ABSTRACT

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Contamination from a human presence on Mars could significantly compromise the search for extraterrestrial life. In particular, the difficulties in controlling microbial contamination, the potential for terrestrial microbes to grow, evolve, compete, and modify the Martian environment, and the likely microbial nature of putative Martian life, make microbial contamination worthy of focus as we begin to plan for a human mission to Mars.

This dissertation describes a relatively simple theoretical model that can be used to explore how microbial contamination from a human Mars mission might survive and grow in the Martian soil environment surrounding a habitat. A user interface has been developed to allow a general practitioner to choose values and functions for almost all parameters ranging from the number of astronauts to the half-saturation constants for microbial growth.

Systematic deviations from a baseline set of parameter values are explored as potential plausible scenarios for the first human Mars missions. The total viable population and population density are the primary state variables of interest, but other variables such as the total number of births and total dead and viable microbes are also tracked. The general approach was to find the most plausible parameter value combinations that produced a population density of 1 microbe/cm^3 or greater, a threshold that was used to categorize the more noteworthy populations for subsequent analysis.

Preliminary assessments indicate that terrestrial microbial contamination resulting from leakage from a limited human mission (perhaps lasting up to 5 months) will not likely become a problematic population in the near-term as long as reasonable contamination control measures are implemented (for example, a habitat leak rate no greater than 1 % per hour). However, there appear to be plausible, albeit unlikely, scenarios that could cause problematic populations, depending in part on (a) the initial survival fraction and death rate of microbes that are leaked into the Martian environment, which depends largely on the possibility for protection from the high UV radiation environment on Mars, (b) organic nutrient availability, and (c) liquid water availability, which is likely to be the limiting survival and growth factor.

A THEORETICAL MICROBIAL CONTAMINATION MODEL
FOR A HUMAN MARS MISSION

By

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Dedication

To my parents, who somehow managed to raise such a geek.

To my wife, Susan, and our new baby girl, Emilee Jayden,

for their patience and inspiration.

To all future space explorers,

may you go boldy and carefully.

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CHAPTER 1:   Background Review

1.1.   Introduction

The search for indigenous extraterrestrial life will be a centerpiece of a human

mission to Mars, and while humans are by most measures the most capable agents to

search for and detect extraterrestrial life, contamination from a sustained human

presence on Mars could compromise that search, and possibly adversely affect or

even cause the extinction of indigenous Martian life. A preliminary question that

needs to be investigated is the extent to which there will be contamination from a

human mission (Lupisella 1999). A subset of this question is the extent to which

there will be biological contamination from many sources, particularly the deposition

and possible growth of microbial organisms, which is the primary focus of this

research. The difficulties in controlling microbial contamination, the likely microbial

nature of possible Martian life, and the potential for terrestrial microbes to grow,

evolve, compete, and modify the Martian environment all make microbial

contamination worthy of focus as part of our planning for a human mission to Mars.

Other sources of contamination should also be investigated, but terrestrial microbial

contamination of Mars may have unusual potential to complicate the search and cause

adverse affects to indigenous Martian life and so is the focus of this research as a first

of many steps in assessing the broad range of potential environmental impacts of a

human mission to Mars.

The amount of biological contamination from robotic missions has been

investigated extensively and standards for controlling such contamination have

changed over the years. The present policy recognizes the need for strict control

when life detection experiments are on-board the landing vehicle because contamination could cause false-positive results. Robotic vehicles can be cleaned and sterilized to meet these requirements, and with so few organisms going to Mars on these vehicles, the probability of producing a viable or runaway population is extremely small (Space Studies Board, National Research Council 1992). However, humans and their associated biological support materials will unavoidably bring many orders of magnitude more organisms and, perhaps more importantly, will be a continuously generating source of biologically relevant contamination, making the problem arguably qualitatively different than that presented by robotic missions. Presently, however, there is no specified planetary protection policy for a human mission, partly because there are many unknowns associated with such a mission that is many years away, and our knowledge may be too limited at present to adequately inform such a policy. However, efforts to address planetary protection policy issues associated with the human exploration of Mars have been initiated by NASA (Race et al. 2003).

A logical starting point is to assess the amount and possible growth of deposited terrestrial microorganisms on Mars, which might then allow an assessment of what kind of impact the contamination could have. At first glance, an obvious conservative approach might suggest the need for rigorous contamination control measures under all circumstances. Or, if a population reaches a certain number or achieves sustained exponential growth, that that would suggest the need for rigorous contamination control procedures. It may also be possible to obtain enough confidence that such a population would not sufficiently compromise the search for

life enough to warrant spending what is likely to be a large fraction of the overall mission budget on contamination control. The latter is complicated by the problem of not having any data regarding the kind of life-form we might encounter.

Nevertheless, in the absence of such empirical data, the primary purpose of this research is to:

(1) Create a theoretical modeling framework which will contribute to an understanding of the potential for terrestrial microbial contamination on Mars.

(2) Facilitate efficient scenario analysis.

(3) Point the way to areas for further research.

Chapter 1 goes on to provide a background review, Chapter 2 covers details of the model, and Chapter 3 applies the model and analyzes results.

1.2. General Mars Background

All Mars data obtained to date suggests the surface of the planet is largely inhospitable to life. The average recorded temperature on Mars is around -60 °C and can get as high as 20 °C and as low as -140 °C. The Martian atmospheric pressure ranges from 6 to 12 mbars and is 95 % carbon dioxide with trace amounts of other gases: nitrogen (N_2): 2.7 %, argon (Ar): 1.6 %, oxygen (O_2): 0.13 %, water (H_2O): 0.03 %, neon (Ne): 0.00025 %. The small amount of water in the Martian atmosphere can condense out and form clouds in the upper atmosphere and localized areas of early morning fog can form in low lying valleys. A thin layer of water frost was seen covering the ground at the Viking 2 Lander site during the winter.

Mars lacks an effective atmospheric ozone layer for UV radiation shielding so the surface is exposed to substanstial UV radiation, namely the far-ultraviolet radiation (UVB (280 nm to 315 nm) and UVC (200 to 280 nm)), including most of the UVC range. Daily UVB and UVC maximum fluxes can reach approximately 13 Watts/m^2 at mid-day, which is approximately 10 times the UVB and UVC flux that reaches the surface of the Earth (Cockell 2000).

The Martian soil is a fine-textured soil which is compositionally similar to the atmospheric dust on Mars which contains silicon, iron, aluminum, magnesium, calcium, titanium, and is relatively rich in sulfur and chlorine compared to terrestrial soils. Amorphous weathered silicate and iron oxide grains make up much of the soil, but clays are lacking (Banin 2005). Salts are also present in the soil and are discussed in detail later since they affect the osmotic water potential of the soil. The soil appears to be lacking in organics which has been identified as a result of unknown oxidizing agents in the soil as well as the high UV radiation flux.

The soil appears to be completely absent of liquid water. However, water does exist in the form of frost and permafrost (both surface and subsurface), and water ice at the poles. It is an open question as to whether liquid water can be stable at any locations and plausible environmental conditions on the surface of Mars. This is discussed in further detail later.

1.3. Terrestrial Analogs

The environments on Earth that are closest to Mars (terrestrial analogs) are significantly different than any locations on Mars due primarily to the characteristics

noted above for atmospheric constituents, high UV radiation exposure, temperatures, apparent oxidizing agents, soil composition, and lack of liquid water. However, for decades, scientists have used extreme environments on Earth to better understand Mars and inform the possibilities for life.

The purpose of studying terrestrial analogs has generally been two-fold: one, to better understand possible past ecosystems on Mars, and two, to better understand how to detect life on Mars (McKay 1993). The soils of the Ross Desert (McMurdo Dry Valleys) are thought to be the most barren and stressed soils on Earth, but nevertheless, this cold (often below freezing) dry desert environment contains a variety of microorganisms and so is a useful terrestrial Mars analog environment for detection techniques (Vishniac 1993; McKay 1993). Such environments also help us understand the limits of terrestrial life, which can then inform the plausibility of survival and growth of terrestrial microorganisms on Mars.

Vishniac (2003) provides a review of Antarctic soil microbiology and notes that *Corneybacterium* and *Micrococcus* were the most prominent bacteria in Ross Desert soils, but at low levels such that organic carbon was usually no more than 0.05 %. Viable bacteria were most abundant at the permafrost level or the top centimeter of the permafrost and on the surface. The snowfall on the Ross Desert generally does not accumulate due primarily to sublimation making the availability of free water scarce with an upper value of 5.1 % water content. The high mineral salt content of the soils further reduces water availability.

The cryptoendolithic microbial ecosystems found in Antarctica provide a particularly compelling terrestrial microhabitat analog for Mars because organisms

are found living just below the surface of sandstone rocks at high elevations where peak summer air temperatures rarely rise above freezing. When the surfaces of rocks are sufficiently heated, snowfall melts and penetrates the rock where it remains inside the pores of the rock, creating a liquid water habitat (Friedmann et al. 1987).

Navarro-González et al. (2003) suggest that the soils in the Atacama Desert in Chile are among the driest on Earth and can serve as a good terrestrial analog for Martian soil. They sampled along a north-south transect with the number of culturable bacteria decreasing substantially to very low values at the driest site. However, results from Atacama have varied substantially and many questions remained unanswered (Maier et al. 2003).

1.4.   Prospects for microbial survival and growth

Two basic assumptions of this research are that prospects for survival and growth of deposited terrestrial microbes from a human mission are sufficiently plausible that modeling the potential population survival and growth of terrestrial microbes is justified, and that the possibility for indigenous life on Mars is also sufficiently plausible to warrant contamination concerns associated with a human Mars mission. While there is a clear difference between assessing the possibility for *terrestrial* microbial survival and growth vs. survival and growth prospects for *indigenous* life, there is enough overlap in the basic arguments to treat them both in this one section which, combined with the context of the previous section on terrestrial analogs, provides an overview of the evidence and reasoning that supports the above assumptions.

Generally, the fact that evolution finds innovative solutions to a dazzling myriad of seemingly insurmountable problems, justifies a conservative approach when considering extraterrestrial life issues. The past few decades, in particular, the existence of so-called extremophiles, demonstrate the surprising tenacity of life, specifically, microorganisms. As an example, chemolithoautotrophic biota seem to exploit every ecological niche. There is evidence to suggest that many or most of these metabolic capabilities evolved early in life's history (Stetter 1994) and so may have on an earlier warmer, wetter Mars as well.

The Martian surface environment presents difficult challenges for life as we know it. What we know about the surface of Mars today suggests that (a) extreme ultraviolet radiation, (b) oxidizing soil compounds and atmospheric species, (c) low level of nutrients, (d) cold climate, and (e) lack of liquid water appear to be among the most challenging environmental factors, the latter obviously being the greatest challenge to population growth.

1.4.1.   Ultraviolet Radiation

The lack of an effective ozone shield on Mars exposes the Martian surface to far-ultraviolet radiation, including most of the UVC range (190 to 280 nm). While photons with these energies can clearly break chemical bonds, they can also produce dangling bonds which can lead to cross-linking and hence increased resistance to further damage (Clark 1998). On the early Earth, when there was little ozone shield protection, terrestrial phototrophs would have had to find a way to survive high UV exposure. Indeed, Cockell (1998) suggests that based on Archaen Earth UV flux,

present day UV Martian flux may not be a limiting factor for the origin and evolution of life.

There are several potential strategies for coping with high UV exposure, such as developing damage repair mechanisms, developing resistant materials such as resistant organics and/or absorbing pigments or minerals, using environmental shielding materials that permit visible light, and using non-photonic energy sources. Indeed, while on-orbit experiments demonstrate high lethal potency of UV, shielding can maintain viability for at least 5 years (Horneck 1998). Unusually high resistance of *Bacillus subtilis* is observed when exposed to a combination of high UV radiation, vacuum, and low temperature (Weber and Greenburg 1985).

While terrestrial life has developed DNA repair mechanisms, a low nutrient, low energy mode of life on Mars may not be able to capitalize on repair mechanisms due to high metabolic costs (Clark 1998). Resistant organics have high metabolic costs, but other inorganic materials such as minerals and pigments are more plausible strategies since biomineralization does not have a high physiological cost in many biological systems (Bengtson 1994). Nitrate, nitrite, sulfate salts, and Fe-containing quartz/silica glass naturally attenuate UV while permitting the transmission of visible light, and the latter has been proposed as a mechanism for containing and hence protecting cells (Vishniac et al. 1996; Pierson 1994). Also, the combination of high Fe content in the soil (which will absorb UV as noted above) and calculations that show Martian soil would scatter visible light efficiently to perhaps several millimeters, referred to as the "euphotic zone" by Sagan and Pollack (1974), suggest

that UV protection and availability of visible light could be available just below the exposed surface.

Chemical sources of energy are an alternative to phototrophy, and several possibilities have been proposed. Chemolithoautotrophy is a plausible theoretical alternative on Mars that could utilize photochemical products such as H_2, CO, and O_2 which appear to be present in sufficient amounts. Specifically, H_2 chemolithoautotrophy (e.g. methanogens, sulfate reduction (Clark 1978)) appears to be plausible given the availability of H_2 and sulfate. Other gaseous reducing equivalents could come from energetic UV interactions with dissolved ferrous iron (DeDuve 1990) as well as from volcanic sources (Clark 1998).

Also, there is the possibility that microbes could find protection just under the surface, or under other shields such as rocks, material deposited by mission activities, or by layers of dust or microbes. In fact, research has shown that minimal protection can maintain the viability of *B. subtilis* spores– perhaps as little as a mere monolayer of dead cells can adequately protect bacterial spores when exposed to Martian UV levels (Mancinelli and Klovstad 2000).

1.4.2. Oxidants

Evidence from Viking suggests the existence of unknown oxidants in the soil (summarized by Zent and McKay 1994), and the lack of organics (see below) as measured by Viking can also be interpreted to suggest the presence of oxidizing agents in the soil. However, there are several reasons why the oxidant(s) should not necessarily preclude the existence of life or the ability of terrestrial microbes to survive and grow. One, it is present in very low concentration, minimizing contact

with distributed microorganisms. Indeed, there is experimental evidence for some terrestrial bacteria surviving substantially higher levels of oxidant than are thought to be on Mars (Mancinelli 1989). Two, water destroys it, providing a potential defense for organisms. Three, oxidation provides a source of metabolic energy (see below).

Of the oxidizing compounds in the Martian atmosphere, ozone probably presents the greatest challenge for microbes at even shallow subsurface depths, but protection by a cell wall, which might also double as a UV protection system, could mitigate the adverse effects of ozone, as well as other highly oxidizing compounds in the atmosphere (Clark 1998).

1.4.3. Lack of Nutrients

While all the essential elements of life appear to be present on Mars in sufficient amounts and usable forms such as gases or water-soluble compounds, organic nutrients appear not to be present based on Viking experiments results that did not detect organic molecules with 1 ppb sensitivity (Biemann et al. 1977). Abiotic synthesis of organic compounds might be possible through photochemical processes reacting with clays (Hubbard et al. 1971), or from meteoritic sources of carbonaceous chondrites, but microorganisms would presumably have great difficulty obtaining such organics given the lack of liquid water for transport and competition from oxidants in the atmosphere and soil. However, as the local environment of a human Mars base is modified, organics and liquid water could increase (see following section on water), which might then provide transport for microbes and destroy much of the oxidant(s) that appear to be present in the soil as noted above.

1.4.4. Cold Climate

The surface of Mars varies in temperature from 20 °C to as low as -140 °C. Only the top few millimeters of soil at moderate latitudes and local summertime are thought to have temperatures above -15 °C. Psychrophiles, organisms with optimal growth temperatures below 20 °C, exist on Earth in cold environments at the poles and in deep marine environments, and some can grow in temperatures around -10 °C (e.g. *Micrococcus cryophilus*, *Vibrio marinus*). Some multicellular organisms can be active at even lower temperatures.

Other strategies for surviving and growing in very low temperatures on Mars might include pigmented absorption of radiation (Vishniac et al. 1966), dormancy (e.g. sporulation), or production of "anti-freeze" proteins (Wharton 2002), production of cryoprotectants and compatible solutes such as glycerol, erythritol, mannitol, sucrose, proline, glutamate glucose, dimethyl sulfoxide, and trehalose (Storey and Storey 1988, Jennings 1990). Methanol or ethanol might also serve as cryoprotectant solvents as well as a source of energy and carbon. Indeed, *Desulfovibrio carbinolicus* can grow using methanol and sulfate as the sole energy source (Thauer 1989) and a 75 % (vol/vol) methanol solution freezes below -51 °C making it a potential compatible solute. Other microorganisms (e.g. methanotrophs) can use methanol as a food source via respiration and fermentation as well.

The practical lower temperature limit appears to be whatever the freezing temperature of the cell contents and surrounding water is. Indeed, living microbes have been observed in temperatures as low as -17 °C in an Antarctic pond that didn't freeze due to high salt content (Simmons et al. 1993).

1.4.5. Lack of Liquid Water

The evidence to date suggests there is no liquid water on the surface of Mars, however water does exist in the form of permafrost (both surface and subsurface), ice at the poles, and small amounts of water vapor in the atmosphere. And it has been suggested that liquid water cannot be present on the surface of Mars today because the atmospheric pressure is at or below the triple-point vapor pressure of water (Malin and Edgett 2000).

But potential exceptions could turn out to be important and should be considered for the purposes of the theoretical model described by this research. At high enough atmospheric pressure locations water could have a liquid phase between ice and vapor, and there are locations on Mars where the atmospheric pressure is above the triple point pressure for water which is 6.1 mbar. The Vikings and Pathfinder landers which went to low regions on Mars never measured surface pressures as low as 6.1 mbar, and Viking 2 actually measured its highest pressure at over 10 mbar (Zurek et al. 1992). The lowest elevations on Mars should have higher pressures than that.

Also, while surface temperatures on Mars are normally well below freezing, there are many potential locations where liquid water could exist (Haberle 2001; Lobitz 2001; Hecht 2002). There are many locations where surface temperatures rise well above freezing during daytime hours– partly because surface materials exposed to sunlight can be warmed to temperatures above freezing– so while water would likely freeze overnight, it is theoretically possible for transient liquid water to exist on the Martian surface during the day (Landis 2001). For example, a polar ecology on

Mars in which cycles of desiccation and liquid water availability drive metabolic cycles of microorganisms is plausible. Transient melting could occur in the upper few centimeters of a 0.1 % dusty, dense coarse-grained snow at atmospheric pressures as low as 7 mbar, and flowing liquid water from dust-contaminated snowmelt could also result if atmospheric pressures reached to as low as 30 mbar (Clow 1987).

Also, any water on Mars today would probably have a lower vapor pressure and a lower melt temperature than pure water because it would likely be saturated salt water since the Martian surface appears to be rich in salts (Clark and Van Hart 1981; Zent et al. 1990; Treiman 1999; Sawyer et al. 1999, Christensen 2004). For example, some aqueous salt solutions can have freezing temperatures as low as -63 °C (Brass 1980). A saturated solution of K_2CO_3 would lower the freezing point of water to below normal water freezing temperatures (Landis 2001). Capillary effects due to micron-scale pores between regolith grains could lower the freezing point as well (Landis 2001). Mellon and Phillips (2001) suggest that salt concentrations of 15 % to 40 % can lower the melting point to allow melting and hence may be a possible mechanism for the formation of recent gullies. Knauth and Burt (2002) also invoke brines as a possible explanation for young seepage features.

So while it is unlikely (although perhaps theoretically not impossible) that the surface of Mars has standing pools of liquid water that persist over long time periods, it does seem reasonable to consider that transient liquid phases of water could exist at certain surface locations on Mars. These qualifications are not meant to suggest that liquid water likely exists on the surface of Mars, but is instead meant primarily to provide plausible theoretical grounds that justify the inclusion of liquid water for the

purposes of this modeling work– especially since a continuous source of leaked water into the environment from a human Mars mission is possible.

Of all the challenges to life on Mars, the lack of liquid water is very likely the critical bottleneck. While many terrestrial organisms are able to tolerate high levels of desiccation for extended periods of time, ultimately, liquid water is needed for metabolism and reproduction. Given that the near-surface zone can have temperatures above –15 °C, organisms will presumably seek out this zone, but sublimation of water to the unsaturated atmosphere occurs here. However, certain surfaces, clay minerals, and other naturally occurring inorganic solids can take up water even under dry conditions (Anderson and Banin 1975). Protoplasts of bacterial endospores appear to contain water in tightly bound states that cannot be frozen. The lichen thallus is a bioorganic surface that can extract water vapor from air at low relative humidity, and water vapor is known to exist in small amounts in the Martian atmosphere but can vary dramatically with obliquity cycles (Clark 1998).

Water can also be important for movement of organisms in an environment, which could be particularly important on Mars given sparse nutrient distributions and limited microbial mobility, although eolian transport might accommodate this need. Fixed-location strategies, e.g. stromatolites, normally require water flow for nutrients to be brought to the organism. Thin aqueous films could accommodate very slow nutrient and waste product transfer via diffusion. Duricrust is prevalent on Mars and is more enriched in salt-forming elements than the loose soil (Clark et al. 1982) and so may be able to accommodate liquid water film transport along mineral surfaces

and across grains, as suggested by salt ion mobility experiments in sub-freezing soils of Antarctica (Ugolini and Anderson 1972).

Dormancy strategies such as sporulation are used by microbes to resist adverse environmental conditions generally, and specifically, desiccation, and are effective at maintaining viability for surprisingly long periods. Viable spore-forming bacteria have been isolated from sediments between 10,000 and 13,000 years old from ice cores at Vostok station in Antarctica.

Similar to the Earth deep hot biosphere first proposed by Gold (1992), a deep subsurface microbial ecology has been proposed by Boston et al. (1992) in which chemolithoautotrophic metabolism is driven by magmatic gases from below and liquid water from permafrost above, perhaps melted by geothermal heat sources. While this research will focus on near-surface microbiology, this possibility nevertheless helps indicate the range of potential possibilities available to life on Mars.

We might also want to be open to the possibility, however unlikely, that terrestrial life could find a way to replicate with very little or possibly no liquid water. Given enough time, and the right conditions, adaptations may occur that allow the use of alternative forms of water and perhaps other replication media altogether. Some speculation has suggested alternative liquids such as liquid SO_2 that may have been prevalent on early Mars (Wanke et al. 1992) and with a freezing point below 0°C, could have acted as a fluid medium or may possibly act as a fluid medium today. Benton Clark (1998) comments that assertions that life's absolute necessity for liquid

water are based on empirical observation and on knowledge of the mechanisms of life

on Earth, and speculates about the possibilities for "quasi solid-state organisms":

> "A revolution in electronics occurred when it finally made the evolutionary leap from electron beams in vacuum to the realm of semiconductors. Life, likewise, might also be capable of a thrust toward the solid state. Indeed, the realization over the past several decades has been that life is more than homogeneous protoplasmic sol in a sack, but rather a highly organized entity combining a fluid medium with rigid structures and numerous solid-like features such as transmembrane charge transport, macromolecular conformational reconfigurations, biological motors, the mechanics of transcription, etc. How far a life left alone, safe from marauding predators, could evolve toward an even more solid-state from of existence is extraordinarily speculative. Has Mars been the testbed?"

While this is obviously highly speculative, it is intended primarily to make the

point that the apparent lack of liquid water on the surface of Mars should not preclude

the kind of research being proposed here– especially since the model allows the user

to vary the amount of water.

1.5.   Why use a modeling approach?

Because we presently have limited data regarding the Martian environment

and no data regarding possible indigenous Martian ecologies, we can start by trying to

assess the basic dynamics of the problem and explore the wide range of possibilities

in an efficient manner by using a model that allows a broad range of circumstances to

be explored. Modeling facilitates an understanding of the basic dynamics of a system

which can lead to more detailed predictive modeling work and empirical studies to

help modify, extend, and refine the model, as well as verify its robustness. Modeling

also facilitates an efficient exploration of the possibility spaces, such as the biotic and

abiotic Martian environmental conditions and mission profile details such as number

of astronauts and kinds and durations of activities. From this theoretical exploration,

we can also assess and prioritize future empirical research based on simulation results and analysis. The power of computer modeling and simulations is that very large possibility spaces can be explored fairly efficiently.

The unique challenge regarding extraterrestrial environments is that attempting to obtain data, i.e. attempting to discover extraterrestrial life with a human mission, may unduly compromise the very data we seek, suggesting perhaps that a modeling approach should be pursued– at least in the near-term as we plan a human mission. Otherwise, the robotic search for life may find the data we need before we send a human mission, in which case, modeling possible interactions could be based on empirical data and perhaps engender more confidence in the results before we implement a human mission.

The overall approach consisted of two phases. Phase I consisted of building a basic model to assess the most important dynamics of the system in order to inform Phase II, which was to build out the Phase I model to include the needed details in those areas that the Phase I model results indicated were most important.

1.6. Science and Mission Planning Relevance

There are a series of contamination related science and mission planning questions that should be addressed before sending humans to Mars. This section will show how this research relates to broad "sequential" questions that are to some extent interdependent, as well as more specific issues and recommendations that were documented in a NASA workshop report on Planetary Protection Issues in the Human Exploration of Mars.

1.6.1. Broad sequential contamination questions

There are a series of questions that if possible, might be best addressed in a serial manner:

To what extent will there be contamination? McKay and Davis (1989) suggest that contamination is inevitable if humans are present. They write: "It may be assumed, a priori, that all space suits and habitats will leak". This is known to have been the case with Apollo since it is thought that there was "significant leakage of gases from the joints of the astronauts' suits" (Cohn 1969). Mckay and Davis also write: "It is arguable that once humans land on Mars, attempts to maintain a strict policy of preventing the introduction of Earth life into the Martian environment will become moot." This suggests the need to assess the extent, preferably quantitatively, to which there will be contamination since the amount and kind will likely be critical to mission planning. The intent of this research is to help directly address a subset of this question, namely to what extent there will be microbial contamination of Mars associated with the first human mission. Microbial contamination deserves particular attention for reasons noted in the Introduction.

If it is thought that there could be contamination to levels that are deemed significant, we should then ask: *Could such contamination compromise indigenous life-forms?* If so, how? What are the chances? Is it even feasible to establish such probabilities with any confidence? We might want to assess the relative probabilities of direct adverse effects given panspermia vs. a separate origin. Is the latter a probability of zero? The Space Studies Board says no (1997). What are the chances for indirect adverse effects via toxins or competition for resources? Could non-

biological elements such as rocket exhaust or industrial chemicals compromise indigenous ecosystems? Given that a single kind of life-form might have caused the extinction of all others early on in the evolution of life on earth (Dyson, personal communication, 1998) could a similar scenario occur if foreign organisms are brought to Mars? This research will help indirectly address these questions by providing a modeling tool to better understand a rough range of magnitudes of microbial contamination that might occur– which relates directly to assessing the potential impacts on indigenous biota since the size of a terrestrial microbial population will likely be an important variable in assessing potential ecological impacts such as competition, predation, environmental modification, etc.

   Could contamination mask the existence of indigenous life-forms? A masking effect could depend on whether the contaminating organisms are dead or viable, either as dormant or active organisms. Dead organisms should not have a significant masking effect for life-detection experiments based on life processes such as metabolism. However, dead organisms might have a masking effect for simple observation based detection devices such as microscopes and robotic life-detection devices– although, presumably, with humans present, detailed genetic sequencing analysis could be done that should reduce this problem– it does however complicate the search, perhaps more than would be desired. While perhaps not the most likely scenario, we might consider that dead terrestrial organisms, after having been on Mars for some time, will not be recognizable as terrestrial organisms. For example, there might only remain fragments of organisms, or the organisms might undergo

physical modification, making it difficult, if not impossible, to rule out an indigenous source. The Space Science Board writes (1997):

> "Contamination with terrestrial material would compromise the integrity of the sample by adding confusing background to potential discoveries related to extinct or extant life on Mars. DNA and proteins of terrestrial origin could likely be unambiguously identified, but other organic material might not be so easily distinguished. The search for candidate Martian organic biomarkers would be confounded by the presence of terrestrial material. Because the detection of life or evidence of prebiotic chemistry is a key objective of Mars exploration, considerable effort to avoid such contamination is justified."

It may also be very difficult to determine if the resident organisms were deposited by the mission or whether they arrived via panspermia– an important scientific question in its own right. This model tracks the amounts of both viable and dead organisms, and so will provide data useful to assessing potential masking effects.

To what extent will we be able to control contamination? Microbial contamination may be difficult to sufficiently control as noted previously. If we cannot properly control microbial contamination from the first human mission, and if microbial contamination is thought to present a sufficient threat, then that will imply waiting to send humans until the biological status of the area in question is assessed. This then implies robotic exploration for the foreseeable future– until the contamination risks can be properly mitigated. The model resulting from this research will help analyze the levels of microbial deposition that will give rise to certain size microbial populations, which will then inform if and how microbial deposition might be controlled. The "area in question" noted above gives rise the next question.

How far could contamination spread? Dead or viable organisms could potentially be distributed over a significant area, perhaps globally, since large,

sometimes global, dust storms are known to occur. James Murphy (personal communication, 1998; and Murphy et al. 1993) suggests bacteria definitely could be spread globally.

The *continuous* source of contamination due to a human presence, the possibilities of subsurface contamination, and other sources of contamination should be considered when exploring this question. If microbial contamination is thought to present a sufficient threat to indigenous life, and if it is thought that sufficient contamination control will not be possible, and if microbial contamination could spread globally, and the criteria for assessing the biological status of the entire planet is complex– which it is likely to be (e.g. large number of missions to a variety of environments, much drilling, etc.)– then contamination dispersal issues will be key in the human exploration of Mars because it could mean the difference between few or many precursor missions, which will directly impact the short and long-term planning, cost, and timeline for sending humans to Mars. Again, this research provides a model that can indirectly inform microbial contamination dispersal since dispersal will likely depend on the size of the terrestrial microbial population in the local Martian environment.

Sharp (1986) has suggested that absolute containment of all terrestrial biology is, in principle, possible and even desirable over the less certain method of obtaining all other relevant data to determine that contamination will not cause adverse effects. Sharp points out that an entire technology has been developed to contain dangerous biological agents, and that while such an effort for the first human Mars mission would be quite expensive, in the long run, it may be the only sure approach as long as

no failures occur. However, given the expense and stringent requirements of such an approach, it makes sense to consider the more realistic suggestion made by McKay and Davis that contamination is likely if humans establish a presence on Mars. This research makes that assumption.

Understanding the amounts and kinds of contamination that are released into the Martian environment will be important for dealing with this overall issue. The Apollo program made some attempts to reduce and inventory contamination. For example, a bacterial filter system on the lunar module was used to prevent contamination of the lunar surface when the cabin atmosphere was released (NASA Manual 1969). NASA also adopted (NASA policy directive 1969), as official policy, aseptic subsurface drilling, decontamination and contained storage of waste materials, and biological and organic material inventory requirements.

1.6.2. NASA workshop results

To further illustrate the relevance of this research to mission planning for the human exploration of Mars, it is instructive to note in detail some of the conclusions and recommendations from a NASA workshop on planetary protection issues associated with a human Mars mission (Race et al. 2003). The workshop noted the following major research areas:

- "Define the spatial dispersion of dust and contaminants on Mars by wind and other means." Again, as noted previously, the size of a local microbial population will inform dispersal.

- "Describe the potential impacts of each of the many human support activities expected in the operation of a human-occupied Martian base, e.g. breathing oxygen, food supply, waste management, etc." These activities and the potential associated microbial deposition into the local environment can be captured in the model.

- "Determine how robotics can best help conduct operations on Mars in a way consistent with planetary protection concerns, both independently during precursor missions and in conjunction with humans in later missions." The model will allow for the amount of astronaut EVA (extra vehicular activity) time to be varied, which can then be used as a method to help assess how much robotic activities will reduce contamination.

- "Improve space suit designs consistent with planetary protection needs, especially for the demands of human activities on the Martian surface located away from pressurized habitats and rovers." Knowing how leakage rates relate to population size will inform suit design requirements.

Workgroup 1, "Protecting Mars and Science", provided more detailed recommendations and areas of work that could be informed by this dissertation:

- "A rigorous but flexible set of guidelines pertaining to forward contamination controls and chemical contamination limits. Guidelines should be updated prior to human missions to reflect new detection and cleaning methodologies as well as advances in knowledge about Mars. Such guidelines are likely to evolve

considerably from precursor missions, to early human exploration, and on through advanced human missions." This microbial contamination model can inform such guidelines and their evolution by showing how certain mission profiles produce certain microbial populations, and by incorporating more knowledge about Mars into the model as more is learned.

- "Determination of levels of filtration that are possible or needed for suits, living or work modules, rovers, etc. Information on release/escape of microbes from suits and development of detection and monitoring procedures." Leakage rates that produce certain levels of contamination can be used to generate suit design requirements.

- "Life support systems (including waste containment and preparation for final departure from Mars)." The model includes microbial sources from the habitat and allows the population to be calculated for a substantial duration after astronauts leave.

- "Determination of how clean the items used on the Martian surface must be (e.g., mobility elements, tools, sampling devices etc.) and how these high level design requirements can be defined."

- "Determination of what levels of chemical cleanliness or sterility will be required and how can they be monitored?"

- "Will the different possible microbial communities, if mixed, become one homogenized community over time, or remain separate? How can this be

monitored? What are the implications for Mars, as well as for future human missions and exploration?" This model can eventually be extended to broader longer-term ecological questions such as how terrestrial and Martian microbial communities might interact.

- "Contamination control technology and procedures consistent with, but not limited to, current planetary protection requirements."

- "Spacecraft cleanliness and isolation of other sources of contamination (humans, life support, etc.)"

- "Levels of biological and chemical residues expected/acceptable on various equipment, surfaces and materials?"

- "Probability of microbes making contact with Mars."

- "Viability, growth, mutations, dispersion and propagation (local vs. global)."

- "Impact on Mars sterility and/or indigenous biota."

- "Impact on life detection experiments."

- "An evaluation scheme and matrix integrating specific measures and items that relate to planetary protection goals. The group suggested that an n-dimensional structuring and analysis of the various items could yield helpful information on relative degrees of difficulty and sensitivity of different missions. In essence, by using overlapping sets of classifications and concerns, individual mission

scenarios can be assessed and planetary protection needs predicted. While details of the matrix and analytical approaches will need considerable future development, the approach may yield useful insights into planetary protection impacts by focusing on the following important categories of concern:

- *"Identification of contaminating microbial communities*: For human missions it will be important to identify, characterize and monitor for distinctive contamination sources and microbial communities whose ecological contexts and physiological properties are sufficiently different to warrant separate assessments. Important communities by origin include spacecraft, robots, humans, life support systems, and other potential sources. In addition to assessing locations within the mission and their microbial characteristics, it will be important to assess the microbes' abilities to survive the journey to Mars and cause possible impacts of importance to Mars, humans, or scientific experiments." This model draws primarily from data associated with *Bacillus subtilis* since related research exists (see Related Work section below). However, this could be extended to accommodate a multitude of diverse microbial species.

- *"Temporal and sequencing issues*: Consideration should be given to the sequencing of operations on Mars as well as contamination monitoring, decontamination and associated procedures. Details will be mission dependent."

- *"Human operations*: In the matrix, critical decisions regarding the exact mix of human, robotic and human-guided robotic sorties will have to be addressed for their differing planetary protection implications."

The mission dependence of the last two recommendations can be captured in the model in terms of number and length of "extra-habitat activities" (EHAs). The model could be further extended to allow for more detailed scenarios.

1.7. Related Work

This section will cover some of the key theoretical and experimental work that relates to microbial population dynamics modeling. To date, there has been no direct theoretical work on modeling potential terrestrial microbial population dynamics on Mars due to a human mission. However, there is extensive research on theoretical and empirical microbial population modeling under controlled laboratory conditions, and there is some work on environmental and soil microbiology modeling. This section will begin by covering the relevant basics of classical general microbial population modeling, followed by more recent general developments and developments in soil microbial modeling.

1.7.1. General background for classical microbial population modeling

This history of microbial population modeling appears to have started with Monod (1942) who first formalized the following hyperbolic relationship (based on basic enzymatic dynamics) between microbial growth rate and limited nutrient concentration as:

$$\frac{dX}{dt} = \mu_{max} \left(\frac{S}{K + S} \right) X \tag{1.1}$$

where X can be either cell biomass or population density, μ_{max} is the maximum growth rate under nutrient saturation conditions, S is the free nutrient concentration, and K is the half-saturation constant (substrate concentration when $\mu_{max} = 0.5$). The basic validity of Monod's model has been well supported and is still widely used today (Dugdale 1967; Powers and Canale 1975; Koch et al. 1998).

A loss of biomass can be captured by adding a biomass loss term, rX (where r is a death rate):

$$\frac{dX}{dt} = \mu_{max} \left(\frac{S}{K + S} \right) X - rX \tag{1.2}$$

This general form is used in the nutrient sector of this model.

Best (1955) developed an equation for the rate of substrate uptake, v, that considered a diffusion process followed by an enzymatic process, or what is known as a diffusion:enzyme model:

$$v = \frac{V(S + K + J) \left(1 - \sqrt{1 - 4SJ/(S + K + J)^2} \right)}{2J} \tag{1.3}$$

where V is the maximum uptake rate, and $J = V/AP$ where A is the surface area of the bacteria and P is the permeability constant defined as the diffusion constant in the membrane divided by the membrane thickness. This formula would be more appropriate for more detailed metabolism modeling and/or individual based modeling which this model does not incorporate.

1.7.2. Recent developments in microbial population modeling

As in much modeling in other domains, there has been both classical mathematical modeling and more direct mechanistic individual-based modeling in attempts to better understand microbial population growth. Classical microbial mathematical population models have been substantially refined over the years, while the newer individual-based modeling approaches (enabled largely by advances in computing power) are beginning to have more influence because of their ability to capture more mechanistic details at the level of individual organisms, from which there emerges global population dynamics.

The research noted in this section tends to focus more on mathematical modeling and bacterial modeling because of the more direct relevance to this dissertation. The first section highlights general developments in microbial modeling, and the second section focuses on soil microbial modeling.

1.7.2.1   General developments in microbial modeling

Jeong et al. (1990) created a detailed mathematical model for examining the growth and sporulation processes of *Bacillus subtillis*. It is a very complex and comprehensive model, containing 35 cellular components, 200 parameters, and 39 nonlinear and coupled differential equations, and was run on a super computer. The practical near-term intent of the model was to integrate sub-cellular processes into whole-cell models by accounting for all of the major interactions of cellular metabolic networks. While the level of detail and cellular component focus of this model are not needed for this dissertation, this work nevertheless informs macro population

growth (including sporulation) dynamics of *B. subtilis* under varying conditions such as low nutrient stresses.

Koch (1993) addressed a controversy regarding linear vs. exponential bacterial growth models when he compared linear and exponential growth models of *B. subtilis* and verified the relevance of exponential growth throughout the cell cycle by showing the global volume growth rate is essentially proportional to cell volume. This justifies the use of exponential growth models such as that developed by Monod and noted above.

Koch (1997) focuses on the physiology and ecology of slow growth couples consumption with uptake to move beyond the single "enzymatic analogue" step developed by Monod to more accurately capture the multiple steps of uptake and metabolism, including relaxing a previous assumption of fast consumption once nutrient has entered the cell. While this is ultimately relevant to the population dynamics sought by this model, multiple stage uptake modeling is unnecessary at this time.

Kreft et al. (1998) developed *BacSim*, a simulator for individual-based modeling of bacterial colony growth, that uses the Best equation (noted previously) for substrate uptake. The model integrates cellular processes into a generic population model and so is more relevant to this dissertation than are more detailed cellular metabolic models such as Jeong et al. (1990) noted previously. However, the details of individual level modeling are still unnecessary for the initial purposes of this dissertation, especially because *BacSim* itself indicates that cell shape and cell surface play only a minimal role in overall population growth. However, *BacSim* has

the potential to be extended to natural environments if detailed microbial growth patterns are of interest– which may indeed eventually be desirable as NASA mission requirements evolve.

Diaz et al. (1999) developed a hybrid on-line estimator to detect and quantify the growth phases in microbial cultures, based on biomass concentration. It is considered "hybrid" because it chooses different models for each growth phase. The significance of this work is that it suggests the need for modeling different phases seamlessly, but separately, with different models. This hybrid approach will ultimately inform future development of this model as heterogeneous modeling and biomass concentration becomes more important.

Patnaik (2001) emphasizes individual-based modeling as a methodology for modeling how microorganisms respond to changing environmental conditions, such as varying multiple substrate conditions. The individual-based approach can account for evolutionary factors that allow cells to regulate their metabolic processes in response to a changing environment, but this is not critical to this modeling work at this time.

Many mathematical models are deterministic and so often lack what are arguably more realistic representations of real world situations. Stochastic models can capture some of the random influences on systems by incorporating random fluctuations where appropriate. Poschet et al. (2004) used Monte Carlo analysis to correlate the sensitivity of microbial growth model parameters with data quality and quantity. They report a linear relation between data quality and model parameter uncertainty. Data quantity also has a substantial influence on model parameter

uncertainty. Not only does this have implications for optimal experiment design, as they note, but it could affect model design as well. However, as discussed below, stochasticity was not deemed to be important enough to include in this model at this time.

Continuing with the theme of the role of stochastic modeling, Ponciano et al. (2005) recently used a fairly simple stochastic model (a stochastic Ricker model (Dennis and Taper 1994)), combined with statistical analysis using an extensive data set, to show how certain environmental factors explain the variability of *E. coli* growth data from deterministic theoretical predications. They used statistical analysis to characterize the effects of 5 different nutrient sources and were able to show that stochastic influences explained how interactions of only two of those nutrients could explain the uncertainties of experimental data relative to deterministic models. While this model uses two nutrients, this application of stochasticity appears to be an unnecessary level of complexity for the purposes of this model as discussed further in Chapter 2.

1.7.2.2   Soil Microbial Modeling

There is little comprehensive soil microbial population modeling, in part because the terrestrial soil environment is so complex (Blagodatsky and Richter 1998). As Rockold et al. (2005) point out, there has been little work on mechanistic approaches in more complicated unsaturated porous media systems such as soils. They note that related studies in soils have not directly considered microbial processes. However, there have been several attempts to model certain aspects of microbial population dynamics in soil.

Cheng (1999) developed a microbial growth model that quantitatively linked decomposition to increased carbon input to the rhizosphere, and mechanistically illustrated the interactions among nitrogen availability, substrate quality, and microbial dynamics when the rhizosphere was exposed to elevated CO_2. The focus on the rhizopshere makes this work only indirectly relevant to this dissertation, but increases in CO_2 may very well occur during a mission to Mars and so the basic dynamic, while premature for use in this present model, could be useful for future applications.

Bosatta and Ågren (1994) developed a model that incorporated microbial mortality as well as a "quality" function that described the availability of soil organic matter to microbes for decomposition. This model, particularly the "quality" function, offers a way to treat the soil organic matter essentially as a variable unit whose changing accessibility to microbes is reflected over time and over varying conditions. However, while the quality function is potentially useful, it would have to be developed in conjunction with detailed Martian soil experiments.

Blagodatsky and Richter (1998) introduce a state variable to classical Monod dynamics that captures microbial activity globally in the model. This "microbial activity factor" ultimately controls microbial growth, death, and decomposition rates of organic matter because it is simply multiplied by the entire microbial biomass expression that is based primarily on Monod kinetics. This "master variable" has the appeal of simplifying the model while simultaneously accounting for the slowing of all life processes due to low nutrient content. However, it is unclear if or how such a variable would be applied to Martian soil conditions given the lack of empirical data.

Trefry and Franzmann (2003) note that some kinetic microbial growth models often do not apply to certain substrate mineralization processes because of observed significant delays in metabolite production. Mohn and Stewart (2000) found lag times of up to 66 days in the mineralization in Arctic soils– an environment directly relevant to this dissertation. Trefry and Franzmann extended a model by Brunner and Focht (1984) to explain non-ideal kinetics by introducing a general time-dependent lag factor that can be applied to biomass growth. A lag factor will likely be relevant for microbial growth in Martian soil, but it is not critical for the purposes of this modeling effort at this time.

To quantify and model the impact of bacterial growth on the hydraulic properties of variably saturated sand, Rockhold et al. (2005) developed a coupled numerical model to describe experiments exploring the interactions between microbial and transport processes (such as water flow, bacterial cell growth and transport, substrate consumption, and gas dynamics) in variably saturated porous sand columns. They used Monod type kinetics for microbial population growth and time-dependent first-order reversible cell attachment-detachment kinetics (based on particle filtration theory and time-dependent detachment rate coefficients) to model sand associated (attached) biomass concentrations. This model uses the same Monod type formula as Rockhold et al. but does not incorporate attachment and detachment dynamics since this is an unnecessary complexity and would require detailed Martian soil experiments.

CHAPTER 2: Model Development

This chapter will cover basic aspects of the methodology used to build this

model (including an overall two-step process of creating a basic "primary" model

leading to a secondary model), decisions regarding simplicity vs. complexity of the

model, the kind of model, modeling tools, the model organism, details of the primary

model, and details of the secondary model.

2.1.   Primary and Secondary Models

Swinnen et al. (2004) note that a two-step modeling process is often useful for

predictive microbiology. Primary models describe basic rules of how population

numbers change over time, from which secondary models can be built that take into

account a range of factors that affect microbial population growth. This reflects a

basic approach taken for this dissertation. Namely, Phase I involved building a

simple model with basic growth dynamics to explore overall model structure and

assess the potential for interesting growth possibilities on Mars and to assess potential

areas of sensitivity for further model build out which was done in Phase II, the details

of which are discussed in those sections.

For both models, steps were taken along the way to ensure accuracy of the

results. As each element was incorporated into the model, extreme parameter value

modifications were done to make sure the model behaved as expected. For example,

when liquid water was not available (either indigenously or from leaked sources)

there was no growth as expected. Similarly no growth occurred when nutrients were

zeroed out. Additionally, as model elements were added, manual calculations were performed to confirm results and to build confidence that the new elements added, the overall model structure, and the model output behaved as expected. Also, more generally, the two phase approach allowed for a high level of confidence in model structure and output to be obtained (via the methods noted above) by ensuring that the primary model acted as expected before going on to additional details of the secondary model development. Fairly extensive sensitivity analysis was done on the primary model (some of which is noted in the Primary Model section) to ensure expected sensitivities such as liquid water and also to inform more detailed build-out of the secondary model for Phase II.

Each model has a baseline set of parameters and values from which deviations were explored to produce population results and analyze model dynamics. Single parameters were varied first to assess single parameter sensitivity, followed by more complex multi-parameter modifications to help analyze how different parameters affected each other, and under what circumstances interesting dynamics and population results emerged, with an emphasis on finding those conditions under which substantial microbial population growth occurred.

2.2. Simplicity vs. Complexity

Blagodatsky and Richter (1998) write of soil microbial modeling: "The conceptual schemes of models look quite simple, but the underlying processes at the levels of biochemistry and microbial population dynamics are prohibitively complex." This is a fundamental challenge of modeling most phenomena, especially in biology, and so a central challenge is often to strike the right balance of simplicity

vs. complexity to realize the purposes of the modeling exercise. The two-step process noted above was intended to help strike this balance so that only the most influential factors would be built-out in the model to accommodate an appropriate amount of detail.

Because the primary intent of this modeling work is to create a first-order theoretical framework to explore basic population dynamics and explore microbial population growth possibilities, the focus was to (a) emphasize critical model elements such as microbial load shedding, water, and nutrient uptake, and (b) simplify microbial heterogeneity and dispersal by assuming population growth of one potentially successful organism (discussed below) in an environmentally uniform area around a habitat.

It was noted previously that while the Best equation incorporates factors such as cell size and surface area, other research (Kreft et al. as noted above) has shown that these are minimal factors and so not necessary for the purposes of this model at this time. Indeed, despite the relevance of demographic structure and demographic stochasticity to small populations (Meffe and Carroll 1997), the model is demographically unstructured since demographic variation such as body size and sex is not relevant, and age structure in microbial populations is not an important factor in microbial reproduction given the short life-cycle spans.

2.3. Numerical Modeling and Stochastic/Deterministic Considerations

While individual-based modeling is arguably more mechanistically and physiologically accurate, it tends to emphasize complex cellular functions which are not necessary for the macro population assessments that are of interest for this work.

A relatively simple numerical mathematical model, for example, incorporating modified Monod dynamics (as used by Rockhold et al. (2005) noted above) suffices for capturing an acceptable level of mechanistic factors while simultaneously allowing for computational efficiencies.

As recently demonstrated by Ponciano et al. (2005), and noted previously, stochastic influences are often useful for obtaining more accurate models. However, this model does not necessarily require stochastic features given the objectives and the parameters involved. While stochasticity would be useful for probabilistic risk assessment that could directly inform mission planning, this model appears to not be sufficiently affected by stochastic influences. Stochasticity was introduced into several parameters (e.g. half-saturation constants and the maximum growth rate) with no notable impact on overall population results. However, randomization did slow the performance enough to be a concern. Also, a deterministic model has the advantage of allowing easier tracking of the variety of simulations results and small deviations (in results and parameter values), in part by making them repeatable to a high degree of precision.

2.4. Modeling Tools

Several modeling packages were assessed. Stella and Madonna are graphically based modeling package that are fairly intuitive to use. Madonna has the ability to use more functions than Stella and has some other interface enhancements, but Stella is more widely used and supported, has more user-friendly model control interface features, and is sufficient for the purposes of this model. Both are capable of handling the modeling proposed here and have the further advantages of

facilitating easy additions to the model by other team members and also allowing for a model interface to be created so that model users can very easily modify parameters of the model to explore further dynamics and management/policy implications without having any modeling software experience. A version of Stella is available for free that allows potential users of Stella models to run the models, but not modify them. Stella was chosen to build this model for the reasons noted above.

Several other modeling packages were considered. *Populus* is a robust comprehensive ecological public domain software package provided by the National Science Foundation. It allows for easy construction of differential growth equations and the display of state-space graphs and isoclines. New evolutionary modules have been added which would have been potentially useful for the mutations aspect of the model that was investigated, but given that mutations are not explicitly captured by this model, this modeling package was not necessary. Regardless, Stella is a general enough modeling tool that is capable of modeling mutations.

Ramas was designed for the analysis of population growth models and provides ease of incorporating demographic and environmental stochasticity. *Ramas* is packaged with the book, Applied Population Ecology, and is a commercial product. The cost and emphasis on demographics and environmental stochasticity rendered this tool unnecessary since the model pursued here does not include demographics, and only minimally incorporates environmental stochasticity. *EcoBeaker* facilitates exploration of models in population and community ecology, including interspecies interactions and disturbances. The models are individual based and output appears in sophisticated displays that show population change as interactions and disturbances

occur. *EcoBeaker* is a commercial package. Its individual-based nature make it unnecessary for this model since this model does not require individual level modeling as noted above. *Mathematica* is an all-purpose math software package that allows for solutions to complex mathematical problems, but is not as user-friendly at Stella.

2.5.   Model Organism

Although the model is not limited to the use of a single bacterium, *Bacillus subtilis* was selected as a "model organism" for this model for a number of reasons. *Bacillus* species are common bacteria that undergo sporulation in response to environmental stresses such as low nutrient and water conditions and high UV radiation, and have been recovered from spacecraft surfaces, and so may be transported to Mars and be capable of surviving the Martian surface environment (Schuerger et al. 2003; Newcombe et al. 2005). There is an extensive body of literature on *B. subtilis* in the general microbiological literature (e.g. note the sporulation model of Jeong et al. (1990) and the Kreft et al. (1998) BacSim model highlighted previously, as well as Koch (1993) and peroxide stress research from Helmann et al. (2003) the latter of which is relevant to the oxidizing Martian surface) and is a favorite organism for research in the space life sciences community (Weber and Greenburg 1985; Koike et al. 1995; Baltschukat and Horneck 1991; Horneck 1993, 1998; Horneck et al. 1994, 2001; Nicholson 2000; Mancinelli and Klovstad 2000; Rettberg et al. 2002; Cockell et al. 2002a, 2002b, 2003; Schuerger et al. 2003; Newcombe et al. 2005). Schuerger et al. (2003) chose *B. subtilis* to study survival under Martian conditions because of the literature base, its ability to resist harsh

environments, and its occurrence as a microbial contaminant found on spacecraft surfaces.

It should be noted that while *B. subtilis* is primarily an aerobic organism, it can grow in anaerobic conditions (Nakano and Zuber 1998). Strictly speaking, the Martian surface is considered to be primarily an aerobic environment since oxygen is present in the atmosphere, albeit in small amounts as noted previously. Microaerophiles and facultative anaerobes are therefore good candidates for survival and possibly growth on the Martian surface, and could very well survive and grow better than obligate aerobes since the amount of oxygen in the Martian atmosphere is low (Brewer 1980). The subsurface of Mars is likely to be an anaerobic environment and so will also be a possible environment in which anaerobic microbes might survive and grow, more so than on the surface on Mars. However, this would require survival and transport via a surface to subsurface transport mechanism. Related to this is the likelihood that most organisms that will leak into the Martian environment will have to pass through an aerobic environment (such as a habitat or space suit), unless there is a direct breach of a support biomass containment facility or process (e.g. waste management) directly into the Martian environment– something that mission design specifications will undoubtedly mitigate. This may put a limit on the number of viable anaerobic organisms that make it into the Martian environment. Finally, aerobic organisms are energetically more efficient, and so from this perspective, given the cold temperatures and minimal liquid water, aerobic organisms have an advantage over anaerobic organisms. Given that many microbes (e.g. *E. coli* and *B. subtilis* as noted above) can survive and grow in both aerobic and anaerobic

environments, the presence of a low amount of oxygen in the Martian atmosphere might not be a critical factor in whether terrestrial microbes will survive and grow on Mars.

2.6. Primary Model

This section will highlight features and results of the basic primary model on which the secondary "built out" model was based. Basic assumptions and model structure are followed by a description of the general approach and preliminary results.

2.6.1. Model Assumptions and Structure

There were three main assumptions for the Phase I preliminary model: (1) most organisms will die due to inhospitable Martian surface conditions, (2) a small fraction of terrestrial microbes exposed to the Martian environment will survive and reproduce, and (3) the primary source of nutrients will come from the microbes introduced into the environment.

The basic structure of the primary model included 5 sectors: (1) total organisms released to the environment, (2) viable organism population, (3) nutrients, (4) water, and (5) mutations. The heart of the model is the viable organism population sector which calculates the population as:

$$X_t = x_s\sigma + X_{t\text{-}dt} + (\mu - r(t))X_{t\text{-}dt} \tag{2.1}$$

Where X_t is the total microbial population number at time t, x_s is the number of organisms that are deposited per unit time, σ is the survival fraction of organisms that

initially survives exposure to the Martian environment (so $x_s\sigma$ is the term capturing the number of viable organism that are added to the environment per unit time), μ is the growth rate and is a function of both water and nutrient availability (functions that were manually drawn and modified to explore consequences), and r is the death rate which is a function of the number of mutations (again, a function that was manually drawn, initially as a decreasing hyperbolic function, and changed to reflect different relationships between death rate and mutations.)

The total organisms introduced to the environment is the sum total of all major sources of microbial organisms that might be leaked into the environment. This includes sources from astronauts while they are in the field in space suits, sources from the habitat including astronauts and other biomass, and a very small fraction that might be brought in from the outside by astronauts. The viable organism death rate is a function of the number of mutations and in the baseline configuration is represented by a manually drawn function approximating an exponential decrease that starts at 0.5 and goes to 0.02.

The birth rate is a sum of two fractions, the nutrient birth rate factor and the water birth rate factor. The nutrient birth rate factor is a measure of how the birth rate increases as a function of nutrients (microbes and their associated nutrients only) into the environment and is a slightly increasing linear function from 0 to 0.1 for the baseline configuration. The water birth rate factor is a measure of how the birth rate increases as a function of the amount of water in the environment and is also a slightly increasing linear function, from 0 to 0.1 for the baseline configuration. These birth rate functions were meant to capture the possibility that as nutrients and water

are increased in the environment, the birth rate of the population will also increase slightly. Both of these functions are manually drawn graphs that can be adjusted within the model.

The mutations sector calculated the total number of mutations as the product of a mutation rate and total number of viable organisms. The baseline mutation rate was 1×10^{-6} per replication which represents an average mutation rate for bacteria on Earth (Atlas and Bartha 1998).

The nutrients sector calculates the total number of nutrients introduced to the environment as the sum of nutrients from dead organisms as well as from viable organisms. The nutrients from dead organisms are calculated as the product of a nutrients per dead organism fraction and total dead organisms. The nutrients from live organisms are calculated as the product of a nutrients per live organism fraction and total viable organisms.

The water sector calculated the total water introduced into the environment as the sum of water introduced from astronauts and from other biomass associated with the habitat. The purpose of the basic model is to try to capture the general dynamic of introducing some liquid water and its effect on population growth.

2.6.2. General Approach

One purpose of the basic primary model was to assess simple population dynamics and the potential for population growth relative to a conservative or "best case scenario" parameter set shown in Table 2.1 below ("best case" meaning conditions likely to result in minimal growth and hence contamination), which assumed minimal microbial leakage and available indigenous nutrients (hence

assumption 3 above), low initial survival fraction after initial exposure to the

environment, minimal water availability, and average mutation rate based on

terrestrial mutation rates of microbes.

As noted, the baseline configuration was used initially as a conservative

reference point representing a plausible set of parameters that would result in a

minimal population number. The baseline values shown in Table 2.1 are separated

into controllable and uncontrollabe parameters– those that we might have some

control over, and those that are not likely to be controllable. The baseline parameter

set in Table 2.1 produced a negligible population of 20 organisms.

Table 2.1: Phase I Primary Model Baseline Configuration Parameter Values

CONTROLLABLE PARAMETERS	
Number of astronauts (A):	6
Microbial suit shed/astronaut (M_A):	240 microbes/day
Microbial shed from biomass (M_B):	1,440,000 microbes/day
Habitat leakage fraction (h):	0.01
UNCONTROLLABLE PARAMETERS	
Survival fraction (σ):	0.0001
Nutrient birth rate factor (v):	0 to 0.1 (linear function of total nutrients)
Water birth rate factor (ω):	0 to 0.01 (linear function of total water)
Viable organism death rate ($r(t)$):	0.5 to 0.02 (approx. exponential decrease)
Mutation rate (μ):	1×10^{-6} mutations per replication

Due to high levels of ultraviolet radiation and oxidants on the surface of Mars,

the model assumed that most organisms would die immediately upon exposure to the

Martian environment. The baseline parameter set had an initial survival fraction of

0.0001, the surviving population of which is subjected to a subsequent initial death

fraction of 0.5, which is a function of the mutation rate that decreases as the number

of mutations goes to 100,000 per replication.

As noted previously, the general approach was to modify all parameters of the

preliminary model relative to the baseline parameter set noted above to explore how

sensitive the population growth was to each parameter and what, if any, set of parameters might cause a viable or runaway population. Results were obtained for the 100 days of a 100-day mission as well as for an additional 200 days past the end of the mission to assess possible population growth after the mission ends. This distinction could be important depending on mission planning objectives. For example, while there may not be a significant microbial population in place during the 100-day mission, hence not compromising the search for life while astronauts are at Mars, subsequent growth thereafter may unduly compromise the search for life for future missions. Or, it may be determined that life likely does not exist if astronauts do not find life within the first few months of a mission, suggesting future contamination growth would be tolerable.

2.6.3.   Preliminary Model Results

This section highlights results from single parameter modifications as well as multi-parameter modifications which are captured in the preliminary results themes section.

2.6.3.1   Single parameter change from baseline

The approach for running simulations was to first make runs changing only one parameter at a time to see if and how sensitive the population was to any one parameter. The baseline configuration resulted in only a population of 20 organisms over the 100-day mission, which then dropped to 0 thereafter. The largest population achieved was 20,000 after changing the viable death rate from 0.0001 to 0.1. So the

general conclusion was that any reasonable change to just one parameter, relative to the baseline, does not cause significant microbial population.

Next, the four mission elements or parameters that are controllable from a mission planning and operations point of view, namely the number of astronauts, the microbial shed per astronaut, the habitat leakage fraction, and the microbial shed from other biomass, were maximized. The number of astronauts was set to 20, the microbial shed per astronaut in a space suit was increased 3 orders of magnitude from the baseline to 240,000 microbes per day, the microbial shed from habitat was increased 3 orders of magnitude to 1.44×10^9, and the habitat leakage fraction was increased by 1 order of magnitude to 0.1. Perhaps surprisingly, the population reached only 50,000 and went to approximately 0 after the mission. This suggested that rigorous controls on the sources of biological contamination might not be needed if the other uncontrollable, or "environmental", factors, such as birth and death rates, are close to the baseline values.

However, given the above maximum controllable parameters, increasing the water birth rate factor function from 0.1 to 0.5 produces a population on the order of 30 million over the 100-day mission and substantial runaway growth thereafter. A similar dynamic existed for nutrient birth rate factor as well. Changing the viable organism death rate function from an exponential decrease of 0.5-0.02 to a more linear decrease of 0.1-0.02 has a similar effect but is perhaps a less likely possibility. Merely making the viable organism death rate function steeper makes little difference.

2.6.3.2   Themes of the preliminary results

The model was sensitive to several factors such as the survival fraction of the organisms when they are initially exposed to the Martian environment, leakage from the habitat (total number and fraction leaked), the initial value of the death rate function, and the water and nutrient factor functions. A change of one order of magnitude in just one of these factors, or a 5 fold increase in the nutrient and water factors, or in the case of the death rate, a 0.015 difference, could cause the population to go from tens of thousands to tens of millions and even hundreds of billions. However, most parameter sets, indeed, the more likely parameter values (based on the limited knowledge of Mars we have to date), did not result in a substantial microbial population.

Any change to just one parameter, relative to the baseline, did not cause a significant microbial population. Rigorous controls on the sources of biological contamination might not be needed if the other uncontrollable environmental/external factors such as birth and death rates are close to the baseline values. However, increasing a birth rate can cause runaway growth. This suggested that it could be important to better understand the uncontrollable factors and especially the birth rate functions and how close the baseline values will be to actual values. There was a sensitivity to the astronaut number when leakage was not controlled and birth rates are higher than the baseline (e.g. a linear function going from 0-0.3).

Combined scenarios involving relative increases in almost all parameters suggest a high sensitivity for mutation rate (one order of magnitude), habitat source and leakage fraction (one order of magnitude), and the initial value for the viable

organism death rate function. Regarding the latter, the very small change, from 0.500 to 0.483 to 0.475 hints that the death rate of those organisms that initially survive could be very important.

2.7. Secondary Model

The preliminary model results highlighted in the previous section suggested the need for building out the water and nutrient sectors as well as further investigation into the possible role of mutations. This section will discuss the details of the model used to generate the results discussed in the Results chapter. After much investigation it was determined that handling mutations in the model would be quite difficult and computationally intensive, so a mutations sector was not developed further. However, mutations effects can be indirectly captured with a user-modified death rate function as will be discussed in this section.

2.7.1. Assumptions

Microbial, water, and nutrient leak rates are assumed to be constant. Also, it is assumed that the location of a human mission base will be in a location that has the higher end of the atmospheric pressure range on Mars, namely around 12 mbars (Haberle et al. 2001; Landis 2001; Zurek 1992a). This allows for the possibility that liquid water could be stable for extended periods time depending on temperature. In addition, while subsurface contamination is possible, the model will primarily be a surface model in that it is meant to model microbial contamination within a volume that includes a shallow depth into the soil. The subsurface of Mars may indeed be a possible abode for life as noted previously, but this model will focus on the more

likely near-term possibility of microbial contamination of the Martian surface soil. This is in part because there is very little understanding of the subsurface of Mars. Aquifers and permafrost will likely provide a more hospitable environment for terrestrial microbial life to survive and grow in subsurface environments, especially because a subsurface environment will provide protection from the high UV radiation on the Martian surface. However, at least initially (i.e. during the first months of the first human mission) the number of microbes that are transported to sufficient depths are assumed to be minimal. This is discussed further in the section on future research.

Initially, most organisms will die due to inhospitable Martian surface conditions. Because of the high levels of ultraviolet radiation and oxidants on the surface of Mars, it is assumed that most terrestrial organisms will die immediately upon exposure the Martian environment (Mancinelli 1989; Mancinelli and Klovstad 2000; Schuerger et al. 2003, Newcombe el a. 2005). The model has been built with the proper flexibility to allow this assumption to be relaxed and significantly altered by the user if necessary.

Even though *B. subtilis* is the model organism, it is assumed that many different kinds of organisms will have a chance to survive and reproduce and therefore the entire microflora of astronauts and the habitat are considered to contribute to the total leakage into the Martian environment around the habitat. The model assumes homogenous distribution of water and microbial leakage, and no water or microbial transport in the contamination volume surrounding the habitat– a

volume which can be specified by the user. Prospects for incorporating spatial

modeling are discussed further in the future research section.

2.7.2. Model Structure

The model has the following 6 sectors: (1) total organisms introduced to the

environment, (2) water, (3) nutrients, (4) contamination volume, (5) viable microbial

population, and (6) total dead and viable microbes. Detailed model structure

diagrams are shown in Appendix A and the model equations are shown in Appendix

B. Appendix C shows a graphical representation of the user interface.

As a baseline, DT = 1 hr, representing a 1 hour interval which allows cycles of

liquid water transience to be calculated since those cycles will most likely be only a

few hours in duration. The minimum doubling time of a terrestrial bacterium in the

Martian environment is likely to be substantially slower than the usual few hours

doubling time of terrestrial microorganisms on earth (Stolp 1988) so a DT of 1 hour is

adequate from a lifetime cycle perspective. As was done with the primary model, a

baseline parameter value set is used initially as a conservative reference point

representing a plausible set of parameter values that might result in a minimal

population. The baseline parameters values are listed in Table 2.3. The controllable

parameters (i.e. those that we will likely have some measure of control over) are

shown in italics.

2.7.2.1   Total organisms introduced into the environment

The total organisms introduced to the environment is the sum total of all

major sources of microbial organisms that might be deposited into the environment.

This includes sources from inside the habitat and sources from astronauts while they are outside the habitat. The three primary sources are: (a) microbial shedding of individual astronauts while they are in the habitat, (b) microbial shedding from astronauts that escapes their space suits while they are in the field, and (c) other habitat sources including waste (e.g. feces, urine) and biomass from supporting elements such as food, water (including waste water), and greenhouse sources (e.g. greenhouse soil).

The baseline value for the number of astronauts is 6, which is likely to be the lowest number of astronauts for the first human mission to Mars (Hoffman and Kaplan 1997). In the habitat, astronauts could shed anywhere from tens to tens of thousands of microorganisms per minute depending on the individual, dispersal control measures, and activity. Based on counts found by Riemensnider (1966) and Whyte and Bailey (1985), and assuming some degree of contamination control measures (e.g. such as clean room suits), the baseline astronaut in-habitat microbial dipsersal parameter value assumes that an active astronaut in the habitat will shed an average of 1000 microbes per minute (or 60000/hr). Riemensnider measured approximately 3,000 microbes per minute being shed from a person wearing a sterile scrub suit, socks, and cap and standing in a small chamber (although one of two measurements in street clothes was approximately 6,000). Whyte and Bailey compared various kinds of clothing (including underwear only) and found shed rate counts ranging from less than 10 to 1,000 microbes per minute depending on the material worn by the subject.

Based on the possibility that a positive pressure space suit can be designed to reduce human microbial release to the environment by two orders of magnitude, the baseline value for astronaut leakage while outside in a spacesuit is baselined at 10 microbes per minute or 600/hr. Whyte and Bailey found that a 2 to 3 order of magnitude reduction was possible with certain fabrics (e.g. gortex) and special closures. However, reports from the U.S. Department of Health (U.S. Department of Health, 1967 and 1966) that tested positive pressure Gemini space suits found only a 1 order of magnitude reduction. The baseline value suggested here assumes that the Gemini suit leak rates will be improved by an order of magnitude.

The average number of "extra habitat activity" (EHA) astronauts per day is assumed to be two for an average of 4 hours. This is probably close to a minimum since optimizing exploration time will be a priority. However, safety will be the first priority and the first human mission will likely take a very conservative approach regarding field exploration and other activities that require being outside the habitat.

It is also assumed that biomass associated with the habitat, including for example, astronaut waste (solid and liquid), food, water, greenhouse sources, etc. will generate approximately 1,000 microbes/minute or 60,000/hour inside the habitat. That is, while these sources will likely be well contained, they may not be perfectly contained and so certain number of microbes may leak into the wider habitat via physical imperfections in containment (e.g. filters), air circulation distribution, management of cleaning processes and cleaning agents, etc. These assumptions could change dramatically depending on the mission details, especially the amount of biomass and the amount of contamination control. The total number of dead

organisms released to the environment is also tracked in this sector. The baseline value assumes the proportion of dead to live microbes leaked is 1.

The habitat leak fraction (representing the fraction of organisms that could leak from the habitat) is baselined at 0.01 of the total organisms produced by a habitat leaking under partially controlled conditions. Microbes may escape a habitat through a number of mechanisms such as slow structural leaks (from what will be a pressurized habitat), water seepage, outgassing, spills, etc.

Lastly, as with the basic model, this sector also has a mission duration time parameter that allows a mission duration time to be entered by the user, after which the deposition of organisms stops, but population growth could still continue. This allows an assessment of population growth for the duration of the mission as well as for some period thereafter. The latter is important because although a minimum mission time for the first mission is likely to be less than a few months, population growth of an established microbial population could continue after the mission is over. This could have implications for subsequent missions. The baseline value for the mission time is 60 days. When the mission time plus one day has elapsed, the daily deposited organisms goes to zero. Further modifications to this model might account for the possibility that some organisms will still continue to be deposited after humans have left because the habitat could continue to produce a microbial population, especially if it is intended that the habitat be partially autonomously maintained for use in a subsequent mission.

2.7.2.2   Water

The water sector calculates (a) the amount of water in the environment, ultimately as water content (defined as the fraction of the contamination volume that contains liquid water), (b) its availability to microorganisms, and (c) an associated reaction rate for microbial activity. The amount of ice and liquid water in the contamination region is the sum of mission sources such as discharge and leakage from astronauts and the habitat, and indigenous sources of liquid water which might exist in the form of transient liquid phases from permafrost melting.

2.7.2.2.1   Amount of water in the environment

The user has the ability to specify the amount of water leaked per astronaut suit (baselined at 0.0001 liters/day, which is then multiplied by the number of astronauts and EHA time), the water leak rate from the habitat (baselined at 0.1 liters/day), and the amount of indigenous liquid water (baselined at 10 liters spread over the contamination volume in question perhaps in the form of shallow permafrost).

As the soil temperature (baselined as Figure 2.1, taken from Smith et al. (2004)) rises above a specified melt temperature (baselined at 270 K (-3 °C) which is slightly lower than a pure water melting point due to slight brine concentration) liquid water forms and accumulates as a function of the fraction of ice that melts (baselined as a function shown in Figure 2.2).

Figure 2.1: Diurnal Soil Temperature Cycle

Hours	temperature K
0.000	197.60
2.392	192.10
4.784	195.95
7.176	210.80
9.568	267.45
11.96	280.65
14.35	279.55
16.74	268.55
19.14	225.65
21.53	208.60
23.92	197.60
26.31	191.00
28.70	195.95

290.00

temperature K

190.00

0.000   28.70

0.000   720.00

Hours

Data Points: 302
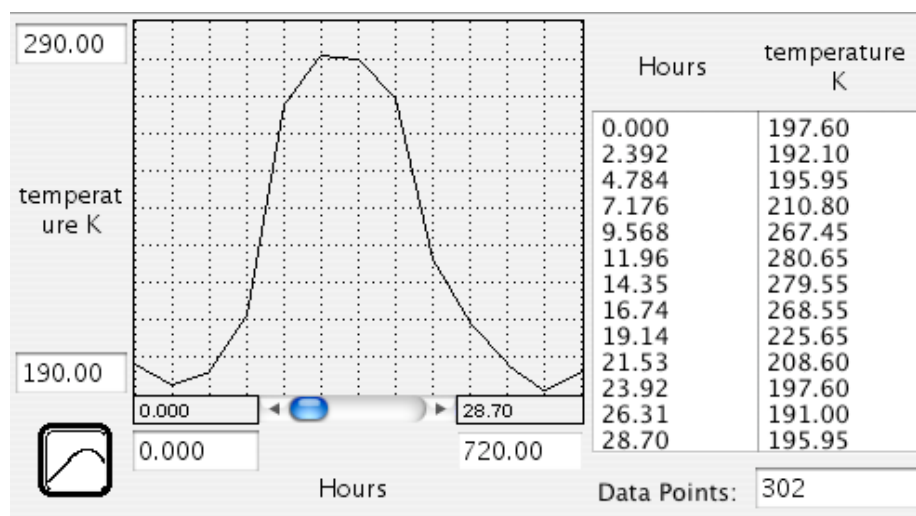
Figure 2.2: Melt Fraction

temperature K	fraction of ice that melts
270.00	0.200
273.00	0.230
276.00	0.280
279.00	0.325
282.00	0.375
285.00	0.430
288.00	0.480
291.00	0.525
294.00	0.580
297.00	0.650
300.00	0.705

1.000

fraction of ice that melts

0.000

270.00   300.00

temperature_K

Data Points: 11

The total liquid water is determined by subtracting the amount of water evaporated which is a fraction determined by the product of the evaporation rate and the thickness of the water layer. The evaporation rate is baselined at 0.5 mm/hr which is based in part on the evaporation rate measured recently by Sears and Moore (2005) of 0.73 mm/hr ($\pm$ 0.14 mm/hr) in Martian conditions. The evaporation rate

they measured was for pure water from a beaker so the baseline value is decreased to 0.5 to account for a possible decrease in evaporation rate due to solutes and soil adhesion effects. The water layer thickness is baselined at 1 mm, giving a baseline evaporation fraction of 0.5.

The total liquid water in the soil is then used to calculate the total water content in the area of interest as the total soil water divided by the contamination volume (specified in the contamination volume sector) to give the fraction of the soil volume of the contamination region that contains water.

2.7.2.2.2 Water availability

The availability of water for microbial activity is calculated as the total soil water potential, Ψ, which is a thermodynamic term that describes the availability of water, or more specifically, the energy or suction pressure required to withdraw water from the system (Atlas and Barth 1998). The total soil water potential can be calculated as the sum of two separate water potential components that can be identified with forces that act on the water to affect its ability to be withdrawn from a system, namely, the matric potential (Ψ_m), and osmotic potential (Ψ_o) (Papendick and Campbell 1981):

$$\Psi_{tot} = \Psi_m + \Psi_o \qquad\qquad (2.2)$$

Water potential is normally a negative number but the model changes it to a positive number in order to use it more easily in the modeling package's graphical functions feature.

The matric potential, Ψ_m, is determined as a function of water content (defined above) for sandy soil, taken from Papendick and Campbell (1980) and Klinger and Vishniac (1988) and shown below in Figure 2.3 as represented in the model. This function provides a value for the matric potential (in bars) as a function of water content for predominantly sandy soils which is a reasonable approximation for Martian soil. Matric potential is normally a negative number, but again, it is converted to a positive number to be used more easily with the modeling package's graphical function feature.

Figure 2.3: Matric Potential

water content	matric potential bars
0.000	90.00
0.050	10.00
0.100	0.500
0.150	0.200
0.200	0.250
0.250	0.030
0.300	0.020
0.350	0.010
0.400	0.000
0.450	0.000
0.500	0.000

Data Points: 11
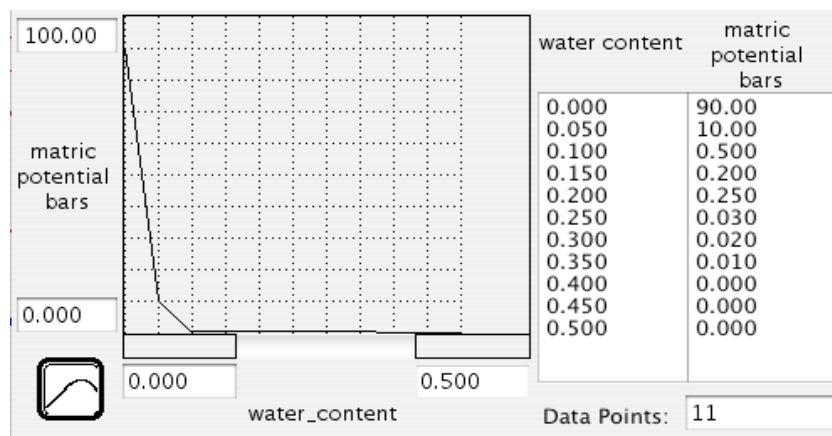
The osmotic potential, Ψ_o, is a measure of potential difference due to solutes in water. Because there is the possibility of brines on Mars as discussed in the background, osmotic potential should be incorporated into the total water potential calculation. Osmotic potential can be calculated using:

$$\Psi_o = \phi\,\gamma\,c\,R\,T \tag{2.3}$$

where ϕ is the osmotic coefficient, γ is the number of osmotically active particles per molecule of solute, c is the concentration (moles/kg) of the solute, R is the gas constant (0.0831 bar L mole^{-1} K^{-1}), and T is the temperature in Kelvins (K). For most purposes, the osmotic coefficient, ϕ, can normally be assumed to be 1 for most solutes– certainly most salts (Papendick and Campbell 1980). For higher accuracy, a table of osmotic coefficients can be used (Robinson and Stokes 1965; Papendick and Campbell 1980). Data suggests that ϕ is not strongly dependent on temperature ($\pm$ 2 % over a range of 0 to 50 C for KCl, for example, (Campbell and Gardner 1971)), and temperature dependence of osmotic potential is accounted for in equation 2.3.

For Mars, the recently detected $CaSO_4$ and $MgSO_4$ rich outcrops (Christensen, P.R. et al. 2004) require unique osmotic coefficients. While $CaSO_4$ has the highest concentrations over time, it is generally not soluble in water, so the model uses the osmotic coefficient for what might be expected to be the predominant salt in a brine that might be found on Mars or be created on Mars by the leaking of water into the environment, namely $MgSO_4$, for which the osmotic coefficient, ϕ, is 0.6 (Robinson and Stokes 1965), and the number of osmotically active particles, γ, is 2.

The solute concentration, c, is calculated from results found by Moore and Bullock (1999) who exposed Mars analog igneous minerals (based on the SNC (Shergottites, Nakhlites, and Chassigny) Martian meteorites group) to a simulated Martian atmosphere, and pure water. The ion concentrations they found over time are shown below in Table 2.2, with the key concentrations shown in bold italics.

Table 2.2: Measured Ion Concentrations (mg/liter)

DAYS	1	42	84	168	336
Calcium	*30.2*	*95.5*	*49.9*	*2950*	*460*
Manganese	0.75	3.02	0.06	1.22	0.46
Magnesium	*11.3*	*52.6*	*66.1*	*38.2*	*5.2*
Iron	0.23	1.52	0.739	10	0.51
Aluminum	1.86	0.42	0.219	39.4	10
Potassium	0.46	0.85	0.522	4	1.2
Sodium	0.12	0.74	0.87	5.8	4
Chloride	5.8	8	12.2	4.9	6.01
Fluoride	0.764	1.3	1.78	1.41	1.82
Nitrate	3.58	4.8	3.29	1	1.05
Sulfate	*2.55*	*10*	*2.81*	*60*	*13.64*

For sulfate, the average concentration between 42 and 84 days (as an approximation to the baseline mission duration of 60 days) is $(2.81 + 10)/2 = 6.4$ mg/liter. The formula weight for SO_4 is 96, so the moles/liter concentration for sulfate is $0.0064/96 = 6.7 \times 10^{-5}$ moles/liter. For magnesium, the average concentration is $(52.6 + 66.1)/2 = 59.3$ mg/liter. The formula weight for Mg is 23, so the moles/liter concentration for Mg is $.0593/23 = 0.0026$ moles/liter. So the total baselined brine concentration of $MgSO_4$ is approximated at 0.003 moles/liter.

The data from Moore and Bullock shown in Table 2.2 above indicates a trend interruption at day 84 which the authors acknowledge is difficult to explain. They acknowledge the possibility of a systematic error in the sample analysis. They also suggest that declines might be explained by the formation of some precipitate.

2.7.2.2.3   Water reaction rate

The last main component of the water sector calculates a water reaction rate, which is a general measure of microbial activity (such as decomposition reaction

rates) as a function of total water potential shown below in Figure 2.4. The curve is representative of soil microbes collectively in reactions such as composition (Paul and Clark 1996). The water reaction rate is used to calculate the overall growth rate in the nutrient uptake sector as noted below in the Nutrients section.

Figure 2.4: Water Reaction Rate

total water potential	water reaction rate
0.000	0.995
10.00	0.665
20.00	0.405
30.00	0.100
40.00	0.075
50.00	0.055
60.00	0.040
70.00	0.020
80.00	0.010
90.00	0.000
100.00	0.000

Data Points: 11

Edit Output:
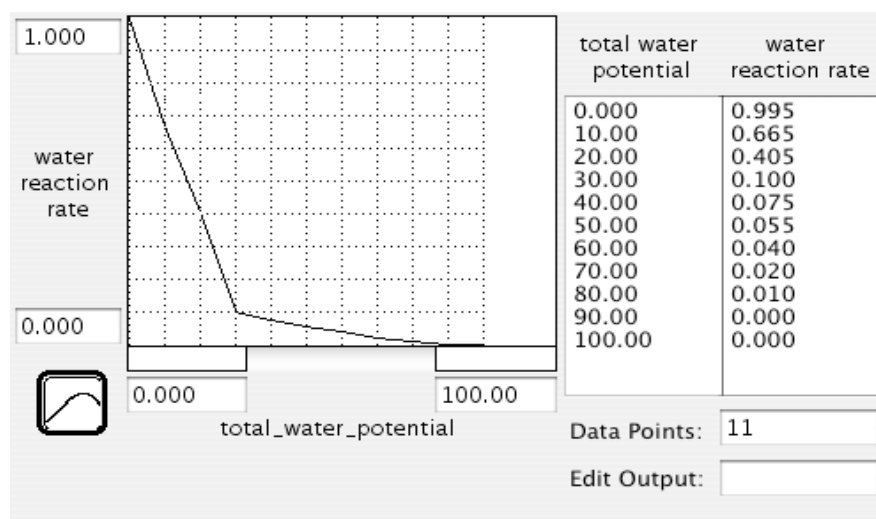
2.7.2.3   Contamination volume

The contamination volume sector calculates the potential contamination volume of interest. It is calculated as:

$$[\pi (r_2 + r_1)^2 - \pi r_1^2)]\, d \qquad\qquad (2.4)$$

where r_2 is the contamination distance outside the habitat (baselined at 1 meter, based partly on personal communication with Andrew Schuerger (September 2005) whose preliminary results from field studies indicate a contaminated zone out to

approximately 2 meters), r_1 is the habitat radius (baselined at 10 meters), and d is the potential depth into the soil that microbial contamination might penetrate (baselined at 0.01 meters). This gives a volume outside the habitat out to a distance r_2 from the habitat that might be contaminated.

2.7.2.4   Nutrients

The nutrient sector calculates (a) the total amount of nutrient as the sum of indigenous nutrient, leaked nutrient from sources associated with the mission, and nutrient from dead and living microbes leaked into the environment, and (b) the growth rate of the microbial population.

2.7.2.4.1   Total Nutrients

The indigenous nutrient is assumed to be oxygen, which is present in trace amounts in the atmosphere (0.13 %), and also in soil oxidants, and in water (Clark 1998) and may be a limiting nutrient since it is available in small amounts as atmospheric O_2. The surface density of Martian atmosphere is 0.02 kg/m^3, or 20 mg/liter, so the concentration of atmospheric oxygen is approximately 0.026 mg/liter. It is unknown how much oxygen will be in the soil oxidants or soil water, but for purposes of establishing a baseline value, the atmospheric concentration is doubled resulting in a baseline oxygen concentration available to microbes of approximately 0.05 mg/liter.

The leaked nutrient from the mission could be from many sources including human waste, food stuffs, and other organic substances. It is unknown what this

concentration will be, but it is baselined at a fairly low value of 0.01 mg/liter, which assumes some degree of containment.

This sector calculates the total number of organisms available as nutrient by multiplying both the total number of dead organisms and the total viable population by the fraction of the population that might be available as nutrients. This fraction is baselined at 0.1 to reflect the possibility that $1/10^{th}$ of the population may be available at any given time as a source of nutrient for other microbes. A high rate of sporulation will keep this number low.

The nutrient from dead organisms is then calculated as the product of individual microbe mass, nutrient per dead organism fraction (baselined at 0.1), and total dead organisms. The nutrient from live organisms is calculated as the product of individual microbe mass, a nutrient per live organism fraction (baselined at 0.1) and total viable organisms. Gonzalez-Pastor et al. (2003) found a decrease of 70 % of viable cells due to cannibalization, a process whereby *Bacillus subtilis* forces lysis of sibling cells to obtain internal nutrients and hence delay sporulation which is a time-consuming process that otherwise conveys a disadvantage when cycling through sporulation morphogenesis because of cycling nutrient availability– a condition relevant to this model. The disadvantage results from the time and energy required to complete sporulation relative to other bacteria that do not, or may not have already entered the sporulation process and are hence better able to capitalize on the appearance of new nutrients. It assumed that approximately 10 % of the cannibalized cell mass are used as nutrients by the cannibalizing bacteria. The cannibalized microbes are accounted for in the Viable Microbial Population sector detailed below.

The concentration of the leaked nutrients is determined by first multiplying the total nutrients by the water content to give an approximation of the total nutrients exposed to water, and then dividing by the total amount of water to give an average concentration distributed throughout the contamination volume.

2.7.2.4.2 Growth Rate

The growth rate is calculated using a multiplicative Monod-type kinetics model (Rockhold et al. 2005, MeGee et al. 1970):

$$\mu = w_r \mu_m \left(\frac{C_I}{K_I + C_I} \right) \left(\frac{C_L}{K_L + C_L} \right) \qquad (2.5)$$

where w_r is the water reaction rate, μ_m is the maximum growth rate (baselined at 0.5/hr, taken from Rockhold et al. (2005)), C_I is the concentration of indigenous nutrient (in this case oxygen, baselined at 0.05 mg/liter as noted above), K_I is the half-saturation growth constant for the indigenous nutrient, oxygen (baselined at 1.5 mg/liter, taken from Rockhold et al. (2005)), C_L is the nutrient concentration of leaked nutrient (in this case organic sources from leaked microbes and other leaked nutrient sources), and K_L is the half-saturation growth constant for the leaked nutrients (baselined at 10 mg/liter, taken from Rockhold et al. (2005)).

2.7.2.5   Viable organism population

The viable population is calculated as:

$$X_t = x_s\sigma + X_{t-dt} + (\mu - r(t) - c)X_{t-dt} \qquad\qquad (2.6)$$

X_t is the total microbial population number at time t, x_s is the number of organisms

that are deposited per time step, σ is the survival fraction of organisms that initially

survives exposure to the Martian environment (making $x_s\sigma$ the term capturing the

number of viable organism that are added to the environment every time step), μ is

the growth rate given by the growth rate equation in the nutrient sector shown above,

$r(t)$ is the death rate given by a manually drawn function that allows a user to have

more precise control over the population dynamics by capturing many kinds of death

rate dynamics separate from growth rate dynamics (including, for example, a

decreasing rate due to mutations), and c is the cannibalization rate.

Experimental evidence shows that virtually all microbes will be inactivated

within minutes (certainly after a full day) by the incident UV on the Martian surface

(Schuerger et al. 2003; Necombe et al. 2005). The survival fraction, σ, therefore

essentially amounts to the probability that a leaked microbe will be protected from the

high UV radiation environment. Very little protection is needed for a microbe like

Bacillus subtilis to survive the high UV environment– in some cases, as little as a

monolayer of dead bacteria might provide enough protection (Mancinelli and

Klovstad 2000), although Schuerger et al. (2003) found that only .5 mm of dust layer

completely protected *B. subtilis*. Given the lethality of the UV environment, but

combined with the potential survivability due to minimal protection, the estimation

for a baseline value for σ is 0.0001– which essentially suggests the possibility that 1

in a 10,000 microbes will be protected once they are leaked into the environment, or

that there is a 1 in 10,000 chance that any given microbe will have adequate

protection once leaked into the environment.

The death rate function is baselined at a constant of 0.1 per hour. Most

organisms will already be dead, suggesting that those that survive will be able to do

so for some period– especially since they will likely be protected from the UV

radiation. The cannibalization rate is also baselined at a relatively low value of 0.1

because the chance for microbial interaction that will allow for the relatively

sophisticated interaction of cannabilization will likely be small due to low

temperatures and limited amounts of liquid water.

Lastly, this sector also captures the total number of births and calculates the

microbial population density in order to give what is perhaps a more practical

measure of assessing the contamination levels and potential impacts.

2.7.2.6   Total Dead and Viable Microbes

This sector calculates the total dead and viable microbes in the environment

by adding the total dead microbes tracked in the Total Organisms Released to the

Environment sector and the Total Viable Microbes from the Viable Microbial

Population sector. The total dead organisms are a sum of dead organisms leaked

from the habitat, astronauts in the field, and those microbes that die after being leaked

into the environment.

2.7.3. Parameter Table

Table 2.3 lists the baseline parameter values. The "controllable parameters",

i.e. those that mission planners could have some control over, are shown in italics.

Table 2.3: Baseline Parameter Values

Parameter Description	Baseline Value
TOTAL ORGANISMS LEAKED TO ENVIRONMENT	
Number of astronauts (A)	*6*
Astronaut microbial source in habitat (M_A)	*60,000 microbes/hr*
Microbes from biomass (M_B)	*60,000 microbes/hr*
Habitat leak fraction (h)	*0.01*
Number of EHA astronauts (M_{EHA})	*2*
Astronaut EHA leak rate (M_{EHA})	*600 microbes/hr*
EHA time (t_{EHA})	*4 hrs*
Proportion of dead to living microbes leaked (p)	1
WATER	
Water leak rate per astronaut (for EHA) (W_A)	*0.0001 liters/hr*
Water leak rate from habitat (W_H)	*0.001 liters/hr*
Indigenous water source (W_i)	10 liters
Surface temperature (T)	Figure 2.1 (function)
Melt temperature (T_m)	-3 °C (270 K)
Fraction of ice that melts (I_{mf})	Figure 2.2 (function)
Evaporation rate (E)	0.5 mm/hr
Water layer thickness (W_t)	1 mm
Matric potential (Ψ_m)	Figure 2.3 (function)
Osmotic coefficient (ϕ)	0.6
Osmotically active particles (γ)	2
Solute concentration (for $MgSO_4$) (C_s)	0.003 moles/liter
Water reaction rate (w_r)	Figure 2.4 (function)
CONTAMINATION VOLUME	
Contamination distance from habitat (r_2)	1 meter
Habitat radius (r_1)	*10 meters*
Contamination depth (d)	0.01 meters
NUTRIENT	
Indigenous nutrient concentration (C_I)	0.05 mg/liter
Non-microbial leaked nutrient (N_L)	*0.01 mg/hr*
Fraction of population available as nutrient (n_{pop})	0.1
Nutrient fraction per dead organism (n_{dead})	0.1
Nutrient fraction per live organism (n_{live})	0.1
Max growth rate (μ_m)	0.5 /hr
Half-saturation constant for indigenous nutrient (K_I)	1.5 mg/liter
Half-saturation constant leaked nutrient (K_L)	10 mg/liter
VIABLE ORGANISM POPULATION	
Initial survival fraction (σ)	0.0001
Death rate function ($r(t)$)	0.1 (constant)
Cannibalization fraction (c)	0.1

CHAPTER 3: Model Application

The overall approach was to find a set of plausible parameters (referred to as a parameter set, or parameter configuration) that produced a significant population, which is defined as population that results in a population density of 1 microbe/cm^3 or greater. The reason for this threshold is discussed in the Discussion section. The first step was to generate results by first modifying parameters within one sector only and one parameter at a time within each sector to assess sensitivity, followed by selected combinations of parameters within each sector. The selected combinations were chosen based on sensitivity (some details are shown in Appendix D), the uncertainty level of each parameter, whether the parameter will be controllable or not, and knowledge of how the parameters are used in the model to calculate total population.

The tables that follow show results for the total viable microbes, the population density (i.e. viable microbes per cm^3), the growth rate per hour, and total births. All of the numbers are maximum values for the run. The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than 1×10^{-5}. The total births value is shown as 0 if no births occurred during the run. This applies to all tables. Noteworthy runs are shown in italics. The tables are followed by plots of some of the runs (not only those runs producing a population density of 1/cm^3, but other runs that may serve as good examples, or have interesting features), which are then discussed in the Analysis section. Some plots also show the number of births per time step as the fifth parameter in the plots. To save space and repetition

in the tables, the runs include the previous accumulation of parameter values unless otherwise noted.

Table 3.1 shows these "sector only" results, with each sector in capitalized italics in the table.

Table 3.2 lists results that use a new baseline with the survival fraction (one of the most sensitive parameters) going from 0.0001 to 0.001, and with multiple cross-sector parameter combinations, again based on sensitivity found in the initial run set shown in Table 3.1 and Appendix D, and knowledge of how the parameters are used to determine the total population. This new baseline has a run number of 1, distinguishing it from the original baseline that has configuration number 0 shown in Table 3.1.

Two subsequent tables (Table 3.3 and Table 3.4) list results that were obtained in search of a parameter set that might cause continued growth after the mission duration ended. Table 3.3 shows runs that focused on modifications to the death rate, and Table 3.4 shows runs from additional parameter combinations involving several parameter modifications including, evaporation fraction, death rate, half-saturation constant for leaked nutrients, water leak rate from the habitat, and indigenous water.

Table 3.4 shows results when the survival fraction is 0.01. While this may not be the most plausible possibility, it is nevertheless worth exploring because there may be a set of circumstances in which 1 in 100 leaked microbes could survive. Regardless, this helps explore the boundaries of the model.

The total organisms, both and dead and alive, could also be an important contamination factor. Dead organisms could confound the search for life, so total

microbe results are shown in Table 3.6 (60-day mission) and Table 3.7 (150-day

mission), as well as the microbial density which will help assess the potential

difficulty for life detection experiments.

Table 3.8 shows results obtained from continuing the run past the 60-day

mission up to 150 days in order to assess if and what circumstances might produce

continued population growth after the mission is over.

Table 3.9 lists results from extending the mission to 150 days.

3.1.   Sector-Only Modifications

Table 3.1 results, specifically runs 1and 5, indicate that the model is most

sensitive to the initial number of surviving organisms, (i.e. the survival fraction) and

the death rate. The baseline death rate is a constant of 0.1. The survival fraction

could plausibly be an order of magnitude higher, so the series of runs shown in Table

3.2 assumes that parameter modification.

Table 3.1: Parameter modifications for each sector only [1]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth Rate	Total Births
0	Baseline	4	0	0	0
	TOTAL VIABLE ORGANISMS				
1	Survival fraction: 0.0001 to 0.1	$4.0x10^3$	0	0	0
2	Fraction of pop avail as nutr: 0.1 to 1	4	0	0	0
3	Cannibalization fract: 0.01 to 0.0001	4	0	0	0
4	Death rate: 0.1 to 0	$4.4x10^1$	0	0	0
5	Death rate: 0 Survival fraction: 0.1	$4.4x10^4$	0	0	0
6	Death rate: 0 Survival fraction: 1 Fract avail as nutr: 1 Cann fract: 0	$6.3x10^6$	10	0	0
	TOTAL ORGANISMS TO ENV.				
7	Astr source in hab $6.0x10^5$ to $6.0x10^6$	$3.3x10^2$	0	0	0
8	Microbes from biomass $6.0x10^5$ to $6.0x10^6$	$5.8x10^1$	0	0	0
9	Hab leak fract 0.01 to 0.1	$3.8x10^1$	0	0	0
10	Astr source in hab: $6.0x10^6$ Microbes fr biomass: $6.0x10^6$ Habitat leak fraction: 1	$3.8x10^3$	0	0	3
11	Above, and: Number of astronauts: 6 to 12 EHA astronauts: 2 to 4 EHA time: 2 to 4 EHA leak rate: 600 to 6,000	$7.1x10^3$	0	0	6
	WATER				
12	Indigenous water: 10 to 10,000	4	0	0	0
13	Above, and: Evap fraction: 0.5 to 0	4	0	0	0
14	Above, and: Water leak rate: 0.001 to 0.1	4	0	0	0
15	Above, and: Melt temperature: 270 to 250	4	0	0	0
16	Above, and: Matric potential max: 90 to 50	4	0	0	0
	NUTRIENTS				
17	Non-micr nutr leaked: 0.01 to 0.1	4	0	0	0
18	Max growth rate: 0.5 to 0.9	4	0	0	0
19	Half-sat const for leaked nutr: 10 to 1 Half-sat const for ind nutr: 1.5 to 0.5	4	0	0	0
20	Non-micr nutr leaked: 0.1 Max growth rate: 0.9 Half-sat const for leaked nutr: 1 Half-sat const for ind nutr: 0.5	4	0	0	0

[1] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than $1x10^{-5}$. The total births value is shown as 0 if no births occurred during the run.

3.2. Initial Cross-Sector Modifications, Survival Fraction 0.001

As noted above, Table 3.2, assumes a new baseline that assumes the survival fraction is 0.001 (an order of magnitude greater than the original baseline). Results for the 60-day mark are shown. These runs are intended to inform more detailed runs in Table 3.3 and Table 3.4.

Table 3.2: Initial cross-sector parameter combinations, survival fraction 0.001 [2]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth Rate	Total Births
1	Baseline, survival fraction 0.001	$4.2x10^1$	0	0	0
2	Astro micr source in hab: $6.0x10^6$ Microbes from biomass: $6.0x10^6$ Indigenous water: 1000	$3.8x10^3$	0	0	0
3	Evap fraction: 0.5 to 0 Indigenous nutr concentration: 0.05 to 0.1	$3.8x10^3$	0	0	27
4	Half-sat const for leaked nutr: 10 to 1	$3.8x10^3$	0	0	$2.7x10^2$
5	Non-micr: leaked nutr: 0.01 to 0.1	$3.9x10^3$	0	0	$2.5x10^3$
6	Non-micr leaked nutr: 1	$4.1x10^3$	0	$1.5x10^{-2}$	$1.5x10^4$
7	Death rate: 0.1 to 0.05	$7.7x10^3$	0	$1.5x10^{-2}$	$2.8x10^4$
8	Death rate: 0.05 to 0.02	$1.6x10^4$	0	$1.5x10^{-2}$	$5.8x10^4$
9	Death rate: 0	$6.7x10^4$	0	$1.5x10^{-2}$	$2.2x10^5$
10	Nutr fract per dead microbe: 0.1 to 1 Nutr fract per live microbe: 0.1 to 1	$6.7x10^4$	0	$1.5x10^{-2}$	$2.2x10^5$
11	Half-sat const for leaked nutr: back to 10	$4.6x10^4$	0	$3.2x10^{-3}$	$2.9x10^4$
12	*Cannibalization fraction: 0.01 to 0*	*$9.4 x10^5$*	*1*	*$3.2x10^{-3}$*	*$3.4x10^5$*
13	Death rate: 0 to 0.1	$4.3x10^3$	0	$3.2x10^{-3}$	$2.8x10^3$
14	Half-sat const for leaked nutr: 1	$4.5x10^3$	0	$1.5x10^{-2}$	$1.6x10^4$
15	Death rate: 0.1 to 0.05	$9.4x10^3$	0	$1.5x10^{-2}$	$3.3x10^4$

In an attempt to find conditions that result in continued growth after the 60-day (1440 hour) mission, and subsequently, more plausible parameter sets that create substantial populations, Table 3.3 and Table 3.4 also have a survival fraction of

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[2] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than $1x10^{-5}$. The total births value is shown as 0 if no births occurred during the run.

0.001, both show results from runs with a duration of 2000 hours with more variations of parameter modifications, but the results shown are values at the 60-day mark. Table 3.3 modifies the death rate, and Table 3.4 goes further in trying to make the parameter sets more plausible by modifying evaporation, water leaked from the habitat, indigenous water, half-saturation constant for leaked nutrient (microbial and non-microbial sources of organics), and death rate modifications. This provides preliminary guidance to help inform runs that extend to 150 days (or 3600 hours) as shown in subsequent tables.

3.3.   Runs Just Beyond 60 days (up to 2000 hours)

The intent of this section was to test further parameter combinations that also ran a little beyond the 60-day mark (up to 2000 hours) in order to assess which configurations might continue to grow beyond the 60-day mission. Results for the 60-day mark are shown.

Table 3.3: Runs beyond 60 days (2000 hours): death rate modifications [3]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth Rate	Total Births
1	*Above, from Table 3.2, which is:* *Survival fraction: 0.001* *Astro micr source in hab: $6.0x10^6$* *Microbes from biomass: $6.0x10^6$* *Indigenous water: 1000* *Evap fraction: 0* *Indigenous nutr concentration: 0.1* *Non-micr leaked nutr: 1* *Half-sat const for leaked nutr: 1* *Nutr fract per dead microbe: 0.1 to 1* *Nutr fract per live microbe: 0.1 to 1* *Cannibalization fraction: 0.01 to 0* *And:* *Death rate: 0.05 to 0* *Growth continued past 60 days*	$1.0x10^7$	$1.5x10^1$	$1.5x10^{-2}$	$9.6x10^6$
2	Death rate: 0.02	$2.6x10^4$	0	$1.5x10^{-2}$	$9.2x10^4$
3	Death rate: 0.01	$6.7x10^4$	0	$1.5x10^{-2}$	$2.2x10^5$
4	Death rate: 0.005	$2.4x10^5$	0	$1.5x10^{-2}$	$6.0x10^5$
5	*Death rate: 0.002* *Growth continued after 60 days* *(Figure 3.1)*	$1.5x10^6$	2	$1.5x10^{-2}$	$2.2x10^6$
6	*Death rate: 0.003* *Growth continued after 60 days* *(Figure 3.2)*	$7.2x10^5$	1	$1.5x10^{-2}$	$1.3x10^6$
7	Death rate: 0.004	$3.9x10^5$	0	$1.5x10^{-2}$	$8.4x10^5$

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[3] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than $1x10^{-5}$. The total births value is shown as 0 if no births occurred during the run.

Figure 3.1 and Figure 3.2 correspond to runs 5 and 6 in Table 3.3 above and are shown below as examples of the most noteworthy results– i.e. the most plausible parameter values with populations that have a density of 1 microbe/cm^3 or more (see Analysis section for more detail). Figure 3.1 and Figure 3.2 show the time plots for the total number of viable microbes (plot line 1 shown in pink), total births (plot line 2 shown in green), total number of viable microbes per cm^3 (plot line 3 shown in black) and the growth rate (plot line 4 shown in orange).
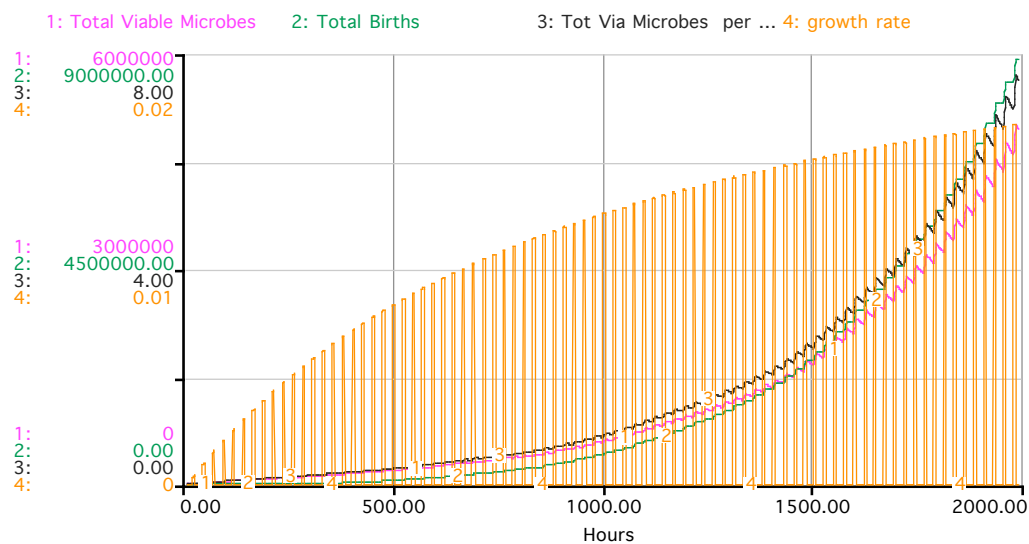
Figure 3.1: Run 5 from Table 3.3
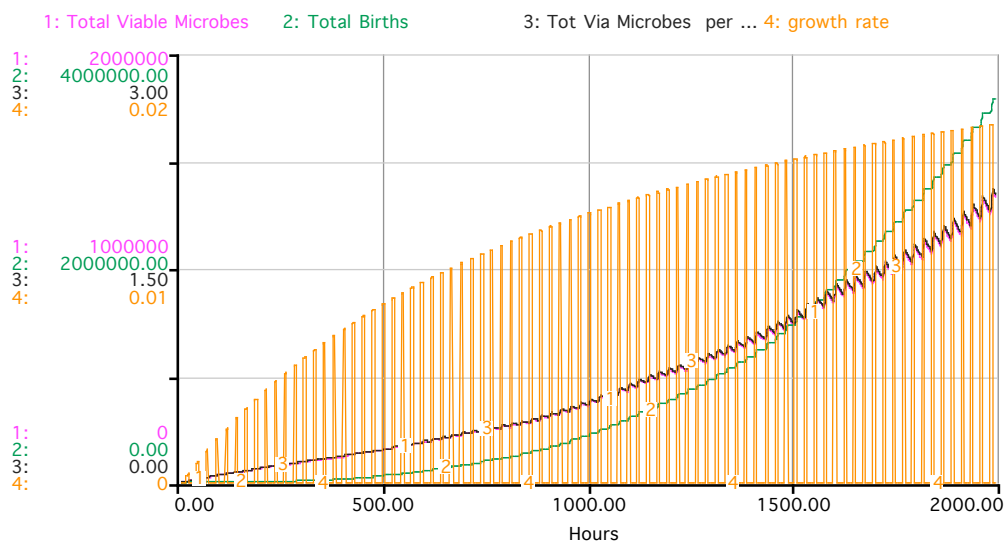

Figure 3.2: Run 6 from Table 3.3

Table 3.4: Runs beyond 60 days (2000 hours): modifications of evaporation fraction, death rate, half-saturation constant for leaked nutrients, water leak rate from the habitat, and indigenous water [4]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth Rate	Total Births
1	Above, from Table 3.3, which is: Survival fraction: 0.001 Astro micr source in hab: 6.0x10^6 Microbes from biomass: 6.0x10^6 Indigenous water: 1000 Evap fraction: 0 Indigenous nutr concentration: 0.1 Non-micr leaked nutr: 1 Half-sat const for leaked nutr: 1 Nutr fract per dead microbe: 1 Nutr fract per live microbe: 1 Cannibalization fraction: 0.01 to 0 + Death rate 0 Evap fraction 0 to 0.1 (Figure 3.3)	6.1x10^5	0	5.0x10^{-3}	6.2x10^3
2	*Evap fraction to 0.01* *Growth continues past 60 days*	*2.5x10^7*	*3.8x10^1*	*1.6x10^{-2}*	*2.5x10^7*
3	*Death rate: 0.001* *growth continues slightly past 60 days* *(Figure 3.4)*	*8.7x10^6*	*1.3x10^1*	*2.0x10^{-2}*	*1.0x10^7*
4	*Half-sat constant back to 10* *growth continues slightly past 60 days* *(Figure 3.5)*	*5.7x10^5*	*0*	*4.7x10^{-3}*	*3.5x10^5*
5	Death rate: 0.005 Half-sat constant back to 1	3.7x10^5	0	2.0x10^{-2}	1.0x10^6
6	Water from hab 0.001 to 0.01 Growth continues slightly past 60 days	3.8x10^5	0	2.0x10^{-2}	6.8x10^5
7	Evap fraction 0.1	8.4x10^4	0	5.2x10^{-3}	4.6x10^3
8	Evap fraction 0.01 Indigenous water 100	1.3x10^5	0	9.5x10^{-3}	1.4x10^5
9	Evap fraction 0	3.8x10^5	0	1.9x10^{-2}	1.0x10^6
10	Half-sat const for ind nutr back to 10 Indigenous water 1000 Evap fraction 0.01	1.1x10^5	0	4.9x10^{-3}	9.4x10^4

Figures 3.3, 3.4 and 3.5 shown below are time plots for total viable microbes

(plot line 1 shown in blue), total viable microbes per cm^3 (plot line 2 shown in red),

total births (plot line 3 shown in green), and growth rate (plot line 4 shown in orange).

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[4] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than 1x10^{-5}. The total births value is shown as 0 if no births occurred during the run.
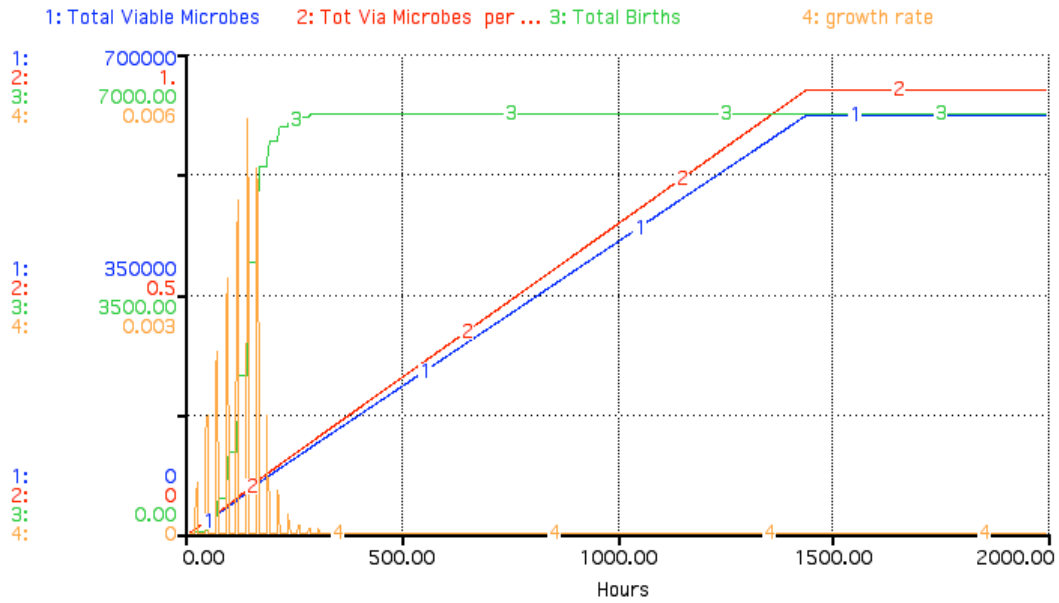
Figure 3.3: Run 1 from Table 3.4
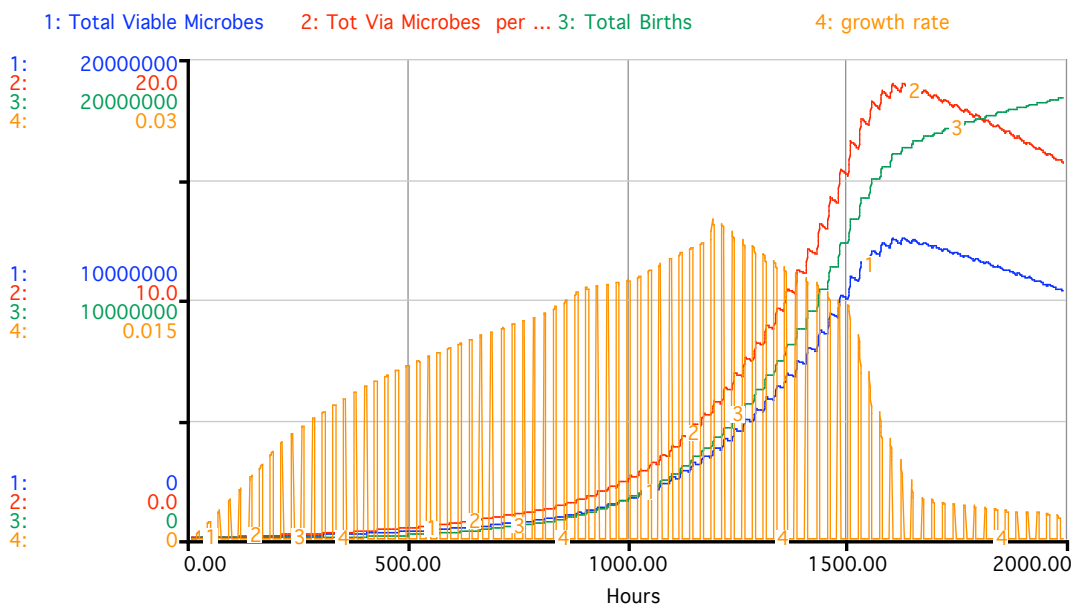

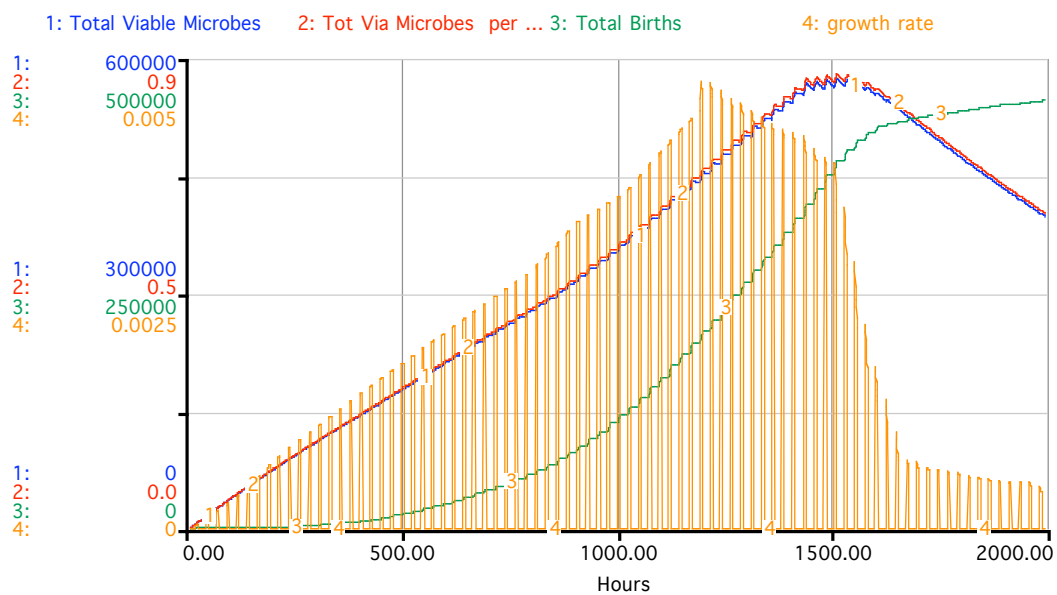Figure 3.4: Run 3 from Table 3.4

Figure 3.5: Run 4 from Table 3.4

3.4. Survival Fraction 0.01

Table 3.5 below assumes a survival fraction of 0.01, which could be realistic depending on how leaked microbes will be shielded from UV radiation. Five graphs of some noteworthy results follow.

Table 3.5: Survival Fraction 0.01 [5]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm3	Growth rate	Total Births
1	Baseline with surv fraction 0.01.	$4.0x10^2$	0	0	0
2	Astro micr source in hab: $6.0x10^6$ Microbes from biomass: $6.0x10^6$	$3.8x10^4$	0	0	0
3	Indigenous water: 1000	$3.8x10^4$	0	0	3
4	Evap fraction: 0	$3.8x10^4$	0	$2.0x10^{-5}$	$1.4x10^2$
5	Half-sat const leaked nutr: 10 to 1	$3.8x10^4$	0	$2.0x10^{-4}$	$1.4x10^3$
6	Non-micr leaked nutr: 0.01 to 0.1	$3.9x10^4$	0	$1.6x10^{-3}$	$1.3x10^4$
7	Death rate: 0.05	$7.1x10^4$	0	$1.6x10^{-3}$	$2.3x10^4$
8	Death rate: 0.02	$1.4x10^5$	0	$1.6x10^{-3}$	$4.7x10^4$
9	Cannibalization fraction: 0.01 to 0	$2.2x10^5$	0	$1.6x10^{-3}$	$7.6x10^4$
10	Death rate: 0.01	$4.6x10^5$	0	$1.6x10^{-3}$	$2.8x10^5$
11	*Death rate: 0.005*	*$9.8x10^5$*	*1*	*$3.2x10^{-3}$*	*$5.8x10^5$*
12	Evap fraction: 0.01	$4.7x10^5$	0	$4.7x10^{-3}$	$4.4x10^5$
13	*Death rate: 0.001 (Figure 3.6)*	*$5.7x10^6$*	*8*	*$4.7x10^{-3}$*	*$3.4x10^6$*
14	*Half-sat const leaked nutr: 10 (Figure 3.7)*	*$3.4x10^6$*	*5*	*$6.0x10^{-4}$*	*$2.9x10^5$*
15	*Evap fraction: 0.1 (Figure 3.8)*	*$3.2x10^6$*	*4*	*$6.0x10^{-5}$*	*$6.6x10^2$*
16	*Evap fraction: 0.5*	*$3.2x10^6$*	*4*	*0*	*4*
17	*Non-micr leaked nutr: 0.01 (Figure 3.9)*	*$3.2x10^6$*	*4*	*0*	*0*
18	Astro micro source in hab: Baseline value of $6.0x10^4$	$4.9x10^5$	0	0	0
19	*Astro micro source in hab: $6.0x10^5$*	*$7.3x10^5$*	*1*	*0*	*0*
20	*Water hab leak rate 0.01*	*$7.3x10^5$*	*1*	*0*	*0*
21	*Evap fraction 0.1 (from 0.5)*	*$7.3x10^5$*	*1*	*0*	*15*
22	*Non-micr leaked nutr 0.1 (from 0.01)*	*$7.3x10^5$*	*1*	*$6.0x10^{-5}$*	*$1.5x10^2$*
23	*Water parameters at baseline Nutrient parameters at baseline (Figure 3.10)*	*$7.3x10^5$*	*1*	*0*	*0*

[5] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than $1x10^{-5}$. The total births value is shown as 0 if no births occurred during the run.
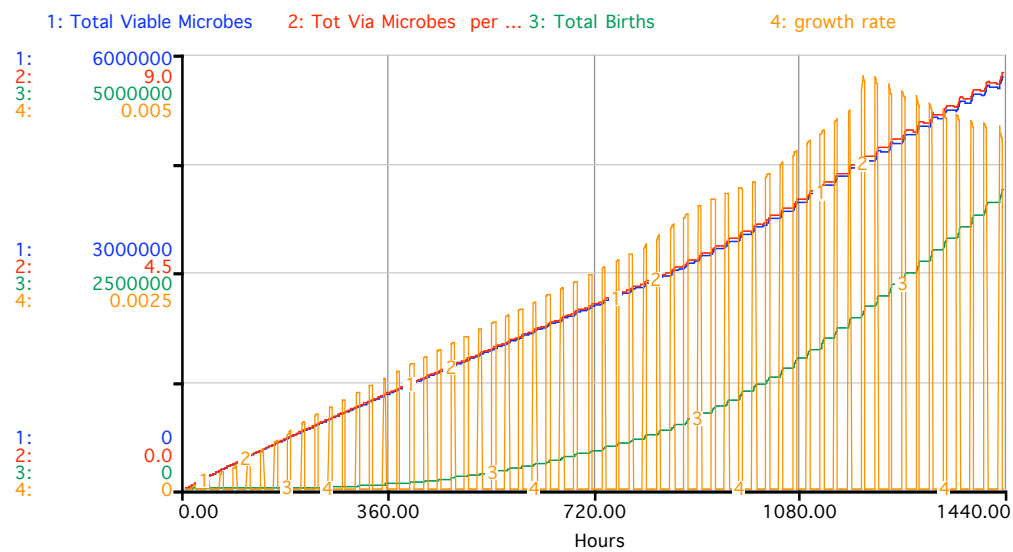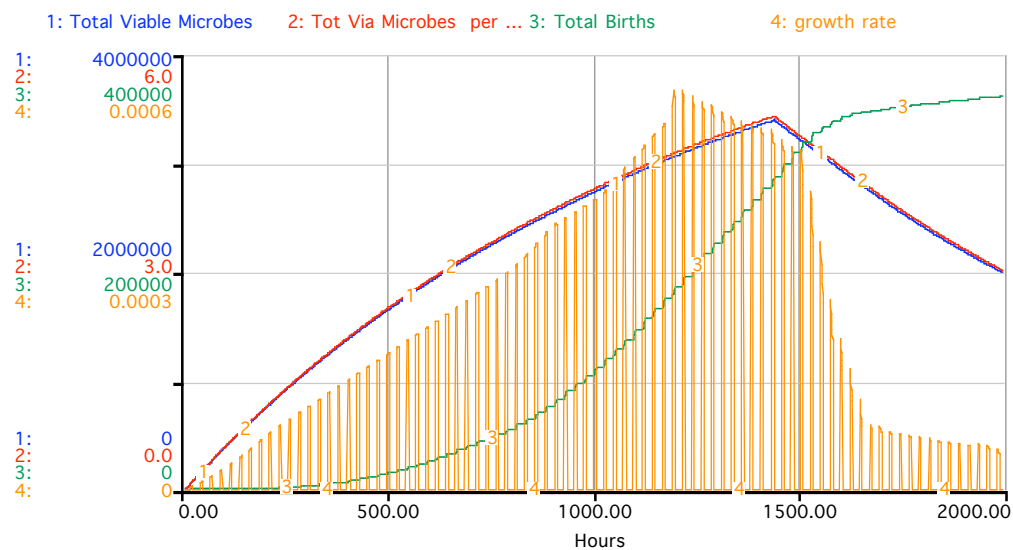
Figure 3.6: Run 13 from Table 3.5

1: Total Viable Microbes   2: Tot Via Microbes per ... 3: Total Births   4: growth rate

Hours

Figure 3.7: Run 14 from Table 3.5

1: Total Viable Microbes   2: Tot Via Microbes per ... 3: Total Births   4: growth rate

Hours

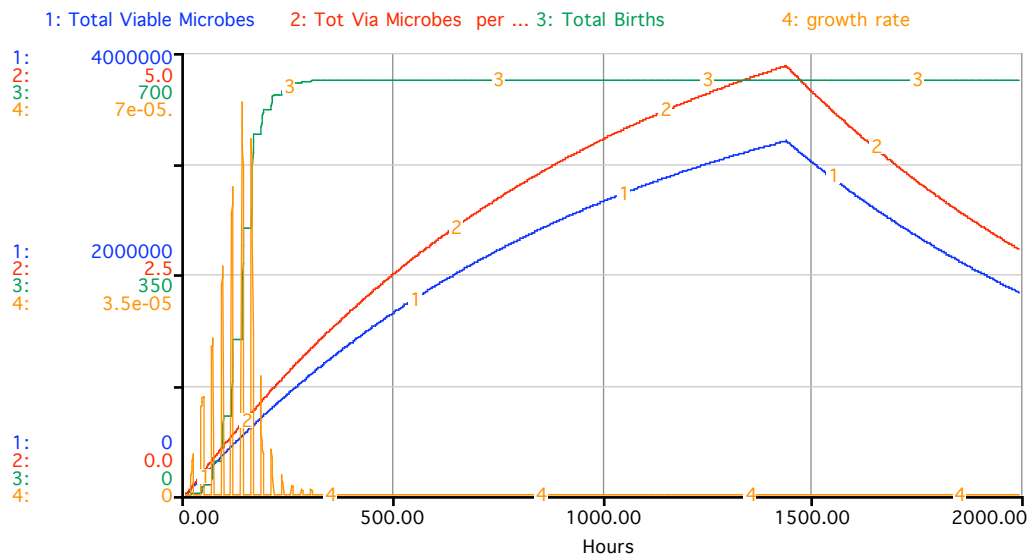Figure 3.8: Run 15 from Table 3.5
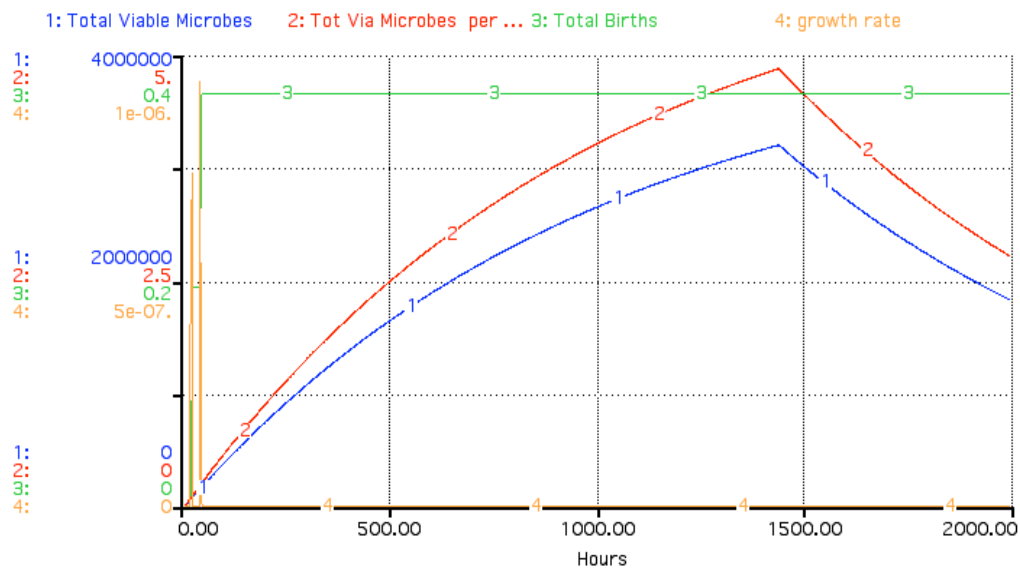

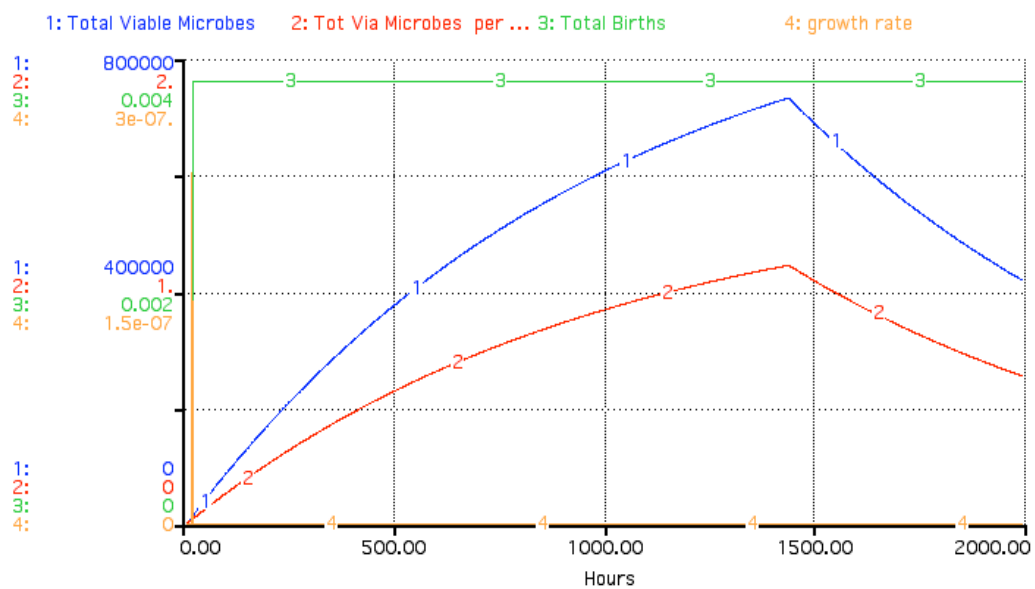Figure 3.9: Run 17 from Table 3.5

Figure 3.10: Run 23 from Table 3.5

3.5. Total Dead and Viable Microbes

Results shown in Table 3.6 indicate that even the baseline configuration could

present a problem given the population density of 19 microbes/cm^3. The only

additional results shown are for increased sources from astronauts (an order of

magnitude increase from the baseline) and other microbial sources in the habitat (two

orders of magnitude increase from the baseline), which is the most plausible

parameter set beyond the baseline. Both of these parameter sets are plausible and do

create a potentially problematic population since the population density is greater

than 1 microbe/cm^3. Similarly, Table 3.7 shows the results from the same parameter

sets but for a 150-day mission instead.

Table 3.6: Total dead and viable microbes, 60-day mission

	Parameter Configuration	Total Microbes	Total Microbes per cm^3
0	Baseline	$1.3x10^7$	$1.9x10^1$
1	Astro micr source in hab: 600,000 Microbes from biomass: 6,000,000 (Figure 3.11)	$2.8x10^8$	$4.2x10^2$

Table 3.7: Total dead and viable microbes, 150-day mission

	Parameter Configuration	Total Microbes	Total Microbes per cm^3
0	Baseline	$3.2x10^6$	$4.8x10^1$
1	Astro micr source in hab: 600,000 Microbes from biomass: 6,000,000 (Figure 3.12)	$6.9x10^8$	$1.1x10^3$

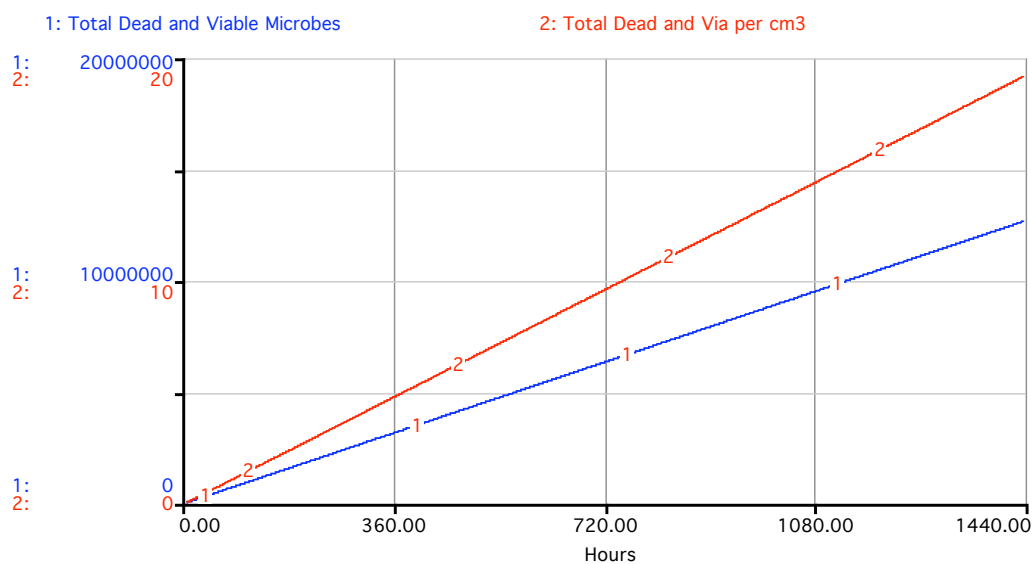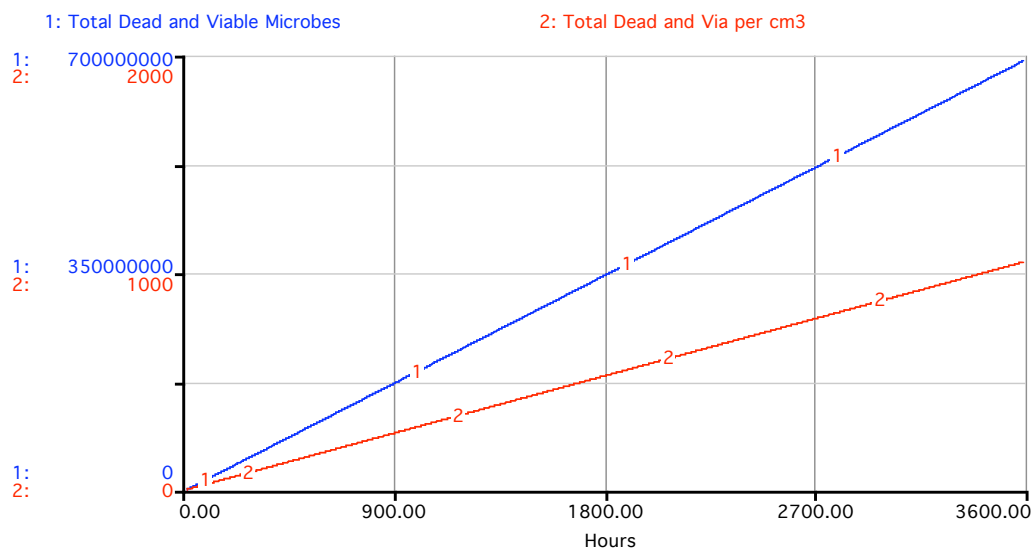Figure 3.11: Run 0 from Table 3.6

Figure 3.12: Run 1 from Table 3.7

3.6.   60-Day Missions, 150-Day Runs

Table 3.8 shows results from runs that have a mission duration of 60 days, but a run time of 150 days. Maximum values are listed. Plots of noteworthy results follow.

Table 3.8: 60-day mission, 150-day run [6]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth Rate	Total Births
1	Baseline + Astro micr source in hab: 6.0x10^6 Microbes from biomass: 6.0x10^6 Indigenous water: 1000 Evap fraction: 0 Indigenous nutr conc: 0.1 Cannibalization fraction: 0 Nutr fract per dead microbe: 1 Nutr fract per live microbe: 1 Non-micr leaked nutr: 1 Half-sat const for leaked nutr: 1	4.3x10^2	0	1.5x10^{-2}	1.6x10^3
2	*Death rate: 0.001*	*1.3x10^9*	*1.6x10^3*	*2.0x10^{-2}*	*1.3x10^9*
3	Death rate: 0.005 (Figure 3.13)	2.4x10^4	0	1.9x10^{-2}	2.0x10^5
4	*Death rate: 0.003*	*2.7x10^6*	*4*	*2.0x10^{-2}*	*7.0x10^6*
5	Half-sat const for leaked nutr: 10 (Figure 3.14)	1.7x10^4	0	7.0x10^{-3}	1.9x10^4
6	Death rate 0.001	9.3x10^4	0	6.6x10^{-3}	1.9x10^5
7	*Survival fraction 0.001 (Figure 3.15)*	*9.3x10^5*	*2*	*6.6x10^{-3}*	*1.9x10^6*
8	*Evap fraction 0.01 (Figure 3.16)*	*5.7x10^5*	*0*	*4.7x10^{-3}*	*4.7x10^5*
9	Water hab leak rate to 0.01	*5.8x10^5*	0	4.9x10^{-3}	5.3x10^5

[6] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than 1x10^{-5}. The total births value is shown as 0 if no births occurred during the run.
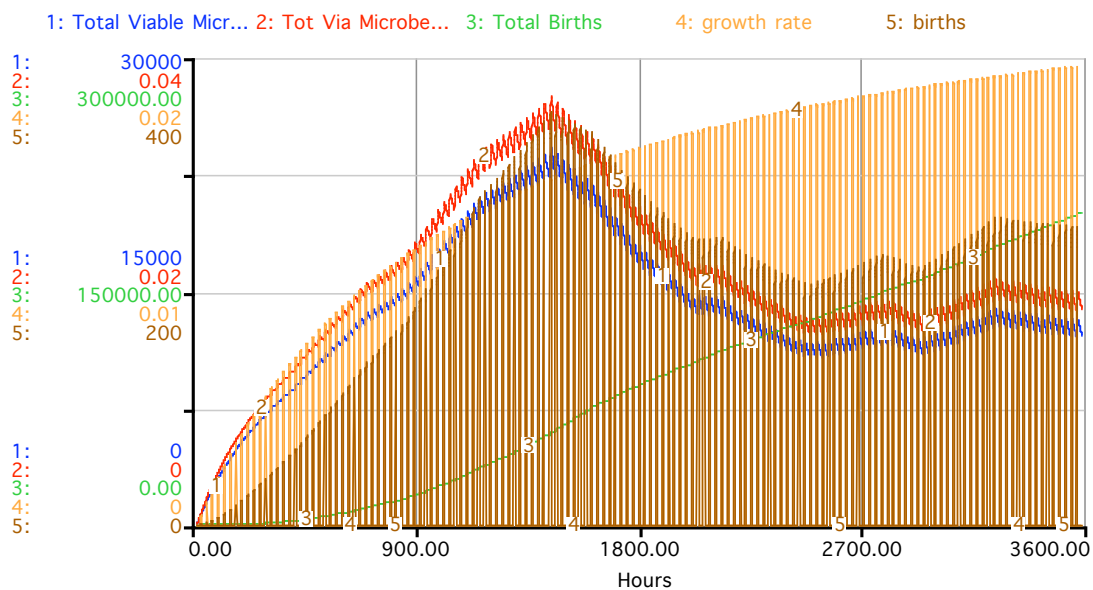
Figure 3.13: Run 3 from Table 3.8

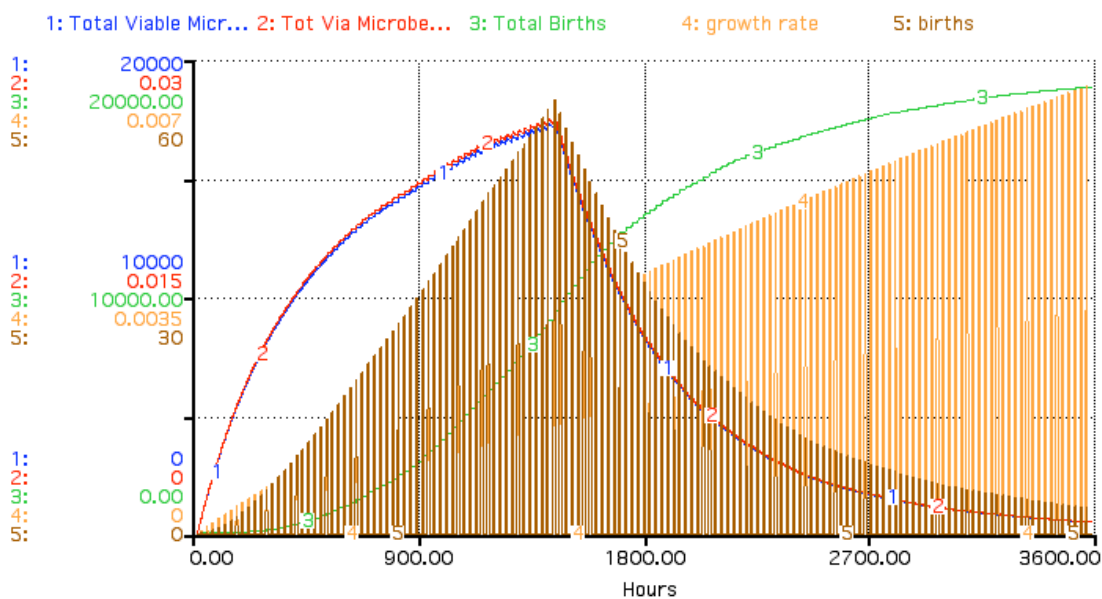Figure 3.14: Run 5 from Table 3.8

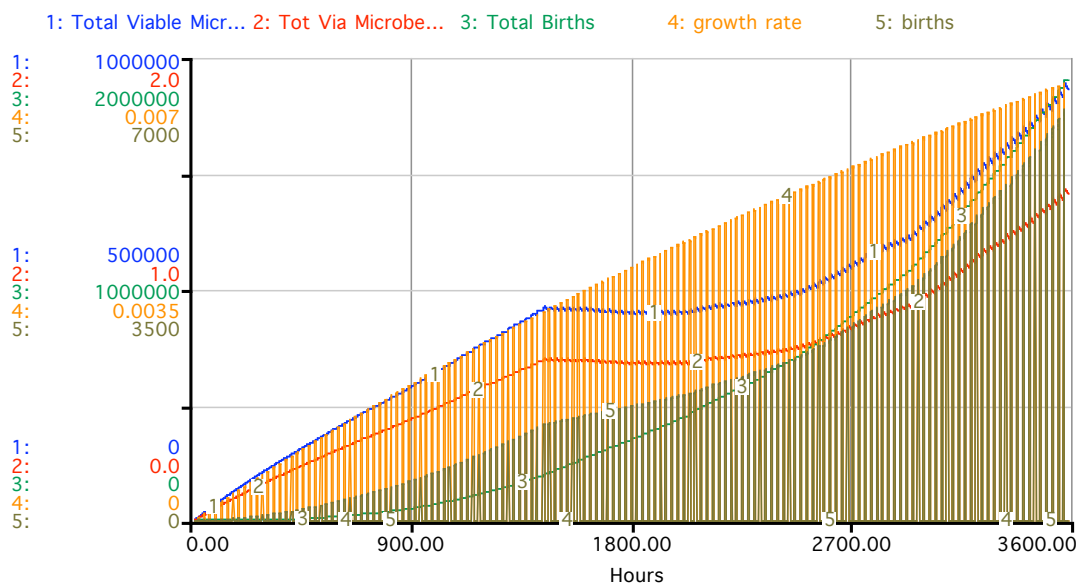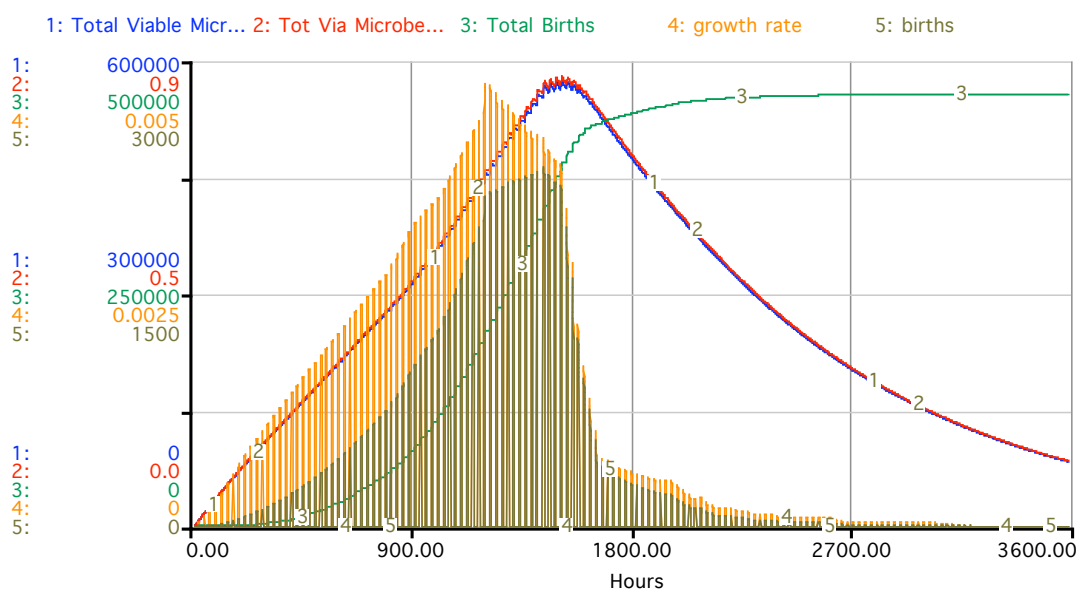Figure 3.15: Run 7 from Table 3.8

Figure 3.16: Run 8 from Table 3.8

3.7.   150-Day Mission

 This section shows results for runs representing mission durations up to 150

days. Graphs of noteworthy results follow.

Table 3.9: 150-day mission [7]

	Parameter Configuration	Total Viable Microbes	Viable Microbes per cm^3	Growth rate	Total Births
0	Baseline	4	0	0	0
1	Baseline + death rate 0.02	1.5×10^1	0	0	0
2	Astro micr source in hab: 6.0×10^6 Microbes from biomass: 6.0×10^6 Indigenous water: 1000	1.4×10^3	0	0	0
3	Evap fraction 0 Surv fraction: 0.001 Indigenous nutr concentration: 0.1	1.4×10^4	0	9.0×10^{-5}	6.0×10^2
4	Cannibalization fraction: 0.01 to 0 Nutr fract per dead microbe: 0.1 to 1 Nutr fract per live microbe: 0.1 to 1 Half-sat const for leaked nutr: 10 to 1 Non-micr leaked nutr: 0.01 to 1	2.7×10^4	0	2.0×10^{-2}	3.7×10^5
5	Death rate: 0.01	8.0×10^4	0	2.0×10^{-2}	1.0×10^6
6	*Death rate 0.005* *(Figure 3.17)*	*9.4×10^5*	*1*	*2.0×10^{-2}*	*6.1×10^6*
7	*Death rate 0.003* *(Figure 3.18)*	*3.9×10^7*	*5.9×10^1*	*2.0×10^{-2}*	*9.7×10^7*
8	*Death rate 0.001*	*1×10^{10}*	*1.6×10^4*	*2.0×10^{-2}*	*1.4×10^{10}*
9	*Astro micr source in hab: 6.0×10^5* *Nutr fract per dead microbe: 0.1* *Nutr fract per live microbe: 0.1* *Half-sat const for leaked nutr: 10*	*5.7×10^5*	*0*	*6.6×10^{-3}*	*8.8×10^5*
10	*Nutr fract per dead microbe: 1* *Nutr fract per live microbe: 1* *(Figure 3.19)*	*5.7×10^5*	*0*	*6.6×10^{-3}*	*8.8×10^5*
11	*Evap fraction 0.01* *Astro micr source in hab: 6.0×10^6* *Nutr fract per dead microbe: 0.1* *Nutr fract per live microbe: 0.1* *(Figure 3.20)*	*6.2×10^5*	*0*	*4.7×10^{-3}*	*4.6×10^5*

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[7] The population density is 0 if the value is less than 1 microbe per cm^3. The growth rate is 0 if it is less than 1×10^{-5}. The total births value is shown as 0 if no births occurred during the run.
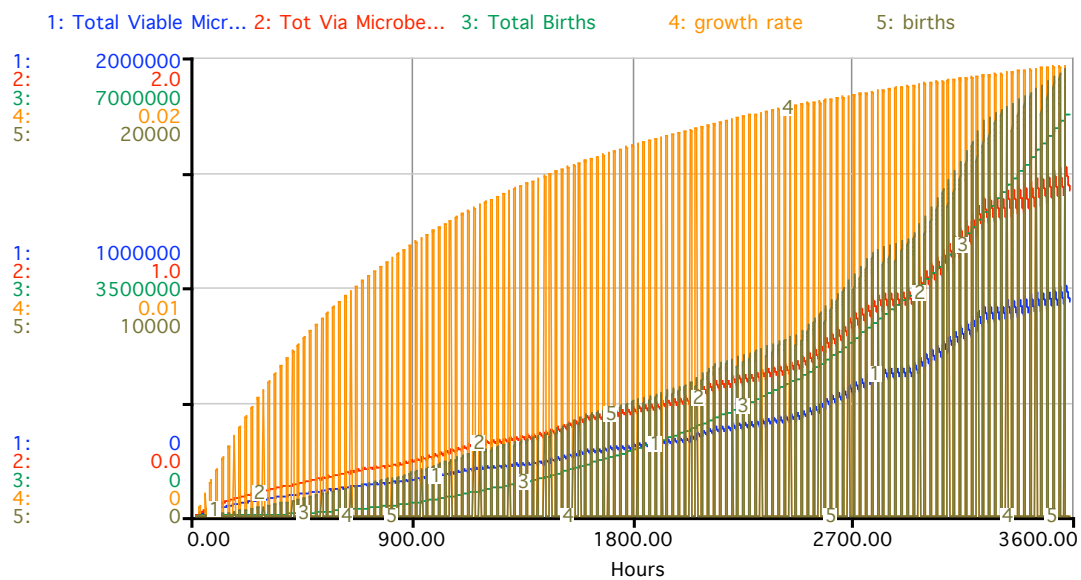
Figure 3.17: Run 6 from Table 3.9

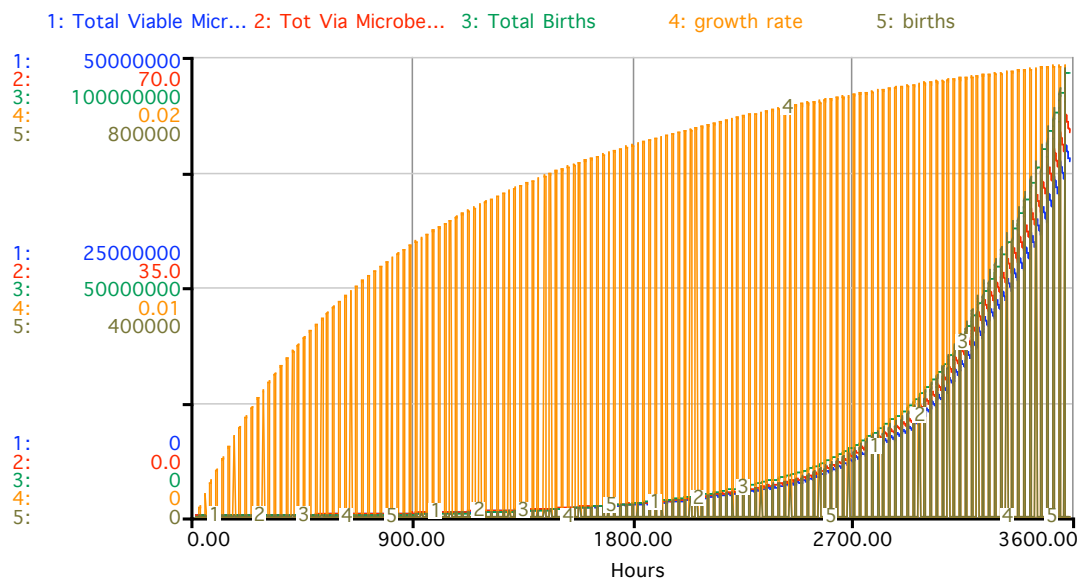Figure 3.18: Run 7 from Table 3.9

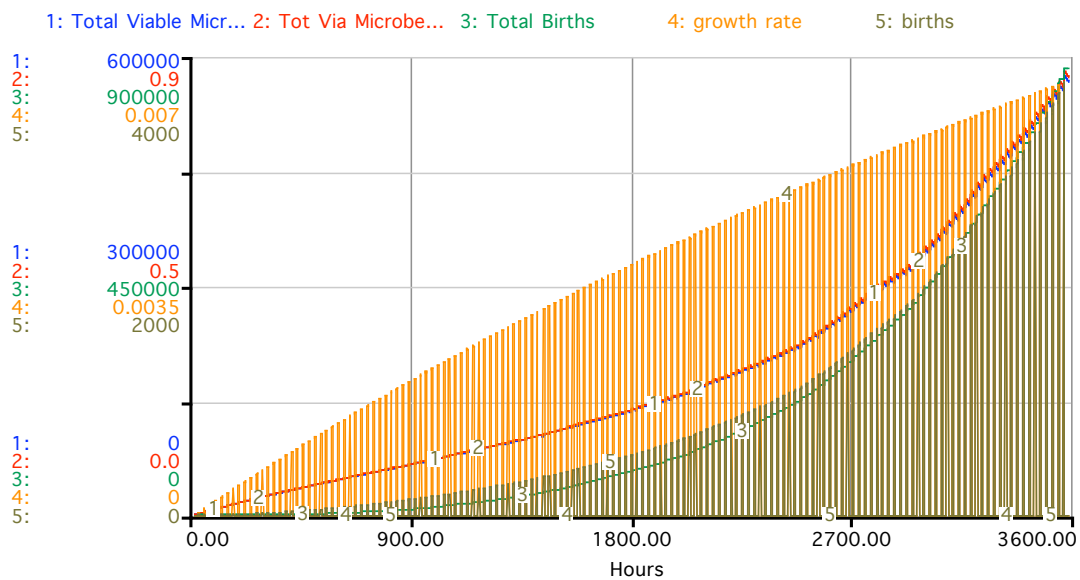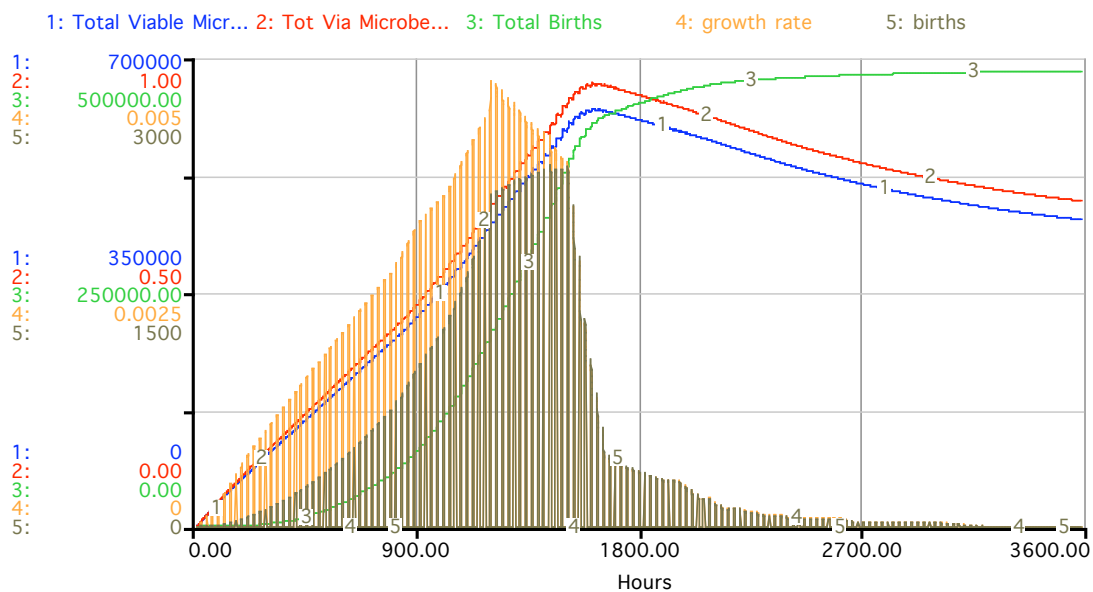Figure 3.19: Run 10 from Table 3.9

Figure 3.20: Run 11 from Table 3.9

3.8. Analysis

This section analyzes results shown in the previous tables and graphs

3.8.1. Baseline Parameter Set

The baseline parameter set results in a population of 4. The baseline

represents a plausible set of parameter values– perhaps the most plausible set– and so

to the extent this is a realistic set of conditions, this result suggests that it will be

difficult, if not impossible, for terrestrial microbes to survive and grow on Mars.

There are several factors that explain this. One, the amount of initial survived

organisms is very small, essentially 1 per time step, after the survival fraction is

applied. Two, the birth rate is effectively zero because the water reaction rate is zero

since there is very little water and it evaporates very quickly given the evaporation

fraction of 0.5 (which assumes a water layer thickness of 1 mm).

3.8.2. "Sector-only" Parameter Modifications

Table 3.1 indicates that no plausible modifications to the baseline set of

parameters in one sector only will result in a significant population, again defined as a

population creating a microbial density of 1 microbe/cm^3 or greater (see below

discussion regarding this threshold). The only exception was run 6 which assumed all

microbes would survive after being leaked into the environment (survival fraction of

1), and none would subsequently die (death rate of 0). This is not a plausible

scenario, but was meant as a run to understand the boundaries of the model. Run 5

produced a population of approximately 44,000 with a survival fraction of 0.1 and

death rate of zero. This is also not likely a plausible result.

3.8.3.   Cross-Sector Parameter Modifications, Survival Fraction 0.001

Table 3.2 shows results from combining extreme parameter values from each sector. Only 1 run (run 12) produced a population density of 1. This parameter set includes a death rate of 0 and an evaporation fraction of 0 and so is probably not a very plausible result. An evaporation fraction close to zero may indeed be reasonable under some circumstances given that the relative humidity on Mars can reach 100 %. However, this is likely to be at night and so would not correlate with the warm part of the day when water may be in a liquid form. This series of runs was intended to guide more detailed future runs that are shown in Tables 3.3 and 3.4, where the intent was to find more realistic parameter sets associated with significant populations and parameter sets that resulted in continued growth past the 60-day mission, which would then inform runs to be made for longer runs past the 60 days, and 150-day mission runs.

Table 3.3 shows 3 runs with significant populations, (runs 1, 5, 6). Run 1 differs from run 12 in Table 3.2 only by the half-saturation constant of the leaked nutrient, which went from 10, its baseline value, to 1. This, combined with a death rate of 0 and evaporation fraction of 0, make this an implausible result. Runs 5 and 6 are more plausible in that they have non-zero death rates, however they are also arguably implausible given the evaporation rate of 0 and the half-saturation constant being an order of magnitude lower than that used in the baseline (which, as noted previously, comes from a recent terrestrial application (Rockhold et al. 2005)). The half-saturation constant for organic sources of nutrients in the Martian soil environment is unknown, but it is not likely to be lower than the value for terrestrial

conditions. However, it may not be possible to rule this out until empirical evidence is obtained regarding the half-saturation constant for organic nutrients in Martian soil conditions.

Table 3.4 picks up from Table 3.3 and attempts to find significant populations with further modifications to cross-sector parameter combinations that are closer to more plausible values. For example, run 1 (shown in Figure 3.3– note that the high evaporation fraction causes the growth rate to decline rapidly to zero) produced a boderline population that has a plausible evaporation fraction of 0.1 (which would result from an evaporation rate of 0.5 mm/hr for a 5 mm thick layer of water), but the death rate is 0 so this is not likely a plausible result. Run 2 has a much more significant population resulting from an evaporation fraction that is an order of magnitude less than run 1 (also plausible perhaps given an evaporation rate of 0.5 mm/hr for a thick layer of water of say, 5 cm). However, again the death rate is 0. When the death rate is increased to a low value of 0.001, the population is reduced by a third, but is still significant (run 3, Figure 3.4). When the half-saturation constant is increased back to 10, the population is borderline (run 4), shown in Figure 3.5. Increasing the water leaked from the habitat by an order of magnitude (run 6) makes little difference because the amount leaked isn't significant.

Figure 3.4 and Figure 3.5 both show a peak in the growth rate prior to the 60-day mark. This is because as the initial saturation of the soil decreases due to evaporation, the water potential approaches 1, which is an optimal value for the water reaction rate (complete saturation inhibits activity slightly). As the water content decreases after this point, so does the water reaction rate, and hence the growth rate.

3.8.4. Survival Fraction 0.01

Table 3.5 shows results based on increasing the survival fraction an order of magnitude to 0.01. This parameter value suggests that 1 in 100 leaked microbes might initially survive after being released into the Martian environment. As mentioned previously, this survival fraction may be possible if microbes are leaked in such a way as to be protected from the UV radiation hitting the soil surface. This could happen under a number of scenarios ranging from being leaked into areas shaded from the sun to being quickly covered by dust or other potential sources of protection, including even other microbes as noted earlier.

Runs 11, 13-17, and 19-23 all show significant populations. The two key results are run 17 and run 23. Run 17 has the baseline evaporation rate as well as several other baseline values shown in previous runs, e.g. the half-saturation constant for leaked nutrient. It also includes a death rate of 0.001, and produces a population in the millions, with a population density of almost 5. Run 23 has more baseline values, namely all water and nutrient parameters, as well as only 1 order of magnitude increase in the astronaut source of microbes (as opposed to two orders of magnitude increase in many previous runs) and still produces a significant population, albeit, borderline.

Note that there is a negligible growth rate for these results as shown in the table and Figure 3.8, Figure 3.9, and Figure 3.10. These figures show a decreasing time span over which there is any growth rate at all (under 500 hours to close to 0, due primarily to the increasing evaporation fraction), and when there is, it is small, certainly in Figure 3.9 and Figure 3.10 where total births are 0. It is primarily the

survival fraction of 0.01 that causes the high population results. This suggests something potentially important, namely that even without growth, a problematic population could be created in the nearby environment surrounding the habitat.

3.8.5. Total Dead and Viable Microbes

Table 3.6 and Table 3.7 show the total number of both dead and viable microbes in the environment. Table 3.6 shows results for a 60-day mission, and Table 3.7 shows results for a 150-day mission. Both tables show that a substantial population and population density result from the baseline parameter set. The population density is increased by a factor of two when the astronaut microbial source is increased to 600,000 microbes/hour and microbes from other biomass is 6,000,000 microbes/hour– both values of which have been used in previous runs as previously noted.

While the number of dead microbes is not the focus of this dissertation, a potentially important implication of these results is that even with the baseline parameter set, a substantial number of microbes could exist in the environment that would confound the search for life. This is discussed further in the Discussion section.

3.8.6. 150-Day Runs, 60-Day Missions

Table 3.8 shows 4 runs that produce noteworthy populations, runs 2, 4, 7, and 8. Run 9 is comparable in number to run 8 but has a habitat leak rate of 0.01 which is less plausible than the baseline leak rate of 0.001 used in run 9. Regardless, the

results are very similar with a slight increase in the results in run 9 as would be expected from leaking more water into the environment.

Run 2 produces a substantial population, but has an evaporation fraction of 0. Run 3 increases the death rate by a factor of 5 and dramatically reduces the population. However, its plot, including the number of births per time step (plot line 5) is shown in Figure 3.13 because this run illustrates a case that appears to produce sustained growth, albeit slow growth. It suggests growth would continue with a longer mission beyond the 150 days. However, this result has a half-saturation constant for leaked nutrient of 1, as opposed to the more likely baseline value of 10. When this change is made, we see in Figure 3.14 that the number of births per time step decreases asymptotically to 0 as the mission progresses.

Runs 7 and 8 maintain the more realistic half-saturation constant and make the death rate and survival fraction 0.001. Run 8 goes further and includes an evaporation fraction of 0.01, making it a somewhat plausible scenario. While run 8 does not quite produce a threshold population of 1 microbe/cm^3, the plausible parameter set makes it a noteworthy run and so is shown in Figure 3.16. The evaporation fraction seems to make an important difference as a comparison of Figure 3.15 and Figure 3.16 indicates. Without evaporation, it appears the population would continue to grow. With even a small evaporation fraction, the growth rate and number of births per hour crashes due to the eventual evaporation of the water and the total population declines rapidly, asymptotically approaching 0, after the mission duration of 60 days.

3.8.7. 150-Day Missions

Table 3.9 lists results from runs with mission duration times of 150 days (3600 hours). Runs 6-11 are noteworthy, with runs 9 and 11 being the most noteworthy runs since they have the most plausible parameter set. Runs 6 and 7 (Figures 3.17 and 3.18) show increasing population growth but have an evaporation fraction of 0 and half-saturation nutrient constant of 1. Run 8 has a low death rate and large population, but again is probably not realistic given the evaporation fraction of 0 and half-saturation constant of 1.

However, run 9 is a more realistic parameter set since it decreases the astronaut microbial source by an order of magnitude to a value that is an order of magnitude above the baseline, and also uses the baseline half-saturation constant of 10. Figure 3.19 shows a plot for run 10 which is almost identical to run 9– which suggests the minimal role of the nutrient fraction per microbe that is used as nutrient. But again, the evaporation fraction for run 9 (and 10) is 0, reducing the plausibility of these results. Relative to run 9, when the astronaut microbial source is raised an order of magnitude to 6 million, the total microbial population is 2.5 million with a population density of almost 4 (result not listed). However, run 9 has an evaporation rate of 0 and when the evaporation rate is increased to 0.01, the maximum population is 105,000 (result not listed). With an evaporation rate of 0.01 and the astronaut microbial source increased to 6 million, the population density is almost 1 as shown in run 11 and Figure 3.20.

3.9.   Discussion

This section will discuss general themes, parameter value modifications, the population density threshold, model validity, and future research.

3.9.1.   Themes

There are several overarching themes of the results, but the primary theme is that while some results show substantial populations, they have parameter values that are unlikely, and so this model suggests that under the most likely set of circumstances and environmental conditions, it is difficult to generate a significant viable terrestrial microbial population on Mars from leaked microbes, water, and nutrients from a human mission. This is a leaning some have suggested previously based on limited empirical data, reasonable intuition, but without comprehensive modeling. In part, because of the latter, it is worthwhile to build a theoretical modeling framework to pursue this question comprehensively and quantitatively to allow scenario exploration and to increase confidence and understanding of possible scenarios.

The low population numbers are due to a number of factors such as a the low initial survival fraction once microbes are exposed to the Martian surface, the subsequent death rate, and the limiting growth factors of liquid water and organic nutrients. Liquid water will likely only exist in transient phases for a small part of the diurnal cycle, allowing for growth during a very limited period, and much liquid water is likely to evaporate regardless. Water leaked into the environment would have to be at unusually high levels and this is unlikely for a well designed habitat and careful mission procedures.

However, the results do suggest sufficiently plausible, albeit unlikely,

possibilities for problematic populations to arise, especially if microbes are leaked

into an area or situation that provides protection from the ultraviolet radiation. But

these scenarios can be mitigated by mission design and procedural measures such as

controlling the microbial shedding of astronauts and other habitat biomass and/or

tightly controlling the number of microbes that are leaked from the habitat.

Controlling water leakage is also an obvious requirement that will reduce

contamination possibilities, however indigenous water sources may make water

leakage control less effective in preventing microbial population growth and so a

confident water assessment of potential habitat locations is critical.

Mission duration can be important, but not as much as intuition might suggest.

The 150-day mission runs do not produce substantially larger populations, and this is

due to an initially low growth rate which only decreases as water evaporates.

However, if longer-term stable sources of indigenous liquid water exist in the

environment, mission duration time could be a much more important consideration.

Lastly, while this model focused primarily on a viable microbe population,

dead microbes were also tracked. Accumulation of dead microbes may very well be

the most plausible microbial contamination concern because if leakage is not

sufficiently controlled, it is almost certain that a large number of microbes and their

associated organic materials will accumulate in the environment to what could be

unacceptable levels.

3.9.2. Parameter value modifications

In many cases, the parameter value modifications that were made in search of significant populations are themselves at the margins of plausibility. For example, the most noteworthy runs assumed 600,000 microbes/min shed by astronauts in the habitat. This is an order of magnitude higher than the baseline, and while it is not unreasonable that this parameter value could be realistic, it is nevertheless something that will likely be sufficiently controlled– if not in the habitat (which might be difficult technically and procedurally), certainly at the level of habitat containment and filtration. This is in part why the habitat leakage fraction was not modified beyond 0.01– that itself is probably a high estimate, and again, can in principle be controlled to achieve a lower number leak fraction.

Not all parameter values were modified– not only because it is impossible to simulate all potentially relevant parameter value configurations, but also because it is not necessary. As noted in the model validity section, sensitivity analysis and detailed knowledge of the model facilitate using the most influential parameters to explore simulation results. The progression of runs shown in the tables helped with this process of narrowing down important parameters and their values to the most relevant values and combinations. Many of the water sector parameters did not need modification because saturation, or a water content close to saturation, was achieved by increasing the indigenous water parameter value and reducing the evaporation rate, often to zero.

3.9.3. Population density "threshold"

For the purposes of analysis, a microbial population density of 1 microbe/cm^3 was suggested as a threshold. This is somewhat arbitrary but is based on the prospect of cubic centimeter size soil sampling (comparable to a gram) and the possibility that very sensitive detection devices, including use by human analysts, might detect even one microbe in that amount of soil, and hence unduly complicate and potentially compromise the search for life. This number is intentionally conservative and hence probably quite low by most microbiological standards for detecting microbes in soil. Clearly, there will be ways to distinguish between terrestrial and indigenous microbes, but time on Mars will be precious and this distinction will be critical to make with high confidence, and since there may be circumstances under which this distinction might not be as easy as one might expect, this conservative approach is probably justified.

The population density threshold is a complex problem that this research did not directly address. The determination of population density threshold values will be based on many complex factors ranging from life detection experiment sensitivity to balancing mission, science, and policy considerations, including broader societal concerns about contaminating other worlds.

3.9.4. Model validity

Model validation is a subtle and fairly broad area with varying definitions and views. Andrew Ford (1999) stresses that a more fruitful, but closely related alternative to model "validation" is model *usefulness*, which he notes is a much more pragmatic approach that can be addressed more concretely. In addition, he notes:

"the key to a model's usefulness is leaving out the unimportant factors and capturing interactions among the important factors" (p. 284). Two aspects of model usefulness he stresses that are related to this model are "face validity" and "extreme behavior", both of which were done with this model.

Face validity is essentially a common sense test that requires, among other things, an assessment that the basic model structure and parameters make sense based on knowledge of the system. This was done step by step, in an iterative fashion as the model was built and run. Ford also suggests that models can become so complex that they prohibit this face validity test. He goes so far as to suggest such "black box" models might be discarded when a face validity test cannot be done with confidence. This model is relatively simple and allows for common sense assessment.

Extreme behavior tests were done with the model at many steps along the way as new elements were added, rearranged, etc., primarily by reducing water and nutrients to 0 to be sure no growth occurred, and that in the abundance of water and nutrients and large numbers of leaked microbes, that large populations resulted. The ultimate test of model usefulness, as Ford and others have suggested, comes over time, often as an arduous process of confidence building based on model use evolution. Model usefulness is not necessarily something that can be discerned immediately after the model is first built and used. This model is certainly the beginning of a longer-term process and so is consistent with that characterization.

Haefner (1996) prefers the word *plausible* as an adjective with which to assess model validity (or model *quality*, as he sometimes refers to it) which allows for degrees of quality. Haefner suggests that "plausibility" is a better adjective to

describe many biological models, in part because biological models can be extremely

complex and difficult to validate with a high level of confidence and predictive

power. He too notes that assessing model quality is a continuous, imperfect, and

unending process, and also, like Ford, stresses usefulness when he writes:

> "Therefore, the system scientists who use the word *validation* use it to mean
> model quality with respect to the objectives of the modeling project."

Haefner provides what he suggests is a complete list of criteria to help assess

model quality: usefulness for system control or management, understanding or insight

provided, accuracy of predictions, simplicity or elegance, generality, robustness

(insensitivity to assumptions), and low cost of model construction and simulation.

If we take the emphasis on model usefulness, and "face validity" and

"extreme behavior" as two aspects of usefulness, along with the full complement of

Haefner's criteria (in which usefulness is number one and supported by the above

quote), we have a comprehensive set of criteria for making a preliminary assessment

of what is arguably a non-traditional theoretical model. As noted in the introduction

the primary model objective was to create a theoretical modeling framework that can

be used to increase understanding of the potential for terrestrial microbial

contamination on Mars. A secondary objective was to act as a preliminary planning

tool to help explore potential scenarios. A third objective was to help point the way

to further research.

With respect to these objectives, the model appears to be useful, although this

will only be known better with time as more is learned about microbial growth on

Mars and as the model evolves to describe more empirical data and make helpful

predictions. Objective 3 will become more obvious in the below section on future research. The model provides increased understanding and insight because it suggests how difficult it will be for a problematic population to arise and helps show explicitly and quantitatively what the driving factors for a potentially problematic population could be and what potential specific conditions might create such a population. The model also provides details about conditions which could produce problematic populations.

It is not the intent of this model to make definitive and precise predictions about population numbers under various conditions. Nor is there data against which this could be compared, although empirical research is increasing and building to a point where this model could be linked better with empirical results within a few years (some empirical results are indeed used in this model as noted previously). Therefore, the model quality as it relates to accuracy of predictions is clearly low. However, again, with respect to the stated objectives, this does not necessarily reduce the model's near-term usefulness.

The model is certainly simple by some standards and as result was low cost to develop and run. Elegance is a much tougher assessment that is best made by others. The intent was to keep things as simple as possible to reduce run time demands and not unduly burden the scenario exploration with too many possibilities– but while maintaining key elements such as water and multi-nutrient availability, as well as more broadly operating parameters such as the survival fraction and death rate.

The model is also fairly general, in part because of its simplicity. It can be applied to almost any set of environmental conditions and was designed to allow

users to have great flexibility with almost all of the parameters. The model is fairly robust in that it is not highly sensitive to parameter variances and assumptions, although a few parameter value combinations clearly create large populations, the reference mode is not dramatically affected.

There are many shortcomings of the model which can be addressed with further research (some are noted here and others are noted in the section on Future Research), but two are worth noting here. One, the model has a loose set of parameter values in that many of them are uncertain for the Martian soil environment. This is not uncommon, and fortunately, the model is not overly sensitive to most of them (with the possibly exception of the survival fraction which varies by two orders of magnitude in the simulations). The survival fraction can be studied further to assess essentially how well leaked microbes might be protected from the UV radiation. This could translate directly into a more certain survival fraction value.

Two, the model assumes uniform distribution of water, nutrients and microbes. Clearly this is an over-simplification that can be overcome with heterogeneous spatial modeling. However, even though this has an averaging effect on the total population and population density, it is still useful relative to spatial modeling (that might incorporate patchiness for example) because much sampling that will be done on Mars will probably be done both systematically and randomly over an area, and so local concentrations of microbial growth (in the absence of significant microbial transport over a large area) would not necessarily have a large impact on search and discovery. Indeed, a better way to manage the problem may be to use averaging affects, such as the population density that this work reports because

this encourages a conservative approach. However, transport mechanisms (e.g. local and global atmospheric circulation patterns and/or water transport) combined with favorable highly localized conditions (e.g. liquid water, nutrients, and landscape variation that allows for variable protection from UV radiation) could create an unpredictable broader microbial ecology in which microbial species interactions could be very important, including potential impacts on indigenous microbial life-forms.

Given the uncertainties, simplifications, and the lack of much data that would help make the model more reliable, some might suggest that this modeling exercise is premature. This probably partly true, but as a start, it has use. This model is still useful as a way of providing a preliminary theoretical framework that can help increase understanding about the system, explore scenarios, and help point the way to further research which can then feed back into the model to improve it.

3.9.5. Future Research

Many potential areas for future research can improve this model and related modeling work. Parameter values such as half-saturation constants, maximum growth rates, and water potentials in Martian soils, would directly improve the reliability of results from this model. The potential for leaked microbes to be protected from the UV radiation could turn out to be a critical research area since the model suggest that initial survival fraction of microbes can make a substantial difference. The extent to which a large number of accumulated dead microorganisms

in the environment could impact discovery needs to be looked at closely. Related to this is the more general question of what population density is acceptable.

Spatial modeling, including patchiness and broader transport and dispersal patterns in soil and air should be modeled to not only understand the potential physical extent of contamination, but its heterogeneous distribution as well– e.g. isolated concentrations of water and microbes, with effective transport mechanisms, could cause problems for discovery. The first step in spatial modeling will be to assume a deposition gradient of both water and nutrients as a function of distance from the habitat. These gradients will likely be highest close to the habitat and decrease substantially as the distance from the habitat increases. In addition to what will likely be a relatively smooth radial gradient, there will also be patchy heterogeneity as a result of natural and non-natural transport. For example, winds on Mars could transport microbial contamination quite far and this should be modeled using the latest Martian atmospheric circulation models. Non-natural transport might occur from outgassing from the habitat or other sources such as in-situ resource utilization devices. Non-natural transport that may be of interest might also occur from astronaut field activities, especially those that are further from the habitat, and those that could result in highly concentrated and localized contamination (e.g. breaches in space suits or other biological material containment devices that could be associated with vehicles and other support devices). Such possibilities will manifest if procedures call for using only certain areas of the Martian landscape for certain activities. For example, there may be what will essentially be designated routes for vehicle and astronaut transport. Modeling contamination along such routes could be

very useful for understanding both localized patchy contamination, and broader regional contamination effects as well. Many of the above suggestions could be partly informed by studying contamination on the Moon that resulted from the Apollo program (Glavin et al. 2004).

In addition, modeling of subsurface contamination could be important because as noted previously, while the subsurface of Mars is not yet well understand, it may be a hospitable environment for microbial survival and growth (e.g. geothermal energy sources, liquid water aquifers, etc. as noted previously). Also, drilling will likely be an important activity of the first mission for several reasons ranging from infrastructural to searching for life. Such drilling activities could provide a microbial transport mechanism from many sources on the surface (e.g. contamination already in the surface soil and human associated microbes for human assisted drilling) to the subsurface of Mars.

Multiple species and multiple nutrients should be modeled to broaden the possibility space and inform effective contamination management procedures and designs. For example, different sources (e.g. human waste) will provide different microorganisms that will behave differently in the Martian environment. One of the next steps in this model's evolution will be to discern key microbial species from key sources and create a multi-species model based on those key organisms. This will require not only modeling each species' population dynamics, but also potential interactions between species (e.g. intraguild predation).

If growth is possible, mutations may play an important long-term role in terrestrial microbial population growth on Mars. The high UV environment could

cause a mutation rate that is higher than normal but sufficiently below an extinction threshold. This dynamic could potentially accelerate microbial evolution to create terrestrial organisms that can survive and grow better in the Martian environment.

Finally, to the extent that we can apply terrestrial ecological modeling techniques to potential extraterrestrial life situations, potential impacts on possible indigenous Martian life should be modeled to assess the range of possibilities we should prepare for.

3.10. Conclusions

As with all modeling, especially more theoretical modeling, caution must be exercised in making definitive conclusions. While the model quality is arguably acceptable in terms of usefulness relative to the objectives, and other measures of quality noted previously, conclusions based on the model should be highly qualified.

In general, it appears that terrestrial microbial contamination resulting from leakage from the first human mission (perhaps lasting up to 5 months) is not a significant risk as long a reasonable degree of contamination control is implemented (e.g. a habitat leak rate lower than 1 % per hour). However, plausible scenarios could cause a problematic population to arise depending on how "problematic" is defined (e.g. acceptable population density thresholds), and depending on UV radiation protection, the initial survival fraction, and water and nutrient availability.

Perhaps somewhat surprisingly, more than doubling the mission time does not have a significant impact on the population for most scenarios. This is due primarily to an overall low growth rate driven in large part by limited water and organic nutrient availability. Depending on discovery constraints, accumulation of dead

microbes could pose a significant problem since dead microbes will accumulate in the environment at a far greater rate than viable microbes, and since the baseline parameter set produced large enough populations to raise concern. Future research should be aimed at understanding this risk as well as a better theoretical and empirical understanding of some of the key parameters in this model such as the survival fraction, half-saturation constants, maximum growth rates, and water potential functions for Martian soil.

APPENDIX A: Model Structure Diagram

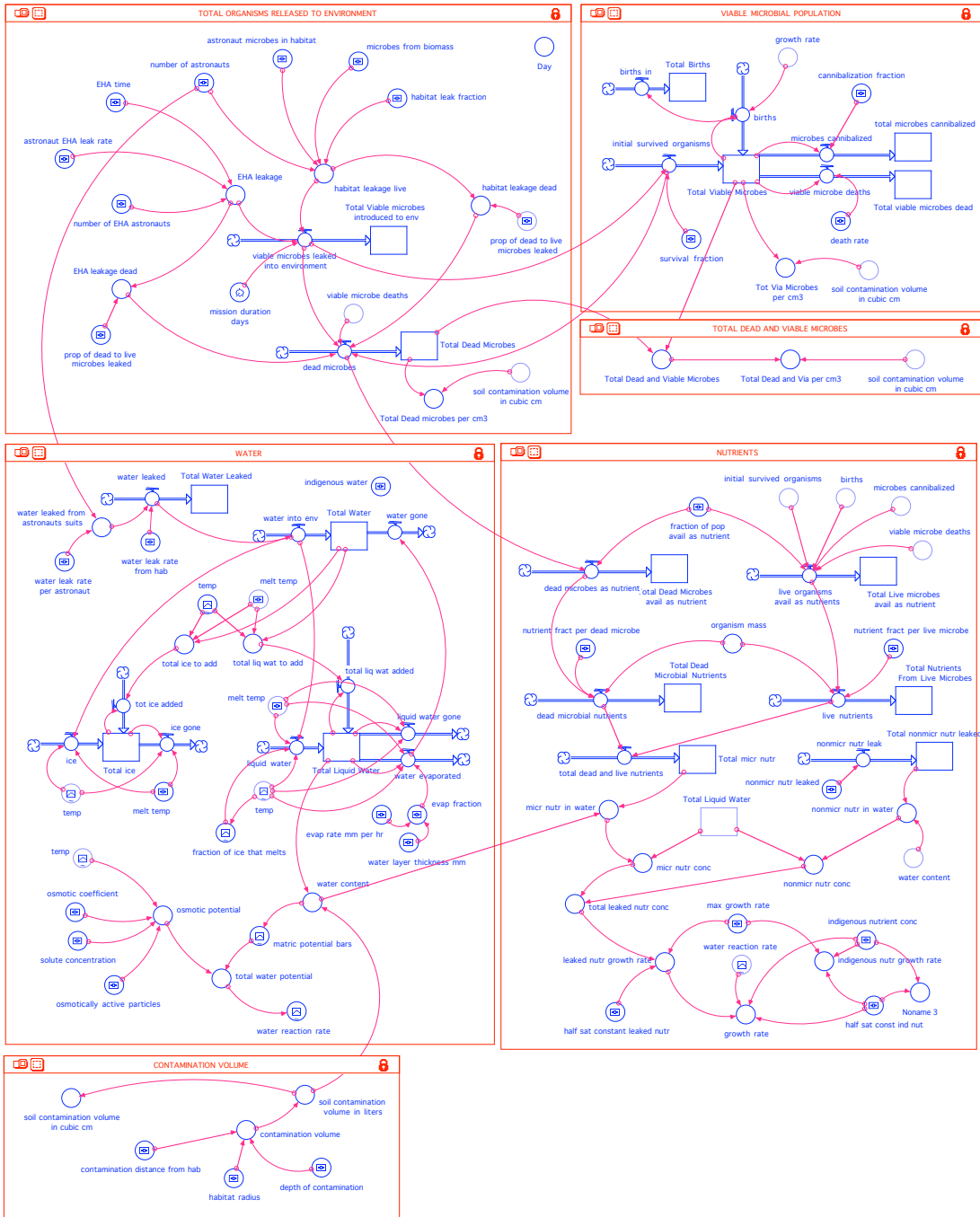Figure A-1: Model Structure Diagram

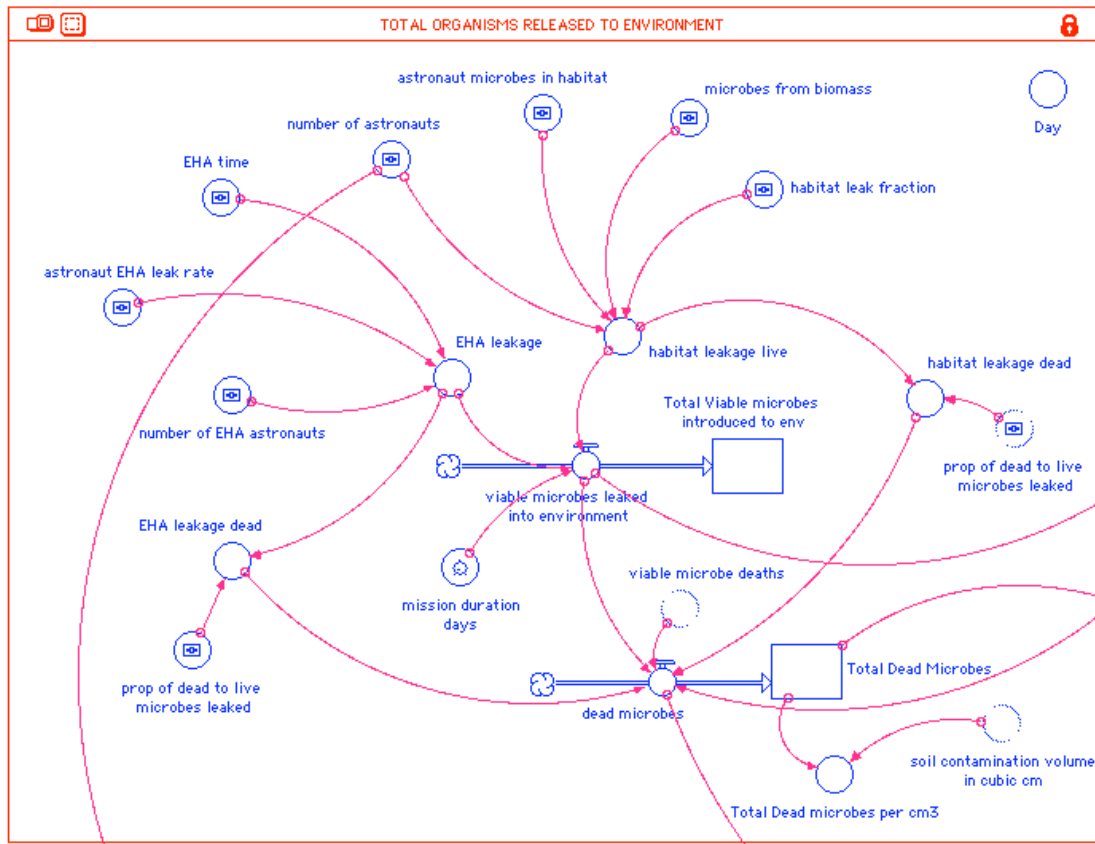Figure A-2: Total Organisms Released to the Environment Sector Diagram

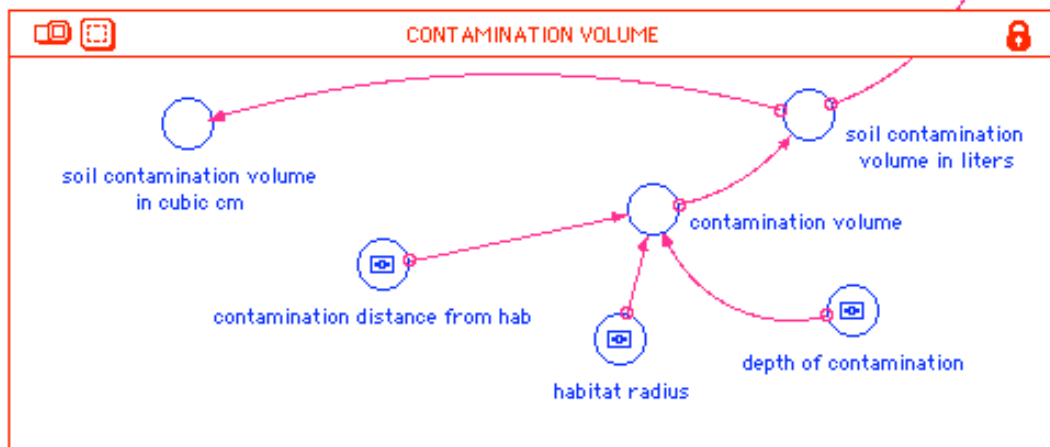Figure A-3: Contamination Volume Sector Diagram
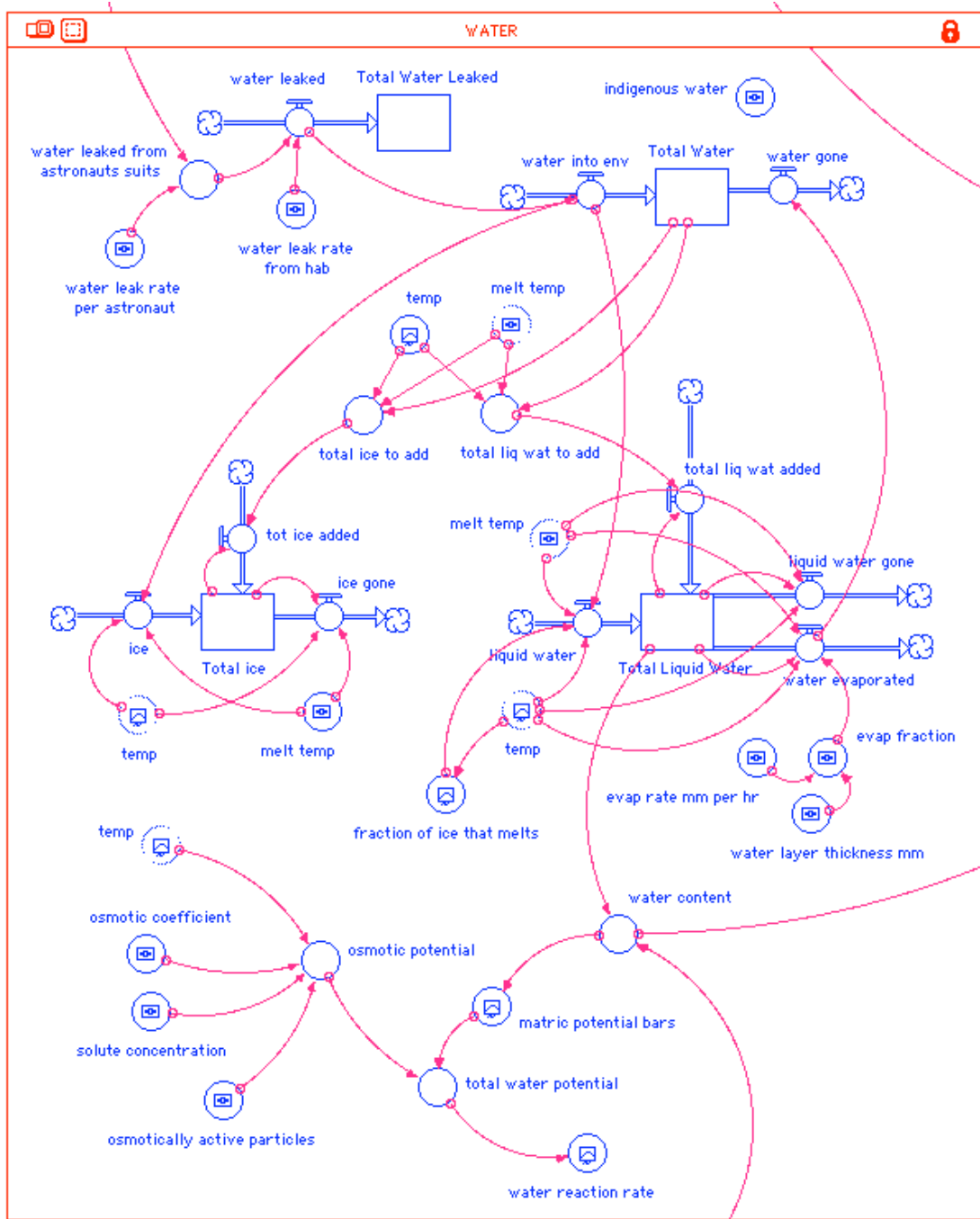
Figure A-4: Water Sector Diagram
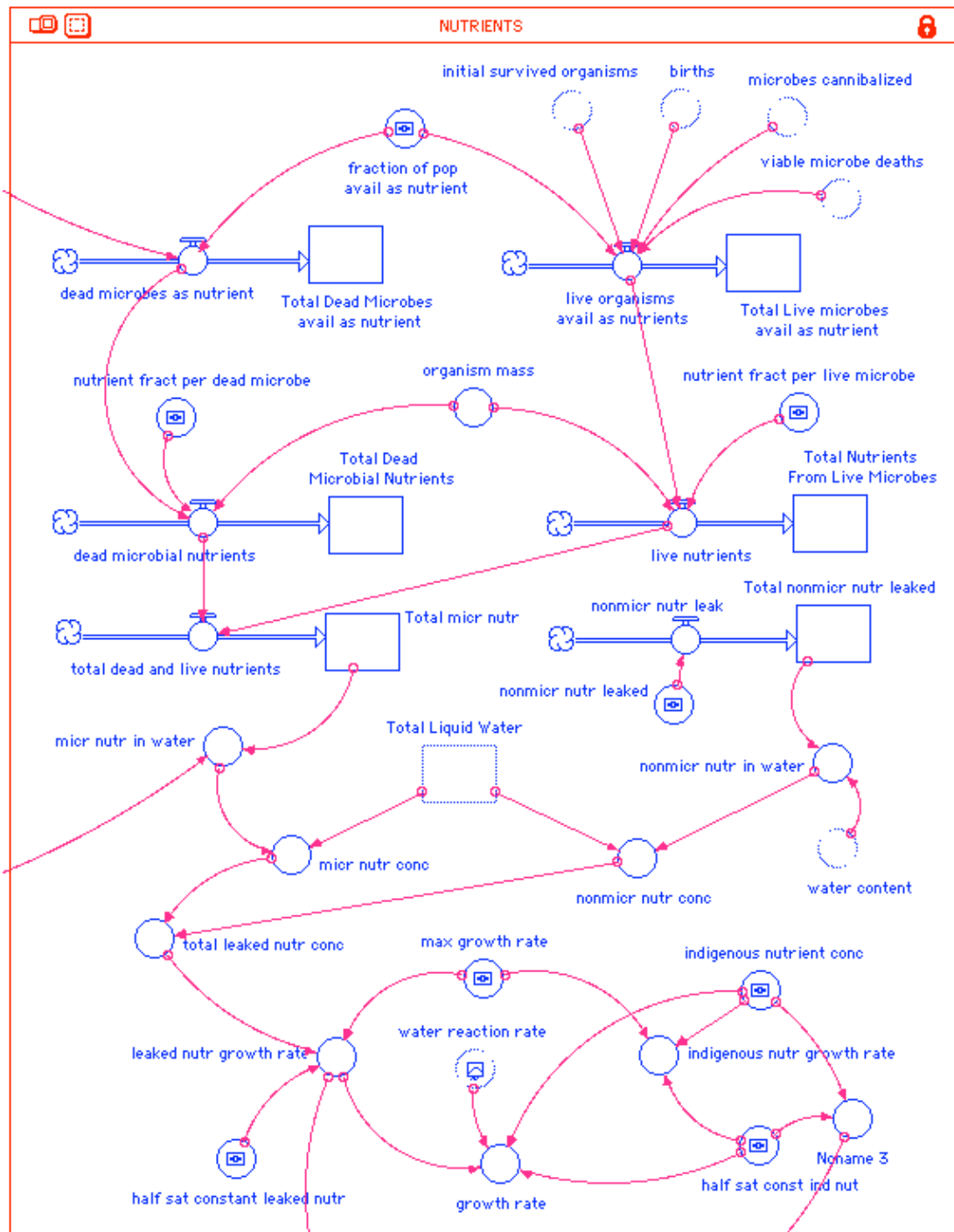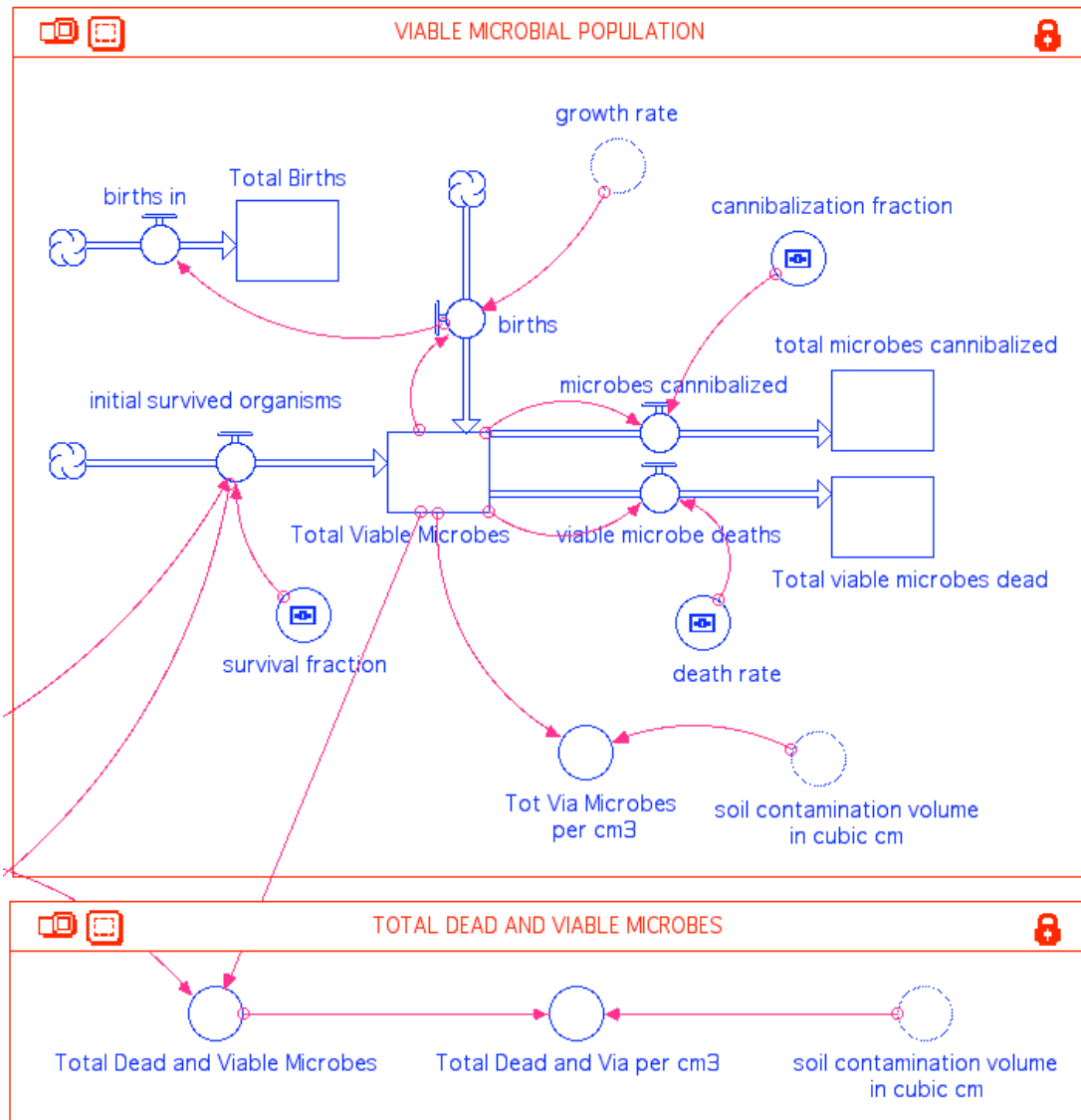
Figure A-5: Nutrient Sector Diagram

Figure A-6: Viable Microbial Population and Total Organisms Sector Diagram

APPENDIX B: Model Equations

TOTAL ORGANISMS RELEASED TO ENVIRONMENT

☐ Total_Dead_Microbes(t) = Total_Dead_Microbes(t − dt) + (dead_microbes) * dt
INIT Total_Dead_Microbes = 0
INFLOWS:

⚙ dead_microbes = viable_microbes_leaked_into_environment−initial_survived_organisms+
viable_microbe_deaths+habitat_leakage_dead+EHA_leakage_dead

☐ Total_Viable_microbes_introduced_to_env(t) = Total_Viable_microbes_introduced_to_env(t − dt) +
(viable_microbes_leaked_into_environment) * dt
INIT Total_Viable_microbes_introduced_to_env = 0
INFLOWS:

⚙ viable_microbes_leaked_into_environment = IF(TIME<mission_duration_days*24+1)THEN(EHA_leakage+
habitat_leakage_live)ELSE(0)

○ astronaut_EHA_leak_rate = 600

○ astronaut_microbes_in_habitat = 60000

○ Day = TIME/24+1

○ EHA_leakage = PULSE(astronaut_EHA_leak_rate*number_of_EHA_astronauts*EHA_time,12,24)

○ EHA_leakage_dead = EHA_leakage*prop_of_dead_to_live_microbes_leaked

○ EHA_time = 4

○ habitat_leakage_dead = habitat_leakage_live*prop_of_dead_to_live_microbes_leaked

○ habitat_leakage_live = ((astronaut_microbes_in_habitat*number_of_astronauts)+microbes_from_biomass)*
habitat_leak_fraction

○ habitat_leak_fraction = .01

○ microbes_from_biomass = 60000

○ mission_duration_days = 30

○ number_of_astronauts = 6

○ number_of_EHA_astronauts = 2

○ prop_of_dead_to_live_microbes_leaked = 1

○ Total_Dead_microbes_per_cm3 = Total_Dead_Microbes/soil_contamination_volume_in_cubic_cm

VIABLE MICROBIAL POPULATION

☐ Total_Births(t) = Total_Births(t − dt) + (births_in) * dt
INIT Total_Births = 0
INFLOWS:

⚙ births_in = births

☐ total_microbes_cannibalized(t) = total_microbes_cannibalized(t − dt) + (microbes_cannibalized) * dt
INIT total_microbes_cannibalized = 0
INFLOWS:

⚙ microbes_cannibalized = Total_Viable_Microbes*cannibalization_fraction

☐ Total_Viable_Microbes(t) = Total_Viable_Microbes(t − dt) + (initial_survived_organisms + births −
viable_microbe_deaths − microbes_cannibalized) * dt
INIT Total_Viable_Microbes = 0
INFLOWS:

⚙ initial_survived_organisms = survival_fraction*viable_microbes_leaked_into_environment

⚙ births = Total_Viable_Microbes*growth_rate

OUTFLOWS:

⚙ viable_microbe_deaths = Total_Viable_Microbes*death_rate

⚙ microbes_cannibalized = Total_Viable_Microbes*cannibalization_fraction

☐ Total_viable_microbes_dead(t) = Total_viable_microbes_dead(t − dt) + (viable_microbe_deaths) * dt
INIT Total_viable_microbes_dead = 0
INFLOWS:

⚙ viable_microbe_deaths = Total_Viable_Microbes*death_rate

○ cannibalization_fraction = .01

○ death_rate = .1

○ survival_fraction = .0001

○ Tot_Via_Microbes__per_cm3 = Total_Viable_Microbes/soil_contamination_volume_in_cubic_cm

WATER

☐ Total_ice(t) = Total_ice(t − dt) + (ice + tot_ice_added − ice_gone) * dt
   INIT Total_ice = Total_Water
   INFLOWS:

   ⚙ ice = IF(temp<melt_temp)THEN(water_into_env)ELSE(0)

   ⚙ tot_ice_added = IF(Total_ice=0)THEN(total_ice_to_add)ELSE(0)

   OUTFLOWS:

   ⚙ ice_gone = IF(temp>melt_temp)THEN(Total_ice)ELSE(0)

☐ Total_Liquid_Water(t) = Total_Liquid_Water(t − dt) + (liquid_water + total_liq_wat_added − liquid_water_gone − water_evaporated) * dt
   INIT Total_Liquid_Water = IF(temp>melt_temp)THEN(Total_Water)ELSE(0)
   INFLOWS:

   ⚙ liquid_water = IF(temp>melt_temp)THEN(water_into_env*fraction_of_ice_that_melts)ELSE(0)

   ⚙ total_liq_wat_added = IF(Total_Liquid_Water=0)THEN(total_liq_wat_to_add)ELSE(0)

   OUTFLOWS:

   ⚙ liquid_water_gone = IF(temp<melt_temp)THEN(Total_Liquid_Water)ELSE(0)

   ⚙ water_evaporated = IF(temp>melt_temp)THEN(Total_Liquid_Water*evap_fraction)ELSE(0)

☐ Total_Water(t) = Total_Water(t − dt) + (water_into_env − water_gone) * dt
   INIT Total_Water = indigenous_water
   INFLOWS:

   ⚙ water_into_env = water_leaked

   OUTFLOWS:

   ⚙ water_gone = water_evaporated

☐ Total_Water_Leaked(t) = Total_Water_Leaked(t − dt) + (water_leaked) * dt
   INIT Total_Water_Leaked = 0
   INFLOWS:

   ⚙ water_leaked = water_leaked_from_astronauts_suits+water_leak_rate__from_hab

○ evap_fraction = evap_rate_mm_per_hr/water_layer_thickness_mm

○ evap_rate_mm_per_hr = .5

○ indigenous_water = 10

○ melt_temp = 270

○ osmotically_active_particles = 2

○ osmotic_coefficient = .6

○ osmotic_potential = osmotic_coefficient*osmotically_active_particles*solute_concentration*.0831*temp

○ solute_concentration = .003

○ total_ice_to_add = IF(temp<melt_temp)THEN(Total_Water)ELSE(0)

○ total_liq_wat_to_add = IF(temp>melt_temp)THEN(Total_Water)ELSE(0)

○ total_water_potential = matric_potential_bars+osmotic_potential

○ water_content = IF(Total_Liquid_Water/soil_contamination_volume_in_liters> 1)THEN(1)ELSE(Total_Liquid_Water/soil_contamination_volume_in_liters)

○ water_layer_thickness_mm = 1

○ water_leaked_from_astronauts_suits = number_of_astronauts*water_leak_rate__per_astronaut

○ water_leak_rate__from_hab = .001

○ water_leak_rate__per_astronaut = .0001

TOTAL DEAD AND VIABLE MICROBES

○ Total_Dead_and_Viable_Microbes = Total_Dead_Microbes+Total_Viable_Microbes

○ Total_Dead_and_Via_per_cm3 = Total_Dead_and_Viable_Microbes/soil_contamination_volume_in_cubic_cm

CONTAMINATION VOLUME

○ contamination_distance_from_hab = 1

○ contamination_volume = ((PI*(contamination_distance_from_hab+habitat_radius)^2)-(PI*habitat_radius^2))* depth_of_contamination

○ depth_of_contamination = .01

○ habitat_radius = 10

○ soil_contamination_volume_in_cubic_cm = soil_contamination_volume_in_liters*1000

○ soil_contamination_volume_in_liters = contamination_volume*1000

NUTRIENTS

☐ Total_Dead_Microbes_avail_as_nutrient(t) = Total_Dead_Microbes_avail_as_nutrient(t - dt) + (dead_microbes_as_nutrient) * dt
INIT Total_Dead_Microbes_avail_as_nutrient = 0
INFLOWS:

⌀ dead_microbes_as_nutrient = dead_microbes*fraction_of_pop_avail_as_nutrient

☐ Total_Dead__Microbial_Nutrients(t) = Total_Dead__Microbial_Nutrients(t - dt) + (dead_microbial_nutrients) * dt
INIT Total_Dead__Microbial_Nutrients = 0
INFLOWS:

⌀ dead_microbial_nutrients = dead_microbes_as_nutrient*nutrient_fract_per_dead_microbe* organism_mass

☐ Total_Live_microbes_avail_as_nutrient(t) = Total_Live_microbes_avail_as_nutrient(t - dt) + (live_organisms_avail_as_nutrients) * dt
INIT Total_Live_microbes_avail_as_nutrient = 0
INFLOWS:

⌀ live_organisms_avail_as_nutrients = (births+initial_survived_organisms-viable_microbe_deaths-microbes_cannibalized)*fraction_of_pop_avail_as_nutrient

☐ Total_micr_nutr(t) = Total_micr_nutr(t - dt) + (total_dead_and_live_nutrients) * dt
INIT Total_micr_nutr = 0
INFLOWS:

⌀ total_dead_and_live_nutrients = dead_microbial_nutrients+live_nutrients

☐ Total_nonmicr_nutr_leaked(t) = Total_nonmicr_nutr_leaked(t - dt) + (nonmicr_nutr_leak) * dt
INIT Total_nonmicr_nutr_leaked = 0
INFLOWS:

⌀ nonmicr_nutr_leak = nonmicr_nutr_leaked

☐ Total_Nutrients_From_Live_Microbes(t) = Total_Nutrients_From_Live_Microbes(t - dt) + (live_nutrients) * dt
INIT Total_Nutrients_From_Live_Microbes = 0
INFLOWS:

⌀ live_nutrients = live_organisms_avail_as_nutrients*organism_mass*nutrient_fract_per_live_microbe

○ fraction_of_pop_avail_as_nutrient = .1

○ growth_rate = water_reaction_rate*leaked_nutr_growth_rate*(indigenous_nutrient_conc/ (indigenous_nutrient_conc+half_sat_const_ind_nut))

○ half_sat_constant_leaked_nutr = 10

○ half_sat_const_ind_nut = 1.5

○ indigenous_nutrient_conc = .05

○ indigenous_nutr_growth_rate = max_growth_rate*(indigenous_nutrient_conc/(half_sat_const_ind_nut+ indigenous_nutrient_conc))

○ leaked_nutr_growth_rate = max_growth_rate*(total_leaked_nutr_conc/(half_sat_constant_leaked_nutr+ total_leaked_nutr_conc))

○ max_growth_rate = .5

○ micr_nutr_conc = IF(Total_Liquid_Water>0)THEN((micr_nutr_in_water)/Total_Liquid_Water)ELSE(0)

○ micr_nutr_in_water = Total_micr_nutr*water_content

○ Noname_3 = indigenous_nutrient_conc/(indigenous_nutrient_conc+half_sat_const_ind_nut)

○ nonmicr_nutr_conc = IF Total_Liquid_Water > 0 THEN (nonmicr_nutr_in_water)/Total_Liquid_Water ELSE 0

○ nonmicr_nutr_in_water = water_content*Total_nonmicr_nutr_leaked

○ nonmicr_nutr_leaked = .01

○ nutrient_fract_per_dead_microbe = .1

○ nutrient_fract_per_live_microbe = .1

○ organism_mass = .000000001

○ total_leaked_nutr_conc = micr_nutr_conc+nonmicr_nutr_conc

APPENDIX C: User Interface

Figure C: User Interface
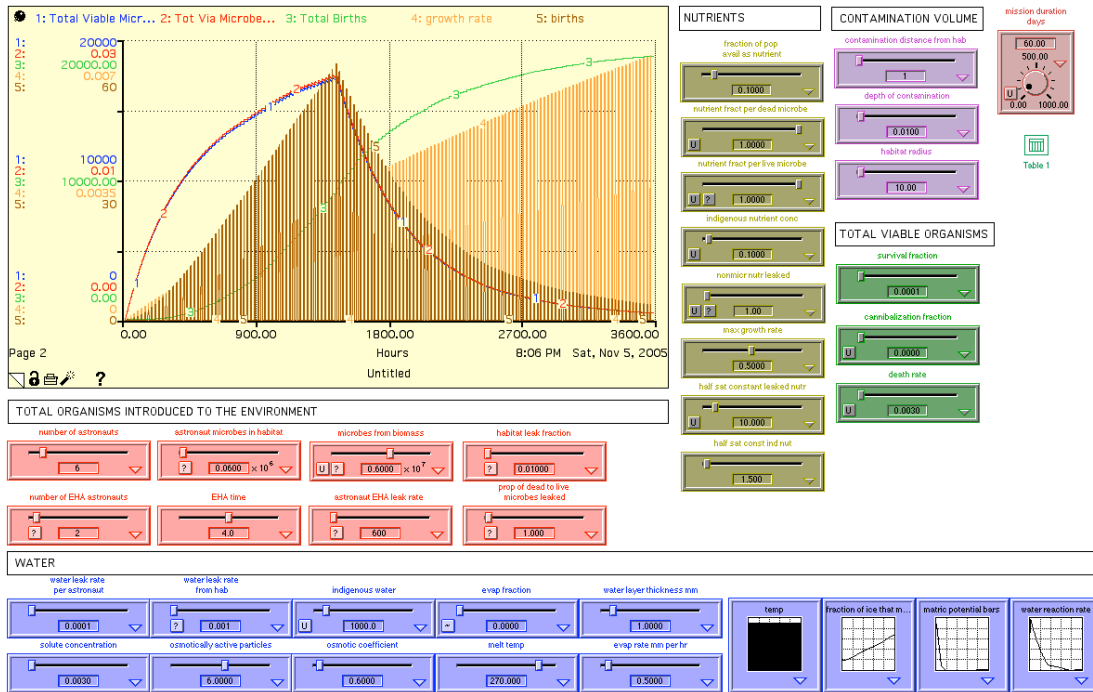
APPENDIX D: Sensitivity Analysis Results

This appendix highlights some of the details of the sensitivity analysis that was performed. Each of the 4 tables has its own baseline, and in total, they represent a progression of increasingly restricted baselines against which the sensitivity of the parameters were assessed. Each table lists the parameters (categorized by sector for the first two tables), the variance (the amount the parameter was varied), the change in the total viable population, and the sensitivity ratio (change/variance). The variances and population changes are shown as multiples– generally shown as order of magnitude changes (e.g. 10^2 indicates the parameter was increased or decreased by a factor of 100, 5 indicates the population change 5-fold). Results are for 60-day runs.

Table D.1: Sensitivity results relative to Table 2.3 baseline

Parameter	Variance	Change in Total # of Viable Microbes	Sensitivity Ratio
TOTAL VIABLE ORGANISMS			
Survival fraction	10^3	10^3	1
Cannibalization fraction	10^2	0	0
Death rate	10^1	5	.5
TOTAL ORGANISMS TO ENV.			
Astr source in habitat	10^2	10^2	1
Microbes from biomass	10^2	10^1	.1
Habitat leak fraction	10^1	10^1	1
WATER			
Water leak rate per astronaut	10^3	0	0
Water leak rate from habitat	10^2	0	0
Indigenous water	$10^2, 10^4$	0, 0	0, 0
Evaporation fraction	$10^2, 10^3$	0, 0	0, 0
Solute concentration	10^1	0	0
Osmotically active particles	3	0	0
NUTRIENTS			
Fract of pop avail as nutr	10^1	0	0
Nutr fract per dead and live microbe	$10^1, 10^1$	0, 0	0, 0
Ind nutr conc	10^1	0	0
Non-micr nutr leaked	10^1	0	0
Max growth rate	2	0	0
Half-sat const for leaked nutr	10^1	0	0

The variances are generally large and justified, in part because there are large uncertainties associated with many parameters. As noted in the main body of the dissertation, 1 in 100 microbes that are leaked out to the Martian environment may be sufficiently protected from UV exposure. This could be due in part to where and how the microbe ends up in the environment, as well as other factors such as dust storms

that may cover the microbe. Similarly, if microbes are protected, they may not die as readily, which is captured by decreasing the death rate an order of magnitude to 1 in 100 dying per hour. Cannibalization may very well not occur at all depending on details regarding the limitations of microbial interaction.

The sources of microbes and leakage from the habitat could vary dramatically depending on mission design and operations details, so variances of 1 order of magnitude for astronauts in the habitat, 2 orders of magnitude for support biomass sources, and 1 order of magnitude for habitat leak rate are probably justified (although a habitat leak rate of .1 is probably not likely). The water parameters could also vary dramatically depending on mission details, including for example the possibilities for structural breaches and large spills. Indigenous water could vary from 0 liters to 10,000 liters depending on the location. The evaporation fraction could vary based on the thickness of the water layer, exposure to the sun, and pressure. A low evaporation rate is not inconceivable in a higher pressure zone with water mixtures and soil adhesion and protection.

Indigenous nutrient could be available in amounts greater than what was determined for atmospheric oxygen depending on location, soil constituents, and the limiting nutrients required. Leaked nutrients (other than from microbes) could also vary depending on the mission details (e.g. outgassing, breaches, spills, etc.) and the half-saturation constant could vary depending on the nutrient.

Table D-2 shows sensitivity results relative to a baseline (referred to as "high baseline") that substantially increases the number of organisms surviving the environment (survival fraction = 0.01, cannabilzation fraction = 0, the death rate = 0.001, astronaut microbial source in habitat = 6×10^5 microbes/hour, microbial source from other biomass = 6×10^6 microbes/hour) and the amount of water in the environment (evaporation fraction = 0.005, water leaked from the habitat .1 liters/hr, indigenous water = 1000 liters).

Table D-2: Sensitivity results relative to "high baseline"

Parameter	Variance	Change in Total # of Viable Microbes	Sensitivity Ratio
TOTAL ORGANISMS TO ENV.			
Hab leak fract	10^1	10^1	1
WATER			
Evap fraction	10^1	0	0
NUTRIENTS			
Fract of pop avail as nutr	10^1	0	0
Nutr fract per dead and live microbe	$10^1, 10^1$	0	0
Ind nutr conc	10^1	0	0
Non-micr: nutr leaked	10^1	0	0
Max growth rate	2	0	0
Half-sat const for leaked nutr	10^1	0	0

Table D-3 and Table D-4 show sensitivity results relative to new baselines (referred to as "nutrient baseline A" and "nutrient baseline B") that are based on the high baseline noted above . Nutrient baseline A adds the indigenous nutrient concentration of .5 to the high baseline, and nutrient baseline B adds the non-microbial leaked nutrient of .1 to nutrient baseline A.

Table D-3: Sensitivity results relative to "nutrient baseline A"

Parameter	Variance	Change in Total # of Viable Microbes	Sensitivity Ratio
Non-micr: nutr leaked	10^1	2.5×10^{-1}	2.5×10^{-2}
Max growth rate	2	0	0
Half-sat const for leaked nutr	10^1	10^1	1
Nutr fract per dead and live microbe	10^1, 10^1	0	0
Fract of pop avail as nutr	10^1	0	0

Table D-4: Sensitivity results relative to "nutrient baseline B"

Parameter	Variance	Change in Total # of Viable Microbes	Sensitivity Ratio
Fract of pop avail as nutr	10^1	0	0
Nutr fract per dead and live microbe	10^1, 10^1	0	0
Max growth rate	2	2	1
Half-sat const for leaked nutr	10^1	10^1	1

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