ENGINEERED BIOREMEDIATION OF WATER OBTAINED FROM IN SITU RESOURCE UTILIZATION. G. A. Roberts Kingman[1], Lynn J. Rothschild[2], and Carol R. Stoker[3], [1]NASA Postdoctoral Program, Astrobiology, Building N239 Room 373, Ames Research Center, Moffett Field, CA 94035, garrett.a.robertskingman@nasa.gov, [2]NASA Ames Research Center, Planetary Systems Branch, Building N239 Room 361, Moffett Field, CA 94035, lynn.j.rothschild@nasa.gov, [3]NASA Ames Research Center, Planetary Systems Branch, Building N239 Room 361, Moffett Field, CA 94035, carol.r.stoker@nasa.gov

Introduction: A key challenge in future human exploration of Mars or the moon is the efficient processing of *in situ* resources, particularly water ice, for use in life support systems, fuel production, and biomass production. Ice on Mars, while widespread, likely contains soluble molecules deleterious to *in situ* resource utilization tasks. In particular, water derived from Martian ice likely contains perchlorate and chlorate[1], which are potent oxidizers and constitute a hazard to human health even at low (ppb) concentrations through inhibition of iodide uptake by the thyroid, hemoglobin oxidation, red blood cell lysis, and renal insufficiency[2]. While at far lower than Mars concentrations, perchlorate and chlorate have been detected in lunar samples[3].

Martian resources and challenges: Ground ice is thought to be accessible within 1-2 meters of the surface within widespread areas of the Northern Hemisphere at latitudes poleward of 40° N. latitude[4]. Vapor deposited ice does not exceed the pore space of the soil; the ice to soil ratio is 50% or less. However, the ice concentration appears to vary. Optically pure ice deposits occur near ice-cemented soils as seen on the high latitude Phoenix mission[5] and in clusters of recent impact craters[6], where some craters show optically pure ice while others do not.

Perchlorate salts are also widespread on the Mars surface. Perchlorates made up ~0.6% of the icy soils sampled by the Phoenix mission at 68° N. latitude[7]. Discovery of perchlorates on Mars was a shock and seen as a serious challenge by those planning human Mars missions involving ISRU. Since perchlorate salts decrease the freezing point of water by as much as 70 degrees C, comparable to or lower than ice table temperatures, Mars ice likely contains some liquid water which allows it to mobilize and even concentrate perchlorate. Consequently, ice derived water on Mars will result in fluids that contain perchlorate as well as other soluble components of the soil mixed with the ice.

Despite being critical parameters for *in situ* utilization of this resource, the range of plausible concentrations of these solutes in Mars ice deposits (either vapor-deposited or optically pure ice) has not been previously studied. Therefore, modeling is needed to understand the compositional challenges to water detoxification systems.

Perchlorate reduction: Traditional water purification approaches require consumable reagents and produce concentrated toxic brines. Additionally, perchlorates are potent oxidizers and increase wear on water purification equipment and membranes. Alternatively, biological enzymes can efficiently reduce these toxic compounds to chloride and oxygen, alleviating these problems.

Perchlorate-reducing bacteria are a diverse collection of microbes capable of reducing perchlorate and chlorate into chloride and oxygen[8], 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 enzymatically catalyzed. Previous study of these microbes has identified the relevant genes for catalyzing perchlorate reduction and demonstrated that they can be readily transferred between even distantly related species[9]. Reduction of perchlorate by naturally occurring microbes is the most efficient method of removing perchlorate from the environment after industrial contamination[10]. However, these microbes are not directly suitable for off-world use, as they deactivate the perchlorate reduction pathway in the presence of oxygen or nitrate, do not express the relevant genes highly under any conditions, and may not be robust under spaceflight conditions.

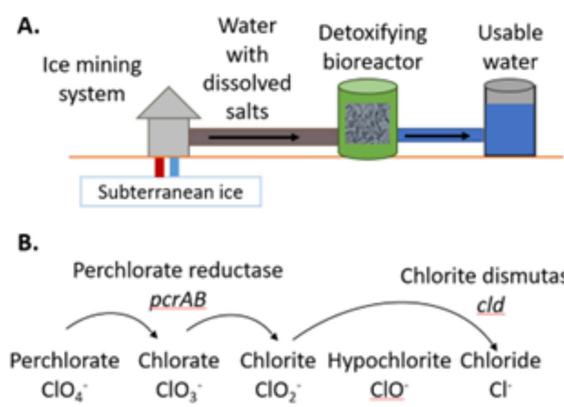

Figure 1: A. Overview of proposed bioremediation system for extracted water ice. B. Summary of genetic pathway reducing perchlorate, chlorate, and chlorite to chloride. Here we use the term "perchlorate reduction" to refer to all steps in this pathway.

Bioengineered perchlorate reduction: An attractive alternative for removing perchlorate and chlorate from water obtained on Mars or the moon is to exploit the prior work studying perchlorate-reducing bacteria by engineering this known pathway into the spaceflight-proven *Bacillus subtilis* strain 168 to solve the issues with using the natural microbes directly. *B. subtilis* has previously demonstrated robustness under spaceflight conditions[11,12] and also forms spores stable for years at room temperature until needed, of obvious utility for Mars or lunar missions.

We are transferring the key genes of this pathway, *perchlorate reductase* (*pcrAB*) and *chlorite dismutase* (*cld*) from *Azospira suillum PS* to *B. subtilis* through genomic integration, and placing them under the control of the strong and space-proven constitutive promoter pVeg[12]. This will induce expression of these genes at high levels, even in the presence of oxygen and other stimuli that deactivate the perchlorate reduction pathway in its original host organisms.

Testing is being performed by adding solutes to either engineered or wildtype *B. subtilis* cultures, followed by quantitation via ion chromatography to quantify remaining perchlorates. We are also testing enzyme secretion through a library of signal peptides, as well as the addition of 6xHis-tags. Secreted enzymes may prove more effective by not requiring perchlorates to enter the cells before being reduced. This approach also enables enzyme purification and lyophilization, opening the door to cell-free treatments, which may enable local water detoxification at smaller scales and without planetary protection concerns.

Compared to traditional solutions for water detoxification, this has the potential to reduce the required consumables such as reverse osmosis membranes, and chemically removes the hazards rather than creating concentrated solutions. As the removal of these potent oxidizers will reduce stress on and design requirements for a traditional filtration system, these two approaches are complementary.

Future steps: Beyond the work currently in progress, next steps for this work broadly fall into two categories. First, continued improvements in the perchlorate reduction pathway include further improvement to catalysis rates (if necessary) through engineering candidate mutations based on comparative sequence analysis across species, crystal structure of the active site[13], and a neural network-based gain-of-function prediction algorithm[14]. Second, maintaining the performance of these strains without the rich Luria Broth medium used to facilitate laboratory experiments, for example using waste materials as biological input, leading to the construction and testing of perchlorate reduction by these strains in a small collapsible bioreactor with minimized input of materials, developing and testing the entire system needed to deploy this technology beyond Earth.

References:

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