

## Hypothesis Paper

# The Possible Origin and Persistence of Life on Enceladus and Detection of Biomarkers in the Plume

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### Abstract

The jets of icy particles and water vapor issuing from the south pole of Enceladus are evidence for activity driven by some geophysical energy source. The vapor has also been shown to contain simple organic compounds, and the south polar terrain is bathed in excess heat coming from below. The source of the ice and vapor, and the mechanisms that accelerate the material into space, remain obscure. However, it is possible that a liquid water environment exists beneath the south polar cap, which may be conducive to life. Several theories for the origin of life on Earth would apply to Enceladus. These are (1) origin in an organic-rich mixture, (2) origin in the redox gradient of a submarine vent, and (3) panspermia. There are three microbial ecosystems on Earth that do not rely on sunlight, oxygen, or organics produced at the surface and, thus, provide analogues for possible ecologies on Enceladus. Two of these ecosystems are found deep in volcanic rock, and the primary productivity is based on the consumption by methanogens of hydrogen produced by rock reactions with water. The third ecosystem is found deep below the surface in South Africa and is based on sulfur-reducing bacteria consuming hydrogen and sulfate, both of which are ultimately produced by radioactive decay. Methane has been detected in the plume of Enceladus and may be biological in origin. An indicator of biological origin may be the ratio of non-methane hydrocarbons to methane, which is very low (0.001) for biological sources but is higher (0.1–0.01) for nonbiological sources. Thus, Cassini's instruments may detect plausible evidence for life by analysis of hydrocarbons in the plume during close encounters. Key Words: Life—Enceladus—Biomarkers—Methanogens—Non-methane hydrocarbons—Ammonia—Origin of life. *Astrobiology* 8, 909–919.

### Introduction

ENCELADUS, A SMALL ICY MOON OF SATURN, was thrust onto the center stage of astrobiology with the discovery by the Cassini spacecraft of Enceladus' dramatic present-day geologic activity. The Cassini Imaging Science Subsystem images of Enceladus have revealed about a dozen jets of fine icy particles that emerge from the south polar terrain (SPT) of Enceladus and feed a giant plume extending thousands of kilometers into space (Porco *et al.*, 2006). Several Cassini instruments were able to sample this plume during the spacecraft's very close flyby in July 2005. *In situ* measurements of the plume found water vapor, simple organic com-

pounds, and some level of N<sub>2</sub> or CO, or both (Waite *et al.*, 2006). Observations at infrared wavelengths by the Composite Infrared Spectrometer instrument on Cassini have also shown the SPT to be anomalously warm (Spencer *et al.*, 2006), and the comparison of high-resolution images of the SPT with the highest-resolution thermal measurements have shown a coincidence between the hottest measured temperatures in the SPT and the "tiger stripe" fractures that straddle the region (Porco *et al.*, 2006; Spencer *et al.*, 2006; Spitale and Porco, 2007).

In this paper, we briefly review the range of geophysical models for the plume on Enceladus. We note that some, but not all, of these models posit a subsurface liquid water

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aquifer. With this caveat understood, we nevertheless focus on such an aquifer and its potential to support an origin of life and a continuing ecosystem. We base our discussions of the possible origin of life on Enceladus on theories for the origin of life on Earth. For ecosystems on Enceladus, we base our discussions on specific existing ecosystems on Earth that survive in conditions similar to those that may be present in an aquifer on Enceladus. Many of the issues related to life on Enceladus mirror previous discussions of life below the ice of Europa, and we make this connection whenever appropriate. Finally, we consider how a signature of biological processes might be present in the plume and detected by the Cassini instruments.

### The Plume of Enceladus

Enceladus is a small world: mean radius 252 km, mass  $1.8 \times 10^{-5}$  Earth masses, and gravity  $0.1 \text{ m/s}^2$ . It is surprising that such a small world, which is relatively distant from its planet (orbital distance is 4 times Saturn's radius), would be one of only three outer Solar System bodies (along with Jupiter's moon Io and Neptune's moon Triton) where active eruptions have been observed. An understanding that would provide a physical framework for assessing habitability is contingent upon answers to the following questions: (1) What is the source of energy that powers the activity on Enceladus? (2) Does the plume originate from a subsurface aquifer? (3) What is the source of  $\text{CH}_4$  and  $\text{N}_2$  in the plume? (4) If there is a subsurface liquid aquifer, how long has it persisted? (5) Is the concentration of solutes in such a liquid aquifer consistent with habitability? We consider each of these questions here.

Several models have been proposed to explain the activity on Enceladus and the associated  $\sim 6$  GW of heat (Spencer *et al.*, 2006). Some models assume the heat is generated within a silicate core (Castillo *et al.*, 2007; Collins and Goodman, 2007). Others assume heating within an outer ice shell (Nimmo *et al.*, 2007). All require heat production at least in part by tidal flexing due to the forced eccentricity of Enceladus by Dione, as the observed heat output far exceeds that produced by radiogenic heating (Porco *et al.*, 2006). However, recent models show that the current rate of heat production observed at Enceladus is inconsistent with a steady-state orbital eccentricity (Meyer and Wisdom, 2007). This result implies that the current warm state of Enceladus is probably the result of a higher eccentricity in the past, though it is presently uncertain how that might have happened. There is not enough data to guide the models, and the source of the heating on Enceladus remains obscure.

The source of the  $\text{H}_2\text{O}$  ice and vapor in the plume is also uncertain. Initial work suggested a shallow liquid water aquifer as the source of the plume (Porco *et al.*, 2006). However, models in which the ice in the plume originates from surface ice have also been proposed (Kieffer *et al.*, 2006).

The  $\text{CH}_4$  and  $\text{N}_2$  in the plume may reflect ancient processes during an early formation period when interior conditions were hot enough to decompose ammonia into  $\text{N}_2$  and produce  $\text{CH}_4$  by thermal decomposition of organics. Matson *et al.* (2007) suggested that this implies internal temperatures at some point in the past on the order of 500–800 K, while Glein *et al.* (2008) suggested 300 K would be adequate. An alternative suggestion for these gases is the formation of the

plume by the decomposition of clathrates in which  $\text{CH}_4$  and  $\text{N}_2$  are present (Kieffer *et al.*, 2006), though this model fails to explain the presence of water vapor in the plume (Ingersoll, 2007).

From an astrobiological perspective, a key question is whether the subsurface aquifer has persisted long enough to allow for the origin of life. Unfortunately, there are no satisfying estimates of the age of the activity on Enceladus although the geologic features and crater density near the plume indicate that they have persisted over several hundred million years (Porco *et al.*, 2006). The plume on Enceladus is clearly the source of the saturnian E ring, but this has been observed only since the 1960s. Another possible constraint on the persistence of the plume comes from the timescale for the hot spot on Enceladus to migrate to the polar position. Nimmo and Pappalardo (2006) suggested that it is not a coincidence that the plume emanates from one of the poles of Enceladus. They point out that, if the plume is originating from a subsurface aquifer, this would represent a mass depletion in the shape of Enceladus, and gravitational torques would act to rotate Enceladus until this mass depletion was located on one of the poles. Unfortunately, there are no estimates, to date, for the timescale for this rotation. The results of Meyer and Wisdom (2007) suggest that the jet activity must reflect residual activity after some unusual event, such as a recent high-eccentricity phase or some sort of storage and episodic release of tidal heat. Roberts and Nimmo (2008) followed up on this logic and suggested that the timescale to freeze up an ocean produced in an episodic event is  $\sim 30$  Myr for a 40 km thick layer of water.

If the plume derives from a liquid water aquifer, then the question arises: Does the solute concentration exceed the habitability limits? Two possible solutes are of interest here: salts and ammonia. Currently, there are no reports of salts in the plume (Schneider *et al.*, 2007), though they would be expected in any geophysical water body. As is often the case on Earth, however, water just below perennial ice covers can be relatively free of salt, while deeper layers are salt rich. Thus, if the plume originates from these shallow layers, it would be free of salt. Also, there are no reports of ammonia in the plume, though there are numerous geophysical arguments for ammonia being the source of N and for its role in maintaining liquid water on small worlds in the outer Solar System (*e.g.*, Squyres *et al.*, 1983; Matson *et al.*, 2007). It is possible that ammonia is present in the subsurface liquid and has been decomposed in the plume to  $\text{N}_2$ . Thus, it may be interesting to consider the possible toxic effects of high concentrations of ammonia.

Figure 1 summarizes the model we consider, from the astrobiological perspective, below. In this model, the jets originate from a subsurface aquifer.

### Origin of Life on Enceladus

Discussions of life on Enceladus must logically begin with a consideration of the possible origin of life on that world. Unfortunately, we do not know how life originated on Earth, nor have we been able to reproduce it in the laboratory. There is not even a consensus theory for the origin of life on Earth. Nonetheless, considerations of the possible origin of life on another world are based on theories for the possible origin of life on Earth. Davis and McKay (1996) attempted to cate-

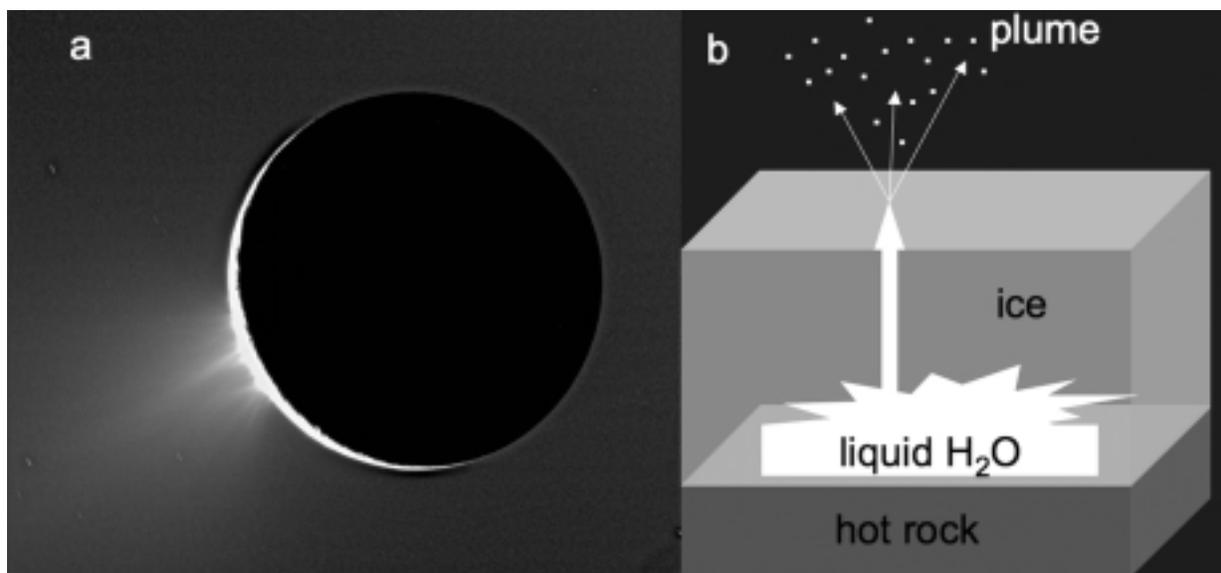


FIG. 1. (a) Cassini image showing the plume of Enceladus (Porco *et al.*, 2006) and (b) the cold jet model for the formation of the plume

gorize theories of the origin of life on Earth and then applied them to Mars. We can follow this same approach for Enceladus. The Davis and McKay (1996) categorization is shown in Fig. 2.

As shown in Fig. 2, it is useful to divide theories for the origin of life on Earth into two main categories, depending on whether life originated independently on Earth or was

carried to Earth from somewhere else. The latter category is usually called panspermia, and versions that involve both natural and directed panspermia have been considered (Davis and McKay, 1996). For application to Enceladus, the more useful distinction is between panspermia that is confined to the inner Solar System, *e.g.*, Mars and Earth, and panspermia that is Solar System wide.

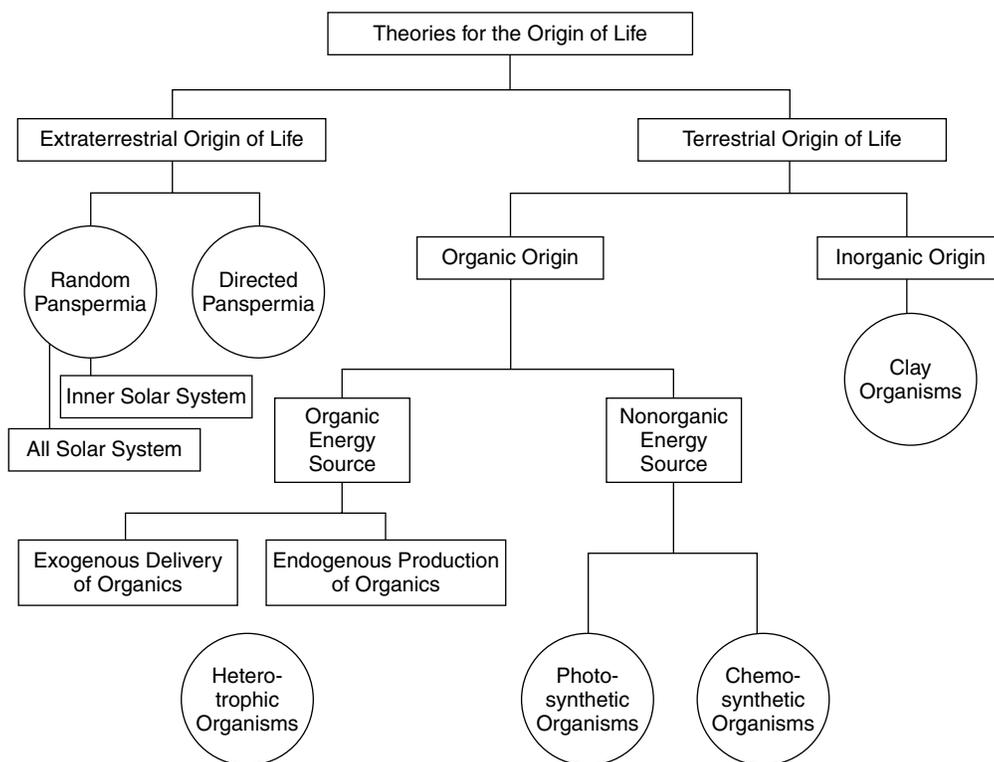


FIG. 2. Categorization of theories for the origin of life on Earth from Davis and McKay (1996) modified and applied to the origin of life on Enceladus by the addition of two subcategories for random panspermia.

The discovery that there are many meteorites on Earth that came from Mars has sparked considerable discussion about the possibility that life originated on Mars and was carried to Earth by meteorites (Melosh, 1988, 2003; Sleep and Zahnle, 1988; Mileikowsky *et al.*, 2000; Weiss *et al.*, 2000). We call this “inner Solar System” panspermia in Fig. 2.

However, even if life did begin on Earth or Mars and successfully transferred from one of these worlds to the other through meteorites, the chance of rocks reaching Enceladus from either of these worlds is much reduced. Furthermore, the timescale for a rock to transit from Mars to Earth can be quite small, 30,000 years or less (Gladman *et al.*, 1996, 2005), which improves the chances of survival against cosmic radiation of a microorganism encased in the meteorite. But transit times to the outer Solar System are presumably much longer, reaching many millions of years (Melosh, 2003). Thus, we conclude that panspermia is unlikely to have infected Enceladus with life from Earth or Mars.

There are possible panspermia schemes that would bring life uniformly to all worlds in the Solar System. Napier (2004) has proposed that life could be carried on dust between stars (see also Weber and Greenberg, 1985). If such dust grains were incorporated into the pre-planetary solar nebula, then every object in the Solar System would be “infected” with life. In Fig. 2, we refer to this case as “Solar System” panspermia. For the case of Solar System panspermia, life on Enceladus would share the same origin and, therefore, the same biochemistry as life on Earth.

Terrestrial theories for the origin of life shown in Fig. 2 posit that life on Earth began on Earth, and we now consider which of these theories could be extended to suggest a separate, independent origin of life on Enceladus. Davis and McKay (1996) suggested that the intrinsic tie of biochemistry to water implies that, just as all life as we know it requires liquid water to grow or reproduce, the origin of life required liquid water. For Enceladus, this would mean that any origin of life would have occurred in a subsurface aquifer maintained in a liquid state by geothermal processes, such as tidal heating. Two of the terrestrial theories for the origin of life seem to apply here: the organic origin with an endogenous source of organics and the chemosynthetic origin of life.

The theory that the origin of life occurred in a “soup” of abiotically produced organic material is arguably the original theory for the origin of life and traces back to Darwin, Oparin, and Haldane. It was given a firm experimental basis with the work of Miller (1953) and has been extended considerably since that time. On Earth, the source of the organics that composed the soup could have been produced on Earth or could have come from cometary and interplanetary dust infall (Chyba and Sagan, 1992). Applied to Enceladus, the organic soup model might involve the formation of Enceladus from organic-rich ices similar to cometary materials. Comets may contain approximately 14–25% organic material by mass (Delsemme, 1988; Chyba *et al.*, 1990). Tidal heating at the present epoch or, soon after Enceladus formed, heating from short-lived radioactive elements such as  $^{26}\text{Al}$ , could result in a subsurface aquifer. The liquid solution produced would be rich in organics comprising a suitable prebiotic soup.

The alternative theory for the origin of life on Earth—the chemosynthetic origin—may apply well to Enceladus. In this scenario, life begins at the interface where chemically rich

fluids heated by tidal dissipation emerge from below the sea floor. This approach is motivated by the chemical and biological properties of deep sea vents in Earth’s ocean (Corliss *et al.*, 1981; Shock, 1990). Wächtershäuser (1990) [see also Pace (1991), and for a review see Holm (1992)] suggested hydrothermal or geothermal environments as promising sites for the subsurface origins of chemosynthetic life. These organisms utilize chemical energy and are represented today by various sulfur-metabolizing organisms and methanogens. These theories require a geothermal source of reduced gases such as  $\text{H}_2\text{S}$ , or  $\text{H}_2$  in contact with a suitable oxidant, which can even be  $\text{CO}_2$ , to form a source of chemical energy (redox potential) for biogenesis (Gaidos *et al.*, 1999).

The fossil record on Earth provides only broad constraints on how long it took for life to start on this planet. Simulations of the formation of Earth suggest that habitable conditions were present no sooner than 3.9 Gyr ago. The earliest indication of possible life is present in the carbon isotope record at 3.8 Gyr ago (Schidlowski, 1988; Mojzsis *et al.*, 1996), and convincing evidence of cellular life is present at 3.4 Gyr ago (Tice and Lowe, 2004). Thus, the origin of life occurred within 100 to 500 Myr after the formation of Earth. This is only an upper limit, however, and the process may have been much faster. In a review of this question, Lazcano and Miller (1994) suggested that, “in spite of the many uncertainties involved in the estimates of time for life to arise and evolve to cyanobacteria, we see no compelling reason to assume that this process, from the beginning of the primitive soup to cyanobacteria, took more than 10 million years.” However, Orgel (1998) criticized this result and stated that we do not understand the steps that lead to life; consequently, we cannot estimate the time required. “Attempts to circumvent this essential difficulty are based on misunderstandings of the nature of the problem.” The problem remains unsolvable with the current data.

The two categories of theories for the origin of life on Enceladus have different implications as to how long habitable conditions would need to persist for life to be probable. For the panspermia theories, life arrives at Enceladus intact and ready to reproduce. In this case, no origination time is required, and life can utilize a pre-existing habitable environment instantly. For the organic soup or chemosynthetic theories for the origin of life within liquid water aquifers in Enceladus, the best we can conclude is that the origination time could be as low as 10 Myr or as long as 500 Myr. As discussed above, there are no direct geophysical estimates of the persistence of the jet activity on Enceladus. However, estimates for the timescale to freeze an ocean on Enceladus are  $\sim 30$  Myr (Roberts and Nimmo, 2008), which may be consistent with the timescale for the origin of life. Thus, there is no reason to conclude that any liquid water on Enceladus is too young to have been a site for the origin of life.

### Possible Ecosystems on Enceladus

Ecosystems require an environment of liquid water, the essential nutrients, and a source of energy. The composition of the plume from the south pole of Enceladus indicates that C and N are present in the source regions of the ice jet. If this source region is a subsurface liquid water aquifer, then the remaining question to establish the possibility of an ecosystem is that of an energy source.

On Earth, the dominant energy source for all ecosystems is sunlight. Even most subsurface ecosystems on Earth derive their energy from photosynthetically produced organic material from the surface. The deep-sea vent communities derive energy from the reaction of  $\text{H}_2\text{S}$  from the vent with  $\text{O}_2$  from the ambient seawater. However, this  $\text{O}_2$  comes from surface photosynthesis. So these ecosystems are also dependent on surface photosynthesis.

To describe a plausible subsurface ecosystem that may exist on Enceladus, consideration must be given to ecosystems on Earth that are completely independent of  $\text{O}_2$  or organic material produced by surface photosynthesis. Three such ecosystems have been reported, two of which are based on methanogens that use  $\text{H}_2$  derived from rock-water reactions (Stevens and McKinley, 1995; Chapelle *et al.*, 2002) and a third on sulfur-reducing bacteria that use redox couples produced ultimately by radioactive decay (Lin *et al.*, 2006).

Stevens and McKinley (1995) discovered the first example of a microbial community completely independent of surface photosynthesis deep below the surface of the Columbia River basalts. The primary producers that sustain the community are methanogens that consume  $\text{H}_2$ , which is produced by the serpentinization of olivine in the rock (*i.e.*,  $\text{H}_2\text{O}$  reacts with iron, oxidizes it, and releases  $\text{H}_2$ ). Chapelle *et al.* (2002) found a similar system in the massive basalts in the Twin Falls area of Idaho. The net chemical reaction for methanogenesis is  $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ . We propose that methanogen-based communities provide a direct analogue for possible microbial ecosystems within a tidally heated liquid water reservoir on Enceladus. Water-rock reactions could produce  $\text{H}_2$ , which, together with the  $\text{CO}_2$  that is expected to be present, would provide the needed energy

source. Interestingly, the  $\text{CH}_4$  detected in the plume could be the product of a methanogenic-based ecosystem inside Enceladus.

McCullom (1999) considered a methanogen-based biota for Europa, and most, if not all, of his analysis would apply to Enceladus. There is also evidence that methanogens can grow over a range of low temperatures. Rivkina *et al.* (2000, 2002) studied methane evolution by a natural community of methanogens in permafrost. Their results confirm metabolic activity in the permafrost organisms down to  $-20^\circ\text{C}$ . The metabolic activity is dependent on unfrozen water that is strongly bound to soil particles. Even at temperatures of  $-20^\circ\text{C}$ , the level of unfrozen water is 1–2%, which is enough for metabolic activity. The methanogen, *Methanogenium frigidum*, has an optimal growth temperature of  $15^\circ\text{C}$ , with decreasing growth down to  $0^\circ\text{C}$  (Franzmann *et al.*, 1997).

Lin *et al.* (2006) reported on another anaerobic chemosynthetic microbial ecosystem in the deep subsurface that derives energy from a redox reaction produced by radioactive decay of long-lived (half life many Gyr) species of U, Th, and K. Primary production in this system is based on sulfur-reducing bacteria, such as *Desulfopropfundis tokoloshe*, and the net chemical reaction is  $4\text{H}_2 + \text{H}^+ + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{S} + 2\text{H}_2\text{O} + 2\text{OH}^-$ , where the  $4\text{H}_2$  is produced by dissociation of  $\text{H}_2\text{O}$  due to radioactive decay. The sulfate is produced by the reaction of the oxidants ( $\text{O}_2$  and  $\text{H}_2\text{O}_2$  also produced by the radiolysis of  $\text{H}_2\text{O}$ ) with  $\text{FeS}_2$  in the host rock.

Gaidos *et al.* (1999) suggested that life in sealed oceans under thick ice cover would rapidly die out due to depletion of redox pairs. Gaidos *et al.* (1999) noted that, for Europa and presumably Enceladus as well, availability of oxidants is the primary factor that limits life in ice-covered oceans. It has

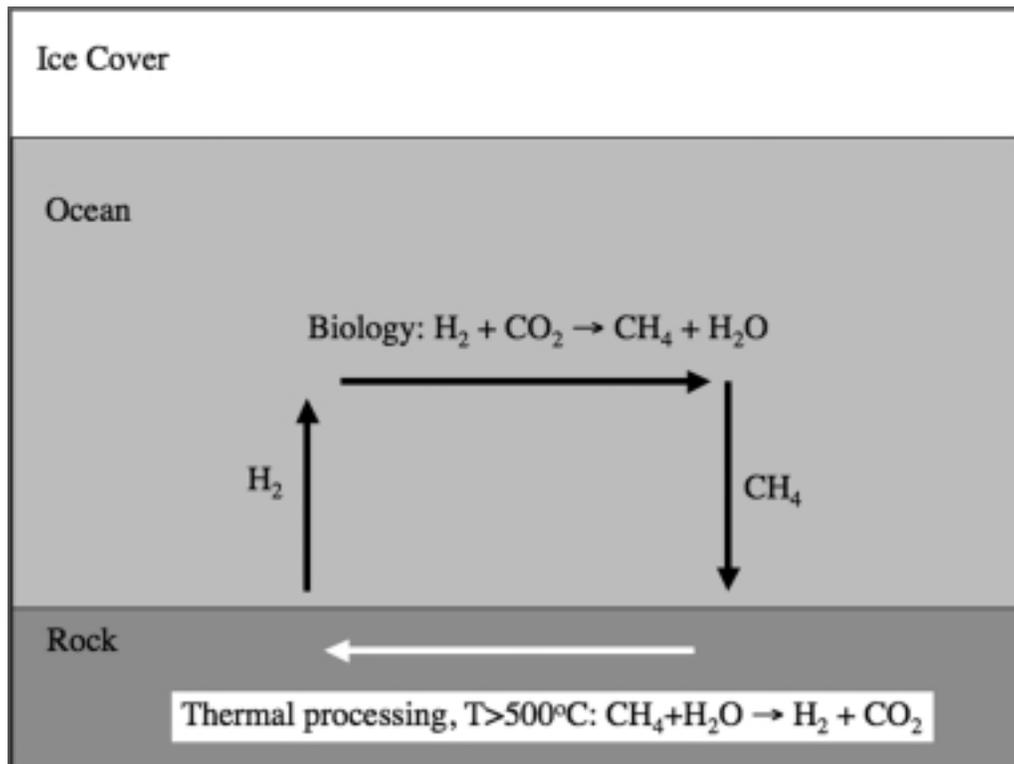


FIG. 3. Possible methane cycle on Enceladus.

been proposed that oxidants produced by radiation on the surface of the icy satellites could be carried to subsurface liquid water reservoirs that may contain reductants (Chyba and Phillips, 2001; Johnson *et al.*, 2003; Cooper *et al.*, 2007; Hand *et al.*, 2007; Moore *et al.*, 2007). Parkinson *et al.* (2007, 2008) considered Enceladus and argued for a similar, strong source of surface oxidants there. This scenario lacks a good terrestrial analogue and is roughly analogous with the oxidants produced by radioactivity as observed by Lin *et al.* (2006).

However, it is not clear whether there is a lack of redox pairs in a subsurface ocean, so the speculative sources and transport of surface oxidants may be unnecessary. The three isolated microbial ecosystems discussed above provide a basis for considering how a long-term biological cycle can persist in an ice-covered ocean.

Figure 3 shows a proposed methane cycle that could support methanogens in a subsurface aquifer on Enceladus or Europa, following the analysis of McCollom (1999). Hydrogen and CO<sub>2</sub> present in the water provide a basis for methanogens that form CH<sub>4</sub> and biomass, which act as the primary producers in the community. Clearly, with no way to recycle this CH<sub>4</sub> and biomass, the system would rapidly die down. However, water entrained into the subsurface and heated to temperatures of 500°C would reform H<sub>2</sub> and CO<sub>2</sub> because of the shift of the stability with temperature. This estimate is based on cometary ratios of C, H, and O (*e.g.*, McKay and Borucki, 1997; Kress and McKay, 2004), and the temperature at which the reformation of H<sub>2</sub> occurs does depend somewhat on the composition. Hydrothermal calculations by McCollom (1999) and Glein *et al.* (2008) suggest even lower temperatures for the reformation of CO<sub>2</sub>. Note that H<sub>2</sub> could also be released from the rock-water reactions as is occurring in the Colombia River Basalt system studied by Stevens and McKinley (1995). The reformation of H<sub>2</sub> and CO<sub>2</sub> complete the cycle, and these gases can enter the water column as nutrients for the methanogens. As Sleep and Zoback (2007) pointed out with regard to Earth, seismic activity—quakes—could open cracks in the silicate core of Enceladus and maintain a finite permeability that allows water to circulate between high- and low-temperature regions. Essentially, the redox cycle shown in Fig. 3 derives its energy from the geothermal gradient and converts this thermal energy to chemical energy in a form useful for microorganisms, in direct analogy with systems described on Earth in which abiotically produced gases originate from the subsurface and provide feedstock for microbial communities (Sherwood Lollar *et al.*, 2006). In this case, the lifetime of the microbial ecosystem is set not by the depletion of available redox pairs but by the geothermal heat source of the object. The interior temperatures in Enceladus are unknown, but given that the surface stripes reach temperatures of 180 K, deeper temperatures of several hundred Kelvin seem plausible. Note that the biogeochemical cycle proposed in Fig. 3 does not require oxidants from the surface.

The subsurface life discovered by Lin *et al.* (2006) provides another model for life under an ice-covered ocean. This is shown in Fig. 4. Radioactive decay produces reduced and oxidized species by the radiolysis of H<sub>2</sub>O. The oxidant produced reacts with sulfide species to produce sulfate, which is then used by sulfate-reducing bacteria such as *Desulfopropionifundis tokoloshe*. This reforms the sulfide species and completes the cycle. Again, the energy-limited lifetime of this

system is set not by the depletion of redox pairs but by the lifetime of radioactive energy sources. McCollom (1999) proposed a similar scenario for Europa with H<sub>2</sub> generated from hydrothermal systems reacting with sulfate in solution.

If the mass 28 peak in the plume of Enceladus represents CO, this would seem to argue against biological processes that might be expected to consume CO. For example, it is known that some methanogens can grow anaerobically on CO (Rother and Metcalf, 2004). As sometimes occurs on Earth, however, biological consumption may not be complete, and some level of leakage could still be consistent with biological consumption.

## Ammonia

Despite the lack of direct detection, there are theoretical arguments for the presence of ammonia on Enceladus. Models of the formation of Enceladus suggest that ammonia may make up ~12% of mass of ice present (Squyres *et al.*, 1983). In addition, ammonia mixed with water would suppress the freezing temperature of water and allow for liquid interiors at lower temperatures. The existence of a subsurface south polar sea has been proposed (Collins and Goodman, 2007); and, indeed, if present, ammonia could potentially play a significant role in its long-term stability as well. Thus, the habitability of ammonia-water solutions warrants consideration.

In solution, ammonia exists as free ammonia (NH<sub>3</sub>-H), ionized-ammonia (NH<sub>4</sub><sup>+</sup>, also called ammonium), or as both (total ammonia), depending upon the pH and temperature of the solution. It is known from previous studies concerning biological systems, including microbes, that the toxic component of ammonia is NH<sub>3</sub>-H. This free ammonia dominates at high pH values; however, as the pH of a solution lowers, the hydrogen ion concentration increases and, thereby, allows NH<sub>3</sub>- to combine with the excess hydrogen and, thus, transition to the non-toxic form, NH<sub>4</sub><sup>+</sup>.

The effect of total ammonia (NH<sub>4</sub><sup>+</sup> + NH<sub>3</sub>-H) on methanogens is of particular interest because, as discussed above, methanogens are a key candidate organism for putative life on Enceladus. There have been numerous studies on the limits of ammonia exposure of various methanogenic species. The majority of these experiments have focused on

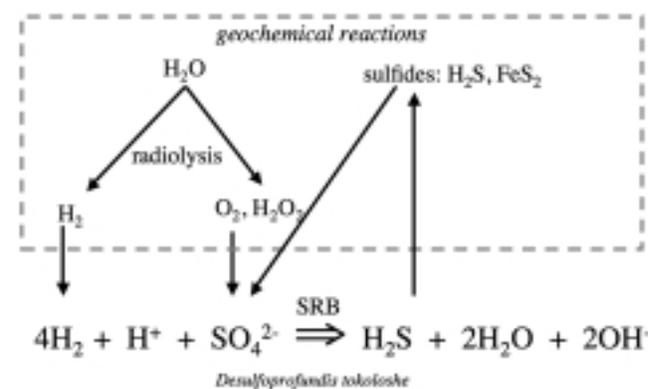


FIG. 4. Proposed biogeochemical cycle in the subsurface of Enceladus powered by radioactive decay as observed on Earth by Lin *et al.* (2006). Figure adapted from T. Kieft.

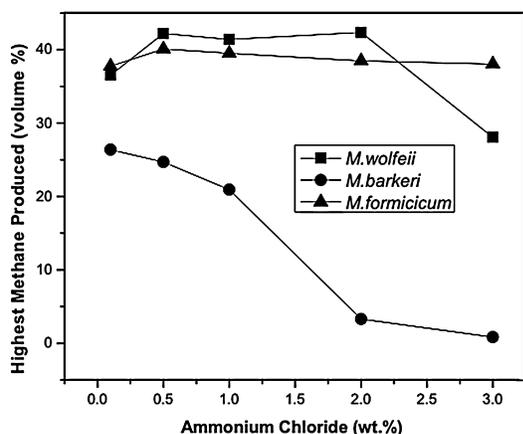


FIG. 5. Tolerance to total ammonia (added as ammonium chloride) for three methanogenic microorganisms. Growth/metabolism was determined by measuring methane production with a Varian benchtop gas chromatograph. Results reported as the highest amount of methane detected inside anaerobic culture tubes.

communities of methanogens involved in anaerobic digestion of industrial or natural waste where individual species were not determined, although studies on pure cultures of methanogens have also been carried out. Concentrations of total ammonia used in these experiments ranged from 200 to 20,000 mg/L (Jarrell and Saulnier, 1987; Koster and Koomen, 1988; Bhattacharya and Parkin, 1989; Hendriksen and Ahring, 1991; Kadam and Boone, 1996; Sawayama *et al.*, 2004).

We report here new experiments to determine the tolerances to total ammonia exposure for three different methanogenic species. Significant methane production (*i.e.*, growth/metabolism) was demonstrated by two of the three organisms even at high levels of ammonia (Fig. 5). The pH in these experiments was between 6.6 and 6.7. We concluded that, while the existence of ammonia on Enceladus is still in question, its presence should not be considered as the limiting factor for the development and persistence of life there, especially considering the range of ammonia tolerances exhibited by methanogenic microorganisms.

### Detection of Biomarkers in the Plume

An important goal for astrobiology that may be achieved with a near-term mission would be the detection of biomarkers in the plume or on the surface of Enceladus. The presence of CH<sub>4</sub> in the plume suggests the possibility that methanogens are present in an anaerobic chemosynthetic ecosystem analogous to those in the Colombia River basalts (Stevens and McKinley, 1995). If that is the case, the CH<sub>4</sub> is biogenic and not thermogenic as suggested by Matson *et al.* (2007).

On Earth the most powerful method to distinguish between biogenic and abiogenic CH<sub>4</sub> is to analyze the carbon isotope difference between the CH<sub>4</sub> produced and the source of C and analyze the pattern of carbon isotopes in non-methane hydrocarbons, such as ethane, ethylene, propane (*e.g.*, Sherwood Lollar *et al.*, 2002, 2006). For example, if methanogens produce CH<sub>4</sub> from CO<sub>2</sub>, there would be a dis-

tinctive shift in the C isotopes between the CH<sub>4</sub> and the CO<sub>2</sub>. The ability to analyze such isotopic shifts would be easily achieved with samples in the laboratory. It would be a challenge to implement in a spacecraft, however, and is certainly not within the capability of the Cassini orbiter.

However, the abundances of other non-methane hydrocarbons relative to CH<sub>4</sub> can also be used to distinguish between biological and other sources. Nonbiological processes that synthesize CH<sub>4</sub> from simpler compounds (H<sub>2</sub> and CO) such as Fischer-Tropsch synthesis and thermal degradation, which produces CH<sub>4</sub> from thermal decomposition of organic material, both tend to produce power law distributions in concentration as a function of carbon number.

Figure 6 modified from Whiticar (1990) and based on data from Bernard *et al.* (1978) shows the difference between CH<sub>4</sub> produced by microbes and CH<sub>4</sub> produced by thermal decomposition in terms of the relative concentrations of C<sub>1</sub> to C<sub>2</sub> + C<sub>3</sub> compounds and the isotope ratio in the CH<sub>4</sub>. Horita and Berndt (1999) showed that these separate regions are indicative, but not conclusive, and variations in formation conditions can change the values, which makes it difficult to determine whether the sources are abiotic or biotic. Allen *et al.* (2006) applied this diagram to the investigation of CH<sub>4</sub> on Mars and concluded that a more convincing case for biogenic sources would rely on the carbon and hydrogen isotope pattern as a function of C number.

McCollom and Simoneit (1999) reported on the abiotic synthesis of hydrocarbons in vent simulations and found a power law with a slope that varied depending on the presence of H<sub>2</sub>O. Sassen *et al.* (2004) reported on a complex source with a primary nonbiological source of organics modified by a biological community. The concentration was also a power law. Proskurowski *et al.* (2008) reported a pattern of concentration versus C number that showed a power law for abiotic processes in the Lost City hydrothermal vent. In supplemental material associated with that paper, the authors suggest that microbial reactions produce a ratio of methane to heavier hydrocarbons that ranges from 2,000 to 13,000 while Fischer-Tropsch synthesis values range from 100 to 1000 and thermogenic processes have ratios less than ~100.

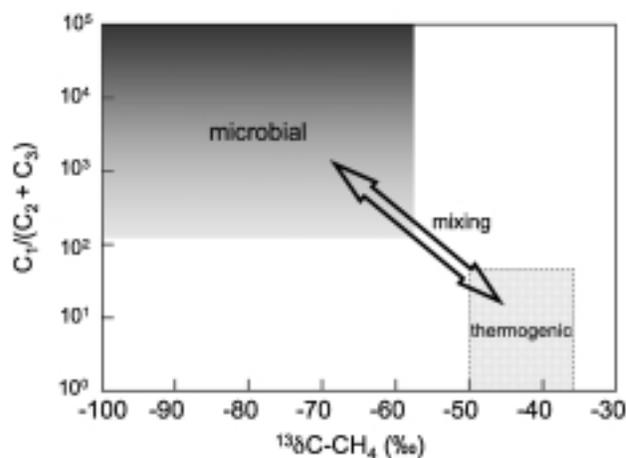


FIG. 6. Concentration and isotope for microbial and thermogenic sources of CH<sub>4</sub>. Figure adapted from Whiticar (1990).

TABLE 1. RATIOS OF NON-METHANE HYDROCARBONS FROM BIOLOGICAL SYSTEMS

Compound	Ratio to CH <sub>4</sub> (gm/gm)	Reference
Ethane (C <sub>2</sub> H <sub>6</sub> )	0.9 × 10 <sup>-3</sup> 0.1 × 10 <sup>-3</sup>	Devai and Delaune (1996) Oremland (1981)
Ethylene (C <sub>2</sub> H <sub>4</sub> )	0.7 × 10 <sup>-3</sup> 0.4 × 10 <sup>-3</sup>	Devai and Delaune (1996) Oremland (1981)
Propylene (C <sub>3</sub> H <sub>6</sub> )	1.5 × 10 <sup>-3</sup>	Devai and Delaune (1996)
Propane (C <sub>3</sub> H <sub>8</sub> )	1.1 × 10 <sup>-3</sup>	Devai and Delaune (1996)
Butane (C <sub>4</sub> H <sub>10</sub> )	0.9 × 10 <sup>-3</sup>	Devai and Delaune (1996)
Isobutane (C <sub>4</sub> H <sub>10</sub> )	1.1 × 10 <sup>-3</sup>	Devai and Delaune (1996)

Results from Devai and Delaune (1996) are taken from the most-reducing conditions reported.

Methanogens, in contrast to a chemical system (Fig. 6), produce low levels of hydrocarbons other than CH<sub>4</sub> but produce them at a level (~10<sup>-3</sup>) that is relatively constant for low C numbers. Table 1 lists the relative concentrations of non-methane hydrocarbons to CH<sub>4</sub> from soil systems. The values are roughly independent of C number for the values up to C<sub>4</sub>, as listed. Figure 7 shows a comparison of the C number concentration profile for biological sources (based on Table 1) contrasted with nonbiological sources from laboratory simulations of hydrocarbon production in deep sea vents (McCollom and Simoneit, 1999).

Methanogenesis is found only in Archaea, not in Bacteria or Eukarya. It is an ancient metabolic pathway (Knoll and Canfield, 1998; Teske *et al.*, 2003; Battistuzzi *et al.*, 2004), and there is evidence that methanogens were ecologically important on Earth early in its history (Ueno *et al.*, 2006). A methanogenic ecosystem operates under strictly reducing conditions and would be inconsistent with any appreciable O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub>. It is also worth noting that, in such a system, the CH<sub>4</sub> derives from the CO<sub>2</sub>, so there would be an important isotopic shift in between these two gases.

The Cassini Ion and Neutral Mass Spectrometer is capable of detecting up to mass number 100 and could in principle measure hydrocarbons up to C<sub>6</sub> and above. This was clearly demonstrated by Waite *et al.* (2007) in measurements

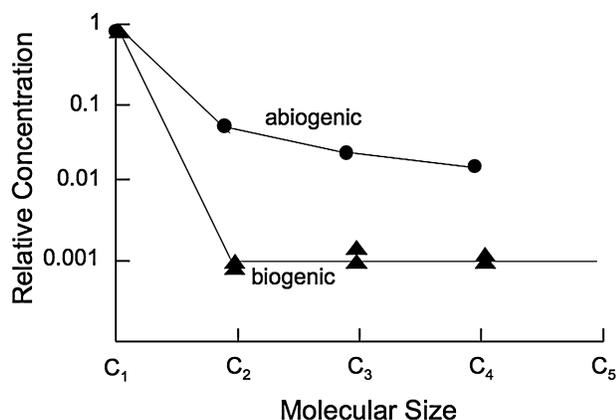


FIG. 7. Comparison between nonbiological production of hydrocarbon from McCollom and Simoneit (1999) and biological production as reported by Devai and Delaune (1996) on Earth.

of Titan's upper atmosphere where hydrocarbons as complex as benzene (C<sub>6</sub>H<sub>6</sub>) were detected at levels of a few ppm at the 1000 km elevation level (total density ~10<sup>10</sup> cm<sup>-3</sup>). If similar detection levels could be achieved in the plume of Enceladus, a determination of the falloff in concentration as a function of C number could be indicative of a biological origin of the CH<sub>4</sub> and other hydrocarbons.

Methane production by methanogens may be accompanied by hydrocarbon production through thermal decay, primary organic matter, or biogenic organic matter. In this case, the ratios and isotopes would reflect the mix of these two sources, as shown in Fig. 6.

It is also important to note that the absence of a biotic source of CH<sub>4</sub> does not preclude the presence of a microbial community; it would just indicate that the CH<sub>4</sub> observed is not a product of a methanogenic-based microbial community.

### Samples and the Search for a Second Genesis

If there are biological processes occurring in a subsurface aquifer on Enceladus and material is carried out into the plume, this opens the opportunity for detailed analysis of the remains of these organisms. Any microorganisms entrained in the plume would probably be dead but would be organically intact. Thus, a detailed analysis could reveal the biochemical nature of this life. Such an analysis could be done with a sophisticated organic analyzer on a future astrobiological mission or on samples returned to Earth.

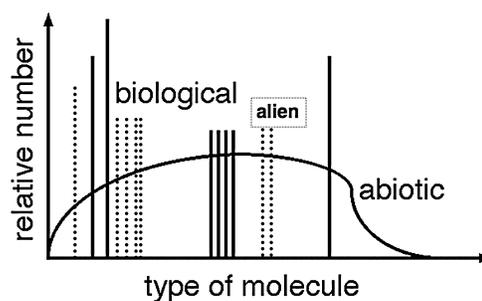


FIG. 8. Organic distribution for abiotic, biological, and possible alien life on Enceladus. Abiotic distributions are smooth. Biology on Earth uses only certain molecules. A second genesis of life on Enceladus may have a different set of basic molecules. Adapted from McKay (2004).

If life exists on Enceladus and is biochemically and genetically related to life on Earth, then detection of the remains of this biology would be straightforward. The sophisticated techniques developed for the study of life on Earth can be applied. These include amplification of DNA by polymerase chain reaction and detection of key molecules such as adenosine-5'-triphosphate (ATP). There are two scenarios by which life on Enceladus may be identical to life on Earth: (1) if Solar System-wide panspermia resulted in a shared origin of life for both Earth and Enceladus and (2) if all life everywhere evolves to the same biochemical and genetic system (e.g., Pace, 2001).

The discovery of life on Enceladus that is identical to life on Earth would be of interest, but it would not be as interesting as the discovery of evidence for life that is different from life on Earth and, thus, represents a second genesis of life in our Solar System (McKay, 2001, 2004). However, if the remains of life are found in the plume of Enceladus, and they are from an alternate type of biochemistry, it would not be clear how we would detect or characterize this life. Finding alien life would certainly be more challenging than finding Earth-like life. McKay (2004) suggested a method by which to detect carbon-based life that represents an alternative biochemistry. The approach, known as the "Lego principle," rests on the observation that life uses just a few discrete molecules to construct the main structures needed for life. For Earth life, these building blocks are the 20 L amino acids used in proteins, the nucleotide bases used in DNA and RNA, the D sugars used in polysaccharides, and the lipids used in membranes.

Figure 8 shows a schematic distribution of organic molecules that might be generated from abiotic processes compared to the distribution from biological sources. In an abiotic distribution, such as that found in meteorites or produced in laboratory simulations, there is a smooth distribution with the abundance of molecules set by their chemical properties. In contrast, in a biological distribution there is selection such that molecules with similar chemical properties may be present in very different abundances. A good example of this is the L and D amino acids. Biological processes on Earth use only the L version in proteins.

We can extend this concept to consider a possible result on Enceladus. Life there would also be expected to select certain molecules at the exclusion of other similar molecules. If life exists on Enceladus and represents an alternative origin from life on Earth, then it is possible that it would use a different set of discrete molecules as the basis for its biochemistry. This concept is represented by the dotted lines shown in Fig. 8.

## Conclusion

We have considered the astrobiological possibilities for a subsurface aquifer on Enceladus. If such a subsurface aquifer exists and is the source of the observed plume, then we conclude the following hypotheses:

- (1) There are plausible scenarios for the origin of life in a subsurface aquifer on Enceladus based on theories developed to explain the origin of life on Earth.
- (2) There are plausible long-lived energy sources for life below the ice on Enceladus that couple suitable redox pairs

to geologic energy sources. Examples of these microbial ecosystems exist on Earth.

- (3) Ammonia is unlikely to be toxic for methanogens in the water solution on Enceladus.
- (4) The CH<sub>4</sub> and other hydrocarbons detected in the plume may be of biological origin. Lacking isotope data, the abundance ratio of heavy hydrocarbons to methane may be an indicator of biological origin. The ratio of ethane to methane for biogenic sources is expected to be 10–100 times less than for abiogenic sources. The extended Cassini mission could characterize the non-methane hydrocarbons and find a ratio consistent with life.
- (5) Samples from the plume would allow for detailed organic analysis, which could detect the presence of a second genesis of life even if all the organisms in the plume were dead.

Perhaps the most enticing aspect of the search for life on Enceladus is that fresh samples of interest are jetting into space ready for collection. Drilling, scooping, melting, or digging may not be necessary. This argues for the plume of Enceladus as a target for a future mission, perhaps a Saturn or Enceladus orbiter, that would be properly equipped for detailed chemical analyses and make repeated and deep flybys through the densest portions of the plume. An extreme possibility is a sample return mission along the lines of the Stardust mission. For astrobiology, Enceladus is a world of possibilities.

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## Abbreviation

SPT, south polar terrain.

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