VENUS'S CLOUDS ARE AN ORDER OF MAGNITUDE BEYOND THE ACIDITY LIMIT, AND TWO ORDERS OF MAGNITUDE BELOW THE WATER-ACTIVITY LIMIT, FOR ACTIVE LIFE. J. E. Hallsworth[1] and C. P. McKay[2], [1]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Northern Ireland, UK. E-mail: johnhallsworth@yahoo.com, [2]Space Science Division, NASA Ames Research Center, Moffett Field, CA, USA. E-mail: chris.mckay@nasa.gov

Introduction: On Earth, the atmosphere contains microorganisms of all domains of life. Many of these are located within the aqueous droplets that make up clouds, and some are known to be metabolically active [1]. This is because both the temperature and the effective concentration of water molecules (water activity) can be permissive for cellular function. There has been speculation that microbial-type life also inhabits the sulphuric acid clouds of Venus [2-7].

Several missions will study Venus in the next few years, sent by NASA (two missions, in 2028 and 2030), Russian Federal Space Agency (2029), and European Space Agency (2031). One of these missions, NASA's 'Deep Atmosphere Venus Investigation of Noble gases, Chemistry, and Imaging' (DAVINCI+), will analyse the Venus atmosphere. The data obtained are likely to confirm measurements made previously.

Here, we use measurements made already: temperature- and pressure data from Venus entry probes, and the water-vapour mixing ratio from *in-situ* and remote observations, to calculate the water activity of the Venus clouds. We determined that these cloud droplets are in equilibrium with the Venus atmosphere with respect to water activity. By analysing the water-activity and acidity limits for active on Earth, we asked whether, based on life as we know it, the Venus clouds are habitable. This study, submitted 15 October 2020, was published 28 June 2021 [8].

Water Activity of Venus's Clouds: Direct observations indicate that Venus's atmosphere is dry. We already know that the water in the small cloud droplets rapidly equilibrate with this dry atmosphere.

Homogeneity of droplets: Venus's Clouds are made up of particles [droplets] that are approximately 1 μm in diameter (see Table 1 of [9]). In equilibrium, the diffusion rates of water molecules into and out of a droplet are equal to each other. For particles of 1 μm, the effect of curvature (aka the Kelvin effect) on the water activity is negligible; see Figure 5.3 of [10]. The equilibrium of water activity between the gaseous phase and the cloud particles is independent of the details of the solution chemistry: H_2SO_4 or S_x or hydroxide salts [11]. The timescale, t, for the particles to exchange water molecules with the atmosphere is ~ 10 s. These calculations are described in [8].

Empirical measurements of the Venus atmosphere. Water activity in the gas phase in the cloud layer can be directly calculated from: temperature and pressure (directly measured by entry probes); small day/night and pole/equator variation [12]; and water-vapour mixing ratio (*in-situ* and remote observations from Venera 11, 13, and 14, Venus Express, and infra-red telescopes) as described in [8].

Water activity of cloud droplets. Water activity (a_w) was calculated according to:

$$a_w = X_{H2O} \times \text{Pressure}/\ \text{Psat}(T)$$

where X_{H2O} is the water-vapour mixing ratio, Pressure is the atmospheric pressure, and Psat is the saturation vapour pressure of water at the atmospheric temperature, T [8]. The parameter 'water activity', which is based on Raoult's Law, is allocated a scale from 0 to 1 (and is dependent on temperature and pressure). For the temperature range pertinent to active life (about 130 to −40°C; i.e., 403 to 233 K), i.e. at altitudes between 40 and 70 km, the water activity of Venus's clouds is in the range 0.00003 to 0.0037 [8].

Stress-tolerance Limits for Active (Terrestrial) Life: Many types of microorganisms are remarkably resilient structures, and are preserved for indefinite time-periods either dry, or within aqueous milieux [13,14]. Indeed, some kinds of microbes are certain to outlast the human race give their metabolic dexterity and stress-tolerant phenotypes [15]. Importantly, the current discourse is concerned with active life—cell division (and metabolism)—rather than survival *per se*.

Acidity limits for cell division (and metabolism). The most acid-tolerant microbe known is the archaeon *Picrophilus torridus* which is able to grow down to a remarkably low pH of -0.06 (at 60°C; 333 K) [16]. Using the model of Clegg et al. [17], we calculated the sulphuric acid concentration and water activity of the *P. torridus* growth medium: 11.5% (w/w) and 0.950, respectively [8].

Water-activity limits for cell division (and metabolism). As described recently by JEH [18]: "Some terrestrial microbes—termed xerophiles—grow optimally at reduced water activity, and some of these—termed halophiles—are adapted to life in brines. Xerophiles have been described as those microbes able to grow below a water activity of 0.850 (under at least two sets of environmental conditions) and must also grow optimally below 0.950 [19]. Remarkably, species such as the ascomycete *Aspergillus penicillioides* are able to flourish at low water-activity in brines, at low relative humidity, or at high concentrations of other solutes including sugars. For such organisms, high-glycerol milieux are the most-permissive for growth and metabolism at low water-activity [20,21], whereas brines are more challenging [22].

Water-activity values, expressed as a fraction of 1, can seem small and insignificant yet cells are sensitive to changes of about ±0.001 [23]. The past 100 years or so of culture-based growth studies has not yielded any verifiable evidence of microbial division below 0.585 water activity (= 58.5% relative humidity) [20,21,24-27]. Furthermore, differentiation and cell division have only been observed at 0.585 water activity for one species, *A. penicillioides*, and only at 24°C (297 K), pH 6.1 [21]. According to reliable studies which yield empirical data for microbial proliferation, the lowest water activity at which growth has been observed in brines is 0.635 (for halophilic archaea), at 37°C (310 K) [26]. No data indicate that any life-form can function at ≤0.585 water activity at temperatures far from 24°C (e.g., sub-zero, or temperatures over 50°C) or at other extremes (e.g., below pH 4 or above 9). Furthermore, there are no empirical data which show cellular metabolism at any water-activity values below 0.585, for any type of xerophile and regardless of the domain of life." For a more-detailed discussion of the water-activity limits for life, see [8,27].

Limits for active life at multiple extremes. The most-xerophilic microbe known, *A. penicillioides*, cannot grow below pH 2.3, which is equivalent to 0.031% (w/w) sulfuric acid; the most-acidophilic microbe known, *P. torridus*, is not known to grow at <0.950 water activity; and the microbe thought to be the most-tolerant to combined acidity and low water-activity is the bacterium *Acidohalobacter*, but its tolerance limits (according to studies of cell division) are pH 2 and 0.955, respectively [8].

Habitability of Venus's Clouds Based on the Limits for (Active) Terrestrial Life: Venus's clouds (77.8 to 99.2% (w/w) sulphuric acid; <0.004 water activity) are an order of magnitude beyond the 11.5% (w/w) acidity limit, and two orders of magnitude below the 0.585 water-activity limit, for active life on Earth; as shown in Figure 3 of [8]. Furthermore, this understates the distance between Earth's functional biosphere and the conditions in Venus's clouds in as much as *A. penicillioides*, can only grow at 0.585 water activity at 24°C, on glycerol-rich media, and circum-neutral pH [21]. Conversely, *P. torridus* is incapable of cell division at <0.950 water activity.

Venus's Clouds Are Inconsistent With the Integrity or Survival of Cellular Structures: Concentrated sulphuric acid is inconsistent with cellular life, and biomacromolecular structure/function, of the kind that occurs on Earth, as shown in Figure 3 of [8].

A Comment on Clouds of Jupiter, Mars, Earth, and Exoplanets: The approach used here to characterise the habitability of Venus's clouds in relation to water activity and temperature was also applied to clouds of Jupiter, Mars, Earth, and exoplanets. For details, see [8].

Concluding Remarks: Based on our understanding of life on Earth, Venus's clouds are not habitable. We would have to evoke a different biochemistry ('life as we do not know it') to imagine life under such conditions [8].

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