DETERMINING FAVORABLE CONDITIONS FOR ENDOLITHIC GROWTH: UV PROTECTION

By

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Table of Contents

Figures

Tables

Abstract

The large UV flux on the Martian surface has been considered an obstacle to the presence of life. However, organisms on earth have had to develop UV protections mechanisms as well. Examining endolithic cyanobacteria from the deserts of Southern Utah has shown that a small amount of iron oxide in the surrounding rock can effectively shield ultraviolet light while still allowing enough light in the photosynthetic zone for the organism to survive. This reopens the possibility of the presence of life at the surface of Mars.

Figure 1 – Mars from the Mars Desert Research Station, August 30, 2003

Introduction

One of the most sought after answers in science is whether or not life is unique or abundant in the universe. Was the genesis of life on earth an improbable event, or does life always evolve given certain circumstances? Currently, we have no evidence one way or the other, but we do have a neighbor that could provide the answer.

Our solar system formed when a cloud of particles, created by the explosion of an earlier star, accreted into the Sun, nine other large bodies that we classify as planets, and moons of varying size that orbit the planets. Of the nine planets, only the Earth and Mars could have evolved life as we know it. Mercury is tidally locked with the sun and is either too hot or too cold, depending on where you are on the surface. Venus has an atmosphere 90 times denser than our own, has an average temperature of 500ºC—hot enough to melt lead on the surface—and has clouds that rain sulfuric acid. Not exactly friendly to life.

The gas giants have no surface to speak of and thus can't support life as we know it. However, Europa, one of Jupiter's moons, is suspected to have an ocean of liquid water underneath a frozen surface. Despite its distance from the sun, it is possible that life could evolve near geothermal hotspots where life is thought to have originated on earth. However, finding life on Europa would require technology that we don't yet have, such as the ability to send a probe capable of drilling through kilometers of ice. Pluto is simply too cold and too small to support life. That leaves Mars as our best chance of finding life elsewhere in the solar system.

The Red and Blue Planets Compared

Earth and Mars may not seem similar at first glance, they may have important life-sustaining traits in common. Mars, the fourth planet, orbits 1.5 AU from the sun (1

AU is the radius of earth's orbit or ~108,000,000 km), has a radius half that of the Earth, has 10% the mass, 39% of Earth's gravity, a rotation period of 24h 36min, and a 687 day long year. Radius and mass are important because larger planets have more internal heat due to radioactive decay, leading to longer geological activity (Mercury and the Moon are relatively small and are both geologically dead). The Earth is large enough to still have geologic activity such as plate tectonics and volcanism after 4.5 billion years. Another similarity between the two planets is that Mars has a day/night cycle roughly equivalent to that of the Earth. While a 24 hour cycle is not necessarily the perfect number for life, we know it's in the range.

Mars is known to have had surface water in the past, implying a thicker atmosphere and higher temperature than now exists. Olympus Mons, the largest known volcano in the solar system at 20 km high, proves without a doubt that Mars has been geologically active. Three and a half billion years ago, about a billion years after the accretion of the solar system, the Earth and Mars were very similar. This is when the first life appeared on Earth in the form of small, single-celled organisms. If life is common under specific conditions, it could have also evolved on Mars during this same time.

The History of Mars Research

Historically, the idea that Mars could be home to life is not new. In the early 20th century, telescopes grew in both size and clarity, allowing a closer look at the face of Mars. Some astronomers, of whom Percival Lowell was the most outspoken, claimed to see a network of water-bearing canals crisscrossing the Martian surface. They concluded that Mars was the home of a noble race of intelligent beings trying to survive on an increasingly harsh planet.

Over the next few decades, the canal theory was discredited, but many still hoped that Mars was a living planet. The Mariner and Viking missions of the 1960's and 70's brought us our first close-up views of Mars. Disappointingly, the satellites returned photos that looked more like the Moon than Earth, a surface pockmarked with craters and no signs of life.

Figure 2 - Mariner 4 Image of the Surface of Mars (NSSDC, 1965)

In 1976, Viking I and II landed on the surface of Mars. The two landers each performed three experiments designed to test for biology: the Viking Gas Exchange (GEX) experiment, Viking Labeled Release (LR) experiment, and Viking Pyrolytic Release (PR) experiment. Though the LR experiment gave a positive response, meaning it detected signs of life, the results were interpreted as being the result of a chemical and not a biological process [1]. Norman Horowitz, head of the pyrolytic release experiment concluded that:

> "Viking not only found no life on Mars, it showed why there is no life there.... the extreme dryness, the pervasive short-wavelength ultraviolet radiation... Viking found that Mars is even dryer than had previously been thought... The dryness alone would suffice to guarantee a lifeless Mars; combined with the planet's radiation flux, Mars becomes almost moon-like in its hostility to life" [2]

Subsequent missions have also failed to turn up any signs of life on the surface of the planet. Most planetary scientists now believe that if we find life on the Red Planet, it will be in groundwater with a geothermal heat source. Therefore, the search for life on the surface of Mars has been largely abandoned as it is thought to be inhospitable to life.

Life as we understand has many limits, among them: lack of liquid water, temperature extremes, ultraviolet radiation, environmental toxicity, and limited energy resources. The discovery in the past 20-30 years of extremophilic organisms on Earth has not eliminated any of the above limitations, but has redefined a range in each case. Extremophiles (literally "extreme loving") are organisms that live under adverse conditions, e.g. environments with high salt content, extreme pH and temperatures, or inside rocks (high pressure, low light conditions). Extremophiles have shown that though temperature is still a limiting factor on life, the range where we have found life continues to increase. In the past, it was thought that organisms couldn't survive much above 60ºC or below freezing. Recently, bacteria have been found near deep sea vents living in 120ºC water as well as in Antarctic snow, metabolizing at -15ºC [3].

Obstacles to Life on Mars

So what are the limitations to life on Mars? The biggest problem is the apparent lack of liquid water. Because of its low pressure and temperature, water can't exist on Mars under ordinary conditions. It simply evaporates into the atmosphere. However, we know that Mars has had standing water on the surface in some periods of its geologic past. So water appears to be possible on the surface, under restricted conditions.

Second, Mars has an atmosphere of 95% CO2 and only 0.13% O2. Abundant oxygen creates an ozone (O3) layer that shields out harmful ultraviolet radiation. UV light is short wavelength, high energy light that is biologically detrimental. Ultraviolet light has a wavelength between 200-400 nanometers (10^{-9} meters) and is grouped into three bands: UVA (400-320 nm), UVB (320-290 nm), and UVC (290-200 nm). Ozone, an important component of Earth's atmosphere, absorbs light below 360 nm. CO2, the main element of Mars' atmosphere, absorbs below 200nm [4]. Mars' carbon dioxide atmosphere acts as somewhat of a shield, but ultraviolet radiation below 290 nm is extremely damaging to most biological systems. The high energy light can destroy biological organisms in many ways. For example, UV rays can create thymine dimers (bonds between adjacent thymine bases) that lead to mutation during DNA replication. Ultraviolet light can also simply break molecular bonds, killing the organism.

So is the lack of an ozone layer a showstopper for life on Mars? Probably not. Before life evolved on Earth, the atmosphere had virtually no oxygen and thus no ozone layer. It wasn't until *after* life evolved and proliferated that we gained an ozone layer. The ozone layer developed with the increase of atmospheric oxygen produced by photosynthesizing early bacteria. So life can (and most likely must) begin without an ozone layer.

Table 1-UV Flux (Watts/m^2) Earth vs Mars [5]

(Irradiances at the equator at a Solar Zenith angle of 0°)	**UVC and B C:200-280nm B:280-315nm**	**UVA A:315-400nm**	**DNA effective Irradiance**
Present Day Earth	**1.86**	**52.81**	**.1**
Present Day Mars	**13.2**	**41.5**	**116.4**
Early Earth	**5.2**	**34.1**	**41**
Early Mars	**4.8**	**22.3**	**33.8**

Early bacteria could have used their habitat to shield them from ultraviolet radiation. Dr. David Allred and I propose that it is possible that endolithic cyanobacteria could have evolved on Mars during a water-rich, warmer past, and that they could have either left fossil evidence or still be living today under rocks that preferentially absorb ultraviolet radiation. By investigating endoliths, or cyanobacteria that live inside rocks, our samples being found in the deserts of Southern Utah, we will show that rocks containing certain minerals can shield out light below a

Figure 3 – A small rock sample with a 1 mm thick endolithic layer.

certain wavelength. Presumably, this result is an effect of the band gap of the material. Our results are consistent with light transmission through Iron Oxide (Fe_2O_3).

At first glance, the high deserts of Southern Utah seem to be almost completely devoid of life. But if you know where to look, you can find life almost anywhere. One of the more interesting desert creatures is a cryptoendolithic bacteria that lives inside rock. We took samples from an area in Southern Utah about 10 miles north of the town of Hanksville. The samples come from the Dakota Formation of the early to mid Cretaceous period.

Figure 4 – Photograph of Dakota Sandstone Boulders lying on the Brushy Basin member of the Morrison Formation. The Dakota Sandstone weathers less quickly than the Brushy Basin siltstone which it caps.

The endoliths grow underneath a millimeter of rock and grow in the rock for about a millimeter, as judged by the naked eye. It is likely that there are cells on either side of this boundary, but microscopic investigation would be necessary to see them.

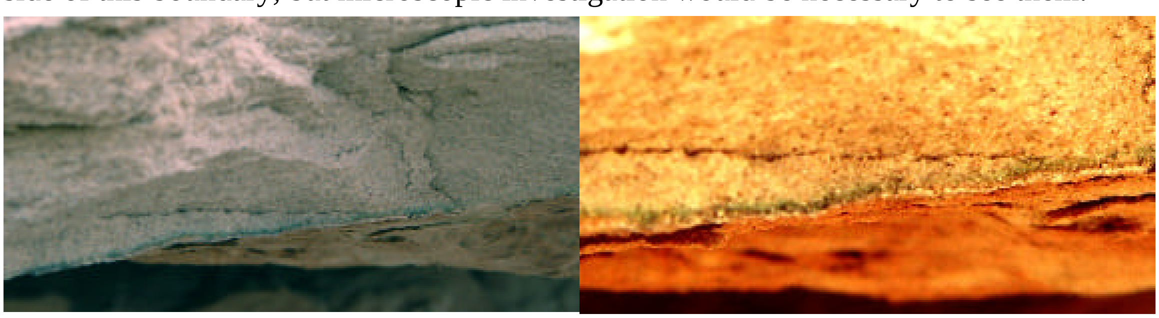

Figure 5 - Photographs of broken edge of a Dakota Boulder; picture is about 8 inches from top to bottom. Note that the endolithic colony is on the *bottom* of the ledge and, therefore, must be receiving light for photo synthesis by scattering from below. The second image is a close up of the colonized area.

Theory

On a microscopic level, energy is quantized. This means that an electron orbiting an atom cannot simply take on any value, but is confined to a certain number of discrete energy levels. In a solid, the electrons of the crystal are similarly bound—they can only have certain energies. Because of interactions with other molecules and other electrons, certain energy levels are forbidden, meaning that they have a very low probability of existing there according to quantum mechanics. The Band Theory of Solids places the electrons in one of two bands, the conduction or valence band. Between the two bands is the band gap, where there are no allowed electron energy levels. For an electron to jump from the conduction to the valence band, it must absorb energy equal to or greater than the energy of the band gap.

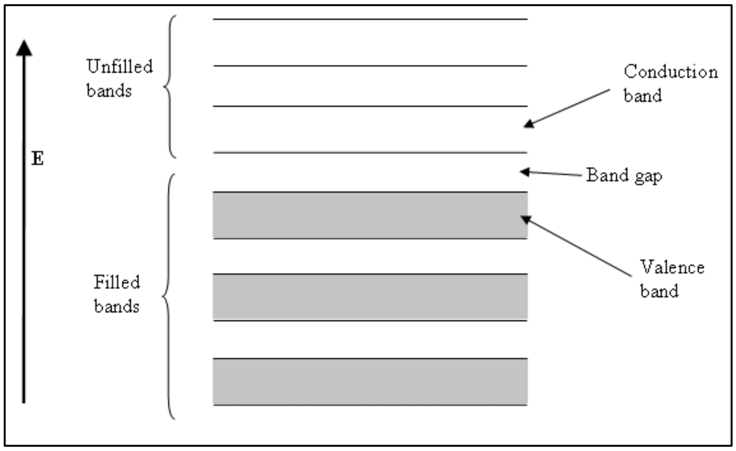

Figure 6 – A graphical representation of bands in materials. The gray is the valence band, the white levels are the conduction band.

The band gap of a material will make the object transparent to low energy, longer wavelength light as the electrons cannot absorb photons of this energy. However, higher energy photons will be absorbed by the electrons as they jump from the conduction to the valence band. Thus, solids are transparent to some wavelengths of light, but absorb others. For life to exist, the material must absorb the harmful, high energy light. For photosynthetic life to exist, the material must both absorb high energy light yet still allow enough light in the photosynthetic zone for the organism to survive. As geologic evidence seems to show that early surface life on the earth was photoautotrophic, we will look for similar life on the surface of Mars.

The Viking missions of the 1970's and the 1997 Pathfinder mission took soil samples from their landing sites with the following results:

Table 2 - Martian Soil Composition [6]

Sample Location	Na2O	MgO	Al2O3	SiO2	SiO3	K2O	CaO	TiO2	Fe2O3
Near Yogi	3.7	8.1	8.9	46.8	6.3	0.2	5.5	1.4	15.6
Scooby Doo	1.9	6.9	8.9	50.3	5.2	0.5	7.1	1.1	14.5
Rocky Flates:crust	--	7.4	7.2	44.1	10	0.9	5.9	0.6	18.3

If we can show that iron oxide is an effective shield against ultraviolet radiation, one of the major barriers to life on the surface of the Red Planet can be overcome under the right conditions.

Materials and Methods

To perform our experiment, we first needed to evaluate what we were looking at. We needed to be sure that the green layer in the rock was in fact biologic in origin. Then, we looked at light transmission through the rock.

Electron Microscopy

To be certain that the green layer in the rocks was biologic the samples were examined with an environmental scanning electron microscope. A few rock samples were broken to expose the green layer and one that presented an abundance of targets was chosen. The sample was placed into the e-SEM chamber and the pressure was reduced to 1 torr, using water vapor as the gas. The images were taken using a 15 kV beam.

Light Transmission

Light emitting diode's (LED's) of different wavelengths were used as a specific energy source of light. All LED's were obtained from All Electronics or Roithner Lasertechnik. The LED's all had 5mm plastic lenses, ranged in wavelength from 350 to 910 nm and have a ? ?$_{fwhm}$ (delta-lambda at full-width, half max) of 10nm. The LED's were mounted at the end of a cylinder wrapped in copper wire to reduce interference effects in the circuit. A Stanford Research Systems™ SR830 DSP Lock-in Amplifier was used to drive the LED at a frequency of 200Hz. The exact frequency is not important, as long as commonly used frequencies such as 60 or 120 Hz, or the frequency of a nearby computer monitor are avoided. The 300 ms setting was used as the time constant on the lock-in amplifier in all cases. The LED was placed onto an optical bench, according to our schematic in Fig. ?.

Although the spectral response of the detector necessitated a different output voltage for each LED, the spectral response of the detector proved unimportant as only the relative intensity at each wavelength was required. To obtain the best results for small amplitude signals (sample present) the driving voltage was adjusted to a value just below the overload value for the input of the lock-in amplifier (around .5V).

A UDT™ PIN-13DI planar diffused silicon photodiode was used to measure light intensity. The photodiode was placed inside a 1 inch long segment of ½ inch copper tubing. The copper was spray-painted white on the inside so that light

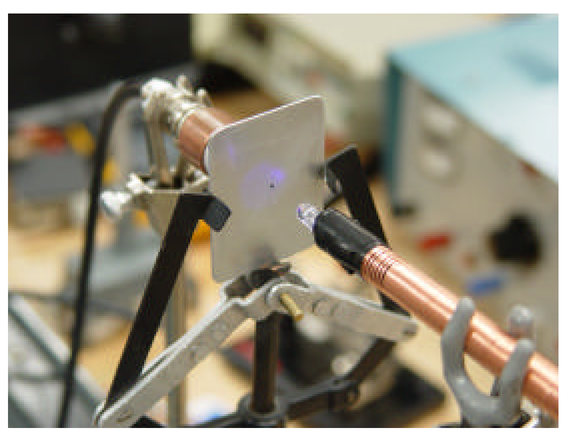

Figure 7 – A photograph of the experimental setup. The LED is shining onto the steel aperture.

entering the tube would reflect off the walls of the tube until hitting the detector (duplicating the effects of an integrating sphere). To avoid maxing out the detector, a steel plate with a 1mm diameter aperture was placed over the copper tubing, effectively enclosing the photodiode.

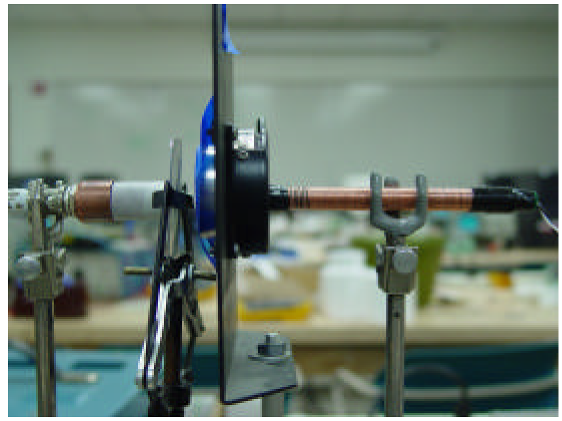

Figure 8 – A side view of our setup. From left to right: photodiode in a copper tube; steel aperture with 1mm diameter pinhole; aluminum mount holding the sample; LED mounted to a tube shielded with copper wire.

A National Semiconductor™ LMC6001 Ultra Ultra-Low Input Current Amplifier was used to increase the signal received by the photodiode (with no sample problem, this would not be necessary, but after passing through 1mm of rock, the current amplifier is necessary). The signal was then read by the lock-in amplifier, reading only signals with a frequency of 200Hz. The phase was adjusted so that channel two read zero, then readings were taken from channel one.

First, a reading of light intensity was taken with no sample present at a sensitivity of 1 V on the lock-in amplifier. Then the sample was inserted with the light source and detector at the same position and a second intensity reading was taken with the sensitivity adjusted to 5mV. The LED and the photodiode were separated by a distance of about 5cm. The sample itself was attached using black electrical tape to the inside of an aluminum mount. Black tape was placed around the edges of the sample to insure that the only light getting to the detector actually passed through the sample. This process was repeated for each wavelength.

Results

Electron Microscopy

We confirmed that the green layer inside the rock was indeed biologic in three

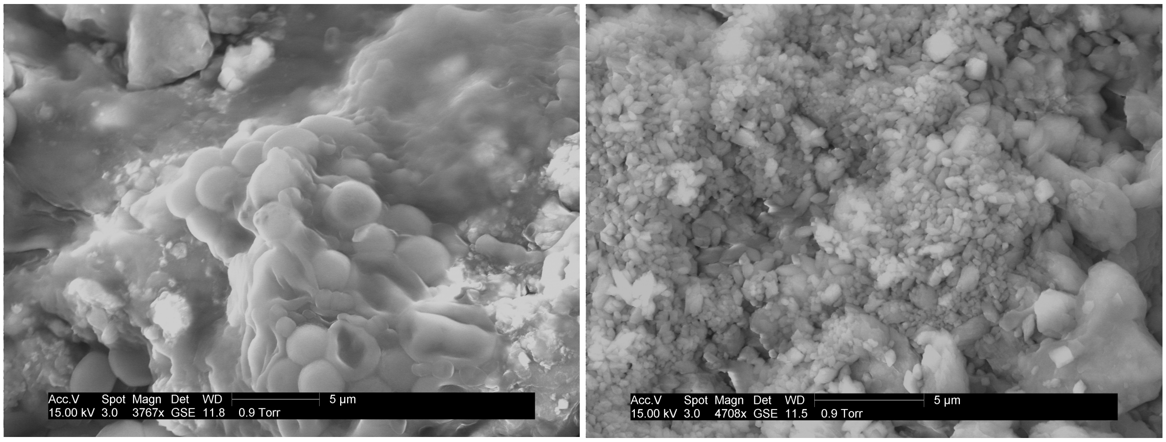

Figure 9 – On the left, an e-SEM photomicrograph of sample showing endolithic colony (2-3 micron spheroidal and associated amorphous structures) on a surface of a rock that fractured at the green endolith level. The organisms appear to be coated by a natural layer which obscures depth information. Angular material can be seen towards the lower corners of the photo. Under higher resolution these are seen to be crystals the chemical analysis of which shows much more Ca C and O than the endoliths or the underlying sand. Carbonates are known to bind sand stones together and may fulfill that role here. An image of a non-organic region of the rock is shown on the right, at the same scale.

ways. First, visually, the circular structure and colony organization as well as the size fits perfectly with known bacteria. Second, figure ? shows an energy dispersive x-ray analysis of the sample, with a small nitrogen peak present. Nitrogen is strongly indicative of life. Third, while taking an energy dispersive analysis of the sample with a stronger beam, we saw one of the cells collapse. The combination of these three facts leads us to the conclusion that our green layer is definitely biologic. We suspect that we are looking at bacteria of the genus *chroococcidiopsis*.

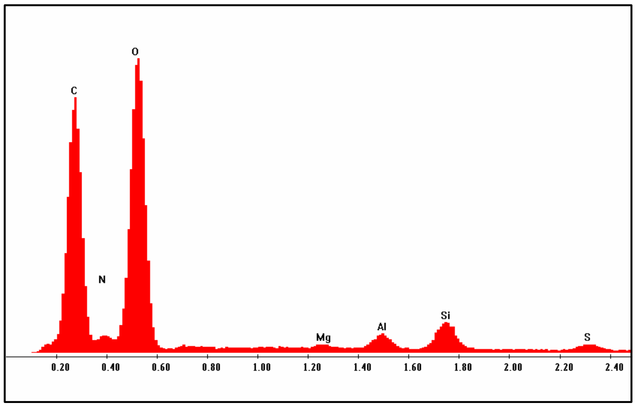

Figure 10 - Energy dispersive x-ray spectra of the elemental composition of a clump of endoliths in the E-SEM photo. Note that nitrogen is seen. N is not seen anywhere else on the samples in the CaCO3 crystals and other clearly inorganic portions of the samples and is thus taken as evidence that the structures we have identified as cells are definitely biological in origin

Experimental Data vs. a Model for Light Transmission

Using a sample of red-orange sandstone with a thickness of 1.83±.254, we obtained the results shown in Figure 11. The absorption maxima of chlorophyll a and b are 662 and 642 nm respectively. Our results show that at these wavelengths, about 5 percent of the incident light is transmitted through the rock. However, everything below 500 nm is essentially cut out.

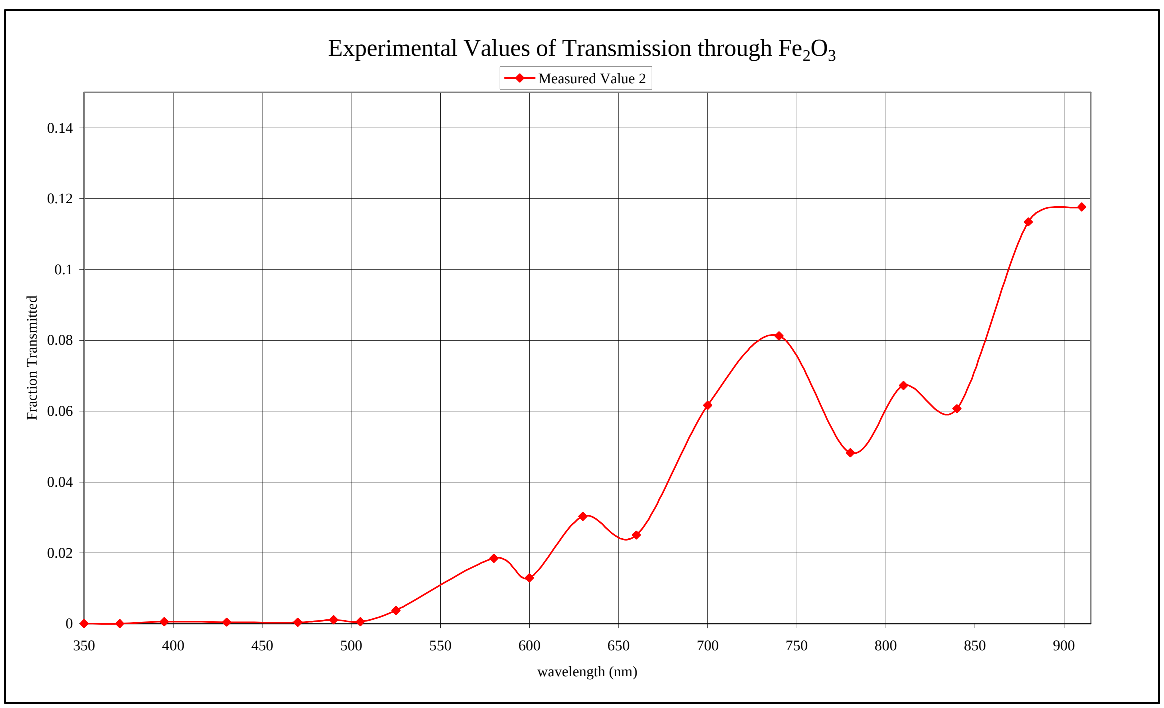

Figure 11 – Experimental results of light transmission through a rock sample

We modeled our results as the transmission of light through a layer of Fe_2O_3. As

$$T = e^{\frac{-4pkd}{l\,(nm)}} \tag{1}$$

a starting point, Model 1 (purple) in Figure 12 uses Eq. 1 and assumes a 1 micron thick layer of iron oxide (d=1 µm) present in the rock. Model 2 (pink) multiplies Model 1 by

$$T_{Fresnel} = 1 - \frac{(n_t - n_i)^2 + k^2}{(n_t + n_i)^2 + k^2} \tag{2}$$

the square of the Fresnel transmission factor (Eq. 2) and a scaling factor. This serves to simulate light striking a surface at normal incidence and imitates the decreased light available due to reflection off of quartz or other minerals in the rock. Model 3 (blue) is simply the square of Model 2, accounting for a second layer of Fe_2O_3, bringing our total depth to 2µm. Model 3 is the best fit for our empirical data.

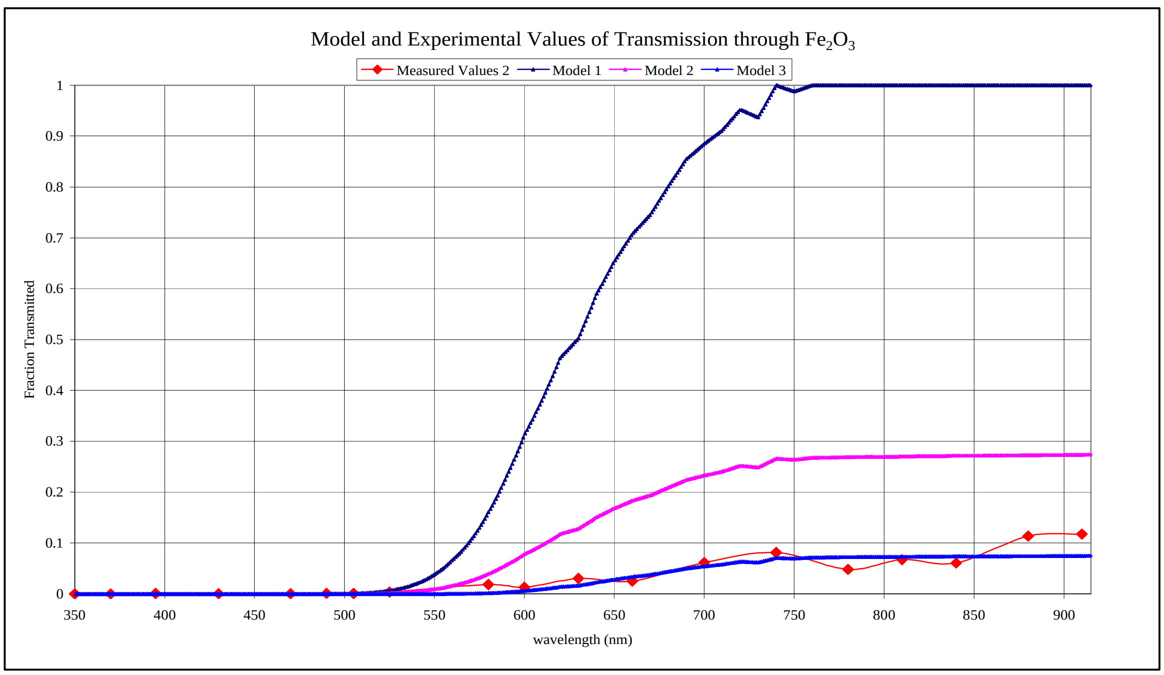

Figure 12 – Compilation of experimental results (red) and modeled data

Discussion

When the Viking missions were sent to Mars in the 1970's, it was hoped that they might discover life on the surface of Mars. Not only did they find no life, they seemed to show that due to the extreme desiccation conditions and intense UV flux, no life was possible at the surface of Mars as mentioned earlier. Since that time, extremophilic organisms have been found that have expanded the definition of where life can exist and even flourish [7].

The endolithic communities of the Southern Utah desert provide an analog for possible biota on Mars. A thin layer of iron oxide acts as an extremely efficient sunscreen, shielding out all solar radiation in the biologically harmful region (<300 nm), while still allowing light in the photosynthetic zone. In most desert environments, light is not the limiting factor on plant growth. Often, it is lack of water or lack of nutrients.

Water on Mars is definitely a problem under current conditions. No matter what the rovers discover about Mars' wet past, current temperatures and pressure make the existence of liquid water at the surface almost completely untenable. However, the small pore spaces may provide an environment where capillary action produces a negative pressure, allowing for liquid water and the presence of life. Desiccation and UV radiation have been considered two of the biggest obstacles to having life at the surface of Mars. As this one habitat can potentially provide a refuge from both, it is definitely worthy of further investigation.

An important point is that we do not know if we will find similar geology on Mars. Endoliths are closely related to their surrounding geology. These particular bacteria can only live in a certain type of sedimentary rock under certain conditions. As yet, we do not know if similar conditions even exist on Mars.

The Opportunity rover has made a discovery pertinent to this topic. In March 2004, the MER team concluded that opportunity had stumbled on sedimentary deposits exposed on the wall of Eagle Crater. The iron oxide content should be no problem at all. Our results are modeled on a very small fraction of Fe_2O_3, and the Red Planet has no shortage of iron oxide.

The 2005 HiRES mission should provide a better answer to the question as it carries cameras that will provide a more detailed view of the surface than we currently have. This will present an opportunity to start looking for a sedimentary rock formation that could be a habitat for cryptoendolithic bacteria.

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