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## MICROBIAL MATS IN ANTARCTICA AS MODELS FOR THE SEARCH OF LIFE ON THE JOVIAN MOON EUROPA

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### 1. Introduction

The discovery of extreme environments with organisms adapted to these conditions have made them useful analogies for the presence of life beyond the Earth, whether autochthonous, or by the transport of microorganisms between different bodies of the Solar System. This latter possibility has been known as the hypothesis of panspermia.

These microorganisms not only tolerate harsh environmental conditions, but even thrive in them. For this reason such organisms are called 'extremophiles'. The occurrence of oxygenic phototrophs in extreme environments has been reviewed extensively (Seckbach and Oren, 2007), including phototrophic thermophiles (adapted to higher temperatures) that are able to survive up to 74°C. Certain archaea may survive in environments up to 114°C. Halophiles (high salt-loving microorganisms) tolerate salt concentration up to saturation. Acidophiles thrive at pH values as low as 0.5. Alkaliphiles living at high pH may survive up to pH 10-13.

The extreme environments and their microbes can thus act as models for extraterrestrial life (Seckbach and Chela-Flores, 2007). Active photosynthetic microbial communities (discussed in Section 2) are found on Antarctica, both in and on ice, in fresh water, in saline lakes and streams and within rocks. In the dry valley lakes of Antarctica close to the McMurdo Base, microbial mats (discussed in Sections 2 and 3) are known to selectively remove a huge quantity of sulfur (Parker *et al.*, 1982). Lake Vostok in Antarctica (discussed in Section 4) possesses a perennially thick (3 to 4 km) ice-cover that precludes photosynthesis below, thus making it a good model system for determining how a potential European biota might survive (Stone, 1999). The presence of liquid water is a prerequisite for life (Oren, 2008).

Jupiter's moon Europa may harbor a subsurface water ocean. This putative ocean lies beneath an ice layer that is too thick to allow photosynthesis. However, that disequilibrium chemistry in the icy surface, driven by charged particles that are

accelerated in Jupiter's magnetosphere could produce sufficient organic and oxidant molecules on an European biosphere (Chyba, 2000).

We restrict our attention to microbial mats that could be thriving under extreme conditions of radiation on Europa. We are especially concerned by the presence of the sulfur patches discovered by the Galileo mission.

## 2. Astrobiological implications of the microbial mats in the dry valley lakes

Microbial mats are stratified microbial communities that develop in the environmental microgradients established at the interfaces of water and solid substrates. The organic laminated multilayered biofilm has hydrated exocellular polymeric substances (EPS) secreted by microorganisms embedded within it (Davey and O'Toole, 2000; Konhauser, 2007) and largely alter the environmental microgradients in the interface as a result of their metabolism.

These microbial mats develop in a wide variety of environments (Cohen, 1984; Cohen and Rosenberg, 1989; Stal, 1995). Therefore we find a wide variation of communities living in them. The Antarctic dry valley lakes are among these ecological sites (Parker *et al.*, 1982). These lakes are unique in that they consistently maintain a thick year round ice cover (2.8-6.0 m) over liquid water. The persistent ice-cover minimizes wind-generated currents and reduces light penetration, restricting sediment deposition into a lake and exchange of atmospheric gases between the water column and the atmosphere. The present lakes are mostly remnants of larger glacial lakes perhaps some 4.6 million years (Ma) before the present (Doran *et al.*, 1994).

The microbial mats found in these environments are composed primarily of cyanobacteria, heterotrophic bacteria, protozoan cysts, eukaryotic algal cells and minerals associated with microbial activity occurring throughout much of the benthic regions of the dry valley lakes (Wharton *et al.*, 1983; Mikell *et al.*, 1984; Vincent, 1988).

There are differences in relative abundances of species that make up the microbial mats (Wharton *et al.*, 1983; Parker and Wharton, 1985). The most remarkable ones concern the distribution of mat morphologies within a lake and between the lakes. The four major categories are: prostrate, lift-off, columnar and pinnacle morphologies. We will discuss further mats that are referred to by their unusual properties simply as "lift-off" mats.

They result from an interesting combination of physical and biological processes. The phenomenon of literally lifting off mats has been observed in every lake that has been studied so far (Parker *et al.*, 1982), with the exception of Lake Vanda (Wharton *et al.*, 1983; Parker and Wharton, 1985).

## 3. Microbial mats: transport agents in subglacial lakes that are found in Antarctica

A lift-off mat is produced when the pressure of dissolved gases inside the prostrate (i.e., flattened) microbial mat exceeds the local hydrostatic pressure at that depth and bubbles from inside the mat causing it to 'lift-off' the lake bottom.

In some cases, lift-off mats tear loose from the lake bottom and float to the undersurface of the ice-cover. Once at the bottom of ice cover, lift-off mats freeze into the ice and through the ablation of surface eventually make their way to the top.

### 3.1 THE ESCAPE MECHANISM OF LIFT-OFF MATS

The escape mechanism is important in the distribution of microbes between the lakes and other environments in the region (Wharton *et al.*, 1983). The process of lift-off mats plays a role in removing nutrients and salts from these lakes (Parker *et al.*, 1982). Areas of the lake bottom in lake Hoare, a dry-valley lake in Antarctica, where lift-off was occurring, received at their surface a range from 0.4 to 1.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , as the amount of photosynthetic available radiation (PAR).

Even at these low intensities sufficient oxygen was generated by photosynthesis to cause bubble formation and mat-lift-off (Parker *et al.*, 1982). At greater depths where intensities were lower, usually beyond 5 m below the lower ice surface, only flattened prostrate mats occurred. These areas received intensities of PAR smaller than 0.10% of that striking the surface (Parker *et al.*, 1980, 1981; Simmons *et al.*, 1979).

Microscopic examination of mat samples reveal that from benthic regions below the ice covers, we always find the *Phormidium frigidum* Fritsch associated with the mats as dominant taxon. In addition, pennate diatoms may be present. Entrapped sediment and precipitated minerals were also abundant in algal mats. This implies that microbial communities are living together and surviving on some synergistic interactions in this microbial world (Schink, 2002) and forming a mutualistic microbial community. If this is happening, we can raise the following question: Can these communities contribute towards the transport of sulfur in various subglacial lakes to their surfaces? Indeed this is the case. Annual removal of sulfur by escaping algal mats in the Antarctic lakes Chad, Hoare and Fryxell, are reported as 104, 56, 40 kg respectively (Parker *et al.*, 1982).

### 3.2 MICROBIAL MATS THAT CONTAIN SULFATE-REDUCING BACTERIA

Versatility of sulfate-reducing bacteria (SRB) at two extremes in anoxic and oxic settings place sulfate respirers to be potential biomarkers in extreme conditions such as Europa. A central role is played by these bacteriae in the biogeochemistry of chemically stratified marine habitats. Such a significant role has been documented both in anaerobic conditions (Jørgensen, 1982a, b), as well as aerobic conditions (Cohen 1984).

Earlier SBR have been recognized as obligate anaerobes in anaerobic marine and terrestrial environments (Widdel, 1988), though they may survive temporary exposure to oxygen and again become active under anaerobic conditions (Canfield and Des Marais, 1991). SRBs can live near the surface region of a cyanobacterial microbial mat (Minz *et al.*, 1999), as well as in depth in syntrophic association with methane producing bacteria. For example, microbial mutualism, namely a syntrophic association has been found in *Desulfovibrio vulgaris* and in *Methanococcus* species (Stoylar *et al.*, 2007). Syntrophy is one form of microbial mutualism that is commonly involved in the degradation of organic substrates by microbial communities (Pernthaler *et al.*, 2008). In syntrophic interactions, the transfer of metabolites between species is essential for growth (Schink, 1997, 2002; Schink and Stams, 2002). Therefore, a contribution of SRB to biogeochemical cycling seems to be significant.

The bacterial sulfur cycle has been extensively reviewed (Pfennig and Widdel, 1982). Under aerobic conditions the reduction of sulfate is assimilatory (e.g., in green plants), whereas the oxidation of reduced sulfur compounds (for example, sulfide minerals) is dissimilatory in many bacteria (as in the case of the colorless sulfur bacteria). Dissimilatory reduction of sulfate is equivalent to the oxidation of organic compounds for energy conserving reactions. Under anaerobic conditions, both oxidized and reduced sulfur compounds are substrates only for metabolic processes of bacteria. Oxidized sulfur compounds, including elemental sulfur, represent counterparts of oxygen as electron acceptors in the terminal oxidation of organic substances and hydrogen; this is true for strictly anaerobic, dissimilatory sulfate- and sulfur reducing bacteria, which form hydrogen sulfide as a product. The sulfate-reducing bacteria thereby drive an important dissimilatory sulfur cycle in which inorganic sulfur compounds serve as extra cellular electron carriers. Microbial sulfate reduction is an energy-yielding process during which sulfate is reduced and sulfide is released, coupled with the oxidation of organic matter or molecular hydrogen (Postgate, 1984). Such a sulfur cycle was even more important during the early Precambrian evolution of the biosphere before molecular oxygen was evolved by oxygenic phototrophs and began to accumulate on Earth. Dissimilatory sulfate reduction (DSR) is a process by means of which sulfate-reducing Bacteria and Archaea are able to use sulfate ions as electron acceptors for anaerobic respiration. This process, DSR, releases large amounts of free sulfides as the sole final product (Widdel, 1988). The turnover rates of sulfur in dissimilatory processes exceed assimilatory reduction by several orders of magnitude (Shen and Buick, 2004; Rabus *et al.*, 2006). These characteristics of sulfate-reducing bacteria suggest that they are possible candidates for life on other extreme environment on Earth as in Lake Vostok, as well as elsewhere in the Solar System such as Europa. In the next section we first consider Lake Vostok as a second environment where some hints may be gained on the possible presence of extremophiles beyond the Earth, especially sulfate-reducing bacteria.

#### **4. Lake Vostok as a model for the emergence of life on Europa**

We assume that if the process of lift-off discussed in Section 3.2 in the dry valley lakes may be rehearsed firstly on Earth, in an Europa-like environment. For example, a good location is Lake Vostok that lies underneath the Vostok station of the Russian Antarctic base. This lake is at about 1,000 km from the South Pole and it is beneath 4 km of ice. In the southern region of Antarctica many bacterial species have been found in zones of accreted ice, about 120 m above the water-ice surface (Christner *et al.*, 2006).

##### **4.1 SULFATE-REDUCING BIOGENIC ACTIVITY IN LAKE VOSTOK**

Bacterial density is found to be two to seven-fold higher in accretion ice than in the overlying glacial ice. This implies that Lake Vostok is a source of bacterial carbon beneath the ice sheath. Phylogenetic analysis of the amplified small subunit ribosomal ribonucleic acid (rRNA) gene sequences in this accretion ice has revealed the presence of *alphaproteobacteria*, *betaproteobacteria* and *gammaproteobacteria* (Christner *et al.*, 2006). With few exceptions, all other characterized species of the *deltaproteobacteria*

are strict anaerobes that respire via the reduction of electron acceptors, such as sulfate, elemental sulfur, iron (III) and Mn (IV) (Lovley *et al.*, 1995). These bacterial communities are diverse and physically associated. Such ecosystems lead to fundamental questions regarding the physiology and metabolism of several microorganisms. Prominent amongst these microbes we have *deltaproteobacteria* and *betaproteobacteria*. *Deltaproteobacteria* are phylogenetically linked with SBR (Shen and Buick, 2004). Detailed analysis on accreted ice has shown that bacterial cells are often associated with organic and inorganic particles (Priscu *et al.*, 1999), implying that a portion of cells within the lake water are not free living. Similar results have been reported for the permanently ice-covered, lakes in the dry valleys (Lisle and Priscu, 2004).

#### 4.2 THE ICY SURFACE OF LAKE VOSTOK

Lake Vostok and its relevance for astrobiology has been extensively reviewed (Christner *et al.*, 2006; Priscu *et al.*, 2003). It has been estimated that the youngest water is at least 400,000 years old. It is a window into life forms and climates of primordial eras. Lake Vostok is the largest of more than 140 subglacial lakes (Siegert *et al.*, 2005). The zone of ice layer up to 3,309 m (referred to as I), and the layer between 3,310 to 3,509 m (zone II) provide detailed information about the paleoclimate record spanning during the last 420,000 years.

The basal portion of the ice core from 3,539 to 3,623 m has many features differing from overlying glacial ice and its geochemical composition indicates that it represents actual lake water that has accreted (i.e., frozen) to the underneath of ice sheet. Despite extremely cold air temperatures above the ice (an average of -55 °C), liquid water is stable in the lake owing to the combined effect of background geothermal heating, the insulating properties of the overlying icy sheet, and adiabatic lowering of the freezing point (Siegert *et al.*, 2003).

#### 4.3 HYDROTHERMAL VENTS IN LAKE VOSTOK

Lake Vostok appears to be harboring hydrothermal vents beneath the water surface. This is suggestive of what may be occurring on Europa. The circulation of pure water in Lake Vostok will be driven by the differences between the density of meltwater and lake water. Geothermal heating will warm the bottom water to a temperature higher than that of the upper layers.

The water density will decrease with increasing temperature resulting in an unstable water column. This leads to vertical convective circulation in the lake, in which cold meltwater sinks down the water column and water warmed by geothermal heat ascends up the water column (Siegert *et al.*, 2001). Similarly, Europa may also have geothermally-heated warm water under its ice-crust. Processes of the type that occur in Lake Vostok may be taking place on Europa, where biogenic sulfur may be reaching the surface.

## 5. Europa and the LAPLACE/EJSM Mission

There is at present a possibility for returning to Europa initially discussed with the project LAPLACE (Blanc *et al.*, 2008), a mission to Europa and the Jupiter System for ESA's Cosmic Vision Programme. This initiative has been promoted to a worldwide collaboration named the Europa Jupiter System Mission, EJSM. In both projects, the earlier LAPLACE Mission and the subsequent EJSM the question of habitability is a major priority.

### 5.1 INSTRUMENTATION FOR PROBING THE HABITABILITY OF EUROPA

The options for approaching the question of selecting the right instrumentation for measuring the more abundant sulfur isotope have been discussed (Chela-Flores and Kumar, 2008). Early discussions, long before the proposed LAPLACE mission, also considered the possibility of exploring Europa's habitability in the future with a submersible called a hydrobot (Horvath *et al.*, 1997). This question is still relevant a decade later, in terms of new NASA autonomous underwater vehicle (AUV) called ENDURANCE for the Astrobiology Science and Technology for Exploring Planets (ASTEP) program (Doran *et al.*, 2007).

Significant papers have more recently addressed the conditions for establishing a stable ecosystem. They include discussions of the biochemistry (Chyba, 2000; Schulze-Makuch and Irwin, 2002), as well as the relevance of sulfur in the biogeochemistry of Europa (Zolotov and Shock, 2003; Chela-Flores, 2006). The radiation of the Jovian magnetosphere may damage traces of biogenic sulfur deposited on the surface. The stopping depth for ionic radiation in the Jovian magnetosphere is expected not to exceed 1 cm (Greenberg, 2005; Baumstark-Khan and Facius, 2002). Thus, organic molecules would not be destroyed below such a thin layer. Penetrators are instruments in the process of development that would impact planetary bodies such as the Moon and bury themselves into the surface. Based on to the preliminary results of the British Penetrator Consortium (Smith *et al.*, 2008), a modest stopping depth of penetrators into the (icy) surface of Europa would be sufficient to obtain samples that can be used to interpret isotopic abundances of sulfur that in the presence of putative S-reducing microbes would show measurable anomalous deviations the  $\delta^{34}\text{S}$  parameter without radiation interference. The concept of 'stopping depth' is discussed in Section 5.2 and in the Appendix.

### 5.2 SULFUR PHYSICS AND CHEMISTRY ON EUROPA

Finding elemental sulfur on Europa may be of special interest. The possibility of such traces of sulfur might have originated from the metabolism of extremophilic sulfur-reducing microorganisms. The relevance of the  $\delta^{34}\text{S}$  parameter is discussed in some detail in the Appendix A2. We have examined the influence of temperature and radiation on Europa's biosignatures, and compare with examples on Earth in Section A2 and A3.

### 5.3. TESTING BIOGENICITY OF THE SULFUR PATCHES WITH PENETRATORS

With the forthcoming missions to explore Europa, a new technology innovation is the penetrator concept that is being developed for early trials on the Moon surface- the Moon-Lite mission (Smith *et al.*, 2008). If the microbial mats that we understand well in the context of the dry valley lakes (cf. Section 2) for the expulsion of a large quantity of sulfur that may be used to introduce tests on the surface of Europa, it is pertinent to evaluate the stopping depths for the European surface. Our main result is shown in Fig. 1.

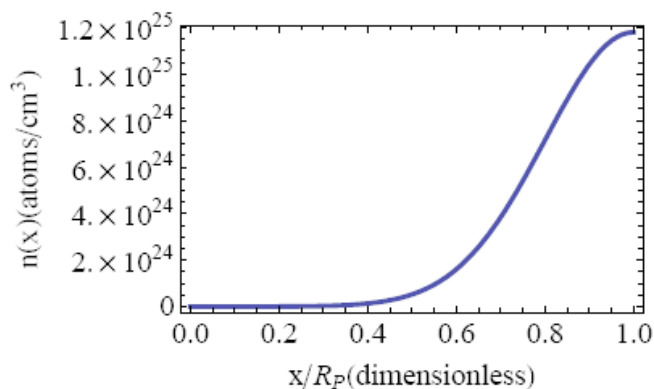


Figure 1: Density distribution of sulfur ions implanted from the Jovian atmosphere as a function of dimensional depth ( $x/R_p$ ) for  $t = 10^6$  years,  $\phi = 9.0 \times 10^6 \text{ (cm}^2 \text{ s)}^{-1}$  and  $R_p = 4.8 \times 10^5 \text{ cm}$ . The maximum density is at the range  $x = R_p$ . The distribution is Gaussian. This Figure is discussed in Appendix A3. The graph is based on the LSS theory (Lindhard-Scharff and Schiot) of ion implantation (Sze, 1988).

We may conclude from these estimates that a penetration of measuring instruments into the icy surface of Europa just beyond the few millimeters of the stopping depth would be sufficient for an accurate estimate of the  $\delta^{34}\text{S}$  parameter (cf., Appendix A1). This would be a possible way for rejecting, or supporting, the hypothesis of biogenicity of the European sulfur patches. The fact that missions such as LAPLACE and others can envisage and undertake such measurements (in a relatively short period) is of the utmost importance for astrobiology's most significant question: Are there other environments in our own Solar System where we could settle the question of habitability?

## 6. Conclusions

The experience we have gained with microbial mats in interaction with ice the subglacial lakes of Antarctica has been shown to be relevant for the future exploration of the Solar System.

### 6.1 FROM THE ANTARCTIC SUBGLACIAL LAKES TO EUROPA

The satellite system of Jupiter has three of its four Galilean satellites locked in the so-called Laplace resonance. Consequently, the energy and angular momentum they exchange among themselves and with Jupiter contribute to various degrees to the

internal heating sources of the satellites. Europa is one of the best candidates for the search for life in our Solar System, for the ‘equation of life’ is fulfilled in this world: not only there is expected to be a supply of biogenic elements, also an energy source we have just described, but in addition there is compelling evidence gathered by the Galileo mission for an internal ocean of salty liquid water, possibly ten times deeper than the deepest point in the Pacific Ocean.

The Jupiter System plays a prominent role in the Cosmic Vision Plan proposed by the European Agency to build its ambitious scientific program over the period 2015-2025 (Blanc *et al.*, 2008). Of the three key questions that have been raised, its habitability is the most urgent. However, if a lander is to be used for a most direct test of biogenicity of the sulfur patches that were discovered during the Galileo mission, new cutting-edge technology has to be envisaged. One such technology has been reviewed in a recent paper (Smith *et al.*, 2008).

## 6.2 TECHNOLOGY REQUIRED FOR THE EXPLORATION OF EUROPA

Penetrators allow key scientific investigations of airless solar system bodies, such as Europa, or the Earth’s satellite via affordable pre-cursor missions. In fact, it is difficult to envisage any other method that allows globally spaced surface exploration of airless planetary bodies that is not prohibitively expensive. Penetrators are small probes that impact planetary bodies at high speed and bury themselves into the planetary surface. Before reaching Europa with LAPLACE, our own satellite, the Moon, can be used as an intermediate stage. We have proposed deployment on the Moon of ~13 kg penetrators that are designed to survive impact at high speed (~ 300 m/s) and penetrate ~ 2-5 m. In earlier work (Chela-Flores and Kumar, 2008), and in the present paper we have presented arguments that militate in favor of introducing mass spectrometry as appropriate instrumentation for the penetrators. The results of this paper shown in Fig. 1 (and its corresponding explanation in the Appendix A3) suggest that with this new technology just a modest penetration of a few millimeters would be sufficient for deciding on biogenicity. Indeed, with the optimum stopping depth we have calculated, sulfate-reducing bacteria would leave a measurable trace of their activity that is not affected by the harsh external environment. Penetrators that are in the process of being developed might succeed in making reliable measurements of the  $\delta^{34}\text{S}$  parameter that are not affected by the presence of harsh radiation of the Jovian magnetosphere.

## 6.3 ON DEEPER IMPLICATIONS OF MICROBIAL MATS

In addition, in this work we have also pointed out the significance of microbial mutualism (a syntrophic association between various genera of bacteria within the microbial mats). This means that we have related sulfur transport in microbial mats that are known to take place in the Antarctica subglacial lakes with a possible mechanism that might rationalize what is taking place on the icy surface of Europa on its sulfur patches. Lake Vostok gave us some further hints from microorganisms in the accreted ice that lies just above the still-unexplored liquid water.

Depending upon the conditions established by the opposing gradients of light intensities from above and sulfide concentrations below, different kinds of bacteria may develop. It is conceivable that once phototrophic sulfur bacteria have evolved under



favorable conditions of pH, salinity, temperature, availability of organic carbon in anoxygenic condition, they could survive with energy conserving processes in the dark. This argues in favor of a biogenic interpretation of the sulfur patches on the European icy surface. Thus, it is suggestive that under such similar environment, the Jovian satellite Europa can harbor life, or may be in process for its emergence. The icy surface of Europa is in the extremely low temperature range 50K to 110K. However, below the ice we may have warm water that could harvest sulfate-reducing bacteria.

#### 6.4 SULFUR, A POSSIBLE BIOMARKER FOR THE ICY SURFACE OF EUROPA

On Europa there is another source of sulfur and that is energetic sulfur coming from the nearby Jovian atmosphere, namely from Jupiter. Now if sulfur from biological origin does exist in the underlying ocean and is carried to the surface by convective currents then this sulfur will mix with the implanted sulfur coming from Jupiter. Now if a probe with appropriate instruments tries to measure the  $\delta^{34}\text{S}$  parameter, then this value would be some average value for that between biogenic sulfur and implanted sulfur. Then any interpretation based on this value would be incorrect. Therefore it is important that we know up to what depth does the sulfur from Jupiter penetrates the surface of Europa. Based on the theory of ion implantation in material science (discussed further in Appendix A3), we have plotted in Fig. 1, the density distribution of implanted sulfur as a function of the  $(x/R_p)$ . Here “x” is the depth in cm and  $R_p$  ( $4.8 \times 10^{-5}$  cm) is the maximum range of implanted sulfur. We have taken on the “X” axis  $x/R_p$ , which is dimensionless just for convenience. It just reduces the scale. As seen from figure, the maximum density is at  $x/R_p = 1$ , which means that  $x=R_p$ . In other words, the maximum density is at  $R_p$ . After distance  $R_p$  the density of implanted sulfur is zero. So this means that any probe that wants to measure sulfur of biogenic origin, it has to go beyond  $4.8 \times 10^{-5}$  cm because beyond this depth only sulfur of biogenic origin will exist.

### Appendix

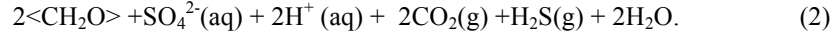
#### A1. SIGNIFICANCE OF THE $\delta^{34}\text{S}$ PARAMETER

Sulfur is one of the key elements of life. Sulfur exists in four stable isotropic forms:  $^{32}\text{S}$  (95.02%),  $^{33}\text{S}$  (0.75%),  $^{34}\text{S}$  (4.21%) and  $^{36}\text{S}$  (0.02%). Sulfur isotope ratios are typically reported in the delta notation, as deviations with respect to the standard that is the troilite of the Cañon Diablo meteorite (CDT):

$$\delta^x \text{S} = \left[ \frac{({}^x \text{S} / {}^{32} \text{S})_{\text{sample}}}{({}^x \text{S} / {}^{32} \text{S})_{\text{std}}} - 1 \right] \times 10^3 \text{ [‰, CDM]} \quad (1)$$

where  $x = 33, 34$  or  $36$ . As pointed out (Kaplan, 1975; Chela-Flores, 2006), metabolic pathways of sulfur bacteria have enzymes that preferentially select the isotope  $^{32}\text{S}$  over  $^{34}\text{S}$ . This implies that where there is an abundance of sulfur bacteria, the value of the  $\delta^{34}\text{S}$  parameter would be negative. Bacterial (dissimilatory) sulfate reduction (BSR) is a naturally occurring process. Under anaerobic conditions, sulfate is used by bacteria as

an electron acceptor for oxidation of organic carbon (from pyruvate, lactate, formate, ethanol, methanol, amongst others), according to the following generalized reaction,



The above reaction is also called ‘sulfate respiration’. The product  $\text{H}_2\text{S}$  in the above reaction is highly enriched in  $^{32}\text{S}$ . According to a model (Farquhar and Wing, 2003, Johnston *et al.*, 2007) the parameter  $\delta^{34}\text{S}_{\text{SO}_4^{2-}} - \delta^{34}\text{S}_{\text{H}_2\text{S}}$ ,

$$\delta^{34}\text{S}_{\text{SO}_4^{2-}} - \delta^{34}\text{S}_{\text{H}_2\text{S}} = \left[ \frac{(^{34}\text{S}/^{32}\text{S})_{\text{SO}_4^{2-}} - (^{34}\text{S}/^{32}\text{S})_{\text{H}_2\text{S}}}{(^{34}\text{S}/^{32}\text{S})_{\text{std}}} \right] \times 10^3 (\text{‰})$$

(The model has been referred to as the "the Rees-Farquhar model".) The above parameter is initially negative, becomes less negative in the process of sulfate respiration. Indeed, by the metabolic activity of sulfate-reducing bacteria, the quantity  $(^{34}\text{S}/^{32}\text{S})_{\text{SO}_4^{2-}}$  becomes less negative, while  $(^{34}\text{S}/^{32}\text{S})_{\text{H}_2\text{S}}$  becomes more negative. This basically means that during the process of sulfate respiration the isotope  $^{32}\text{S}$  increases in the product  $\text{H}_2\text{S}$  and is reduced in  $\text{SO}_4^{2-}$ . Accelerated sulfate reduction by bacterial communities is known to occur in the presence of organic carbon (Fauville *et al.*, 2004).

The relevance of the  $\delta^{34}\text{S}$  parameter for biogenic sulfur on the icy surface of Europa has been reviewed (Chela-Flores, 2006; Chela-Flores and Kumar, 2008). Life requires an input of energy and must be able to control the flow of energy through redox chemistry, which is a universal concept. As life is based on organic chemistry, such chemistry must be allowed to operate. An extremophile must either live within the extreme environmental parameters, or guard against the outside world in order to maintain these conditions. For example, certain cold-tolerant cyanobacteria (Vincent, 2007) have a variety of strategies to minimize stresses of freeze-up. Like sea-ice microbiota, the mat-forming species in the McMurdo Ice shelf form copious quantities of exopolymeric substances already referred to as EPS in Sec. 2). This material shows the flow of liquid water during freeze-up and thaw, and may also force crystal formation to occur well away from the cells. Experiments indicate that EPS is critical to surviving desiccation, as well as freeze-up (Tamaru *et al.*, 2005). Also EPS are the source of organic carbon. We now examine the influence on temperature and radiation on biosignatures suitable for Europa and suggest a comparison with their counterparts on Earth.

## A2. TEMPERATURE

The magnitude of isotope fractionation by microbial sulfate reduction also depends upon temperature (Kaplan and Rittenberg, 1964; Canfield *et al.*, 2006). The isotope fractionation factor (a) is (Ono, 2008):

$$^x\alpha_{\text{SO}_4^{2-}-\text{H}_2\text{S}} = \frac{[^x\text{SO}_4^{2-}]/[^{32}\text{SO}_4^{2-}]}{[^x\text{H}_2\text{S}]/[^{32}\text{H}_2\text{S}]} \quad (4)$$

where  $x = 33, 34$  and  $36$ . The isotope fractionation factor at equilibrium can be derived from the ratios of the partition function ( $Q$ ),

$${}^{34}\alpha_{so_4^{2-}-H_2S} = \frac{{}^{34}Q_{so_4^{2-}} {}^{32}Q_{H_2S}}{{}^{32}Q_{so_4^{2-}} {}^{34}Q_{H_2S}} \quad (5)$$

The partition function is

$$Q = \prod_i u_i \exp\left[-\frac{u_i}{2}\right] [1 - \exp(-u_i)] \quad (6)$$

where  $u_i = hv_i/kT$  ( $h$  is the Planck constant,  $k$  is the Boltzmann constant,  $T$  is the temperature in Kelvin and  $v_i$  is the  $i$ th vibrational frequency of the molecule). A plot (cf., Fig. 2 below) of  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S})$  as a function of temperature shows that as temperature decreases, the value of  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S})$  increases, which is a signature of biological process.

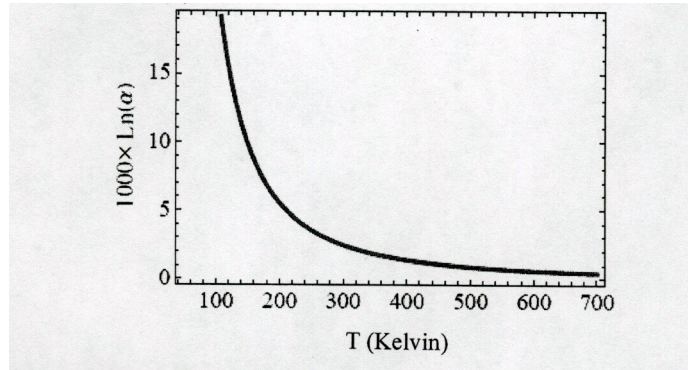


Figure 2: A plot of  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S})$  as a function of temperature shows that as temperature decreases the value of  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S})$  increases, which is a signature of biological process. The temperature on the surface of Europa is about 110K. This corresponds to  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S}) \sim 15$  while the temperature in the sea water below in contact with the ice is 270K which corresponds to  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S}) \sim 4.5$ .

Below the ice, the evidence gathered by the Galileo mission suggests that there is an ocean of liquid water, which could in principle harvest sulfur bacteria. The temperature of such an underlying sea just in contact with the ice is estimated to be near 270K. This temperature is appropriate for BSR as it is evident from Figure 2. A surface temperature on the surface of Europa of about 110K corresponds to  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S}) \sim 15$ , while the temperature in the sea water below in contact with the ice of temperature of the order of 270 K, corresponds to  $1000 \times \ln({}^{34}\alpha_{so_4^{2-}-H_2S}) \sim 4.5$ . In comparison, the average

temperature of the dry valley Antarctic lakes is in the range 273-280K, which means that  $1000 \times \ln(^{34}\alpha_{SO_4^{2-}-H_2S}) \sim 3$ . The algal mats in these lakes are known to exist about 4 meters below the surface of the frozen lakes and are also capable of lifting off, floating and freezing in ice. These mats selectively remove a huge quantity (40-104 kg) of sulfur annually (discussed in Section 3.1). It may be speculated that similar algal mats, if they exist beneath the icy surface of Europa, may eventually be transported to the surface by surficial ablation from above (produced by the micrometeoroids and refreezing form below the ice layer, thus contributing to the surficial sulfur patches. It is interesting that Europa offers a wide temperature range suitable for a wide variety of microorganisms to exist. Moreover, low temperature favors enhanced biological activity of sulfate respiration. This would be reflected in the extremely high values of  $1000 \times \ln(^{34}\alpha_{SO_4^{2-}-H_2S})$ , or by highly negative values of  $\delta^{34}S_{SO_4^{2-}} - \delta^{34}S_{H_2S}$ . These effects are subject to measurement by miniature mass spectrometer in future missions (Blanc et al., 2008). The possibility of highly negative  $\delta^{34}S$  value due to hydrothermal process is ruled out since extremely high temperatures ( $> 550K$ ) is required for such a process. The possibility of bacterial life well below the sea underneath with temperatures exceeding 350K cannot be ruled out since we know examples of sulfur dependent extremophiles such as *Sulfolobus acidocaldarius* an archaea that flourishes at pH 3 and  $> 350K$  in Yellowstone National Park (USA) (Rothschild and Mancinelli, 2001).

There are thermophiles among the bacteria (*Bacillus*, *Clostridium*, *Thiobacillus*, *Desulfotomaculum*), the archaea (*Pyrococcus*, *Thermococcus*, *Thermoplasma*, *Sulfolobus*) and the methanogens that exist in the temperature range 330-390K. In contrast, the upper limit for eukaryotes is 330K, a temperature suitable for some protozoa, algae and fungi.

### A3. SULFUR ION IMPLANTATION ON THE SURFACE OF EUROPA

On Europa, however, there is another source of sulfur, namely, the energetic sulfur ions coming from the nearby Jovian atmosphere. These energetic ions after striking the surface of Europa will penetrate a certain depth into the icy surface. If we assume that there are sulfur bacteria on the surface and below the icy crust of Europa and the initial value of the  $\delta^{34}S$  parameter is negative then this mechanism of ion implantation will change the value of the  $\delta^{34}S$  parameter and make it less negative or even positive. A probe on the surface of Europa that tries to measure the  $\delta^{34}S$  parameter would then wrongly detect the absence of sulfur bacteria. Consequently, it is important that the probe goes well beyond the maximum penetration depth of the ions to measure the  $\delta^{34}S$  parameter. For this it is essential to know the density distribution of the implanted ions as a function of depth as well as time of implantation. Based on the LSS (Lindhard, Scharff and Schiøt) theory of ion implantation, the implant profile in an amorphous material can be described by the equation (Sze, 1988):

$$n(x) = n_o \exp\left(-\frac{(x - R_p)^2}{2\Delta R_p^2}\right) \quad (7)$$

where,  $n_o = \frac{\phi}{t} \sqrt{2\pi} \Delta R_p$ ,  $\phi$  is the implanted dose,  $t$  is the time of implantation,  $R_p$  is the projection range and is equal to the average distance an ion travels before it stops and  $\Delta R_p$  is the standard deviation of  $R_p$  which is roughly  $1/5R_p$  from the known data for different ions and impact surface. The value of  $R_p$  for sulfur ion for the European surface is  $4.8 \times 10^{-5}$  cm and  $\phi = 9.0 \times 10^6$  (cm<sup>2</sup> s)<sup>-1</sup> (Cooper *et al.*, 2001) (cf., Fig. 1 in Section 5.3). It is therefore clear from Figure 1 (in Section 5.3) that the implanted sulfur is heavily distributed around the maximum depth  $R_p$ . This implies that a penetrator has to go beyond  $R_p$  to measure biogenic sulfur without any contamination from implanted sulfur.

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