DETOXIFYING MARS: THE BIOCATALYTIC ELIMINATION OF OMNIPRESENT PERCHLORATES.

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Introduction: Imagine what astronauts could do with unlimited clean water on Mars. Humans are 60% water. Beyond drinking it, water can be turned into oxygen for breathing and propellant for the return to Earth. It is fundamental to all biomanufacturing, farming and aquaculture, all of which are essential for a sustained human presence. Fortunately, Mars has plenty of water to sustain these aspirations in the form of subsurface ice. Unfortunately, it is contaminated by toxic perchlorates.

Perchlorates made up ~0.6% of the icy regolith sampled by the Phoenix mission at 68° N latitude [1] and have subsequently also been detected by the Curiosity Rover (4.6° S) and the Perseverance Rover (18.5° N). Perchlorate salts can form brines by deliquescence and, consequently, Mars ice deposits could contain perchlorate at or above the concentration seen at the Phoenix site. Ice-derived water on Mars will result in fluids that contain perchlorate and chlorate as well as other soluble components of the soil mixed with the ice [2] and could reach beyond 1% in Mars ice deposits. These perchlorates pose a serious challenge to plans for Mars *in situ* resource utilization.

Perchlorate and chlorate are potent oxidizers that cause equipment corrosion. Worse, they are hazardous to human health even at low concentrations. Death occurs at 15 g per astronaut from hemolysis, hemoglobin oxidation, and kidney failure [3-5]. Untreated Martian water contains double this in a single day's drinking water. Chronic exposure even to low levels result in thyroid inhibition and lung toxicity. California limits perchlorate to 6 ppb and chlorate to 800 ppb (CA EPA 2015). As the raw Martian water is expected to contain ~10,000,000 ppb of each, these must be reduced by a factor of ~100,000. This need is aligned with NASA Roadmap technical area TX07.1.2, "Resource Acquisition, Isolation, and Preparation". Further, the NASA Moon-to-Mars Strategy highlights in Section MI-4[M] "the importance of long-lead technology developments demonstrating the use of *in-situ* resources for [...] future sustained presence" [6].

Efficient purification of water will be critical for all future Mars missions in many applications and at many different scales, from human life support systems (discussed below) to large-scale propellant production. For example, a single SpaceX Starship-class lander requires 1200 tons of propellant, of which 600 tons could be derived from *in situ* water containing up to 6 tons of perchlorates. State of the art resins can adsorb 233 mg perchlorates /g resin [7], thus requiring 25.8 tons of resin - a huge fraction of total payload capacity (100 tons). Regenerative resins require input of salts for ion exchange, which will not be readily available. Another approach, vapor distillation, has high power requirements of 20 kWh/ton water [8]. Over a 500-sol conjunction class mission, this requires 1000 W average power (the output of 40 m^2 of solar panels under ideal conditions). Reverse osmosis using semipermeable membranes has lower power and consumable demands but is highly prone to membrane fouling from some salts and other contaminants, so "pretreatment is widely used in practice" [9] to avoid these problems. Additionally, reverse osmosis removes only 90-95% of solutes, so a complex multi-tiered system would be required to achieve the required 100,000-fold reduction in perchlorates. All the above systems also generate perchlorate wastes, which must be transported for dumping away from the work site, and so also waste precious, hard-won water.

Using life to transform perchlorates into oxygen: What if we could make the perchlorates vanish? This is the innovative solution we present here, taking advantage of the fact that while chlorate and perchlorate are kinetically stable, their reduction to chloride and oxygen is thermodynamically favorable and can be readily catalyzed. Imagine a system that generates perchlorate reduction catalysts from waste materials, self-regulates, self-repairs, and can grow from <1 g of initial material. This is achieved with no toxic chemicals and is already demonstrated to be safe and effective. *This is the promise of our regenerative perchlorate reduction system leveraging synthetic biology to take advantage of - and improve upon - natural perchlorate-reducing bacteria.*

This work exploits prior studies by engineering this known pathway for biocatalyzing reduction of perchlorates (Figure 1) into the spaceflight-proven *Bacillus subtilis* strain 168, used on the LDEF [10] and PowerCell [11] missions, which also forms spores stable for years at room temperature until needed, of obvious utility for Mars missions. This approach also allows for stricter control over expression and activity of these enzymes, which are expressed only weakly or not at all under a variety of relevant environmental

conditions in their original host microbes. Compared to traditional solutions for water detoxification, this has the potential to reduce consumables and power demand and chemically removes the hazards rather than creating concentrated solutions.

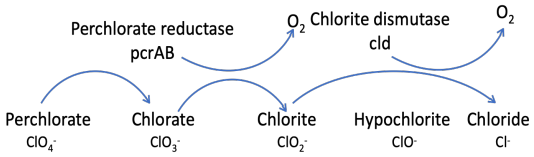

Figure 1: Overview of perchlorate reduction pathway and key genes.

We have engineered several variants of this pathway into *Bacillus subtilis*. The simplest approach, introducing perchlorate reductase and chlorite dismutase with no additional supporting genes, yields reduction of perchlorate, chlorate, and chlorite by >200 ppm per day. We will present additional results from three refinements to this strategy: introduction of additional chaperone and electron transport proteins from the perchlorate reduction locus, upregulation of the closely related and functionally overlapping nitrate reductase pathway, and an engineered increase in production of the key bis-MGD cofactor that coordinates the biochemical reduction of (per)chlorate in the active site of the enzymes.

Integration into mission design: Water is critical to future human Mars missions, including (but not limited to) potable water for crew consumption and agriculture, one component used for ascent propellant production, fuel cell reactants, and radiation protection [12]. Acquiring water from Martian sources saves significant transportation costs versus if this water were to be brought from Earth. Each potential use of this water will have its own unique purification requirements.

"Water rich" mission scenarios using Mars ISRU can be accomplished efficiently if relatively pure ice, as is identified in several locations on Mars [13], can be accessed with a drilling system to construct a Rodwell water mine [14,15]. A similar system currently provides water to operate research stations in Antarctica. Figure 2 shows notionally how bioengineered perchlorate reduction would integrate with this critical technology. Extraction of water via a Rodwell followed by biological perchlorate decomposition offers the simplest and lowest mass and power solution to obtaining sufficient water on Mars to support crew activities and propellant production.

The systems and operations depicted in Figure 2 would need to be demonstrated at subscale before human missions would rely on this concept for mission success. NASA's current "Moon to Mars Strategy (6) specifically calls out an objective to "Demonstrate Mars ISRU capabilities to support an initial human Mars exploration campaign" – Mars Infrastructure objective number 4). One option for accomplishing this demonstration is to include the systems as part of an early human mission, allowing for crew participation in setup and operation and, if required, intervention should contingencies occur. A recent human Mars mission description envisions an early crewed Mars mission features a short, 30-sol duration on the surface of Mars (16). This short-surface-stay mission description is agnostic to specific science or utilization activities carried out by the crew or any pre-deployed robotic assets, providing guidelines for the resources - such as research payload mass, power, crew time, etc. - likely to be available for this mission. Details related to a set of demonstration systems, focusing on key subsystems related to the biocatalytic elimination of perchlorates, consistent with (16) will be described.

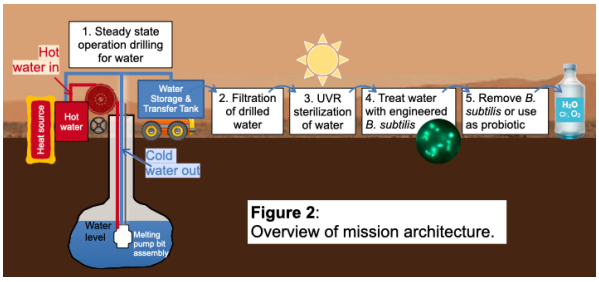

Figure 2. Water acquisition and processing concept

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