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# Water retention of selected microorganisms and Martian soil simulants under close to Martian environmental conditions

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ABSTRACT

Based on the latest knowledge about microorganisms resistant towards extreme conditions on Earth and results of new complex models on the development of the Martian atmosphere we quantitatively examined the water-bearing properties of selected extremophiles and simulated Martian regolith components and their interaction with water vapor under close to Martian environmental conditions. Three different species of microorganisms have been chosen and prepared for our study: *Deinococcus geothermalis*, *Leptothrix sp.* OT_B_406, and *Xanthoria elegans*. Further, two mineral mixtures representing the early and the late Martian surface as well as montmorillonite as a single component of phyllosilicatic minerals, typical for the Noachian period on Mars, were selected. The thermal mass loss of the minerals and bacteria-samples was measured by thermoanalysis. The hydration and dehydration properties were determined under close to Martian environmental conditions by sorption isotherm measurements using a McBain-Bakr quartz spring balance. It was possible to determine the total water content of the materials as well as the reversibly bound water fraction as function of the atmospheres humidity by means of these methods. Our results are important for the evaluation of future space mission outcomes including astrobiological aspects and can support the modeling of the atmosphere/surface interaction by showing the influence on the water inventory of the upper most layer of the Martian surface.


## 1. Introduction

Recent results and observations of Mars missions regarding the detailed mineralogy and the occurrence of water on the surface of Mars especially in the equatorial regions (Feldman et al., 2004; Bibring et al., 2005; Poulet et al., 2005) and their implications for the conditions on early Mars have again stimulated the discussion about the development of life on the planet. Latest knowledge about extremophiles on Earth (Davila et al., 2010; Cangalla and Wiegel, 2011) and results of new complex models on the development of the Martian atmosphere (Lammer et al., 2013) improves the basis of this debate.

An important issue in this research is the interaction of moisture of the Martian atmosphere with soil components and possibly existing organisms of the planet's surface. Therefore, we quantitatively examined the water vapor interaction and water-bearing properties of simulated Martian soil components and selected microorganisms in a broader expanse of surface temperature and pressure. The aim thus is to contribute to an improved understanding of astrobiological aspects on Mars.

## 2. Experimental

### 2.1. Samples

Two mineral mixtures representing the early basic and the late acidic Martian surface (P-MRS, Phyllosilicatic Mars Regolith Simulant and S-MRS, Sulfatic Mars Regolith Simulant) have been selected for this study based on OMEGA results (Bibring et al., 2005, Poulet et al., 2005, Gendrin et al., 2005). Both samples have been produced and provided by the Museum für Naturkunde, Berlin. Details of the composition can be found in Böttger et al. (2012). P-MRS contains, with respect to water-bearing compounds, montmorillonite (45 wt%), chamosite (20 wt%), kaolinite (5 wt%)

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and hydromagnesite (5 wt%) as main components. The water inventory of S-MRS is determined by 30 wt% gypsum and 7 wt% goethite (source Salchendorf, Siegerland, Germany) beside other "dry" components such as igneous rocks and anhydrous iron oxides.

For comparison, in particular for the water adsorption properties, the smectite Ca-montmorillonite (STx, Gonzales County, Texas) has been included in this study as a single component, also typical for the early period on Mars (Poulet et al., 2005). Another goethite (source mineralogical collection, Institute for Geosciences, J.G. University Mainz) served as example of a water bearing iron oxide for comparative measurement with the iron compound deposited by iron oxidizing strain OT_B_406.

Three different microorganisms have been selected for our study: *Deinococcus geothermalis*, *Leptothrix sp*. OT_B_406, and *Xanthoria elegans*. Fig. 1 shows some typical features of these microorganisms. These organisms can be cultivated under standard laboratory conditions, but at least *D. geothermalis* und *X. elegans* are known to live and survive under extreme conditions.

*D. geothermalis* (DSM no. 11300, type strain), an extremely radiation- and desiccation-resistant bacterium capable of forming biofilms (Kolari et al., 2002; Makarova et al., 2007), was cultivated in oligotrophic organic medium (R2A broth) with or without 5 wt% of the Martian soil simulants until stationary phase was reached. The soil particles and bacteria ($5\times10^{10}$ $g^{-1}$ soil, determined by microscopic cell counts) were sedimented by centrifugation, the supernatant removed, and the resulting pellet used for the experiments.

Strain *Leptothrix sp*. OT_B_406 has been isolated from a biofilm sample taken from a stagnating water body in the national park Lower Oder Valley and was assigned to the *Sphaerotilus-Leptothrix* group of bacteria (description in preparation). OT_B_406 forms large aggregates of sheathed cells and is capable of oxidizing iron (II) and manganese(II).

For TG/DTG analyses OT_B_406 was cultivated in modified manganese medium (Mulder and van Veen, 1963). The modified medium contained 2 g/l manganese(II) carbonate and 0.15 g/l ammonium iron(II) sulfate, which resulted in manganese(IV) oxide and Fe(III) oxides deposition onto the sheaths by strain OT_B_406. Samples for isotherm measurements were obtained from R2A broth cultures, containing neither Mn(IV) nor Fe(III) precipitates. Cell aggregates were harvested by centrifugation and dried over silica gel (RH = 30–40%) until the mass remained constant.

The lichen, *X. elegans* was collected in areas around the Sanetsch Glacier, Switzerland. The thalli were removed from the rocky substrate and stored at 248 K before use in the present experiments. It is an evolved symbiotic and eukaryotic extremophile from polar and alpine regions. It survived space exposure and is able to photosynthesize under simulated Martian conditions (de Vera et al., 2010, de Vera, 2012, de la Torre et al., 2010). The lichen has a gelatinous and mucilage matrix with similar characteristics of EPS and contains a cocktail of secondary metabolites enabling both of the symbionts (alga and fungi) to be resistant to UV- and space radiation, desiccation and freezing.

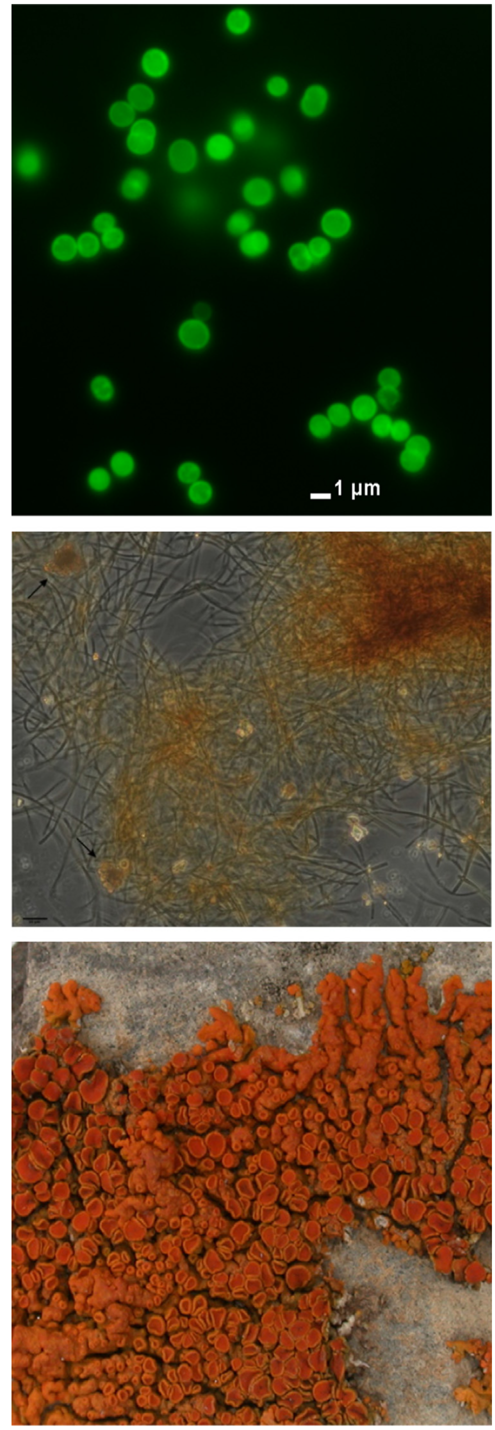


**Fig. 1.** (1) Epifluorescence microscopic image of *D. geothermalis* stained with the nucleic acid dye SYTOX Green. (2) Phase contrast micrograph of *Leptothrix sp.* OT_B_406 flakes; arrows indicate $MnCO_3$ grains. (3) Lichen *Xanthoria elegans*, Sanetsch Glacier, Switzerland.

### 2.2. Methods

The dehydration, dehydroxylation and, in case of the organisms, partial decomposition was investigated by thermogravimetry (TG) differential thermogravimetry (DTG) and differential thermoanalysis (DTG) using a Netzsch STA 409 apparatus. The heating rate was 10 K/min to $T = 873$ K in nitrogen as the carrier gas. Prior to the TG experiments the *D. geothermalis*-samples were preconditioned at controlled atmosphere with RH = 30–40% (6 days over silica gel in a desiccator) and *Leptothrix sp*. OT_B_406 was dried over silica gel as well but stored afterwards in closed containers at ambient air. The lichen was subject to hydration for several weeks in an evacuated desiccator at RH = 79% (over saturated ammonium chloride solution).

The $H_2O$ sorption isotherms were measured gravimetrically from 257–293 K in a pressure range of $10^{-2}$–20 mbar with a McBain-Bakr quartz spring balance (sensitivity 4 mg/mm) equipped with MKS Baratron pressure sensors covering a range of $10^{-5}$–$10^{3}$ mbar. The resolution in respect to the amount water taken by the sample (100 mg) was 0.0004 g/g. Before the sorption experiment, the samples

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