{\mathbf{0}}/_{\mathbf{00}}$, PDB] parameter is defined as follows:

$$\textbf{delta}\ ^{13}\mathbf{C} = [(^{13}\mathbf{C}/^{12}\mathbf{C})_{sa} / (^{13}\mathbf{C}/^{12}\mathbf{C})_{st} - 1] \times 10^{3}\ [^{0}/_{00}, \mathbf{PDB}]$$

The value of delta ^{13}C is close to zero when the sample coincides with the PeeDee belemnite standard (PDB) in which (^{13}C/^{12}C) = 88.99 and delta ^{13}C is defined as equaling 0.00 $^{o}/_{oo}$. This parameter can be used as a good biosignature. On the Earth biota, for instance, there is ample evidence that photosynthetic bacteria, algae and plants have typical significant deviations that yield values of up to - 30 and beyond, due to biological processes (Schidlowski *et al.,* 1983). These results are analogous to the deviations shown by fractionation due to bacterial sulfate reduction. Yet, without prior knowledge whether we are in the presence of life in a given environment, negative values of the delta ^{13}C parameter do not arise exclusively from biogenic sources. For this reason we have mentioned above that sulfur is more relevant for studying possible biosignatures. But carbonates that the Galileo Mission detected on the icy Europan surface may add some further information on the evaluation of the redox state of the Europan ocean. Although inorganic input to the fractionation of carbon and sulfur is possible, the very extreme values of the delta ^{34}S parameter, up to 70$^{o}/_{oo}$ are clearly associated with biogenic processes (cf., Sec. 6). For instance, for abiogenic sources:

- Thermochemical sulfate reduction (TSR) of organic compounds that are gaseous has been characterized for gas condensates where TSR delta ^{13}C for CH_4 ranges from – 46 to -35 (Machel, 2001), which are ranges comparable to values of biogenic organic carbon (Strauss *et al.,* 1992).

In comparison with the isotopes of hydrogen, carbon or nitrogen, only sulfur shows fractionation with a relatively narrow distribution range in meteorites, as well as the Moon fines and breccias **(*)** fine-grained basalts retrieved by the Apollo missions earlier (Kaplan, 1975, Chela-Flores, 2006; 2007). In the case of meteorites these values are about 2$^{o}/_{oo}$ relative to the standard CDT average (Farquhar and Wing, 2005).

(*) Amongst the materials recognized on the lunar regolith we can distinguish breccias that is rocks composed of fragments derived from previous generations of rocks, and ‘fines’ that is soils with grain sizes down to dimensions smaller than one micrometer.

6. DISCUSSION OF DISTINGUISHING CRITERIA FOR LIFE AND NONLIFE

6.1. S REDUCTION OF BSRs AND THERMOCHEMICAL SULFUR REDUCTION

Some criteria are necessary to distinguish reaction products of the action of BSR. Bacteria Sulphur Reducers and TSR. In the case of sulfur isotopic ratios during the most important redox reactions have been discussed earlier (Machel *et al.,* 1995). In various cases where the fractionation has been studied it is generally agreed that isotopic equilibrium exchange is irrelevant or at any rate small. For instance,

- The kinetic isotope fractionation during oxidation of sulfide to sulfur whether inorganic or microbial is very small in most cases. Thus elemental sulfur would tend to have an isotopic composition similar to its sulfide parent. Besides, the fractionation between H_2S and S^0 is so close to zero at temperatures compatible with inorganic sources ($>80^0$ that isotopic equilibrium exchange is irrelevant [Tudge and Thode, 1950]).
- The kinetic fractionations during microbial and abiological oxidation of S^0 to sulfate are close to zero, as demonstrated earlier for mixed cultures (Nakai and Jensen, 1964) and $0^0/_{00}$ for *Thiobacillus* spp. (Fry *et al.,* 1986). The general trend is that very large initial fractionations ($>> - 20^0/_{00}$) are realized by BSRs, whereas much smaller ones ($< - 20^0/_{00}$) are ascribed to TSRs. However, in some cases small, or no kinetic differences, are realized. In this case, the effects of TSR cannot be distinguished from those produced by BSRs. Thus, for small kinetic differences, isotopic data alone of inorganic sulfur compounds cannot distinguish between life and no-life. Supplementary instrumentation will be needed in the case of Solar System exploration that focuses on sulfur as biomarkers on the icy surfaces of satellites and of the Outer Solar System (cf., next paragraphs.)

6.2. RETURNING TO EUROPA

We are in the process of planning which kind of mission will return to Europa as a collaboration of ESA with the other main space agencies. With all the previous experience (Ball et al., 2007) it is timely to raise the following question: Which are the appropriate payloads that will have the best opportunity to distinguish life from nonlife on the icy surface of Europa?

Although the sulfur patches seen on its icy surface might bear significant biomarkers, to distinguish biogenic from abiogenic sulfur, we should indeed go back to Europa. As in the case of the Earth sulfur cycle, the wide range of sulfur isotope values may imply that several different isotopic fractionation processes may have taken place. These include non-biological sources and ocean water sulfate reduced to sulfide by sulfate reducing bacteria, and then reoxidized by sulfide oxidizers, before being reduced again by sulfate reducers, if such chemothrophs are in existence in the Europan ocean (Grassineau *et al,* 2001). Any biogenic sulfur probably has been recycled many times. *In situ* stable isotope analysis may contribute to providing a significant biomarker. Clearly, on Europa complications due to repeated high-temperature volcanic recycling and fractionations due to global volatile loss have not occurred. Coexisting dissolved sulfide and sulfate from hypersulfidic interstitial waters sediments show a large isotopic difference of up to 72 ‰ that is caused by *in situ* microbial sulfate reduction (Wortmann *et al,* 2001). Such a large effect is not generated by non-biological processes (Machel, 2001, Machel *et al.,* 1995, Shen and Buick, 2004). Some points should be kept in mind in this context, including an assessment of non-biological sulfur isotopic fractionation (Rollinson, 2007), such as:

- Hydrothermal sulfate reduction by Fe^{2+}-bearing minerals (Shen and Buick, 2004),
- Magmatic hydrolysis of SO_2, (Shen and Buick, 2004) and
- TSR by hydrocarbons in diagenetic environments (Machel, 2001, Machel *et al.,* 1995, Ohmoto, 1992).

For the above reasons S isotope analysis is a valuable tool to be used in conjunction with other approaches for planetary exploration. *In situ* analysis of the sulfur patches, especially its carbon signatures could yield a clearer interpretation of the sulfur isotopes signatures. Geological and biogeochemical data from many sources of the Precambrian demonstrate that pyrites and evaporates were formed biologically by dissimilatory sulfate reduction (Schildowski *et al*, 1983, Konhauser, 2007). Rocks of Archean age [older than 2.5 Gyr BP] provide the best evidence of early metabolic processes. Their study allows reconstruction of the biogeochemical cycle for sulfur since the origin of life on Earth. The remarkable sulfur icy patches on the Europan surface will inevitably be targets for future space missions that are expected to return to Europa in the next decade. With landers, or low-cost penetrators that could first of all be tried out on the Lunar surface (Smith and Gao, 2007), we would be in a position to test the redox state of the Europan ocean. Alternatively, the imprint of the possibly biogenic signature of the surficial sulfur would be retained in the dust cloud that surrounds this singular jovian satellite (Kruger *et al.,* 2003). The arguments in the present paper militate in favor of mass spectrometry as the instrumentation for future dust detectors in Europan orbit (Taylor *et al.,* 2007): the payloads should however be complemented with additional instrumentation to single out the sulfate reducer biomarkers (such as gas chromatography-mass spectroscopy, GCMS).

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