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Laser spectroscopic real time measurements of methanogenic activity under simulated Martian subsurface analog conditions

Janosch Schirmack [a,*], Michael Böhm [b], Chris Brauer [b], Hans-Gerd Löhmannsröben [b], Jean-Pierre de Vera [c], Diedrich Möhlmann [c], Dirk Wagner [d,*]

[a] Alfred Wegener Institute for Polar and Marine Research, Research Unit Potsdam, Telegrafenberg A45, 14473 Potsdam, Germany
[b] University of Potsdam, Department of Chemistry, Physical Chemistry, innoFSPEC Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Golm, Germany
[c] German Aerospace Center (DLR), Rutherfordstraße 2, 12489 Berlin, Germany
[d] Helmholtz Centre Potsdam, German Centre for Geosciences (GFZ), Section 4.5 Geomicrobiology, Telegrafenberg, 14473 Potsdam, Germany

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ABSTRACT

On Earth, chemolithoautothrophic and anaerobic microorganisms such as methanogenic archaea are regarded as model organisms for possible subsurface life on Mars. For this reason, the methanogenic strain *Methanosarcina soligelidi* (formerly called *Methanosarcina spec.* SMA-21), isolated from permafrost-affected soil in northeast Siberia, has been tested under Martian thermo-physical conditions. In previous studies under simulated Martian conditions, high survival rates of these microorganisms were observed. In our study we present a method to measure methane production as a first attempt to study metabolic activity of methanogenic archaea during simulated conditions approaching conditions of Mars-like environments. To determine methanogenic activity, a measurement technique which is capable to measure the produced methane concentration with high precision and with high temporal resolution is needed. Although there are several methods to detect methane, only a few fulfill all the needed requirements to work within simulated extraterrestrial environments. We have chosen laser spectroscopy, which is a non-destructive technique that measures the methane concentration without sample taking and also can be run continuously. In our simulation, we detected methane production at temperatures down to −5 °C, which would be found on Mars either temporarily in the shallow subsurface or continually in the deep subsurface. The pressure of 50 kPa which we used in our experiments, corresponds to the expected pressure in the Martian near subsurface. Our new device proved to be fully functional and the results indicate that the possible existence of methanogenic archaea in Martian subsurface habitats cannot be ruled out.

1. Introduction

Although Mars is considered to be the most Earth-like planet of our solar system, its present conditions are characterized as extreme cold and dry. However, there is evidence that 3.8 Ga ago the environmental conditions of early Mars and early Earth have been very similar (Carr, 1989, 1996; Durham et al., 1989; McKay and Davis, 1991; McKay et al., 1992). Considering this, there is the given possibility of life emerging on Mars during the same time as life first appeared on Earth. After its hypothetical initial evolution, life on Mars either might have become extinct resulting in only fossil records being found today, or it might have adapted to the drastically changing conditions and still may be present in some ecological niches.

Spectroscopic observations from the ESA Mars Express spacecraft (Formisano et al., 2004; Geminale et al., 2011) and ground based spectra analysis from Earth (Krasnopolsky et al., 2004; Mumma et al., 2009) have detected trace amounts of methane in the Martian atmosphere. Direct photolysis by solar UV radiation and homogenous oxidation by OH and O (^{1}D) are believed to cause a relatively short lifetime of methane on Mars of approximately 340 years (Gough et al., 2010; Krasnopolsky et al., 2004). This leads to the assumption, that the observed methane must have a recent origin on the planet. At the time of writing latest measurements performed with the Tunable Laser Spectrometer on board of the Curiosity Rover indicated no definitive detection of methane on Mars, with a measured value of only 0.55 ± 1.46 ppbv, at least for Gale Crater region (Webster et al., 2013). This stands in contrast to the predicted global average value of about 10–15 ppbv methane (Formisano et al., 2004; Geminale et al., 2011). Nevertheless

* Corresponding author. Tel.: +49 331 288 2142; fax: +49 331 288 28802.
* Corresponding author. Tel.: +49 331 288 28800; fax: +49 331 288 28802.
E-mail addresses: Janosch.Schirmack@awi.de (J. Schirmack), Dirk.Wagner@gfz-potsdam.de (D. Wagner).

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sources of methane on Mars could be abiotic either via active volcanism, which has not yet been observed on Mars (Krasnopolsky, 2006), or formation via Fischer–Tropsch reactions associated with serpentinization (Michalski et al., 2013; Oze and Sharma, 2005; Vance et al., 2007). Another possible source for methane would be biogenic production. Although the spectroscopic detection of methane on Mars has been discussed critically (Zahnle et al., 2011), the observed spatial and temporal variation of methane in the Martian atmosphere (Formisano et al., 2004; Geminale et al., 2008, 2011) and its correlation with the presence of water vapor are supporting the biogenic production theory. On earth aerobic methane production by plants is also known (Bruhn et al., 2012), but the main biological source of terrestrial methane is anaerobic methanogenesis (methane production by methanogenic archaea e.g. Barbier et al., 2012; Bischoff et al., 2012). For this reason several scientific publications have previously dealt with methanogenic archaea as model organisms for possible life on Mars (Boston et al., 1992, Jakosky et al., 2003, Kral et al., 2004, 2011; Krasnopolsky et al., 2004; Morozova et al., 2007; Morozova and Wagner 2007; Ulrich et al., 2012; Weiss et al., 2000).

Methanogenic archaea from Siberian permafrost environments have shown extraordinary tolerance against different environmental stresses. However, previous tests were not able to reveal if the microorganisms could be metabolically active in a Mars-like environment or merely were able to survive by entering a dormant state. The methanogenic strain we are using in our recent experiments is *Methanosarcina soligelidi* (formerly called *Methanosarcina spec.* SMA-21), which has been isolated from the active layer of a permafrost-affected soil in northeast Siberia (Wagner et al., 2013). In direct comparison to reference strains from non-permafrost environments *M. soligelidi* has shown high resistances against long term freezing for up to two years at −20 °C, high salt concentrations up to 6 M NaCl and starvation (Morozova and Wagner, 2007), as well as high doses of UV and ionizing radiation (Wagner, D., unpublished data). Along with two methanogenic strains isolated from the Lena Delta, *M. soligelidi* survived three weeks of simulated Martian thermo-physical conditions (Morozova et al., 2007). This was done with a diurnal profile in a Mars-like atmosphere (95.3% CO_2, 0.6 kPa) with temperature fluctuations from +20 to −75 °C and varying humidity with a_w-values between 0.1 and 0.9 corresponding to the Mars average equivalent water vapor pressure of 0.1 Pa. Three in parallel tested reference strains from non-permafrost environments did not show any activity after the simulation period, whereas *M. soligelidi* had almost the same activity than before.

The aim of this study is to present a method to measure the methane production of methanogenic archaea under defined thermo-physical conditions in real time without affecting the simulation conditions through removal of gas samples as it would be necessary for gas chromatography. We developed a system in which we combine laser spectroscopic methane detection with a simulation chamber. In this first study we are focusing on simulated Mars analog subsurface conditions regarding temperature and pressure like would be found in a potential deep biosphere.

2. Methods

2.1. Wavelength modulation spectroscopy

With laser spectroscopy one can very specifically measure the absorption of methane in the gas phase. With careful selection of the absorption line, cross sensitivities to other gases can be ruled out. Here, we choose the strong absorption line at 1653.45 nm (6047.95 cm^{-1}), to avoid cross sensitivity to water or carbon

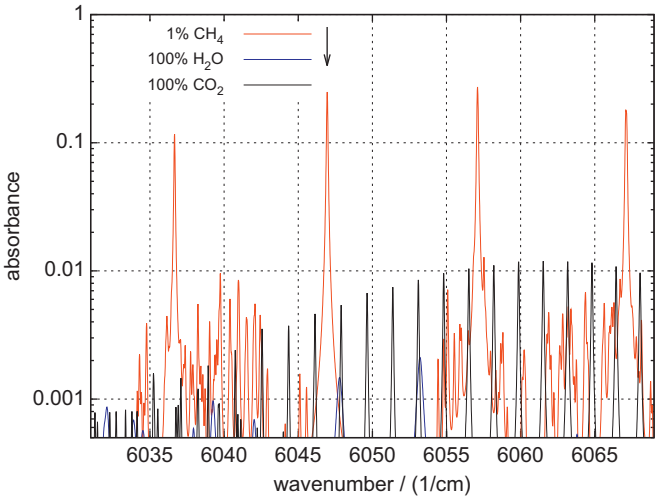

Fig. 1. Comparison of the absorption of 1% CH_4 (red curve), 100% CO_2 (black curve) and 100% H_2O (blue curve) for 1 m optical path at 50 kPa and 296 K according to the Hitran database. The marked line was chosen to detect methane, because it is strong and avoids cross sensitivites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dioxide (HITRAN database: Rothman et al., 2009), as shown in Fig. 1. Although there are stronger absorption lines in the mid infrared, the near infrared spectral region is better addressable because of readily available laser-based telecommunication equipment. For instance, the distributed feedback laser, which was used in this work, is from the telecom provider Anritsu (GB6B5004BDP; Japan). This fiber coupled laser emits cw-light with several mW of optical power, which is tunable across the entire methane line. A highly sensitive InGaAs-photodiode is used (Thorlabs PDA10CS-EC; Newton, New Jersey, USA) to detect the light. Such a near infrared system is less expensive than mid infrared ones, which use e.g. quantum cascade lasers. A near infrared system, like the one presented here, is also better when building up several such devices in parallel. Such a parallel detection scheme seems to be necessary for a systematic analysis of methane production under various conditions.

Due to laser power fluctuations, simple absorption spectroscopy is not sensitive enough and thus a more sophisticated method is needed. Here we use wavelength modulation spectroscopy (Demtröder, 2007). The current of the laser diode is controlled to vary the wavelength of the emitted light (Thorlabs ITC 502; Newton, New Jersey, USA). With lock-in detection technique (lock-in amplifier from Stanford Research System SR830 DSP; Sunnyvale, California, USA), strong rejection of laser fluctuations, background light and noise is achieved.

The experimental setup is shown schematically in Fig. 2. For a given center wavelength, the laser output wavelength is varied sinusoidal. This modulated light passes the measuring chamber and is analyzed by the lock-in amplifier after detection. The lock-in signal is measured as a function of center wavelength. The received data were recorded every 6 min, and the concentration was determined by fitting to the data, as described in more detail below (see Section 2.2).

The production of methane takes place in the liquid phase, but the detection is performed in the gas phase. Therefore, the methane must out-gas from the water, which is a slow process. To increase the out-gassing rate, our growth chamber has a high surface to volume ratio (see Section 2.3). For an effective measurement a large optical path length is needed. The presented device has an optical path length of 1.7 m with a gas volume of 590 ml and is shown in Fig. 3a. A better method for increasing the beam path length to gas volume ratio would be given by an approach

presented by Cubillas et al. (2009) for measuring methane. However, due to the complexity of this approach it was not investigated here.

2.2. Calibration

Although there are methods for quantitative calibration-free and reference-free wavelength modulation spectroscopy (Zakrevskyy et al., 2012), we calibrated the whole setup using a reference gas (10,000 ppm methane in N_2). Calibration was performed for different pressures and different dilutions, which is shown in Fig. 4. The received data were fitted assuming a Gaussian shaped spectral line. As a measure for the concentration the area retrieved from the fit is used. Since this kind of fitting is prone to systematic errors, we used 210 calibration points with known concentration, pressure and temperature of the gas resulting in a nonlinear calibration curve (Fig. 4). For each concentration and pressure three measurements were performed to check reproducibility. A fit to the calibrated data is used to derive a conversion function for the measured data with unknown concentration. Additionally, before and after a measured simulation sequence, we cross checked the validity of the calibration with a single point measurement using the reference gas. This procedure ensures, that even in the case of a residual systematic error in the absolute calibration an increase of the methane concentration is not observed due to a wrong calibration.

To calibrate the wavelength, a wavelength meter WA1100 (Burleigh; Victor, New York, USA) was used achieving a resolution in the order of several pm.

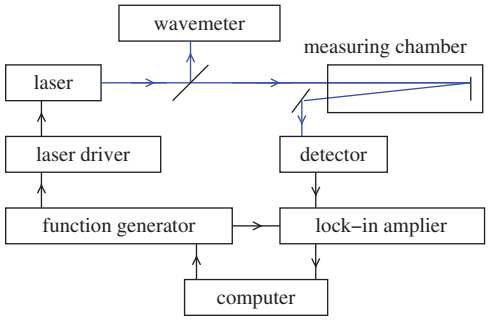

Fig. 2. Schematic diagram of the used wavelength modulation spectroscopic technique: a function generator is used to modulate the laser wavelength via a laser driver, while the actual wavelength is monitored with a wavemeter. The light is, after passing through the measuring chamber, detected with an InGaAs-photodiode. The signal from the detector is then analyzed with lock-in technique. The lock-in signal is finally recorded as a function of the optical center wavelength.

2.3. Two chamber simulation system

Since the methane production should be investigated for different temperatures and pressures, a growing chamber (made of glass), which is surrounded by a controllable temperature flow of oil (Lauda Pro Line RP845; Lauda-Königshofen, Germany) was used, as shown in Fig. 3b. The pressure was set and recorded with a pressure sensor (Vacuubrand DVR5; Wertheim, Germany). The growing chamber was in gas flow contact with the measuring chamber (Fig. 3a). The measuring chamber was thermally isolated from the lab conditions and was kept at a constant temperature of 22 °C (Lauda Ecoline RE 306; Lauda-Königshofen, Germany) for easy spectroscopic calibration of the methane concentration. The scheme of the two chamber setup is shown in Fig. 5.

2.4. Organism and growth media

The strain *Methanosarcina soligelidi* was isolated from the active layer of permafrost-affected soil on Samoylov Island in the delta of the river Lena in northeast Siberia (72°22′N, 126°28′E; Wagner et al., 2013). The cells of *M. soligelidi* were incubated under strict anaerobic conditions in 125 ml serum bottles sealed with a butyl rubber stopper of 12 mm thickness. The medium used for growth had the following composition (l^{-1}): NaCl, 1.0 g; KCl, 0.5 g; $MgCl \cdot 6\,H_2O$, 0.4 g; NH_4Cl, 0.25 g; $CaCl \cdot 2H_2O$, 0.1 g; KH_2PO_4, 0.3; $NaHCO_3$, 2.7 g; $Na_2S \cdot 3H_2O$, 0.3 g; cysteine hydrochloride, 0.3 g; trace element solution (Wolin et al., 1963), 10 ml; vitamin solution (Bryant et al., 1971) 10 ml; and 2 ml resazurin indicator solution. The bottles were flushed with H_2/CO_2 (80:20 v/v, 100 kPa), which served as a substrate and were pressurized with N_2/CO_2 (80:20 v/v, 200 kPa) to ensure anaerobic conditions. The culture was incubated at 28 °C in the dark. Growth was monitored by analyzing gas samples with gas chromatography (Agilent GC 6890, with a Flame

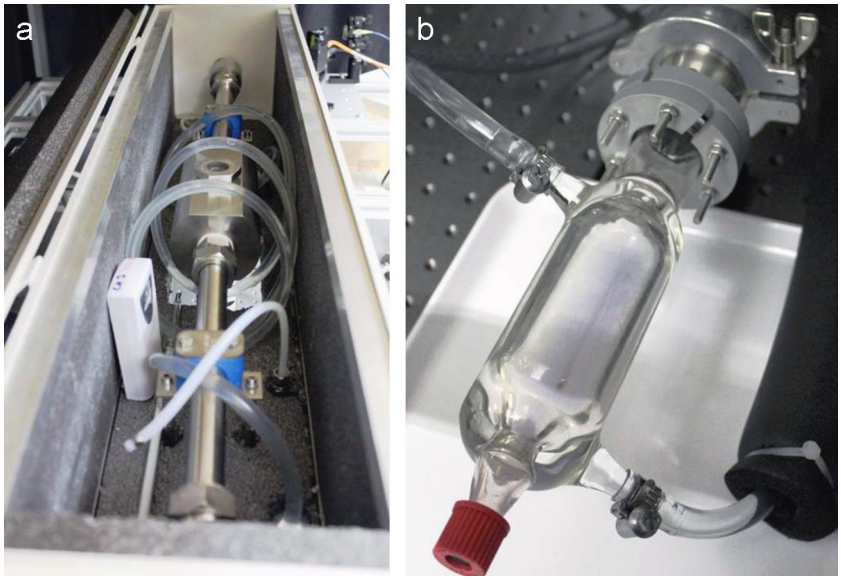

Fig. 3. (a) Photograph of the measuring chamber with the optical path length of 1.7 m. It is placed inside a thermos box and tempered with a water flow. (b) Photograph of the growing chamber. The inner 50 ml cell is connected to the measuring chamber to allow gas and pressure exchange. It is surrounded by an outer oil flow cell, which keeps the temperature constant to the set value.

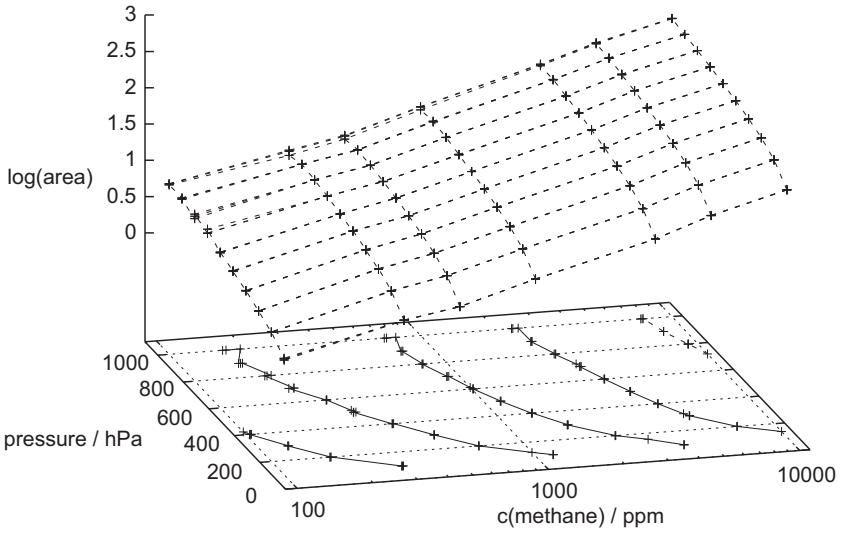

Fig. 4. The measured area (a.u.) of the spectral line is shown for different concentrations of methane and different pressures. This calibration data is used to derive the unknown concentration during a simulation sequence, while measuring the area of the spectral peaks in the same way.

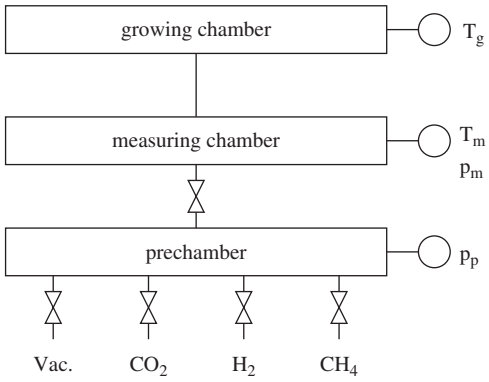

Fig. 5. Schematic diagram of the gas flow between the different simulation system chambers. The temperature T_m of the measuring chamber is held constant at 22 °C due to stable measuring conditions. The temperature of the growing chamber T_g is varying over time (as shown in Figs. 5 and 6). The gas mixture (H_2/CO_2, 80:20% v/v) is prepared in the prechamber, p_p and then transferred to the measuring chamber, p_m. The initial pressure of the measuring chamber is adjusted to 50 kPa, the pressure of the growing chamber is according to that. Methane gas (CH_4) was filled in from an external source only during calibration measurements.

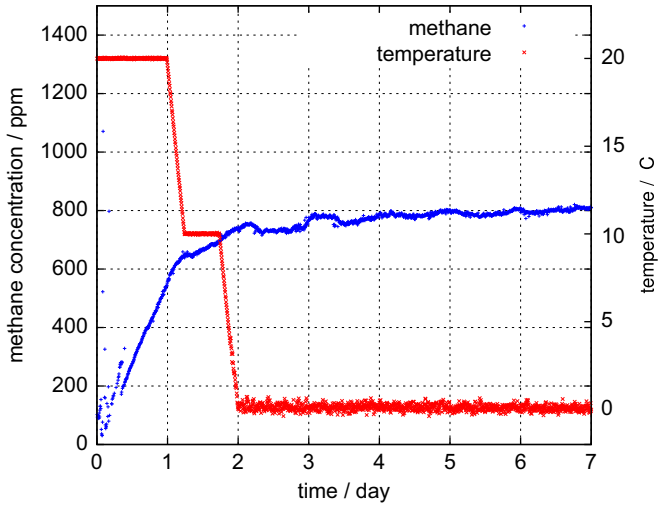

Fig. 6. Methane concentration (blue curve) as a function of time shown stepwise by cooling down from 20 °C to 0 °C (red curve) within 24 h with a intermediate step at 10 °C for 12 h. The initial pressure was 50 kPa (H_2/CO_2 80:20% v/v). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Ionization Detector and equipped with a Plot Q column, diameter: 530 μm, length: 15 m; Agilent Technologies, Germany).

2.5. Preparation of cells of Methanosarcina soligelidi

After growth in the exponential phase, the cells were harvested through centrifugation (Sigma 6K15-Z3; Sigma-Aldrich, Germany) for 45 min with 4200*g* at 4 °C, resolved in a fresh sterile medium and transferred to a new serum bottle according to the previous description (see Section 2.4). All preparation steps were performed under strict anaerobic conditions. At the start of each experimental run in the simulation chamber, the cell density was adjusted between 1.0×10^8 and 5.0×10^8 cells ml^{-1}. Cell numbers were determined by Thoma cell counts with a Zeiss Axioscop 2 microscope (Carl Zeiss, Germany).

2.6. Preparing the initial simulation conditions

The removable growing chamber was autoclaved and assembled to the measuring chamber (see Section 2.3). The entire system was evacuated for at least 8 h and the gas mixture of H_2/CO_2 (80:20 v/v) was prepared in the pre-chamber before filling the measuring chamber. The pressure of 50 kPa was adjusted after two flushing steps with the gas mixture. 16.5 ml of the prepared methanogenic archaea cell suspension (see Section 2.5) was injected through a septum using a syringe under sterile conditions. The temperature was automatically varied as a function of time and at the given pressure of 50 kPa the liquid phase stabilized over the measuring period.

3. Results

Our developed simulation chamber and measuring system (described in Sections 2.1–2.3) was set up and is operating reliably. In both presented experimental simulation sequences (Figs. 6 and 7) an increase of the methane concentration inside the measuring chamber was detected as a function of time and for different temperatures. Within one week of simulation the initial pressure of 50 kPa decreased to approximately 48 kPa. This effect did not

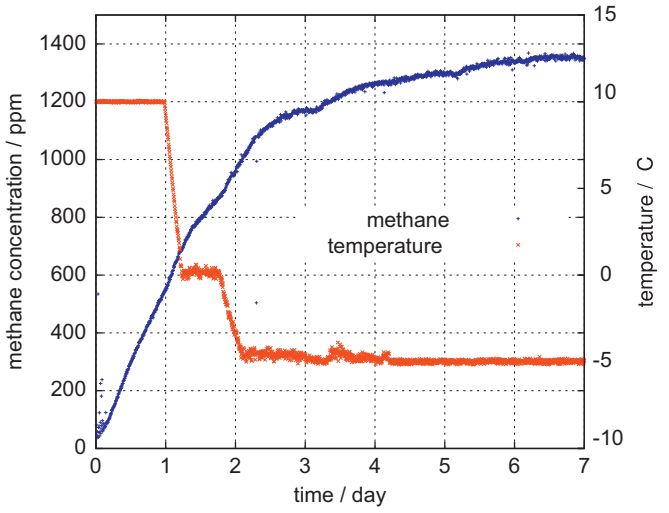

Fig. 7. Methane concentration (blue curve) as a function of time shown stepwise by cooling down from 10 °C to −5 °C (red curve) within 24 h with a intermediate step at 0 °C for 12 h. The initial pressure was 50 kPa (H_2/CO_2 80:20% v/v). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Methane production rates calculated from the increase in the methane concentrations at different temperature intervalls of the two presented experimental simulation sequences shown in Figs. 5 and 6.

Temperature [°C]	CH_4 production rate [nmol h^{-1} ml^{-1}]	
	Test 1 (Fig. 5)	Test 2 (Fig. 6)
20.0	17	n.d.
10.0	4	19
0.0	0.4	12
−5.0	n.d.	1

n.d.=not determined in the specific test.

occur in tests without organisms, so the biogenic methane formation ($4H_2+CO_2 \rightarrow CH_4+2H_2O$) might explain this observation. After an initial temperature step of 20 °C (Fig. 6) and 10 °C (Fig. 7) for 24 h, the temperature of the growing chamber was decreased to 0 °C (Fig. 6) and −5 °C (Fig. 7), with an intermediate step at 10 °C (Fig. 6) and 0 °C (Fig. 7). Each temperature decrease was conducted continuously within 6 h and the intermediate temperature steps lasted 12 h. Decreasing temperatures resulted directly in decreasing methane accumulation rates, as it can be seen in the stepwise flattening of the methane concentration curves at each temperature step. However, an increase in the methane concentration over time was still observed down to −5 °C. The methanogenic cell suspension inside of the growing chamber remained liquid at a temperature of −5 °C. In Table 1, the calculated methane production rates for each temperature step of both experiments (Figs. 6 and 7) are shown. Although the cell samples for both experiments were treated similarly, a variation in the rates for 10 °C and 0 °C occurred. Both experiments were performed separately with separately grown microbial cultures. Hence, slightly different initial cell densities, or cells harvested in not absolutely the exact same phase of growth could be an explanation for the observed differences. One should also mention that the measured data show a small variation of the methane concentration with the periodicity of 24 h. From separate measurements we pinned down the origin of this effect in the imperfect complete thermal isolation of the measuring chamber from the environment. A delay between the temperature variation of the growing cell and the methane growth rate is also visible. This effect can be derived from the slow diffusion and outgassing of the methane. All these effects unfortunately limit the methane rate determination for these measurements. Nevertheless, a significant change of the methane accumulation rate for the different temperature regimes was observed.

4. Discussion

Previous experiments have shown that the archaeal strain *Methanosarcina soligelidi* is able to survive simulated Martian thermo-physical surface conditions (Morozova et al., 2007). However, during these experiments it was only possible to measure the methane production and survival rates of the cells before and after the simulation sequence. Our results show, that the constructed simulation system is fully functional and methane production could be observed under the tested conditions.

The first experiments show a methanogenic activity of *M. soligelidi* at temperatures down to −5 °C; lower temperatures were not tested so far. Methane production by a pure culture at temperatures below 0 °C was only reported previously from *Methanogenium frigidum* (Franzmann et al., 1997). This strain has been isolated from Ace Lake in Antarctica and was used as one of the reference organisms in the previous study done by Morozova et al. (2007). No methane production of *M. frigidum* was observed after three weeks exposure to Martian thermo-physical surface conditions shown during the experiment of Morozova et al. (2007), whereas the strain *M. soligelidi* showed nearly the same methane production rate after the simulation compared to the rate measured before the experiment. Incubation experiments done by Wagner et al. (2007) with Holocene permafrost deposits obtained from Samoylov Island in the Lena Delta, northeast Siberia (the same investigation area *M. soligelidi* was isolated from) have shown methanogenic activity at temperatures down to −6 °C. The methane production rates reported in that study, with incubation with hydrogen as a supplemented energy source, resulted in rates of 0.78 ± 0.31 nmol CH_4 h^{-1} g^{-1} for −3 °C and 0.14 nmol CH_4 h^{-1} g^{-1} for −6 °C and match the calculated rates for decreasing temperatures presented here (see Table 1). Other tests performed with soil samples obtained from permafrost of the Kolyma lowland from northeast Eurasian Arctic tundra, reported methanogenesis of the native microbial community even at temperatures down to −16.5 °C (Rivkina et al., 2004). However, the presented observation of methanogenic activity of a pure culture at −5 °C was not shown before.

The surface of Mars is an extremely cold place with seasonal and diurnal temperature fluctuations reaching from −138 °C to +30 °C (Jones et al., 2011). The lowest temperature of −5 °C tested in this study would only be found on Mars in temporarily variation on the surface, or the first meter below the surface, in mostly equatorial or mid-latitudes. An example for such a periglacial landscape is Utopia Planitia (Ulrich et al., 2012). In persistent timeframes −5 °C would merely occur in a depth of several kilometers (Jones et al., 2011). However, *M. soligelidi* was found to at least survive down to temperatures of −80 °C, even repeatedly and reversibly within the three weeks Martian simulation experiment (Morozova et al., 2007; Morozova and Wagner, 2007). Additionally, active microbial metabolism, albeit only close to the maintenance level, was reported from an Antarctic ice-lake even at −13 °C (Murray et al., 2012). If it comes to terms of stable conditions, the low and highly alternating temperature on Mars seems to be a critical factor. Nevertheless, our experimental temperature is consistent with the temperature maxima in diurnal and seasonal fluctuations in the shallow subsurface and also with the assumed stable temperatures in the deep subsurface of Mars.

The overall atmospheric pressure on Mars is as low as 0.06 kPa. The pressure of 50 kPa used in our experiments equals the hydrostatic pressure which is believed to be found on Mars at depth of approximately 10–20 m below the surface, according to Kral et al. (2011) and to the PT-diagrams for a potential Martian biosphere presented by Jones et al. (2011). Even lower pressures would not be a problem for methanogenic archaea. In the simulation experiment of Morozova et al. (2007) *M. soligelidi* survived the overall Mars like low pressure of 0.06 kPa for three weeks and was almost unaffected. Also, Kral et al. (2011) have shown that several archaea strains were able to produce methane at pressures down to 0.5 kPa. Such a pressure is expected in the shallow first few meters of the subsurface of Mars. In deep subsurface environments, the pressure would of course be higher than that in the shallow subsurface, reaching about 10,000 kPa at 1 km depth (Jones et al., 2011). On Earth, living methanogens have been isolated from deep subsurface habitats (Kotelnikova et al., 1998; Lever et al., 2013; Shimizu et al., 2011), where the pressure is understandably higher compared to Mars. In addition, the activity of the marine methanogen *Methanococcus jannaschii* has been proved at a pressure of up to 75,000 kPa (Miller et al., 1988). Therefore, the pressure at either the shallow or in the deep subsurface of Mars would not be a problem for the existence of methanogenic archaea and thus the pressure chosen for this simulation study is suited over this observed pressure regime.

Liquid water was present during the whole simulation sequence of our experiments and thus was easily accessible for the archaeal cells. However, in general the presence of liquid water is a major limiting factor for life as we know it from Earth which includes methanogens. Although, the present day Mars is known to be a dry planet, water might become available periodically at or below the surface. Changing diurnal and seasonal temperatures in addition to pressure conditions result in liquid water in terms of water vapor, interfacial water or in cryobrines (Möhlmann, 2010a, 2010b; Möhlmann and Thomson, 2011). Furthermore, liquid-like adsorption water in potential subsurface habitats could support life (Möhlmann, 2005). In a previous study, *M. soligelidi* has shown high desiccation tolerance (Morozova and Wagner, 2007). During the three week long Martian thermo-physical simulation experiment done by Morozova et al. (2007), the water activity was varying between a_w 0.1 and 0.9 due to diurnal water vapor pressure fluctuations, which had no influence on the viability of *M. soligelidi* tested before and after the exposure. Other methanogenic strains, especially *Methanosarcina barkeri*, have also been shown to survive long term desiccation for more than 300 days (Kral et al., 2011). Hence, even solely periodically available liquid water does not rule out the possible existence of methanogens in the Martian subsurface and the permanent liquid phase during the presented simulation experiments is not necessarily a contradiction to Martian conditions.

As chemolithotrophic microorganisms, methanogenic archaea are able to grow with carbon dioxide and molecular hydrogen as the only carbon and energy sources. Therefore, we used a gas mixture of H_2/CO_2 (80:20% v/v) within the simulation, which serves as a good stoichiometric ratio for hydrogenotrophic methanogenesis ($4H_2+CO_2 \rightarrow CH_4+2H_2O$). Carbon dioxide is commonly distributed in the Martian atmosphere at about 95%, but hydrogen could be detected only in trace amounts (Oze and Sharma, 2005). On Earth, molecular hydrogen is not available in such high concentrations as it is not on Mars, but it has been reported to be dissolved in groundwater at mM concentrations for subsurface and deep subsurface microbial ecosystems (Sherwood Lollar et al., 2007). Not all of these habitats were characterized by hydrogen produced from geothermal activity, whose occurrence on Mars is uncertain (Krasnoplosky, 2006), but it was produced through alteration processes of basaltic crust in stable ancient Precambrian cratons, which serve as good Earth analogs for a single-plate planet like Mars (Sherwood Lollar et al., 2007). In the Martian subsurface, direct interaction with liquid interfacial water may also be a source of hydrogen due to the release of protons. Furthermore, it has been shown on Earth that with interspecies hydrogen transfer (e.g. Conrad and Babbel, 1989; Ishii et al., 2005), electrons (as hydrogen or formate) which are generated in one microorganism species, can be transferred to another. This might also be a possibility for methanogens in a potential Martian ecosystem to obtain hydrogen as an energy source in a hydrogen limited environment. In addition, methanogens on Earth are also able to substitute hydrogen with carbon monoxide (O'Brien et al., 1984), which has been detected in the Martian atmosphere in varying concentrations reaching from 400 to more than 2500 ppm (Sindoni et al., 2011). Accordingly, high concentrations of molecular hydrogen as substrate for methanogenesis are consistent with Martian subsurface simulation conditions.

5. Conclusion

We have shown that the developed simulation and measuring system is fully functional and ready to operate with Mars (subsurface) analog conditions. At temperatures down to −5 °C metabolic activity of the pure archaeon strain *Methanosarcina soligelidi* could be detected, which has not been reported for a single methanogenic strain before. This result and the findings of previous reported studies have shown that methanogenic archaea (especially those from permafrost environments) can be regarded as ideal model organisms for possible life on Mars. Although, they are able to survive the harsh thermo-physical conditions found on the Martian surface (Morozova et al., 2007) and also high doses of UV and ionizing radiation (comparable to *Deinococcus radiodurans*; Wagner et al., 2013) as they would occur on the surface of Mars, the subsurface seems to be a more suitable habitat due to more stable temperature conditions and the higher probability of liquid water. The conditions of potential Martian (deep) subsurface habitats were initially tested in our experiments. Further tests will be needed to check the potential of our model organism *Methanosarcina soligelidi* for methanogenic activity under simulated Martian shallow subsurface conditions. These can be conducted with presence of Mars analog soil simulates, under colder temperature, lower pressure and humidity, also over diurnal variations which are representative for other possible habitats on Mars.

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