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Mining Moon & Mars with microbes: Biological approaches to extract iron from Lunar and Martian regolith

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Abstract

The logistical supply of terrestrial materials to space is costly and puts limitations on exploration mission scenarios. *In-situ* resource utilization (ISRU) can alleviate logistical requirements and thus enables sustainable exploration of space. In this paper, a novel approach to ISRU, utilizing microorganisms to extract iron from Lunar or Martian regolith, is presented. Process yields, and kinetics are used to verify the theoretical feasibility of applying four different microorganisms. Based on yields alone, three of the four organisms were not investigated further for use in biological ISRU. For the remaining organism, *Shewanella oneidensis*, the survivability impact of Martian regolith simulant JSC-MARS1 and Mars-abundant magnesium perchlorate were studied and found to be minimal. The payback time of the infrastructure installation needed for the process with *S. oneidensis* on Mars was analyzed and the sensitivity to various parameters was investigated. Water recycling efficiency and initial regolith concentration were found to be key to process performance. With a water recycling efficiency of 99.99% and initial regolith concentration of 300 g/L, leading to an iron concentration of approximately 44.7 g/L, a payback time of 3.3 years was found.

1. Introduction

The next step in human space exploration will revolve around the Moon, as a stepping stone for an eventual human presence on Mars (NASA, 2018). In the context of longer stays on the Moon's surface, the supply and maintenance of a functional habitat requires the input of resources. Transport of these resources is a major cost for an extraterrestrial base, and reducing this cost will bring us closer to realizing a sustained human presence on another celestial body (Carpenter et al., 2016). *In situ* resource utilization (ISRU), the use of local resources for production and maintenance, can help us bring down long-term transport requirements and brings us closer to colonizing another celestial body (Culbert et al., 2015). In this paper, we help to address this challenge by investigating the use of microorganisms for the extraction of metals from Lunar and Martian regolith.

Microorganisms as used in production processes can be described as self-reproducing modifiable nanofactories, catalyzing a wide range of chemical conversions. Some branches of microbial life on earth have developed a metabolism around the use of metal oxides as electron donors or acceptors (Weber et al., 2006). A subsection of these organisms can utilize solid metal oxides as substrate, converting them to more soluble forms,

32 which makes them interesting for use in mining operations (Valdés et al., 2008). Such use of microorganisms
33 on earth is widespread and is actively used in the biomining of copper, cobalt, gold, uranium and other metals
34 (Rawlings, 2002; Schippers et al., 2013). In the case of copper, biomining accounts for more than 20% of the
35 yearly worldwide production (Yin et al., 2018). These facts give a promising outlook on the application of
36 biomining in space exploration (Cousins and Cockell, 2016). Lab-scale experiments on the interaction between
37 bacteria and lunar regolith simulant confirm this expectation (Navarrete et al., 2013). However, so far, no design
38 for full-scale biomining operations in space has been presented.

39 Iron is one of the most utilized metals on Earth, most of our building materials rely on it in some way. It
40 can be hypothesized that the construction and maintenance of an extraterrestrial base will also rely on iron.
41 Considering the abundance of iron in both Lunar and Martian regolith, at 5-22 wt% and 17.9 ± 0.6 wt%,
42 respectively (Halliday et al., 2001; Lawrence et al., 2002), this element is likely to be useful in construction-
43 oriented ISRU.

44 With this work, we show a general setup for a biological iron extraction process, we investigate the
45 feasibility of several candidate organisms and perform a sensitivity analysis for the biological process.
46 Combined with a lander concept (Lehner et al., 2019), this provides a framework for future evaluations of
47 biomining processes in space exploration and a basis for evaluation of other bioprocesses.

48 **2. Materials & Methods**

49 *2.1. Kinetic models*

50 Growth kinetics for *Escherichia coli*, *Magnetospirillum gryphiswaldense*, *Shewanella oneidensis* MR-1
51 and *Acidithiobacillus ferrooxidans* were derived from literature (Table 1 & SI) and combined with mass
52 balances for the relevant chemical compounds. The resulting system of differential equations was solved with
53 the MATLAB ode15s or ode45 solver. Similar initial concentrations were chosen for all simulations (Table 2).
54 Growth on lactate was considered for all heterotroph organisms (*S. oneidensis*, *M. Gryphiswaldense*, *E. coli*).
55 Only *A. ferrooxidans* uses CO₂ as a carbon source and grows autotrophically. Production of acetate by both *S.*
56 *oneidensis* and *E. coli* is indicated in table 1, but inhibitory effects are not considered in their models.
57 *A. ferrooxidans* grows aerobically and shows oxygen-limited behavior at concentrations below 1 mg/L ($3.1 \cdot 10^{-2}$
58 mM) (Liu et al., 1988). The current kinetics hold if the concentration remains above this level. The inhibition
59 by Fe³⁺ is considered in the model.

60 *Table 1.* Kinetic characteristics and expected byproducts for each proposed organism. Both *E. coli* and *M. gryphiswaldense*
 61 are intended for accumulation of dissolved iron in magnetic forms, while *A. ferrooxidans* and *S. oneidensis* are utilized for
 62 the extraction of iron from minerals.

Organism	Byproducts	Kinetics	Parameters
<i>E. coli</i> (Hua et al., 2007; Núñez et al., 2002)	Acetate	$\mu = \mu_{max} * \frac{c_{Lac}}{c_{Lac} + K_{Lac}} * \frac{c_{O2}}{c_{O2} + K_{O2}}$	$\mu_{max} = 0.22 h^{-1}$ $K_{Lac} = 10 \mu M$ $K_{O2} = 0.01 mM$
<i>M. gryphiswaldense</i> (Naresh et al., 2012)		$\mu = \mu_{max} * \min(f_{NO_3}, f_{Lac})$ $f_{NO_3} = 0.5 + 0.5 * \frac{c_{NO_3}}{c_{NO_3} + K_{NO_3}}$ $f_{Lac} = \frac{c_{Lac}}{c_{Lac} + K_{Lac}}$	$\mu_{max} = 0.15 h^{-1}$ $K_{Lac} = 1 mM$ $K_{NO_3} = 0.2 mM$
<i>S. oneidensis</i> (Feng et al., 2012; Kostka et al., 2002, 1996; Liu et al., 2002; Pinchuk et al., 2010)	Acetate	$\mu = \mu_{max} * \frac{c_{Lac}}{c_{Lac} + K_{Lac}} * \frac{c_{Fe^{3+}}}{c_{Fe^{3+}} + K_{Fe^{3+}}}$	$\mu_{max} = 0.1 h^{-1}$ $K_{Lac} = 19.4 mM$ $K_{Fe^{3+}} = 0.55 mM$
<i>A. ferrooxidans</i> (Liu et al., 1988; Molchanov et al., 2007; Navarrete et al., 2013)		$\mu = \mu_{max} * \frac{c_{Fe^{2+}}}{c_{Fe^{2+}} + K_{Fe^{2+}}} * \frac{K_{Fe^{3+}}}{c_{Fe^{3+}} + K_{Fe^{3+}}}$	$\mu_{max} = 0.082 h^{-1}$ $K_{Fe^{2+}} = 0.072 mM$ $K_{Fe^{3+}} = 2.5 mM$

63 *Table 2.* Initial conditions used for solving the kinetic models of the different organisms. Concentrations in mol / L, biomass
 64 concentration in Cmol / L.

	Lactate	Fe ²⁺	Fe ³⁺	NH ₄ ⁺	O ₂	CO ₂	Biomass
<i>E. coli</i>	0.08	0.15	0	0.02	2.5*10 ⁻⁴	0	4.0*10 ⁻⁴
<i>M. gryphiswaldense</i>	0.08	0.15	0	0.035	2.5*10 ⁻⁴	0	4.9*10 ⁻⁴
<i>S. oneidensis</i>	0.08	0	0.15	0.001	2.5*10 ⁻⁴	0	4.8*10 ⁻⁴

<i>A. ferrooxidans</i>	0	0.15	0.005	0.001	2.0*10 ⁻⁴	1.5*10 ⁻⁴	3.7*10 ⁻⁴
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65

66 The biomass mass balance was set up as follows:

$$68 \quad \frac{dc_X}{dt} = \mu * c_X \quad (1)$$

67 And for the relevant chemical species in *i*:

$$70 \quad \frac{dc_i}{dt} = Y_{i/X} * \mu * c_X \quad (2)$$

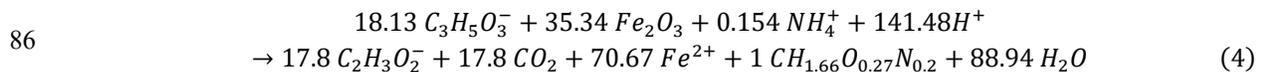
69 In the case of gaseous components, a mass transfer term was included:

$$71 \quad \frac{dc_i}{dt} = Y_{i/X} * \mu * c_X + (k_L a)_i * (c_{i,i}^* - c_{i,i}) \quad (3)$$

72 The internal pressure is assumed to be 1 bar. When the process requires O₂ and CO₂, a gas composition
73 with 21% O₂, 0.05% CO₂ and an inert gas such as N₂ for the remainder is assumed. In the anaerobic processes,
74 the medium will be continuously sparged with an inert gas to induce mixing. The composition could be further
75 tuned if an otherwise feasible process is slowed down significantly due to the gas-liquid mass transfer (Doran,
76 2013).

77 The maximum concentration dissolved gas ($c_{i,i}^*$) was determined by Henry's law. The values for $k_L a$, which
78 combines interfacial area and diffusivity, were assumed to be 10.8 and 8.6 h⁻¹ for O₂ and CO₂, respectively. The
79 value for CO₂ is slightly lower because of the larger molecule size, which slows diffusion. These values are
80 chosen on the low end of typical $k_L a$ values for slurry bioprocesses on earth (Neale and Pinches, 1994; Schumpe
81 et al., 1987; Van Weert et al., 1995; Zokaie-Kadijani et al., 2013) to account for the decreased volumetric gas-
82 liquid mass transfer in a reduced gravity setting (Pettit and Allen, 1992).

83 The process stoichiometry for the *S. oneidensis* process, which was found to be the only feasible process
84 under our current conditions, is presented in equation 4. In Table 3 this stoichiometry is combined with the
85 precipitation of magnetite, counteracting the acid requirements of the *S. oneidensis* reaction.



87 2.2. *Shewanella* growth

88 *Shewanella oneidensis* MR-1 (ATCC® 700550™) and *Shewanella oneidensis* ANA-3 (Wang et al., 2011)
89 were aerobically cultured in Tryptic Soy Broth (TSB) media overnight at 30°C under continuous shaking (250
90 rpm). Different concentrations and compositions of JSC-MARS1 (0.5 g/L or 5 g/L) and Mg(ClO₄)₂ (0.06 M

91 or 6 M) were added. Thereafter, the growth behavior was observed for 48 hours by optical density (O.D.)
92 measurements at a wavelength of 650 nm using a 96-well plate in a plate reader.

93 2.3. Payback time analysis

94 For analysis of the payback time, the approach to the kinetics and mass balances was repeated. The mass
95 of the lander was approximated by first estimating the mass of the basic components (Volger et al., 2018). The
96 mass of the 1 m³ cylindrical reactor was assumed to be 250 kg, filled with 700 L of water. Measuring devices
97 and peripherals were assumed to add 100 kg. The equipment for water recovery was estimated to weigh 100
98 kg. The rover collecting regolith was assumed to weigh 300 kg. Power supply is covered with an RTG of 500
99 kg, which covers peak power consumption of the bioprocess, and can be used to power other processes at a
100 Martian colony when the bioprocess needs less power. The resulting mass of the basic components (1950 kg)
101 was combined with the variable mass of water and nutrients and multiplied by 3 to account for structural
102 components and a maturity margin. To get to an estimated launch price, SpaceX's listed price for the launch of
103 up to 8.000 kg to GTO with a reusable rocket (SpaceX, 2019) was multiplied by a factor five to get an estimate
104 for a launch to Mars at maximum capacity, resulting in a price of about \$25.000 per kg. The SLS rocket aims
105 at a cost of about \$15.000 per kg transported to Mars (Dumbacher, 2014; Potter et al., 2018). With these
106 estimations in mind, a launching cost of \$ 20.000 per kg was assumed, which would correspond to a total cost
107 of \$ 117 million. The mass of nutrients and water was minimized to obtain the minimal payback time. With this
108 approach, the nutrient supply runs out when the extracted iron mass equals the total initial lander mass
109 (including nutrients). Increased nutrient and water supplies result in a slightly longer payback time, but a final
110 iron production exceeding the initial lander mass.

111 The system provides its own power through the addition of an RTG, which is sized for use during the
112 evaporation phase of the process and thus has an overcapacity during the other process phases. In terms of
113 maintenance, the system is intended to be an autonomous reactor system requiring no astronaut intervention.

114 3. Results & Discussion

115 3.1. Biomining process

116 The general process of using bacteria for mining applications in space, is the dissolution and accumulation
117 of specific resources from Lunar or Martian regolith (Fig 1A). The biological methodologies can be split up in
118 two categories (Fig 1B, 1C):

119 1. Accumulation of dissolved iron in concentrated form, allowing for magnetic extraction.

120 2. Leaching of iron from mineral ores, bringing it in a dissolved state, allowing for precipitation of
121 magnetic particles and ores.

122 These categories could be combined to allow for the magnetic extraction of iron from a variety of ferrous
123 mineral ores. The leaching process alone can also be combined with electrochemical manipulation of the
124 solution to promote magnetite precipitation (Bale et al., 2016; Lozano et al., 2017; R. Dasgupta and L. Mackay,
125 1959).

126 The considered candidates for the accumulation are a genetically modified *Escherichia coli* strain and the
127 magnetotactic wildtype bacterium *Magnetospirillum gryphiswaldense*. The constructed *E. coli* overexpresses a
128 modified ferritin complex, has a dysfunctional iron export mechanism and an improved iron import mechanism
129 (He et al., 2016). The combination of those modifications leads to a high intracellular iron concentration.

130 *M. gryphiswaldense* accumulates iron to produce magnetosomes, intracellular vesicles filled with magnetite
131 that form a backbone for the organism (Lefèvre et al., 2011). The resulting capability to swim along magnetic
132 field lines is commonly known as magnetotactic behavior (Schüler and Baeuerlein, 1998). *M. gryphiswaldense*
133 readily uses lactate as electron donor and carbon source.

134 For the bioleaching category, *Shewanella oneidensis* and *Acidithiobacillus ferrooxidans* were considered.
135 *S. oneidensis* is well-known for its capability to use a wide range of electron acceptors (Myers and Nealson,
136 1988), one of which is Fe^{3+} , both in aqueous and solid state (Kostka et al., 2002, 1996). Fe^{3+} from mineral
137 sources is converted into Fe^{2+} , which is both excreted in aqueous form and precipitated on the cell surface in
138 magnetite (Bennett et al., 2015; Perez-Gonzalez et al., 2010). In the current work, the use of lactate as electron
139 donor and carbon source is considered, which is then oxidized to acetate and CO_2 (Fig 1C). Other possible
140 electron donors are pyruvate, formate, amino acids or N-acetylglucosamine (Kane et al., 2016; Lovley et al.,
141 1989).

142 *A. ferrooxidans* is often used in biomining operations on earth (Valdés et al., 2008). It preferably grows in
143 very acidic conditions (pH 1-2) and can fix both carbon and nitrogen from atmospheric sources. For the current
144 work, nitrogen from ammonium is considered instead of atmospheric nitrogen. *A. ferrooxidans* requires oxygen
145 as electron acceptor when it oxidizes Fe^{2+} to Fe^{3+} .

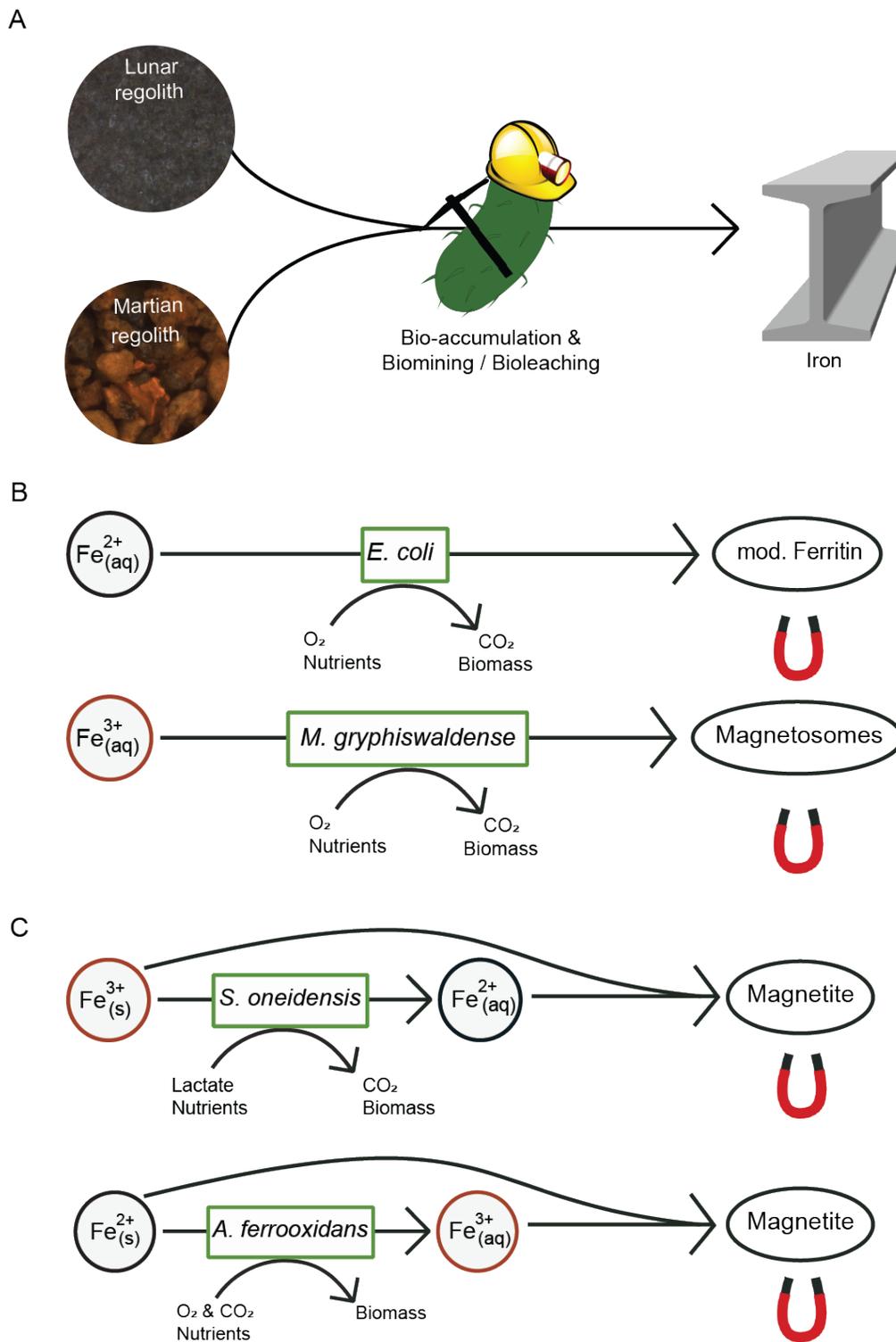


Figure 1. Workflow of the bacterial iron extraction. (A) The concept of using bacterial mining processes for the extraction of elemental iron, which can be used for material production. (B) Bio-accumulation processes using *E. coli* to bind aquatic Fe^{2+} in a modified Ferritin molecule, and *M. gryphiswaldense* to combine aquatic Fe^{3+} molecules in magnetosomes. (C) Biomining and Bioleaching approaches using *S. oneidensis*, to reduce in ore bound Fe^{3+} to Fe^{2+} and allow for magnetite precipitation, and *A. ferrooxidans* to oxidize Fe^{2+} to Fe^{3+} and allow for magnetite precipitation.

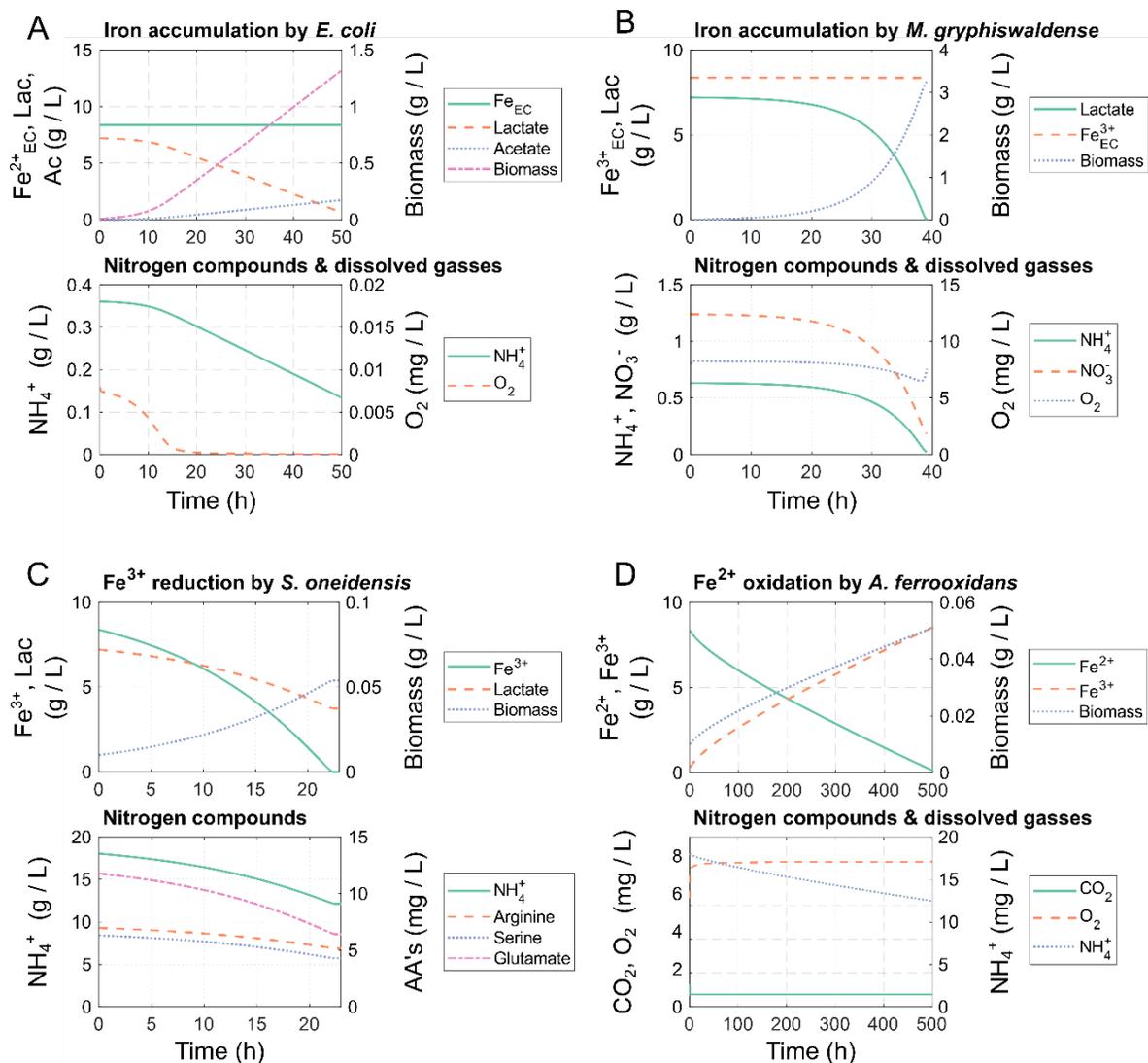


Figure 2. Literature-based growth models for *E. coli*, *M. gryphiswaldense*, *S. oneidensis* and *A. ferrooxidans*, showing nutrient consumption, dissolved gasses and iron dynamics. (A) Predicted growth and iron accumulation by *E. coli* with an overexpressed ferritin complex. After 10 hours, the oxygen transfer rate is predicted to be the limiting factor. The uptake of extracellular iron is negligible. (B) Predicted growth and iron accumulation by *M. gryphiswaldense*. The exponential growth rate is maintained until lactate is depleted. The uptake of extracellular iron is negligible. (C) Predicted growth and iron conversion for *S. oneidensis* reducing Fe^{3+} to Fe^{2+} from solid mineral substrates. Exponential growth is maintained until iron is depleted. AA's: Amino Acids (D) The predicted growth and iron conversion for *A. ferrooxidans* oxidizing Fe^{2+} to Fe^{3+} . Product inhibition by Fe^{3+} results in a linear growth profile.

147 3.1. Nutrient consumption

148 The kinetics (Table 1) were used to set up mass balances, which were solved over time to the point where
 149 one of the nutrients was used up (Fig 2). For an ideal process, the growth is expected to resemble batch growth,
 150 with an exponential increase in biomass concentration. In the accumulation processes by *E. coli* and *M.*
 151 *gryphiswaldense*, significant uptake of extracellular dissolved iron is expected. For the leaching processes by

152 *S. oneidensis* and *A. ferrooxidans*, the iron conversion is a key part of the organism's metabolism, so growth
153 should be accompanied by a rapid iron conversion.

154 The accumulation process in *E. coli* (Fig 2A), takes slightly over 50 hours to consume the initially provided
155 lactate (7.2 g/L). The predicted growth profile is initially exponential, but changes into a linear profile after 15
156 hours. The decreasing dissolved oxygen concentration indicates that the growth is limited by the oxygen transfer
157 rate. If the process would show otherwise favorable results, methods for an increased oxygen transfer rate could
158 be explored to decrease process time. However, the extracellular iron concentration does not decrease notably
159 over the course of the simulation and thus the process does not fulfill its main purpose of accumulating dissolved
160 iron. The overexpression of the encapsulated ferritin leads to an estimated 20-50 encapsulated ferritin
161 complexes per cell, which corresponds to an iron concentration in biomass of only 8-20 $\mu\text{g} / \text{g}_x$. This value is
162 too low to have a significant effect on the extracellular concentration.

163 The model for *M. gryphiswaldense* (Fig 2B) predicts batch growth for the full process and takes 39 hours
164 to fully consume the initial lactate and ammonium. The decreasing level of dissolved oxygen indicates its
165 consumption, but the oxygen transfer rate is not the limiting factor. Again, the concentration of extracellular
166 iron does not decrease notably. *M. gryphiswaldense* reportedly accumulates iron to a concentration of 4.4 mg /
167 g_x (Naresh et al., 2012), three orders of magnitude more than the proposed *E. coli* strain. Still, this is not enough
168 to have a substantial impact on the extracellular iron concentration.

169 For the bio-accumulation of iron to be a feasible approach to ISRU activities, the amount of iron
170 accumulated in one gram of biomass should outweigh the amount of transported nutrients required to generate
171 that one gram of biomass. In the case of *M. gryphiswaldense* growing on lactate, that means that one gram of
172 lean biomass should contain 2.8 grams of iron (Table 2), i.e. 74% of total biomass should be iron. For *E. coli*
173 growing on lactate, these values are 7.73 gram and 88.5%. This requirement will become more lenient when
174 the process is integrated with other biological systems. These systems can provide nutrients for the mining
175 operation or make use of the byproducts of the process.

176 The process for Fe^{3+} reduction by *S. oneidensis* (Fig 2C) takes 22 hours to reduce all the initial Fe^{3+} and
177 doesn't follow a full exponential growth curve. The growth rate slowly decreases from 80 to 70% of the μ_{max}
178 value in the first 20 hours, and rapidly drops further after that. The reduction of the growth rate occurs due to
179 a decrease of both the lactate and iron concentration. The acetate concentration (not shown) will increase over

180 the course of the process, mirroring the concentration profile of lactate. Acetate has an inhibitory effect on *S.*
 181 *oneidensis* (Tang et al., 2007), but the extent of that effect in anaerobic conditions has not been quantified.

182 The Fe²⁺ oxidation by *A. ferrooxidans* (Fig 2D) is by far the slowest process in the analysis and requires
 183 500 hours to fully consume the initial iron. The slow process is mainly due to the inhibitory effect of Fe³⁺. In
 184 the current model, no further reactions consuming dissolved Fe³⁺ or precipitation are considered, and the
 185 resulting continuous accumulation is detrimental for the growth rate of *A. ferrooxidans*. The dissolved gasses
 186 in the bottom panel show that no O₂ or CO₂ limitation is expected with the current growth rates.

187 *Table 3.* Mass-wise yields for the considered components in each process, normalized for the production of 1 gram biomass.
 188 All values in gram / gram biomass. Iron uptake in both *E. coli* and *M. gryphiswaldense* is multiple orders of magnitude
 189 smaller than the consumption of other nutrients.

	<i>E. coli</i>	<i>M. gryphiswaldense</i>	<i>S. oneidensis</i> *	<i>A. ferrooxidans</i> *
Fe²⁺	-2.1*10 ⁻⁵			
Fe³⁺		-4.4*10 ⁻³		200.7
Fe₂O₃ (Fe)			-817.74 (566)**	
FeO				-258.22
Fe₃O₄ (Fe)			790.45 (566)**	
Lactate	-4.95	-2.22	-78.90	
Acetate	1.31		51.64	
NH₄⁺	-0.24	-0.19	-0.13	-0.13
NO₃⁻		-0.33		
CO₂	3.62	0.65	37.84	-1.65
O₂	-2.61	-0.05		-27.60
H₂O	1.83		15.85	96.64
Arginine			-41.1*10 ⁻³	
Serine			-45.9*10 ⁻³	
Glutamate			-0.12	
HCl			-0.006	-392.9

190 *The *S. oneidensis* process considers the biological conversion of Fe³⁺ and precipitation of magnetite (Fe₃O₄)
 191 simultaneously, since both these processes occur at a similar pH-level. The *A. ferrooxidans* process considers
 192 just the biological conversion due to the low pH required for *A. ferrooxidans* growth.

193 ** Equivalent Fe mass.

194 The yields and nutrient requirements of the four organisms were analyzed in further detail (Table 2). The
195 large difference between iron uptake and consumption of nutrients for both *E. coli* and *M. gryphiswaldense* is
196 emphasized once more. In the case of *S. oneidensis*, its essential amino acids were added to the equation to
197 evaluate their impact on the mass yield. Even though the amino acids are essential for growth, their impact in
198 terms of mass is negligible. In the *S. oneidensis* process, performed at a pH level of 7, the reduction of iron is
199 combined with the precipitation of magnetite. In this combination, the precipitation of magnetite is assumed to
200 be non-limiting and it is assumed to consume all bio-reduced iron. Since the biological process consumes
201 protons, but the precipitation produces protons, the combination results in only a very small acid consumption.

202 In the *A. ferrooxidans* process, performed at a pH level of 2, this combination is not possible, and thus a
203 large amount of acid is required to maintain the process pH. The resulting acid requirement, converted to a mass
204 of HCl, is larger than the amount of leached iron, which makes this process unfeasible.

205 There are several key changes for bioprocesses carried out on the moon or mars as opposed to those carried
206 out on earth. Radiation doses on the Moon and Mars are significantly higher than those on earth. However,
207 comparison of the yearly Martian surface dose of 0.242 Sv (Simonsen et al., 1990) with the minimal effects of
208 an acute radiation dose of 12 Sv on the growth profile and biomass yield of *S. oneidensis* (Brown et al., 2015)
209 leads to the conclusion that radiation effects are likely negligible (Volger et al. manuscript submitted).
210 Furthermore, the process will be carried out under lower gravity, which will impact fluid dynamics, but might
211 also have an impact on bacterial performance (Demey et al., 2000; Kacena et al., 1999). On top of that, the
212 composition of the local regolith can have a negative effect on survival rates of some organisms, such as the
213 case with perchlorates in the Martian soil.

214 3.2. Bacterial survivability on Martian regolith

215 The bacterial survivability if they are mixed with Martian regolith is a key factor for the successful
216 appliance of bacterial *in situ* resource utilization (BISRU). *S. oneidensis* was, therefore, added and grown in two
217 different concentrations of JSC-Mars1 intermixed with magnesium perchlorate, which was reported of being
218 toxic to a variety of organisms (Al Soudi et al., 2017; Wadsworth and Cockell, 2017) and abundant on the
219 Martian surface. First, the bacteria were observed via 3D microscopy while growing in a high concentration
220 setting with about 50 g/L JSC1-MARS1 (Fig 3AB). To quantify the so-obtained data and test the effect of the
221 perchlorates optical density (OD) measurements at a wavelength of 600 nm were performed. The concentration
222 of the iron (and so the entire regolith simulant) had to be lowered to 0.5 g/L and 5 g/L for the planktonic culture

223 to limit the effect of light reflection by the ore-particles at this wavelength. The growth curves of two different
224 sub strains of *S. oneidensis* (MR1 and ANA 3) showed no differences between the tryptic soy broth (TSB)
225 control, the 0.5 g/L JSC-MARS1 control and the 0.5 g/L JSC-MARS1 mixed with 0.05 M magnesium
226 perchlorate (Fig. 3C). The no-bacteria control of the JSC-MARS1 medium together with the 0.05 M magnesium
227 perchlorate showed a strong fluctuation. This might be due to the acidity of perchloric acid and its interaction
228 and oxidation of the regolith ores in the aquatic solution (Jackson et al., 2006). The increased baseline and
229 fluctuations of the OD₆₅₀ curves was even more severe in the samples with 5 g/L JSC-MARS1 (Fig. 3D). The
230 ΔOD₆₅₀ and, therefore, the growth of the bacteria seems to be lower in the JSC-Mars1 and JSC-
231 MARS1+Mg(ClO₄)₂ samples, but these results are not significant because of the high fluctuations. With the
232 lower concentration of regolith simulant and perchlorate *S. oneidensis* MR1 and ANA3 growth is not influenced
233 for high concentrations of regolith a quantification is barely possible but the visual check of 3D microscopy
234 showed normal growth activity.

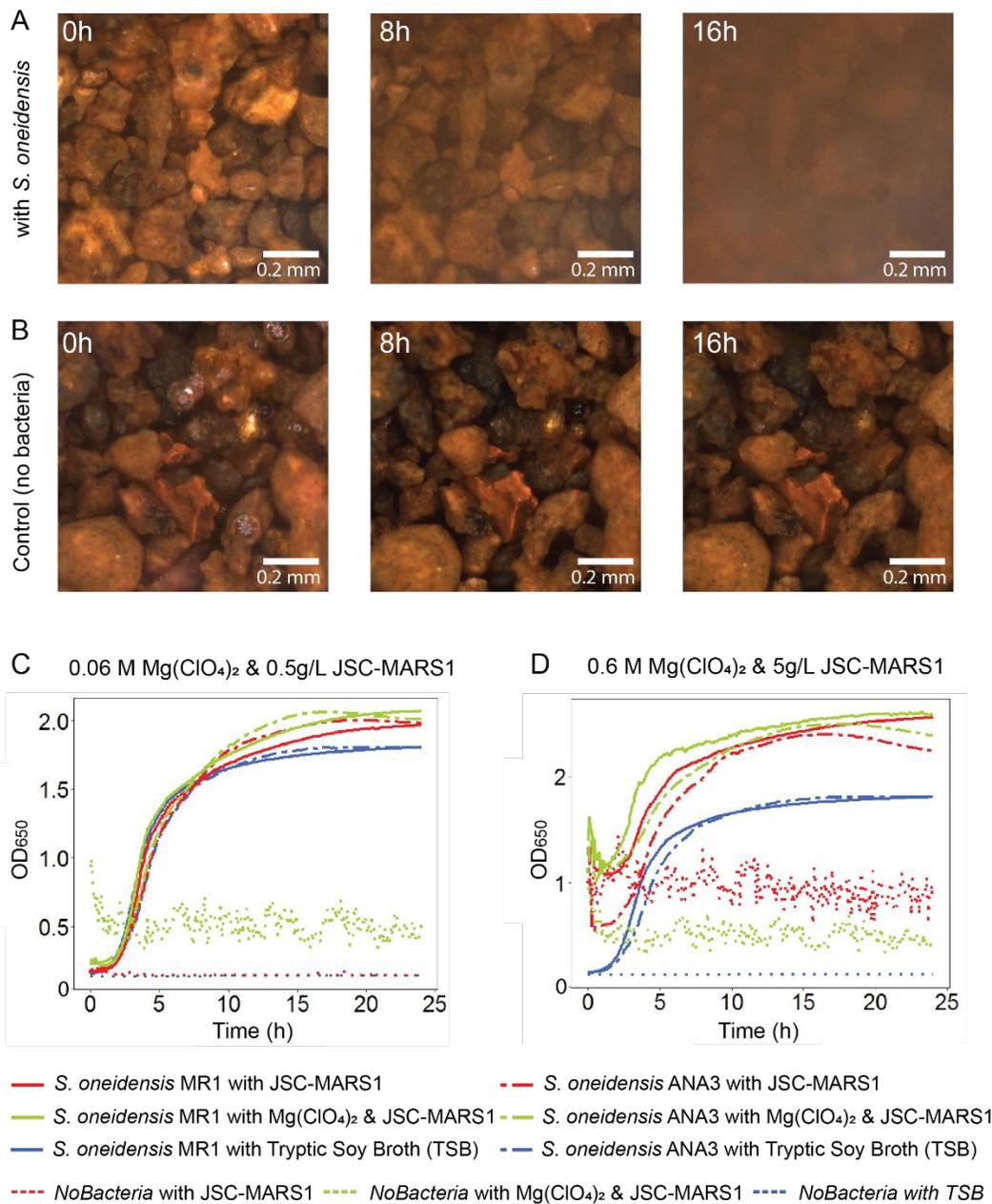


Figure 3. Growth of *S. oneidensis* MR1 and ANA3 in Martian regolith simulant (JSC-MARS1). (A) Bacterial growth observed via a 3D microscopy approach. After 8 hours we see a increase in the bacterial sample and after 16 hours the regolith particles are barely visible any more. (B) Control experiment without bacteria. No change in the 3D microscopy pictures was observed. (C) Bacterial growth of *S. oneidensis* ANA3 & MR1 under the influence of 0.5 g/L Mars regolith simulant (JSC-MARS1) and 0.06 mol/L magnesium perchlorate. No differences in the growth behavior was seen. (D) Bacterial growth of *S. oneidensis* ANA3 & MR1 under the influence of 5 g/L Mars regolith simulant (JSC-MARS1) and 0.6 mol/L magnesium perchlorate. The baseline of the control was shifted due to the higher number of particles in the solution. The absolute growth rate was not influenced.

235 3.3. Payback time analysis

236 The *S. oneidensis* process was used for a further analysis, where the process performance was combined
237 with estimated bioreactor lander characteristics (Volger et al., 2018) to calculate the payback time of the entire

238 process (Figure 4). This process uses ferric iron (Fe^{3+}) as a starting material, and thus is focused on Martian
239 applications. The process consists of three distinct phases:

- 240 1. Intake phase: Fresh regolith is loaded in the reactor, water and nutrients are added and finally inoculated
241 with a small amount of *S. oneidensis* biomass.
- 242 2. Growth phase: *S. oneidensis* grows exponentially and leaches iron from the regolith.
- 243 3. Extraction phase. Magnetite and other magnetically active minerals are separated from the rest of the
244 medium, the water is evaporated and recycled, and the rest of the regolith is sterilized and disposed.

245 The duration of the growth phase is determined with the kinetics from table 1, while the other two phases
246 combined are assumed to take 24 hours. The minimal payback time is defined as the moment where the mass
247 of extracted iron exceeds the initial mass of the lander.

248 The impact of several parameters on the payback time was investigated (Fig 4). An increase in initial
249 biomass concentration will lead to a faster process and increased mass gains per hour, but more inoculate from
250 a frozen stock (assumed $10 \text{ g}_x / \text{L}$) is needed per run, increasing the required transported mass (Fig 4A). These
251 effects counteract each other; when the initial biomass is increased up to $5 \text{ mg} / \text{L}$, the payback time decreases,
252 but any further increase result in a decrease of the overall performance. The optimal initial biomass
253 concentration for the base case was found to be $4.5 \text{ mg} / \text{L}$.

254 The efficiency of the water recycling step was varied, and it was found that a certain minimal efficiency
255 was required (Fig 4B). At lower efficiencies, the process loses more water than it gains in iron. Higher efficiency
256 always leads to a better payback time. For the base case, with an initial iron concentration of $0.27 \text{ mol} / \text{L}$, the
257 minimum required recycling efficiency is 99.79%. For most further analyses, a water recycling efficiency of
258 99.9% is assumed (Fu et al., 2016), unless stated otherwise.

259 If the process starts with a higher iron concentration, a higher amount of iron is extracted at the end of the
260 process, which weighs up against the water loss in the recycling steps. On top of that, a higher initial iron
261 concentration increases the duration of the growth phase, so the productive time per run increases. With the
262 water recycling efficiency of 99.79% (Fig. 4C left), the base case initial iron concentration of $0.27 \text{ mol} / \text{L}$ was
263 also found to be the minimum required for a positive payback time. If the water recycling efficiency was
264 increased to 99.90% (Fig. 4C right), the minimum initial iron concentration decreased to $0.13 \text{ mol} / \text{L}$. The
265 further rise to infinity when approaching this value and asymptote were cut off the figure due to the axis bounds.
266 Higher initial iron concentrations always lead to a reduced payback time. It should be noted that mixing issues

267 due to the resulting slurry and abrasion issues from the increased regolith concentration have not been
268 considered and so no maximum concentration will be found with the current models.

269 The relation between minimum water recycling efficiency and minimum initial iron concentration was
270 further investigated, and a linear relation between the two was found (Fig 4D). The higher the water recycling
271 efficiency, the lower the initial iron concentration needs to be to find a positive payback time. A combination
272 of very efficient water recycling and high initial iron concentration leads to lower payback times.

273 The environment of another celestial body can have an impact on the performance of a bioprocess. The
274 increased radiation and altered gravity can lead to increased stress for the organism, changing its performance.
275 This effect was simulated by changing the maximal growth rate and analyzing the effect on the payback time
276 (Fig 4E). The process was found to be sensitive to low growth rates, with high sensitivity between 0 and 0.3 h⁻¹
277 ¹. The chosen growth rate of 0.1 h⁻¹ is in this regime, so a small change in the growth rate will have a large
278 impact on the payback time. The insights about the different parameters were combined, and a payback time of
279 3.3 years was found at a water recycling efficiency of 99.99%, an initial iron concentration of 0.8 mol / L (300
280 g regolith / L) and an initial biomass concentration of 0.088 g / L. Addition of Martian ice water can reduce the
281 required water recycling efficiency to more achievable levels.

282 Independent of the exact biological properties, the fact that bioprocesses take place in watery solutions
283 results in general process limitations. A key tradeoff is that high concentrations of solids will lead to impaired
284 mixing and reduced gas-liquid mass transfer, but at the same time these high solids concentrations result in
285 more concentrated iron output. Assuming a maximum regolith concentration of 500 g / L with an iron content
286 of ~15%, a maximum of 75 g iron will be extracted with one liter of biological culture. This puts a lower limit
287 on the water recycling efficiency: a 92.5% recycling efficiency is required to extract more iron than the lost
288 water. When ores with higher iron concentration are fed, lower water recycling efficiency is required; at 22%
289 iron the minimum water recycling efficiency is 88.9%. Dependent on the characteristics of mixing in reduced
290 gravity and iron content of the ores, a precise maximum solids concentration and minimum recycling efficiency
291 can be determined.

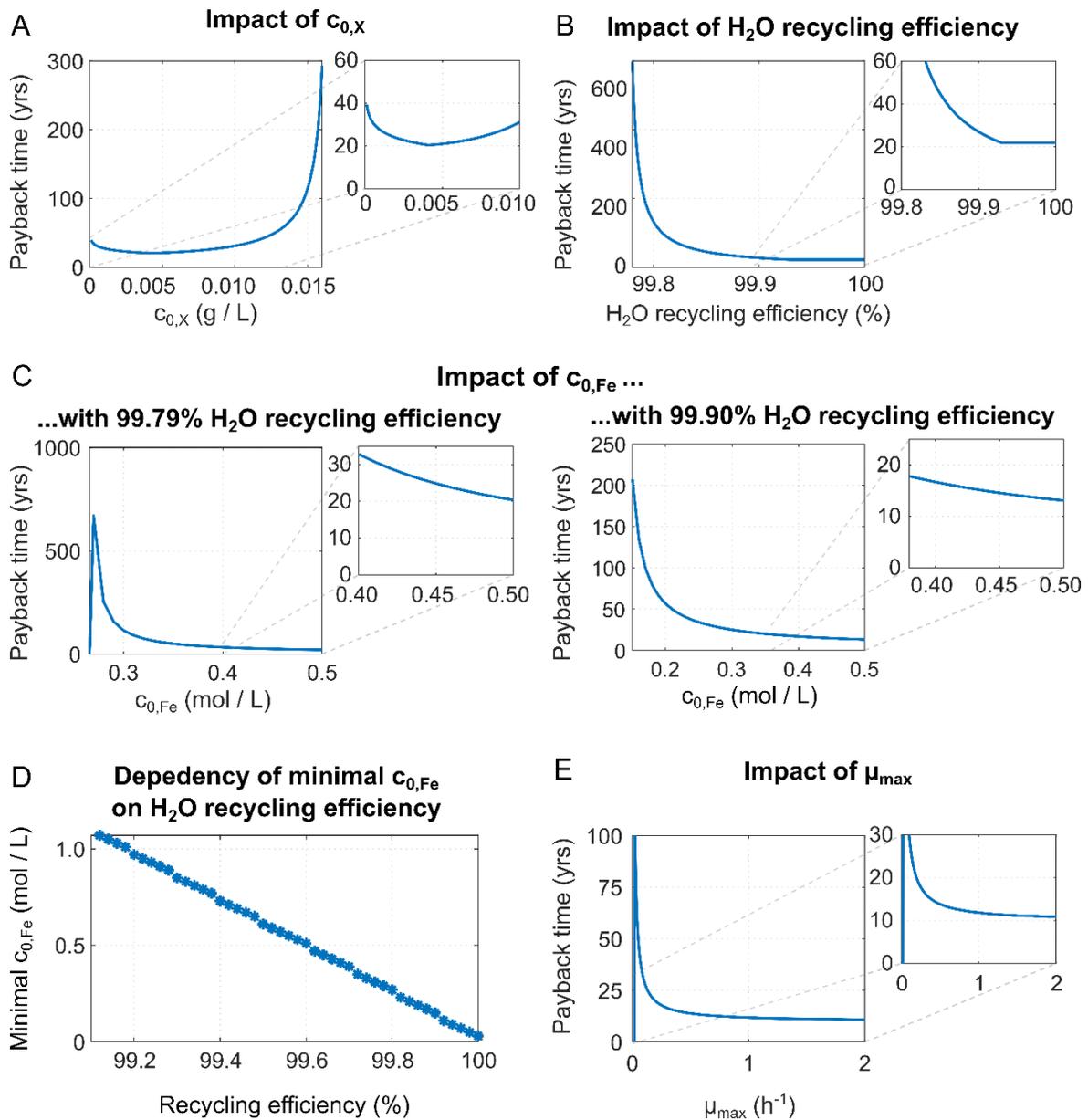


Figure 4. Sensitivity analysis on the mass-dependent payback time. (A) Impact of initial biomass concentration on process payback time, assuming 99.9% water recycling efficiency. Asymptotic behavior is observed when the initial biomass concentration exceeds 0.014 g / L. (B) Payback time shows high sensitivity to the water recycling efficiency. A higher water recycling efficiency leads to lower payback times. Asymptotic behavior is observed when recycling efficiency drops below 99.8%. (C) Impact of initial iron concentration both with 99.79% water recycling efficiency and 99.90% recycling efficiency. The payback time decreases with increasing initial iron concentration and the asymptote shifts with changes in water recycling efficiency. (D) The linear relation between water recycling efficiency and minimum required initial iron concentration for a positive payback time is displayed. (E) The impact of maximum growth rate (μ_{max}) on payback time, assuming 99.9% water recycling efficiency, is shown. A higher μ_{max} leads to shorter payback times and asymptotic behavior is observed when μ_{max} drops below 0.02 h⁻¹. The payback time shows high sensitivity in the range 0-0.3 h⁻¹.

292 **4. Conclusion**

293 In this paper, a general process setup for biological extraction of iron from Lunar or Martian regolith is
 294 presented, consisting of a leaching step and an accumulation or precipitation step. These are combined with a
 295 magnetic extraction to obtain the iron-rich minerals. Four organisms were investigated for their use in either an

296 iron leaching or iron accumulation step. Their yields and kinetics were derived from elemental balancing and
297 literature, and kinetic models were set up. *M. gryphiswaldense*, modified *E. coli* and *A. ferrooxidans* were found
298 to be incompatible with the envisioned process due to a disappointing level of accumulation and low yield.
299 Their nutrient requirements outweigh the extracted iron, which makes the process inherently infeasible with
300 transported nutrients. In the case of *A. ferrooxidans*, the most important factor was the acid consumption,
301 something that needs close attention in the analysis of bioprocesses. If the acids could be provided *in-situ* *A.*
302 *ferrooxidans* would be an optimal organism for bioleaching on the iron(II) rich Moon.

303 *S. oneidensis* was identified as a promising candidate, with an iron yield of 2.5 g / g_{nutrients} for just the
304 biological conversion, and a yield of 7.14 g / g_{nutrients} after combination with magnetite precipitation. This
305 combination also minimizes the acid consumption of the process. The effect of JSC-Mars1 and Mg(ClO₄)₂ on
306 the growth of *S. oneidensis* was found to be small, a promising feature for application for Martian exploration.

307 The payback time of the process utilizing *S. oneidensis* was analyzed, and the sensitivity to various
308 parameters was investigated. Key factors for a feasible process are highly efficient water recycling and a high
309 initial concentration of iron. With a water recycling efficiency of 99.99% and initial iron concentration of 0.8
310 mol / L, a payback time of less than 4 years can be achieved. The current conclusions are based on literature
311 data and will rely on further experimental work with *S. oneidensis*.

312 The process setup assumes a watery or slurry-like solution. Other modes of operation, such as a trickle bed
313 reactor, could provide higher or longer productivity in the same volume of water due to a higher amount of
314 solids per volume of liquid and continuous waste removal. Lower water requirements can counteract the
315 negative effects of water losses and thus increase the potential of biological ISRU processes.

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320 6. References

- 321 Al Soudi, A.F., Farhat, O., Chen, F., Clark, B.C., Schneegurt, M.A., 2017. Bacterial growth tolerance to
322 concentrations of chlorate and perchlorate salts relevant to Mars. *Int. J. Astrobiol.* 16, 229–235.
323 <https://doi.org/10.1017/S1473550416000434>
- 324 Bale, C.W., Bélisle, E., Chartrand, P., Decterov, S.A., Eriksson, G., Gheribi, A.E., Hack, K., Jung, I.-H., Kang,
325 Y.-B., Melançon, J., Pelton, A.D., Petersen, S., Robelin, C., Sangster, J., Spencer, P., Van Ende, M.-A.,
326 2016. FactSage thermochemical software and databases, 2010–2016.
327 <https://doi.org/10.1016/j.calphad.2016.05.002>

- 328 Bennett, B.D., Brutinel, E.D., Gralnick, J.A., 2015. A Ferrous Iron Exporter Mediates Iron Resistance in
329 *Shewanella oneidensis* MR-1. *Appl. Environ. Microbiol.* 81, 7938–7944.
330 <https://doi.org/10.1128/AEM.02835-15>
- 331 Brown, A.R., Correa, E., Xu, Y., AlMasoud, N., Pimblott, S.M., Goodacre, R., Lloyd, J.R., 2015. Phenotypic
332 Characterisation of *Shewanella oneidensis* MR-1 Exposed to X-Radiation. *PLoS One* 10, e0131249.
333 <https://doi.org/10.1371/journal.pone.0131249>
- 334 Carpenter, J., Fisackerly, R., Houdou, B., 2016. Establishing lunar resource viability. *Space Policy* 37, 52–57.
335 <https://doi.org/10.1016/J.SPACEPOL.2016.07.002>
- 336 Cousins, C.R., Cockell, C.S., 2016. An ESA roadmap for geobiology in space exploration. *Acta Astronaut.* 118,
337 286–295. <https://doi.org/10.1016/j.actaastro.2015.10.022>
- 338 Culbert, C., Linne, D., Chandler, F., Alexander, L., Jefferies, S., Kennedy, K.J., Lupisella, M., Metzger, P.,
339 Moore, N., Taming, K., 2015. NASA Technology Roadmaps - TA 7: Human Exploration Destination
340 Systems.
- 341 Demey, D., Hermans, V., Cornet, J.-F., Leclercq, J.-J., Lasseur, C., Delahaye, A., 2000. BIORAT: Preliminary
342 Evaluation of Biological Life Support in Space Environment. <https://doi.org/10.4271/2000-01-2384>
- 343 Doran, P.M., 2013. *Bioprocess engineering principles*, 2nd ed. Elsevier/Academic Press.
- 344 Dumbacher, D., 2014. “Fact checking rumors on NASA’s Space Launch System” by Jason Rhian [WWW
345 Document]. URL <https://www.spaceflightinsider.com/organizations/nasa/fact-check-sls-rumors/>
346 (accessed 10.20.19).
- 347 Feng, X., Xu, Y., Chen, Y., Tang, Y.J., 2012. Integrating Flux Balance Analysis into Kinetic Models to
348 Decipher the Dynamic Metabolism of *Shewanella oneidensis* MR-1. *PLoS Comput. Biol.* 8, e1002376.
349 <https://doi.org/10.1371/journal.pcbi.1002376>
- 350 Fu, Y., Li, L., Xie, B., Dong, C., Wang, M., Jia, B., Shao, L., Dong, Y., Deng, S., Liu, Hui, Liu, G., Liu, B.,
351 Hu, D., Liu, Hong, 2016. How to Establish a Bioregenerative Life Support System for Long-Term Crewed
352 Missions to the Moon or Mars. *Astrobiology* 16, 925–936. <https://doi.org/10.1089/ast.2016.1477>
- 353 Halliday, A.N., Wänke, H., Birck, J.-L., Clayton, R.N., 2001. The Accretion, Composition and Early
354 Differentiation of Mars. *Space Sci. Rev.* 96, 197–230. <https://doi.org/10.1023/A:1011997206080>
- 355 He, D., Hughes, S., Vanden-Hehir, S., Georgiev, A., Altenbach, K., Tarrant, E., Mackay, C.L., Waldron, K.J.,
356 Clarke, D.J., Marles-Wright, J., 2016. Structural characterization of encapsulated ferritin provides insight
357 into iron storage in bacterial nanocompartments. *Elife* 5. <https://doi.org/10.7554/eLife.18972>
- 358 Hua, Q., Joyce, A.R., Palsson, B.O., Fong, S.S., 2007. Metabolic Characterization of *Escherichia coli* Strains
359 Adapted to Growth on Lactate. *Appl. Environ. Microbiol.* 73, 4639–4647.
360 <https://doi.org/10.1128/AEM.00527-07>
- 361 Jackson, W.A., Anderson, T., Harvey, G., Orris, G., Rajagopalan, S., Kang, N., 2006. Occurrence and
362 Formation of Non-Anthropogenic Perchlorate, in: *Perchlorate: Environmental Occurrence, Interactions*
363 *and Treatment*. Kluwer Academic Publishers, Boston, pp. 49–69. [https://doi.org/10.1007/0-387-31113-](https://doi.org/10.1007/0-387-31113-0_3)
364 [0_3](https://doi.org/10.1007/0-387-31113-0_3)
- 365 Kacena, M.A., Merrell, G.A., Manfredi, B., Smith, E.E., Klaus, D.M., Todd, P., 1999. Bacterial growth in space
366 flight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. *Appl. Microbiol.*
367 *Biotechnol.* 51, 229–234. <https://doi.org/10.1007/s002530051386>
- 368 Kane, A.L., Brutinel, E.D., Joo, H., Maysonet Sanchez, R., VanDrisse, C.M., Kotloski, N.J., Gralnick, J.A.,
369 2016. Formate Metabolism in *Shewanella oneidensis* Generates Proton Motive Force and Prevents
370 Growth Without an Electron Acceptor. *J. Bacteriol.* 198, JB.00927-15. [https://doi.org/10.1128/JB.00927-](https://doi.org/10.1128/JB.00927-15)
371 [15](https://doi.org/10.1128/JB.00927-15)
- 372 Kostka, J.E., Dalton, D.D., Skelton, H., Dollhopf, S., Stucki, J.W., 2002. Growth of Iron(III)-Reducing Bacteria
373 on Clay Minerals as the Sole Electron Acceptor and Comparison of Growth Yields on a Variety of
374 Oxidized Iron Forms. *Appl. Environ. Microbiol.* 68, 6256–6262.

375 <https://doi.org/10.1128/AEM.68.12.6256-6262.2002>

376 Kostka, J.E., Stucki, J.W., Nealson, K.H., Wu, J., 1996. Reduction of Structural Fe(III) in Smectite by a Pure
377 Culture of *Shewanella Putrefaciens* Strain MR-1. *Clays Clay Miner.* 44, 522–529.
378 <https://doi.org/10.1346/CCMN.1996.0440411>

379 Lawrence, D.J., Feldman, W.C., Elphic, R.C., Little, R.C., Prettyman, T.H., Maurice, S., Lucey, P.G., Binder,
380 A.B., 2002. Iron abundances on the lunar surface as measured by the Lunar Prospector gamma-ray and
381 neutron spectrometers. *J. Geophys. Res. Planets* 107, 13-1-13–26. <https://doi.org/10.1029/2001JE001530>

382 Lefèvre, C.T., Abreu, F., Lins, U., Bazylinski, D.A., 2011. A Bacterial Backbone: Magnetosomes in
383 Magnetotactic Bacteria, in: *Metal Nanoparticles in Microbiology*. Springer Berlin Heidelberg, Berlin,
384 Heidelberg, pp. 75–102. https://doi.org/10.1007/978-3-642-18312-6_4

385 Lehner, B.A.E., Schlechten, J., Filosa, A., Canals Pou, A., Mazzotta, D.G., Spina, F., Teeney, L., Snyder, J.,
386 Tjon, S.Y., Meyer, A.S., Brouns, S.J.J., Cowley, A., Rothschild, L.J., 2019. End-to-end mission design
387 for microbial ISRU activities as preparation for a moon village. *Acta Astronaut.* 162, 216–226.
388 <https://doi.org/10.1016/J.ACTAASTRO.2019.06.001>

389 Liu, C., Gorby, Y.A., Zachara, J.M., Fredrickson, J.K., Brown, C.F., 2002. Reduction kinetics of Fe(III),
390 Co(III), U(VI), Cr(VI), and Tc(VII) in cultures of dissimilatory metal-reducing bacteria. *Biotechnol.*
391 *Bioeng.* 80, 637–649. <https://doi.org/10.1002/bit.10430>

392 Liu, M.S., Branion, R.M.R., Duncan, D.W., 1988. The effects of ferrous iron, dissolved oxygen, and inert solids
393 concentrations on the growth of *thiobacillus ferrooxidans*. *Can. J. Chem. Eng.* 66, 445–451.
394 <https://doi.org/10.1002/cjce.5450660315>

395 Lovley, D.R., Phillips, E.J.P., Lonergan, D.J., 1989. Hydrogen and Formate Oxidation Coupled to Dissimilatory
396 Reduction of Iron or Manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.* 55, 700–706.

397 Lozano, I., Casillas, N., de León, C.P., Walsh, F.C., Herrasti, P., 2017. New Insights into the Electrochemical
398 Formation of Magnetite Nanoparticles. *J. Electrochem. Soc.* 164, D184–D191.
399 <https://doi.org/10.1149/2.1091704jes>

400 Molchanov, S., Gendel, Y., Ioslvich, I., Lahav, O., 2007. Improved experimental and computational
401 methodology for determining the kinetic equation and the extant kinetic constants of Fe(II) oxidation by
402 *Acidithiobacillus ferrooxidans*. *Appl. Environ. Microbiol.* 73, 1742–52.
403 <https://doi.org/10.1128/AEM.01521-06>

404 Myers, C.R., Nealson, K.H., 1988. Bacterial Manganese Reduction and Growth with Manganese Oxide as the
405 Sole Electron Acceptor. *Science* (80-.). 240, 1319–1321. <https://doi.org/10.1126/science.240.4857.1319>

406 Naresh, M., Das, S., Mishra, P., Mittal, A., 2012. The chemical formula of a magnetotactic bacterium.
407 *Biotechnol. Bioeng.* 109, 1205–1216. <https://doi.org/10.1002/bit.24403>

408 NASA, 2018. The Global Exploration Roadmap 2018.

409 Navarrete, J.U., Cappelle, I.J., Schnittker, K., Borrok, D.M., 2013. Bioleaching of ilmenite and basalt in the
410 presence of iron-oxidizing and iron-scavenging bacteria. *Int. J. Astrobiol.* 12, 123–134.
411 <https://doi.org/10.1017/S1473550412000493>

412 Neale, J.W., Pinches, A., 1994. Determination of gas-liquid mass-transfer and solids-suspension parameters in
413 mechanically-agitated three-phase slurry reactors. *Miner. Eng.* 7, 389–403. [https://doi.org/10.1016/0892-6875\(94\)90078-7](https://doi.org/10.1016/0892-6875(94)90078-7)

415 Núñez, M.F., Kwon, O., Wilson, T.H., Aguilar, J., Baldoma, L., Lin, E.C.C., 2002. Transport of L-Lactate, D-
416 Lactate, and Glycolate by the LldP and GlcA Membrane Carriers of *Escherichia coli*.
417 <https://doi.org/10.1006/bbrc.2001.6255>

418 Perez-Gonzalez, T., Jimenez-Lopez, C., Neal, A.L., Rull-Perez, F., Rodriguez-Navarro, A., Fernandez-Vivas,
419 A., Iañez-Pareja, E., 2010. Magnetite biomineralization induced by *Shewanella oneidensis*. *Geochim.*
420 *Cosmochim. Acta* 74, 967–979. <https://doi.org/10.1016/j.gca.2009.10.035>

421 Pettit, D.R., Allen, D.T., 1992. Unit Operations for Gas-Liquid Mass Transfer in Reduced Gravity

- 422 Environments, in: The Second Conference on Lunar Bases and Space Activities of the 21st Century,
423 Volume 2. NASA. Johnson Space Center, p. p 647-651.
- 424 Pinchuk, G.E., Hill, E.A., Geydebrekht, O. V., De Ingeniis, J., Zhang, X., Osterman, A., Scott, J.H., Reed, S.B.,
425 Romine, M.F., Konopka, A.E., Beliaev, A.S., Fredrickson, J.K., Reed, J.L., 2010. Constraint-Based
426 Model of *Shewanella oneidensis* MR-1 Metabolism: A Tool for Data Analysis and Hypothesis
427 Generation. *PLoS Comput. Biol.* 6, e1000822. <https://doi.org/10.1371/journal.pcbi.1000822>
- 428 Potter, R., Saikia, S., Longuski, J., 2018. Resilient architecture pathways to establish and operate a pioneering
429 base on Mars, in: IEEE Aerospace Conference Proceedings. IEEE Computer Society, pp. 1–18.
430 <https://doi.org/10.1109/AERO.2018.8396506>
- 431 R. Dasgupta, D., L. Mackay, A., 1959. β -Ferric Oxyhydroxide and Green Rust. *J. Phys. Soc. Japan* 14, 932–
432 935. <https://doi.org/10.1143/JPSJ.14.932>
- 433 Rawlings, D.E., 2002. Heavy Metal Mining Using Microbes. *Annu. Rev. Microbiol* 56, 65–91.
434 <https://doi.org/10.1146/annurev.micro.56.012302.161052>
- 435 Schippers, A., Hedrich, S., Vasters, J., Drobe, M., Sand, W., Willscher, S., 2013. Biomining: Metal Recovery
436 from Ores with Microorganisms. Springer, Berlin, Heidelberg, pp. 1–47.
437 https://doi.org/10.1007/10_2013_216
- 438 Schüler, D., Baeuerlein, E., 1998. Dynamics of iron uptake and Fe₃O₄ biomineralization during aerobic and
439 microaerobic growth of *Magnetospirillum gryphiswaldense*. *J. Bacteriol.* 180, 159–62.
- 440 Schumpe, A., Saxena, A.K., Fang, L.K., 1987. Gas/liquid mass transfer in a slurry bubble column. *Chem. Eng.*
441 *Sci.* 42, 1787–1796. [https://doi.org/10.1016/0009-2509\(87\)80183-5](https://doi.org/10.1016/0009-2509(87)80183-5)
- 442 Simonsen, L.C., Nealy, J.E., Townsend, L.W., Wilson, J.W., 1990. Space Radiation Shielding for a Martian
443 Habitat. *SAE Trans.* 99, 972--979. <https://doi.org/10.2307/44472557>
- 444 SpaceX, 2019. Capabilities & Services | SpaceX [WWW Document]. URL
445 <https://www.spacex.com/about/capabilities> (accessed 10.20.19).
- 446 Tang, Y.J., Meadows, A.L., Keasling, J.D., 2007. A kinetic model describing *Shewanella oneidensis* MR-1
447 growth, substrate consumption, and product secretion. *Biotechnol. Bioeng.* 96, 125–133.
448 <https://doi.org/10.1002/bit.21101>
- 449 Valdés, J., Pedroso, I., Quatrini, R., Dodson, R.J., Tettelin, H., Blake, R., Eisen, J.A., Holmes, D.S., 2008.
450 *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. *BMC*
451 *Genomics* 9, 597. <https://doi.org/10.1186/1471-2164-9-597>
- 452 Van Weert, G., Van Der Werff, D., Derksen, J.J., 1995. Transfer of O₂ from air to mineral slurries in a rushton
453 turbine agitated tank. *Miner. Eng.* 8, 1109–1124. [https://doi.org/10.1016/0892-6875\(95\)00076-3](https://doi.org/10.1016/0892-6875(95)00076-3)
- 454 Volger, R., Brouns, S.J.J., Cowley, A., Picioreanu, C., Lehner, B.A.E., 2018. Bioreactor design to perform
455 microbial mining activities on another celestial body, in: 69th International Astronautical Congress.
456 Bremen.
- 457 Wadsworth, J., Cockell, C.S., 2017. Perchlorates on Mars enhance the bacteriocidal effects of UV light. *Sci.*
458 *Rep.* 7, 4662. <https://doi.org/10.1038/s41598-017-04910-3>
- 459 Wang, G., Qian, F., Saltikov, C.W., Jiao, Y., Li, Y., 2011. Microbial reduction of graphene oxide by
460 *Shewanella*. *Nano Res.* 4, 563–570. <https://doi.org/10.1007/s12274-011-0112-2>
- 461 Weber, K.A., Achenbach, L.A., Coates, J.D., 2006. Microorganisms pumping iron: Anaerobic microbial iron
462 oxidation and reduction. *Nat. Rev. Microbiol.* 4, 752–764. <https://doi.org/10.1038/nrmicro1490>
- 463 Yin, S., Wang, L., Kabwe, E., Chen, X., Yan, R., An, K., Zhang, L., Wu, A., 2018. Copper Bioleaching in
464 China: Review and Prospect. *Minerals* 8, 32. <https://doi.org/10.3390/min8020032>
- 465 Zokaei-Kadrijani, S., Safdari, J., Mousavian, M.A., Rashidi, A., 2013. Study of oxygen mass transfer coefficient
466 and oxygen uptake rate in a stirred tank reactor for uranium ore bioleaching. *Ann. Nucl. Energy* 53, 280–
467 287. <https://doi.org/10.1016/J.ANUCENE.2012.07.036>

