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Supplementary information

Water activity in Venus's uninhabitable clouds and other planetary atmospheres

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Supplementary text on biophysical limits of terrestrial microbes.

There have been no reports of cellular metabolism below the acidity or water-activity limits for microbial growth of the most-extremely stress-tolerant terrestrial microbes[4]. For each parameter we therefore identified the most-extreme values at which any definitive determination(s) of microbial growth have been made. To this end, we searched for any empirical evidence; whether via physical (for example, observing cell division by microscopy or measuring biomass increase using a spectrophotometer to quantify turbidity) or biochemical or molecular analyses (for example, rates of respiration; transcriptomics) and identified two seminal studies that represent the biophysical/physicochemical limits of the functional biosphere on Earth based on current knowledge[14,18] (see also main text). We then examined the conditions under which growth occurred at these (the ultimate) values of acidity and water activity for active life. For the water activity at which active life is prevented, we calculated the equivalent sulfuric acid concentration. We also quantified the water activity at the acid limit-of-active life (see 'Determination of habitability for Venus's acid clouds' in Methods). In this way, we looked for evidence that a lack of water (rather than the co-solvent) limits life at high stressor (solute or acid) concentration or vice versa. The goal was to disentangle the mechanisms at play that lead to cell-system failure and consider whether active life might be plausible beyond these apparent limits. The E-AIM[19] was used to calculate the sulfuric acid concentration at 24°C for a water activity of 0.585, and the corresponding pH. The latter was derived from the molality of the H^+ ion, its mole fraction and mole fraction-based activity coefficient, and interconversions given in Jia et al.[63] for calculating the molality-based pH in this solution according to the IUPAC definition[64].

Microbial metabolism has been proposed in the sulfuric-acid droplets of the Venusian clouds[12,13]. However, active life depends on the presence of water as the cell's internal solvent[34] and on the thermodynamic requirement for a water activity high enough to facilitate biotic activities[3,35,94,95]. Whereas desiccated cells can exhibit low-level maintenance functions such as DNA repair, based on the activities of specific enzymes, such processes do not constitute cellular metabolism per se[4]. A few studies have revealed gene-regulated changes in cells considered to be either "dehydrated" or "desiccated" (for references, see[13]). However, these studies did not use lyophilised cells and did not determine the internal water activity or water content, so it seems unlikely that the microorganisms were actually desiccated. Indeed, in much of the published literature, the concepts of i. physiologically active, hyper-osmotically stressed cells, ii. dehydrated cells, and iii. desiccated cells are used as if interchangeable. Here, however, we regard desiccation to be a condition where all non-bound water is absent; according to the definition of "DES′I□□ATE, *v. t*. [L. *desicco*; *de* and *sicco*, to *dry*.]. To dry ; to exhaust of moisture ; to exhale or remove moisture from." ref. [96].

The water-activity limits and acid-tolerance limits for the most-extreme terrestrial extremophiles/polyextremophiles may represent adaptations that are as effective as life is capable of. For instance, subsurface acid brines and surficial acid-brine lakes have acted as large-scale habitats that existed during the history of life on Earth (including Precambrian, Permian, early Triassic, and Modern) so have already provided a sustained selection pressure for the development of halophilic acidophiles[97]. Based on knowledge of the biophysical limits for active terrestrial life, and the data presented here (Table 1; Figure 3), we find it difficult to share the optimism of Limaye et al.[8] who state that there is a biologically permissive amount of available water in Venus's clouds.

Supplementary text relating to the equilibration of droplets.

Walcek and Pruppacher[98] considered SO_2 uptake by liquid drops and concluded that the time was proportional to the inverse radius squared. The diameter of liquid sulfuric acid droplets in the clouds of Venus is of the order 1 μm, implying that equilibration with the ambient gas occurs rapidly – much less than a second. The mass accommodation coefficient, α, is defined as the ratio of the incoming (or outgoing) flux of water vapour to (or from) a droplet compared to the maximum rate of condensation (or evaporation), and is assumed equal for evaporation or condensation[99]. For SO_2 interacting with water, Gardner et al.[68] give a lower limit to α of $(5.4 \pm 0.6) \times 10^{-2}$ and give a time for SO_2 solution equilibrium of 10^{-6} s. Given these values, there is no reason to expect that the water activity in the Venus cloud droplets will depart significantly from the atmospheric value other than a small reduction due to the Kelvin effect.

Supplementary text for Figure 3.

The properties of H_2SO_4–H_2O mixtures vary depending on their composition. Pure water is a weakly self-ionising compound, whilst pure sulfuric acid is a self-ionising substance that has high conductivity[100]. At higher concentrations of sulfuric acid, the water activity of the mixture decreases[20,22,101] and the pH also decreases.

The physical interactions between proteins, DNA, lipids and carbohydrates are dependent on the pH of the surrounding aqueous solution. This is due to the different protonation states of the chemical groups on these molecules at varying values of pH, which alters the balance of intermolecular and /or intramolecular forces and electrostatic interactions. For example, the lyotropic liquid crystal phase behaviour of many lipids is pH-responsive; such that at low pH the lipid bilayer motif, found ubiquitously in cells, is not always stable[102,103]. Protein tertiary and quaternary structure and enzymatic activity vary with pH (ref.[104]), whilst below pH 5.5 the changing stability of DNA conformations increases the probability of base mismatching[105].

The chemical stability of different biomacromolecules in water is also pH dependent. Acidic solutions are frequently used to hydrolyse and dehydrate carbohydrates[106] during

carbohydrate analyses[107] and to break down plant biomass[108]. Similarly, concentrated acids can readily hydrolyse proteins[109] and DNA[110]. The low chemical stability of these biomacromolecules at low pH can be understood from the effects of sulfuric acid on smaller organic molecules like amides[111], esters[112] and ethers[113], which range from protonation, hydrolysis through to complete decomposition as reviewed by Gillespie and Leisten[114].

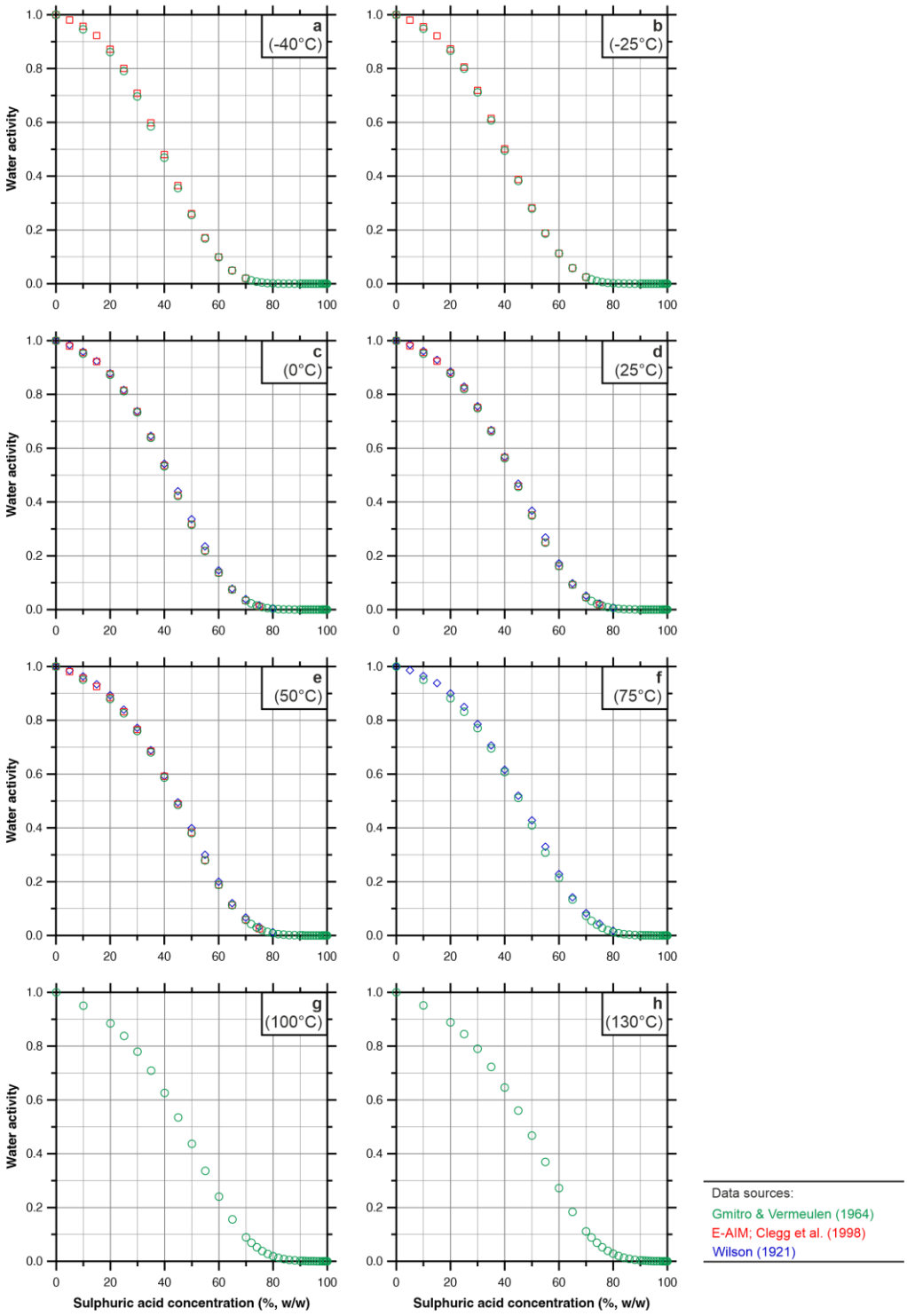
Supplementary text relating to validation of water activity for H_2SO_4–H_2O mixtures.

In order to compare values derived during the current study, data for relative vapour pressure of H_2SO_4–H_2O mixtures were identified in Table II of Wilson[22] which provides values from 0 to 75°C (273 to 348 K). Wilson[22] did not state how the various dilutions of sulfuric acid were produced (mass fraction - %, w/w; volume fraction - %, v/v; mass concentration v/w [or g l^{-1}]). However, contemporary literature citing the same sources (for example, ref. [115]) as well as our own analysis of Wilson's Table V make it clear that mass fraction was the used method. Wilson[22] collected experimental data at 25°C and measured equilibrium relative humidity in the air above H_2SO_4–H_2O mixtures, for various sulfuric acid concentrations. Then he compiled experimental measurements performed by other researchers at different temperatures (0°, 75° and 100°) over a range of sulfuric acid concentrations and compared them with those obtained at 25°C, using the Clausius-Clapeyron equation and other thermodynamic relationships. Wilson[22] tabulated these relative-humidity values for different sulfuric acid concentrations at 0, 25, 50 and 75°C. We converted these values to water activity (see Supplementary Table 2b) and then used them in the construction of Supplementary Figure 1 to compare with water-activity values derived in the current study using both Gmitro and Vermeulen[20,21] (Supplementary Table 1b) and the E-AIM model[19] (see Supplementary Table 2a).

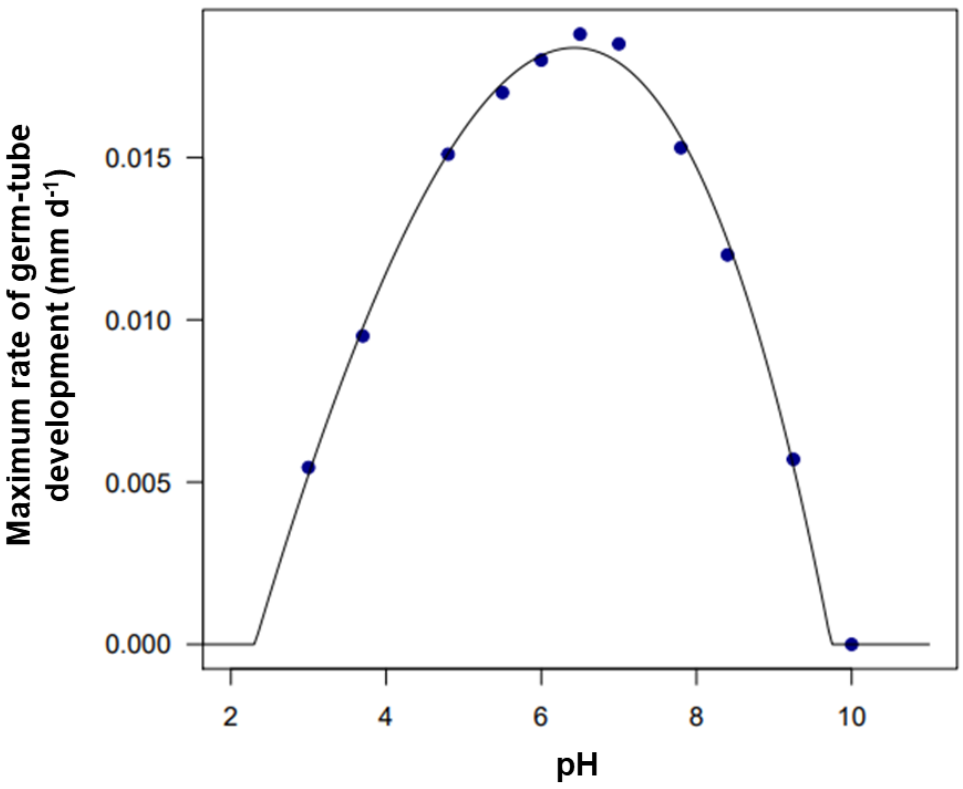
Supplementary text for Figure 6b.

In Earth's atmosphere, we can treat all clouds as being composed of nearly pure water, either in the form of liquid water or as ice, see Supplementary Table 3, (with the exception of type-I polar stratospheric clouds which contain significant amounts of nitric acid, while type-II polar stratospheric clouds consist predominantly of water ice). The water activity of pure liquid water is 1 by definition, and so liquid water clouds have typical water activities of about 0.99 to 1.01, independently of temperature. The slight variation is due to small concentrations of solutes and the Kelvin effect of small, nanometre-sized droplets, particularly during cloud droplet formation[65]. The water activity of crystalline water ice can also be calculated according to basic thermodynamic principles from the ratio of the water-vapour pressure of ice to that of liquid water. At the melting temperature of 0°C (273.15 K), the two are the same and so the water activity of ice is also one, but it decreases at lower temperature because the difference between the vapour pressure of ice and that of supercooled liquid water becomes increasingly large at

lower temperatures. In Supplementary Table 3, we estimate the atmospheric temperature range at which different types of ice clouds occur in Earth's atmosphere, allowing calculations of typical water-activity ranges for these clouds. We show the results of these calculations in Figure 6b, in which the water activity- and altitude ranges of ice clouds are indicated as orange boxes and those of liquid water clouds and fogs as the magenta bar in the bottom right of the plot. This analysis shows that water-activity values in tropospheric clouds, that is in liquid clouds and in higher-temperature cirrus ice clouds, are sufficiently high to support active life. Whereas some enzyme systems may be functional in supercooled water at -30 to -35°C, cellular proliferation is not known to occur for any terrestrial microbes in this temperature range[4].



Supplementary Figure 1. Comparison of the water-activity values of liquid sulfuric acid–water mixtures from three published sources at selected temperatures (**a**-**h**) (within the range -40 to 130°C that is pertinent for the current study). Overall, there is very good agreement between the three datasets. The data from the E-AIM model (Clegg et al.[19]; red squares) are shown only within its range of validity, that is from -40 to 50°C and for sulfuric acid concentrations up to ~80% (w/w) and water-activity values larger than 0.01. Data are given by Wilson[22] (blue diamonds) for the range 0 to 75°C and sulfuric acid concentrations up to 80% (w/w). The data from Gmitro and Vermeulen[20,21] (green circles) are available over the entire temperature range and for concentrations from 10 to 100% (w/w) sulfuric acid. The Gmitro and Vermeulen[20,21] data are the only values in the range of 80 to 100% (w/w) sulfuric acid that is particularly relevant for Venus's clouds.



Supplementary Figure 2. Maximum rates of germ-tube development (mm d^{-1}) versus varying pH for *A. penicilliodes* JH06THJ, grown on malt-extract, yeast-extract phosphate agar (MYPiA) supplemented with glycerol (5.5 M) + NaCl (1.2 M), buffered (pH 3 to 9.80), and incubated at 24°C. Data were obtained from Stevenson et al.[79] and analysed using the Cardinal pH Model (CPM) (Rosso et al.[80]) to determine the theoretical pH minimum. This theoretical minimum for growth lies between pH 1.96 and 2.57, with a 95% confidence level, and was estimated to be pH 2.3 (a value equivalent to 0.031%, w/w sulfuric acid).

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