Hydrogen Dynamics in Cyanobacteria Dominated Microbial Mats Measured by Novel Combined $H_2/H_2S$ and $H_2/O_2$ Microsensors

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Hydrogen may accumulate to micromolar concentrations in cyanobacterial mat communities from various environments, but the governing factors for this accumulation are poorly described. We used newly developed sensors allowing for simultaneous measurement of $H_2S$ and $H_2$ or $O_2$ and $H_2$ within the same point to elucidate the interactions between oxygen, sulfate reducing bacteria, and $H_2$ producing microbes. After onset of darkness and subsequent change from oxic to anoxic conditions within the uppermost $\sim$1 mm of the mat, $H_2$ accumulated to concentrations of up to 40 $\mu$mol L$^{-1}$ in the formerly oxic layer, but with high variability among sites and sampling dates. The immediate onset of $H_2$ production after darkening points to fermentation as the main $H_2$ producing process in this mat. The measured profiles indicate that a gradual disappearance of the $H_2$ peak was mainly due to the activity of sulfate reducing bacteria that invaded the formerly oxic surface layer from below, or persisted in an inactive state in the oxic mat during illumination. The absence of significant $H_2$ consumption in the formerly oxic mat during the first $\sim$30 min after onset of anoxic conditions indicated absence of active sulfate reducers in this layer during the oxic period. Addition of the methanogenesis inhibitor BES led to increase in $H_2$, indicating that methanogens contributed to the consumption of $H_2$. Both $H_2$ formation and consumption seemed unaffected by the presence/absence of $H_2S$.

Keywords: sulfate reducing bacteria, methanogens, survival, fermentation, sediment, microsensor

INTRODUCTION

Photosynthetic microbial mats are highly diverse communities both physiologically and phylogenetically. The mats are stratified communities where different functional groups exploit the microniches created by steep gradients in light and chemistry. They form on top of solid substrates where the photosynthetic primary producers fuel heterotrophic and chemolithoautotrophic bacteria. Environments with little or no disruption from benthic fauna are a prerequisite for formation of microbial mats, and they are thus best developed where fauna is absent such as in hypersaline and high-temperature environments (Stal, 1995; Fenchel, 1998; Bolhuis et al., 2014).

Photosynthetic microbial mats are autotrophic and thus reduce $CO_2$ to organic matter while oxidizing an electron donor. Under certain conditions they may, however, also produce $H_2$. Hydrogen production by photosynthetic bacteria has been reported numerous times (Gest et al., 1962; Benemann and Weare, 1974; Warthmann et al., 1992; Moezelaar and Stal, 1997; Hoehler et al., 2001; Burow et al., 2012; Lee et al., 2014; Nielsen et al., 2015), and such microbial $H_2$
production has recently gained considerable interest due to its potential use in renewable energy supply (Lee et al., 2010).

Photosynthetic bacteria can produce H$_2$ by three different pathways: as a by-product during nitrogen fixation (Benemann and Weare, 1974; Warthmann et al., 1992), by hydrogenases during anaerobic fermentation (Moezelaaar and Stal, 1997; Burow et al., 2012), and by photoproduction through the action of hydrogenases when there is an excess of energy (Warthmann et al., 1992; Appel et al., 2000). Hydrogen is a favorable electron donor for aerobic and anaerobic respiration as well as for anoxygenic photosynthesis (Drutschmann and Klemme, 1983; Hoehler et al., 1998). Even in anoxic environments the H$_2$ concentration is normally kept at few nanomolar levels due to the tight coupling between production and consumption (Lovley et al., 1982; Hoehler et al., 1998; Stams and Plugge, 2009).

A substantial H$_2$ accumulation has, however, been observed in the cyanobacterial layer of both hypersaline (Burow et al., 2012), marine (Nielsen et al., 2015) and hot spring (Anderson et al., 1987; Revsbech et al., 2016) microbial mats during dark incubations following periods with illumination. The H$_2$ production in photosynthetic mats has been suspected to be due to nitrogenase activity, but the H$_2$ production in hypersaline mats seems to be mainly due to fermentation, where the photosynthetic bacteria ferment the photosynthate fixed during the day as a nighttime metabolism (Hoehler et al., 2001; Burow et al., 2012; Lee et al., 2014). The simultaneous production of other fermentation products supports this hypothesis (Burow et al., 2012; Lee et al., 2014). Analysis of the gene expression of bidirectional hydrogenases pointed to cyanobacteria of the genus Microcoleus as the main hydrogenogens in these mats (Burow et al., 2012; Lee et al., 2014). Sulfate is a dominant terminal electron acceptor in marine sediment (Jørgensen, 1982) and the sulfate reducing bacteria Desulfovococcales have been identified as the dominant hydrogenotrophs in hypersaline microbial mats (Burow et al., 2014). In hypersaline and marine sediments sulfate reduction typically outcompetes methanogenesis due to high sulfate concentrations. Some methane production has been observed in saline photosynthetic mats (Hoehler et al., 2001), but methanogenesis appeared to be an unimportant H$_2$ sink as compared to sulfate reduction in a thoroughly investigated hypersaline mat (Burow et al., 2012, 2014). It is generally assumed that the upper limit for sulfate reduction and hence sulfide production is governed by the depth of O$_2$ penetration, although some reports suggest that sulfate reduction may occur under oxic conditions (Canfield and Des Marais, 1991; Fründ and Cohen, 1992). During the day when oxygenc photosynthesis is producing O$_2$, sulfate reduction will thus be confined to the deeper anoxic part of the sediment. After darkening when O$_2$ production is replaced with consumption, sulfide moves closer to the surface and may actually reach the very surface (Revsbech et al., 1983). Sulfate reducing bacteria may contain enzymes protecting them against reactive oxygen species, which reflect their frequent presence close to the oxic zone (Fournier et al., 2003; Dolla et al., 2006), and massive presence of especially Desulfomena has been observed in oxic mat layers sampled during illumination (Minz et al., 1999). Occurrence of nighttime sulfate reduction in layers that were oxic during the day may thus be due to either migration of sulfate reducing bacteria into the formerly oxic zone or resumption of activity by resident sulfate reducers. Tolerance to reactive oxygen species has also been found in methanogens (Horne and Lessner, 2013; Jasso-Chávez et al., 2015).

In this study the H$_2$ dynamics in a photosynthetic microbial mat were described by applying combined H$_2$ – H$_2$S and H$_2$ – O$_2$ microsensors, yielding fine scale measurements and aligned profiles in this system. The alignment of profiles measured during light-dark cycles allowed for accurate positioning of the H$_2$ producing layer relative to the photosynthetically active layer that was oxic during illumination, and also to the occurrence of free sulfide. The application of these sensors was essential, as previous data seem to show that the main H$_2$ producing zone in cyanobacterial mats is at the lower boundary or even below the photosynthetic zone (e.g., Figure 7 in Nielsen et al., 2015), and we hypothesized that this was due to erroneous alignment of profiles measured with different sensors.

**MATERIALS AND METHODS**

**Sampling Site**

Samples were collected from Løgstør Bredning in Limfjorden close to the village of Aggersund (57°00′04.4″N 9°17′18.6″E). The place is subject to changing water levels where the sediment may be either covered by water or exposed to air for hours or days. Due to these harsh conditions the mats can develop without being grazed by larger invertebrates. Sampling was done by coring with Plexiglas cylinders having inner diameters of 3.6 cm and a length of 8 cm. Sampling was done in November 2014, January 2015, June 2015, September 2016, and December 2016. During each sampling campaign ~10 samples were collected and selected cores with well-developed cyanobacterial communities were profiled.

Samples were kept moist and stored at 5°C under a halogen lamp (100 µmol photons m$^{-2}$ s$^{-1}$) with a timer mimicking a diurnal cycle until experiments could be conducted in an aquarium kept at 20°C. Experiments were conducted between 1 and 17 days after sampling.

**Sensors**

A combined H$_2$ and H$_2$S microsensor was applied for measuring H$_2$ and H$_2$S profiles in the mat. This type of sensor has been developed very recently (Maegaard et al., 2017, submitted) and allowed for perfectly aligned profiles of H$_2$ and H$_2$S, as both species enter the sensor through the same membrane-covered opening at the 20–50 µm wide tip. The physical design is identical to the STOX oxygen sensor (Revsbech et al., 2009), with a Clark-type H$_2$ microsensor (obtained from Unisense A/S) housed in an outer sensor capillary with a tip silicone membrane. A gold anode placed in front of the H$_2$ microsensor and bathed in a ferricyanide containing electrolyte (Jeroschewski et al., 1996) serves to quantify the H$_2$ signal. The H$_2$ part of the sensors applied had a sensitivity of 1.2–7.7 pA µmol$^{-1}$ L$^{-1}$ and the H$_2$S part of the sensors had a sensitivity...
of 16.3–32.5 pA µmol⁻¹ L⁻¹. The calibration of the sulfide part of the sensors was done in 0.1 M HCl where O₂ had been removed by flushing with N₂. A 20 mmol L⁻¹ Na₂S solution was prepared from a washed crystal of Na₂Sx9H₂O from which fresh calibration standards of 0, 50, 100, and 200 µmol L⁻¹ H₂S were made. No pH profiles were recorded in the mat, and the total pool of dissolved sulfide thus cannot be calculated. The pKa of H₂S is about 7 (varying somewhat with salinity (Millero et al., 1988) and the total pool of dissolved sulfide was thus substantially higher than the measured H₂S concentration. The calibration of the H₂ part of the sensor was performed in water from the sampling site to which demineralized water saturated with H₂ was added to final concentrations of 0, 50, 100, and 200 µmol L⁻¹. The concentration of the H₂ saturated stock solution was calculated according to Wiesenburg and Guinasso (1979) to contain 805 µmol H₂ L⁻¹. In addition to the combined H₂ and H₂S sensor, an O₂ microsensor (Revbsch, 1989) with a tip diameter of 10 µm was applied. The O₂ sensor was calibrated assuming O₂ saturation (252 µmol L⁻¹ at 20°C and 20% salinity) in the aerated aquarium. The second calibration point for the linear calibration curve was the zero reading obtained in deep anoxic sediment. Further, a combined H₂ and O₂ sensor was constructed according to the same principle as the combined H₂ and H₂S sensor, but where the H₂S anode and ferricyanide electrolyte were substituted with a gold O₂ cathode and bicarbonate electrolyte (Revbsch, 1989). The sensitivity was 5.7–21.8 pA µmol⁻¹ L⁻¹ for O₂ and 0.7–1.0 pA µmol⁻¹ L⁻¹ for H₂. The large current for O₂ is associated with a large stirring sensitivity, amounting to an about 10% signal difference between stirred and stagnant water. Oxygen data obtained with such sensors should thus not be used for calculation of fluxes and rates near mat-water interfaces. The H₂ and O₂ calibrations were performed as described above. Solubility of O₂ in water and diffusion coefficients for O₂, H₂ and H₂S in water were obtained from the tables at http://www.unisense.com/files/PDF/Diverse/Seawater & Gases table.pdf.

Profiling
The mat was placed in an aerated aquarium filled with seawater from the sampling site (20% salinity) with the temperature kept at 20°C. The water depth above the sample was ~2 cm. The depth position of the sensor tips was controlled by a motorized micromanipulator connected to a PC with the data acquisition software Sensor Trace Pro (Unisense A/S). The program also served to collect the A/D converted signals from the sensors. The signal currents from all types of sensors were measured by custom-made picoammeters connected to an 8-channel A/D converter (Unisense A/S). The sensor signals were recorded for every 50 µm depth interval. The recording of a H₂/H₂S profile took about 5 min and recording of a H₂/O₂ profile took about 15 min. The times for recording profiles mentioned in the following are for the start of a profile.

Experiments
The surface of the sediment cores was illuminated at an irradiance of 600 µmol photons m⁻² s⁻¹ in the 400–700 nm range using a halogen lamp equipped with a heat filter. This light intensity corresponds to about a third of maximum sunlight intensity at noon during mid-summer. For all H₂ accumulation experiments the samples received 3 h of light before onset of darkness. Profiles of O₂, H₂ and H₂S were recorded both during illumination and during darkness. O₂ profiles were only recorded at steady-state light and dark conditions, whereas combined H₂/H₂S profiles and H₂/O₂ profiles were measured after 3 h of illumination and at about 20 min intervals throughout the dark period following illumination. For clarity of illustration only some of the measured profiles are shown on figures.

Inhibition of Sulfate Reduction and Methanogenesis in Intact Sediment Core
Sulfate reduction was inhibited by addition of 10 mmol L⁻¹ Na₂MoO₄ during the illuminated period. The molybdate was allowed to diffuse into the mat for 2 h before illumination ceased. When the H₂ profile during the dark period was approaching a steady-state in the presence of molybdate, 20 mmol L⁻¹ 2-bromoethanesulfonic acid (BES) was added to inhibit methanogenesis (Scholten et al., 2000). Profiling ceased when a new steady state was approached.

Hydrogen Dynamics in Isolated Cyanobacterial Layer
To prevent migration of organisms from deeper anoxic layers to the photosynthetic zone of the mat during dark conditions, a thin slice of the mat was placed on top of seawater agar, and the mat was fastened with multiple glass needles. The about 1-mm thick mat slice was cut off the sediment core with a razor blade after 3 h of illumination. Profiling of this thin mat slice during illumination and darkness was performed as described above for the intact sediment core. The importance of sulfate reduction and methanogenesis in the separated mat was evaluated by adding sodium molybdate and BES as described above for the intact sediment core. In addition H₂ profiling was performed on a mat slice where molybdate was added when the H₂ concentration during dark incubation had decreased to about half of the maximum value.

Consumption of External Hydrogen during Illumination and Darkness
Hydrogen was added to the water phase by bubbling with a gas stream controlled by a gas mixer (Brooks Instruments 0260 with Thermal Mass Flow Controllers and Meter GF 40). Profiles were recorded during light and dark conditions until steady-state profiles were observed.

Calculations and Modeling
Profiles of reaction rates of O₂ and H₂ were obtained by use of the diffusion-reaction program "Profile" (Berg et al., 1998). The porosity in this type of mat can be assumed to be 0.75 according to Glud et al. (1995). We estimated the effective diffusion coefficient in the mat (Dₑ) assuming Dₑ = ϕ D (Berg et al., 1998), where ϕ is porosity and D the diffusion coefficient in water. For the values of D for O₂ and H₂ we used the Unisense table values of 2.0 and 3.9.10⁻² cm⁻² s⁻¹, respectively. The boundary conditions were a H₂ or O₂ flux of zero in the water column and below

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the consumption zone. Calculation of activity distributions from H₂ and O₂ profiles were only possible when the profiles were relatively stable, and we were thus unable to do calculations on data obtained just after a light-dark shift. The presence of iron minerals oxidizing and precipitating sulfide and the lack of pH data prevented modeling of H₂S production profiles. It should be stressed that the recording of a H₂ concentration profile required about 5 min, which further stresses the need of near-steady state of modeled profiles. Fick's first law of diffusion (Crank, 1975) was applied for calculation of water – mat diffusional exchange of O₂, assuming that the steepest gradient was in a loose surface layer with diffusion coefficient for O₂ close to the one in water.

RESULTS

Visual Impressions

The sampled mats were 1–2 mm thick rigid, dark-green structures formed on top of sandy sediment. Microscopic examination showed that Microcoleus sp. were dominating the oxygenic photosynthetic community, but other filamentous cyanobacteria and diatoms were present as well. The underlying sediment was black indicating sulfidic conditions. During illumination O₂ bubbles were formed on top of the mat. A white layer of colorless sulfur bacteria was observed at the surface after several hours of dark incubation, indicating that the O₂/H₂S interface was at the very surface of the mat (Jørgensen and Revsbech, 1983).

Oxygen Profiles during Light and Dark Conditions

Oxygen concentrations peaked at ~0.1 mm during illumination and O₂ penetrated to a depth of ~1 mm. Modeling of the profile shown in Figure 1 indicated a net O₂ production zone from 0 to 0.2 mm and a net consumption zone from 0.2 to 1.1 mm. The net O₂ production was calculated to be 0.59 nmol cm⁻² s⁻¹ and the net O₂ consumption below 0.2 mm depth was 0.17 nmol cm⁻² s⁻¹ (Figure 1). Oxygen profiles were measured at 3 additional positions of the mat, and all showed O₂ penetrations of 1.0–1.1 mm and comparable O₂ productions in the zones with net O₂ production. The exact figure for net O₂ production is, however, very dependent on the diffusivity at the mat/water interface. If we assume that the steepest O₂ gradient is in the overlying water, which will be true with a diffuse water-sediment interface, then the estimate of O₂ production in the zone with net production (Figure 1) increases to 0.79 nmol cm⁻² s⁻¹, and the average from all 4 profiles was 0.86 ± 0.16 (SD) nmol cm⁻² s⁻¹. After illumination ceased a new steady state O₂ profile was approached within 5 min, and O₂ penetrated to a depth of only ~0.2 mm. The modeled O₂ consumption in the zone from 0 to 0.2 mm was 0.17 nmol cm⁻² s⁻¹ (Figure 1). With a similar argumentation about the steepest gradient found in a layer with a diffusivity close to the one in water, then the estimate of total diffusive uptake increases to 0.25 ± 0.04 (SD, n = 4) nmol cm⁻² s⁻¹. It should be stressed that the relatively large differences between surface gradient and profile modeling obtained estimates of reaction rates are due to the ill-defined boundary between mat and water. Modeling of rates in deeper layers are not subject to a similar uncertainty.

Hydrogen and Hydrogen Sulfide Profiles under Dark Conditions

Many sets of combined H₂/H₂S profiles were measured in the Aggersund microbial mat, and an example is shown in Figure 2. The extent of H₂ accumulation was highly variable between samples with the maximum H₂ levels after darkening varying from 0 to 40 μmol L⁻¹ (average 12 ± 12 (SD, n = 13)]. We suspect that the variation was caused by differences in the species composition of the cyanobacterial community. The average depth of the H₂ peak was 0.5 ± 0.2 (SD, n = 12) mm. The average H₂ production rate was 0.031 ± 0.027 nmol cm⁻² s⁻¹ (SD, n = 6). Mats that appeared to be very similar could have very different levels of H₂ accumulation, but a prerequisite for substantial H₂ accumulation seemed to be a well-developed cyanobacterial community. The data in Figure 2 show H₂ accumulation higher than average, but the shapes and positions of the profiles are representative. After only 1 min of dark incubation H₂ concentrations of up to ~15 μmol L⁻¹ were observed in the formerly oxic layer of the mat (Figure 2A). Hydrogen sulfide was present from a depth of ~1.3 mm, and in this layer H₂ was consumed as well. Hydrogen and hydrogen sulfide profiles after 20 min of darkening are shown in Figure 2B. Here the H₂ accumulation peaked with a concentration of ~40 μmol L⁻¹ and H₂S was present from a depth of ~0.8 mm. Modeling of the H₂
profile showed a production zone from 0.5 to 1 mm and a consumption zone from 1 to 1.3 mm. The H₂ production in the zone with net production was 0.060 nmol cm⁻² s⁻¹ and the H₂ consumption in the layers with measurable H₂ and net consumption was 0.043 nmol cm⁻² s⁻¹, with the difference of 0.017 nmol cm⁻² s⁻¹ diffusing out of the mat. The peak H₂ concentrations had decreased to ~20 µmol L⁻¹ after 120 min of darkening, and H₂S was present at the surface of the mat (Figure 2C). Modeling of the H₂ profile now showed a production zone from 0.5 to 0.8 mm and a consumption zone from 0.8 to 0.9 mm, where the net H₂ production was 0.032 nmol cm⁻² s⁻¹ and the H₂ consumption was 0.023 nmol cm⁻² s⁻¹, respectively. At 120 min, 0.09 nmol H₂ cm⁻² s⁻¹ diffused out of the mat. After 240 min (Figure 2D), there was no H₂ accumulation and H₂S was present to the very surface of the mat.

**Overlap of H₂ and O₂ under Illumination and Darkness**

To investigate how O₂ affected H₂ metabolism, the mats were analyzed with combined H₂-O₂ microsensors. An example of perfectly aligned data for O₂ and H₂ obtained with such sensors is shown in Figure 3. During illumination, O₂ was produced in a zone from ~0-0.4 mm and consumed in a zone from ~0.4–1.0 mm, and no H₂ was detected (Figure 3). The set of profiles initiated 1 min after darkening showed that O₂ now only penetrated to ~0.25 mm, and H₂ was already accumulating with maximum concentrations of 26 µmol L⁻¹ (Figure 3). It is not possible to model the rates of H₂ metabolism as the data were obtained 1–5 min after darkening and thus are far from a steady state. However, as seen in Figure 2 the peak of H₂ occurs in the layer that was oxic during illumination, and the linear
H$_2$ profile through the ~0.25 mm thick oxic zone indicates no net transformation in this layer. Due to sulfide poisoning of the sensor it was not possible to get H$_2$ profiles for extended periods of dark incubation.

**Consumption of External Hydrogen during Light and Dark**

A series of experiments were conducted where H$_2$ was added to the overlying water, and an example of data from these experiments is shown in **Figure 4**. Illumination resulted in O$_2$ being produced in a zone from 0 to 0.55 mm at a rate of 0.94 nmol cm$^{-2}$ s$^{-1}$ and O$_2$ being consumed in a zone from 0.55 to 0.65 mm at a rate of 0.38 nmol cm$^{-2}$ s$^{-1}$. The main H$_2$ consumption zone was from 0.5 to 0.7 mm, and H$_2$ was consumed at a rate of 0.03 nmol cm$^{-2}$ s$^{-1}$ (**Figure 4**). There was no indication of any H$_2$ consumption in the oxic photosynthetic zone, but a high rate of H$_2$ consumption at the oxic-anoxic interface. This consumption could be due to both photosynthetic (Drutschmann and Klemme, 1985) and non-photosynthetic prokaryotes. Oxygen was consumed in a zone from 0 to 0.15 mm at a rate of 0.17 nmol cm$^{-2}$ s$^{-1}$ during darkness. Hydrogen was consumed down to 0.5 mm at a uniform low rate integrating up to 0.05 nmol cm$^{-2}$ s$^{-1}$ (**Figure 4**). It is not possible to judge if the oxic zone actually had lower activity than the anoxic zone, but modeling of the profile with zero activity in the oxic zone gave just as good a curve fit (data not shown) as the activity distribution shown in **Figure 4B**.

**Hydrogen Accumulation When Sulfate Reduction Was Inhibited by Molybdate and Methanogenesis Was Inhibited by BES**

Substantial H$_2$ concentrations persisted for a long time in the mat when sulfate reduction was inhibited by molybdate (**Figure 5**). After 120 min the H$_2$ accumulation thus peaked with a concentration of ~13 µmol L$^{-1}$. This concentration is much lower than the levels shown in **Figure 2**, but this may be due to a less developed mat. After 210 min the maximum H$_2$ concentration had decreased to ~6 µmol L$^{-1}$, and the methanogenesis inhibitor BES was added. Inhibition of methanogenesis resulted in the maximum H$_2$ concentration increasing to ~10 µmol L$^{-1}$. Hydrogen sulfide profiles are not shown since sulfide forms a complex with molybdate (Newport and Nedwell, 1988). A positive effect of BES on H$_2$ accumulation was also seen in two similar experiments with an average increase in maximum H$_2$ concentrations of 53 ± 9% (SD, n = 3).

**Hydrogen and Hydrogen Sulfide Profiles When Microbial Migration from Anoxic Layers to the Oxic Zone Was Prevented**

Hydrogen accumulation after darkening also occurred when the photosynthetically active part of the mat was placed on agar (**Figure 6A**). The H$_2$ accumulation peaked after ~30 min and H$_2$ was barely detectable after 450 min. No H$_2$S was detected at any time (data not shown). Oxygen profiles were measured at several locations and all data showed that the mat was oxic in all layers during the illumination but became anoxic below about 0.2 mm after 5 min of darkening (data not shown). In another experiment with the surface ~1 mm of the mat placed on agar, the H$_2$ accumulation peaked at ~15 µmol L$^{-1}$ after 20 min of darkness (**Figure 6B**). When the maximum concentration had decreased to ~4 µmol L$^{-1}$ at 165 min, molybdate was added to inhibit sulfate reduction. This resulted in a sizable stimulation of the H$_2$ accumulation so that a maximum concentration of 27 µmol L$^{-1}$ was reached. Sequential addition of inhibitor was also tested on a new slice of mat by adding molybdate 2 h before
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FIGURE 4 | \( \text{O}_2 \) and \( \text{H}_2 \) concentration profiles when \( \text{H}_2 \) was added to the water phase. (A) After 2 h of illumination, (B) after 2 h of dark incubation. Shown are measured \( \text{H}_2 \) concentrations (○), \( \text{O}_2 \) concentrations during illumination (□), \( \text{O}_2 \) concentration during darkness (⋄), modeled \( \text{H}_2 \) and \( \text{O}_2 \) concentrations (---), modeled \( \text{H}_2 \) production rate (green box diagram) and modeled \( \text{O}_2 \) production rate (red box diagram). Data obtained by combined \( \text{H}_2 – \text{O}_2 \) sensor.

FIGURE 5 | Hydrogen accumulation after darkening when sulfate reduction was inhibited with molybdate and after 210 min where methanogenesis was additionally inhibited by addition of BES. Time after darkening: 1 min (■), 120 min (●), 210 min (○) and 240 min (△). Data obtained by a combined \( \text{H}_2 – \text{H}_2\text{S} \) sensor.

darkening and adding BES after 210 min of darkening. The maximum level of \( \text{H}_2 \) of 13 \( \mu \text{mol L}^{-1} \) in the molybdate inhibited mat occurred after 20 min of darkening, after which \( \text{H}_2 \) decreased to a maximum of 5 \( \mu \text{mol L}^{-1} \) at 195 min. When BES was added, it led to an increase of the maximum \( \text{H}_2 \) concentration to 15 \( \mu \text{mol L}^{-1} \) at 315 min (data not shown).

DISCUSSION

When comparing stratification of chemical species and types of metabolism in compact microbial communities it is very important to align profiles obtained by different methods at the same depth scale. Various procedures have been used to get a reasonable alignment of microsensor profiles, including gluing sensors together with tips very close to each other (e.g., Revsbech et al., 1983) and visual determination of “surface” by observing the individual sensor tips during insertion. Until now only the combined \( \text{O}_2 – \text{N}_2\text{O} \) sensor (Revsbech et al., 1988) has allowed for simultaneous measurement of two gasses in the same point. We here present the first comprehensive study of \( \text{H}_2 \) metabolism in a microbial mat where we have totally aligned profiles of \( \text{H}_2 – \text{H}_2\text{S} \) and \( \text{H}_2 – \text{O}_2 \) so that interactions between \( \text{H}_2, \text{O}_2 \) and sulfur cycling can be precisely evaluated.

Position of \( \text{H}_2 \) Producing Layer

The microbial mat was oxic down to about 1 mm during illumination, but after darkening \( \text{O}_2 \) only penetrated to about 0.2 mm. When darkened, \( \text{H}_2 \) accumulated only within the formerly oxic zone of the intact sediment cores, where photosynthates formed during illumination were available for fermentation. The zone of net \( \text{H}_2 \) formation as measured by the combined \( \text{H}_2 – \text{H}_2\text{S} \) sensor was in anoxic layers below 0.4–0.5 mm depth and thus relatively far below the depth of maximum net \( \text{O}_2 \) production as measured by a separate \( \text{O}_2 \) sensor. When the combined \( \text{H}_2 – \text{O}_2 \) sensor was used
we observed, on the other hand, a perfect alignment between the concentration peak of H$_2$ and photosynthetic layers below 0.2 mm that became anoxic by darkening. It thus seems that our surface definition was about 0.2 mm wrong when we estimated the surface from combined H$_2$-H$_2$S data. This illustrates the problems of aligning profiles from separate sensors and the strength of using sensors with dual chemical sensing as used here. By use of the two types of sensors with dual chemical sensing it was possible to determine at very high resolution how H$_2$ production and consumption were associated with the presence of both O$_2$ and H$_2$S. The problem of aligning profiles from different sensors was in our case probably aggravated by the very different tip shapes and dimensions of O$_2$ and combined sensors, where the O$_2$ sensor had a slimline-tip of 10 µm and the combined sensors had blunt tips of 50 µm. Insertion of a microsensor changes the flow pattern above the mat and also the thickness of the diffusive boundary layer (Glud et al., 1994), and this effect is very dependent on sensor dimensions and shape.

**Position of H$_2$ Consuming Layers**

The results shown in **Figures 3, 4** were obtained by use of combined H$_2$-O$_2$ sensors. Microbial mat overlaid with air-saturated water was analyzed in **Figure 3**, and the dark profile initiated 1 min after darkening showed no sign of H$_2$ consumption in the 0.2-mm thick oxic surface layer. Further proof for the very low H$_2$ consumption potential is provided by the data in **Figure 4**, obtained after saturating the overlying water with a mixture of ~10% H$_2$ gas and air. No H$_2$ consumption could be seen in the oxic 0.7-mm layer of the illuminated mat (**Figure 4A**). When the mat had been dark incubated for 2 h there was a uniform low rate of H$_2$ consumption throughout the formerly oxic zone (**Figure 4B**). These data are important as it has previously been suggested that sulfate reduction may occur at high rates in oxic cyanobacterial mats (Canfield and Des Marais, 1991). The absence of any significant H$_2$ oxidation potential in oxic mat counter-indicates that there are active sulfate reducers in the oxic mat, as sulfate reducing bacteria generally use H$_2$ as electron donor (Jørgensen, 1982).

The data obtained with combined H$_2$ – H$_2$S sensors shortly after darkening, but sufficiently long after darkening to approach diffusional equilibrium, all have almost linear profiles between the H$_2$ production zone and the overlying water (e.g., **Figure 2B**). This indicates that not only oxic mat layers, but also mat layers that have a recent history of being oxic are characterized by very little H$_2$ consumption as illustrated by **Figure 4B**. A low H$_2$ oxidation potential of oxic cyanobacterial mats was also suggested by Hoehler et al. (2002).

A high H$_2$ consumption potential was found in the layer that formed the oxic-anoxic interface during illumination (**Figure 2**). This indicates that anaerobic bacteria are the main hydrogenotrophs in the mat, and this is in accordance with the findings of Lee et al. (2014) who identified sulfate reducing bacteria as the main hydrogen consumers. The zone of intense H$_2$ consumption moved 0.2 mm upward from 20 to 120 min after darkening (**Figures 2B,C**), indicating that the anaerobic bacteria were able to migrate. At 120 min there was also a low rate of H$_2$ consumption in the surface layers that were now highly sulfidic, indicating that some anaerobic bacteria had reached these layers by migration, or that dormant bacteria permanently inhabiting these layers had become active. The presence of sulfate reducing bacteria in oxic microbial mat has previously been reported by Minz et al. (1999). The question of migration of bacteria versus revival of inactive hydrogenotrops in the layer that was oxic during the light period was addressed by slicing off the top 1 mm and placing the slice on agar (**Figures 6A,B**). When molybdate was added to the sliced mat after 165 min of dark incubation...
(Figure 6B) there was a rapid and significant increase in \( H_2 \) concentration, indicating that resident sulfate reducing bacteria had become active although all layers of the mat slice were oxic during the preceding light period. The effect of molybdate could also be explained by change in the fermentative pattern, but we are not aware of reports indicating such an effect of molybdate. So after >1 h of dark incubation it seems that both migration from below and revival of resident anaerobic hydrogenotrophic microorganisms caused hydrogen consumption in the surface layers of intact mats.

**Sulfate Reduction versus Methanogenesis as \( H_2 \) Sinks**

Previous investigations (Lee et al., 2014; Nielsen et al., 2015) indicate that sulfate reduction is the major hydrogenotrophic process in dark-incubated cyanobacterial mats, although methanogenesis may also occur in saline environments (Oremland and Taylor, 1978). We investigated the possible role of methanogens as hydrogenotrophs in the mat by sequentially adding molybdate and BES to both intact (Figure 5) and sliced mat (data not shown). Data from experiments with addition of inhibitors should always be interpreted with care as no inhibitor is totally specific. Molybdate thus complexes with \( H_2S \) (and thereby lowers the \( H_2S \) activity) and it also forms complexes with volatile fatty acids (VFAs) (Finke et al., 2007). BES addition caused a significant increase in \( H_2 \) concentration, indicating that significant numbers of active methanogens were present. As expected the effect of BES was smaller than the effect of molybdate, in accordance with the conclusions of Lee et al. (2014) that sulfate reducers are the main hydrogenotrophs in such mats. The high \( H_2 \) concentrations in a dark-incubated mat might, however, make methanogenesis locally and temporally favorable (Hoehler et al., 2002). The effect of BES in the sliced mat provides evidence that methanogens like sulfate reducers could survive long periods of oxic conditions in the mat, as previously described for a hot spring microbial mat (Revsbech et al., 2016) and soils (Angel et al., 2012).

**Absence of \( H_2S \) from Sliced Mat**

It should be noticed that sulfide did not accumulate in the sliced 1-mm mat layer during the dark incubation (Figures 6A,B). That might seem to counter-indicate \( H_2 \) consumption by sulfate reduction, suggested by the increase in \( H_2 \) after addition of molybdate, but the data in Figure 2 may offer an explanation. Here \( H_2S \) was found in high concentrations in layers deeper than 1.1 mm after 1–6 min (the time to record a profile was about 5 min) of darkness, and the situation was basically unchanged after 20–26 min of darkness. The mean diffusion time for a \( H_2S \) molecule over a distance of 1 mm is less than 10 min, assuming a diffusion coefficient of \( 1 \times 10^{-5} \) cm\(^2\)s\(^{-1}\) within the mat (Jørgensen, 2000). By diffusion alone the whole mat should thus have been highly sulfidic already after a few minutes, and that points to extensive sulfide consumption within the previously oxic mat. The consumption of free sulfide may be due to precipitation and oxidation by iron and manganese minerals. The buffer capacity of the iron pool should be very high if it could consume the high sulfide flux from below in the intact mat for more than 20 min. The iron pool might thus be able to keep an isolated 1-mm top layer free of dissolved sulfide for a long time. An alternative explanation for the absence of free sulfide is oxidation by cable bacteria (Pfeffer et al., 2012). The mats from Aggersund have been found to be rich in cable bacteria (L.P. Nielsen, unpublished), and these may be able to oxidize \( H_2S \) within the upper few mm of the mat by transporting the electrons to the surface of mat. In intact mat they may not be able to oxidize all the sulfide diffusing up from below, but the much lower amount formed in a thin slice might all be oxidized.

**Rate of \( H_2 \) Formation as Compared to Net Photosynthesis**

The rates of net \( H_2 \) production deduced from Figure 2B were 0.06 nmol cm\(^{-2}\) s\(^{-1}\) when they were at the highest. This is about 10% of the rate of net \( O_2 \) production in the same mat (Figure 1). Even in the presence of molybdate there is some decrease in \( H_2 \) production with time (Figure 5), but the illumination periods were also limited, so the figures are roughly comparable. One \( O_2 \) corresponds to two \( H_2 \) molecules in terms of electron transfer, so about 5% of the reducing equivalents stored in organic compounds during the light period were liberated as \( H_2 \). Fermentation of glucose by a *Clostridium* that was studied because of a high rate of \( H_2 \) formation found to yield up to 2 moles of \( H_2 \) per mole of glucose (Jo and Kim, 2016). Assuming a loss of glucose equivalents as fast as the rate of build-up during photosynthesis, the 0.6–0.9 nmol \( O_2 \) cm\(^{-2}\) s\(^{-1}\) corresponds to a formation rate of 0.2–0.3 nmol \( H_2 \) cm\(^{-2}\) s\(^{-1}\). The loss rate of glucose in the dark should actually be lower than the rate of glucose production by photosynthesis in the light, and we thus also see only 20–30% of the \( H_2 \) production calculated above. The mass balance considerations do indicate a substantial loss of photosynthates by fermentation during the dark period. This is in accordance with previous estimates of extreme rates of sulfate reduction is such mats (Jørgensen and Cohen, 1977), as the sulfate reducing bacteria utilize the fermentation products.

Based on our data we cannot rule out that there was \( H_2 \) production due to nitrogenase activity, which would invalidate the \( H_2 \) budget outlined above. However, Burow et al. (2012) and Lee et al. (2014) found no effect on \( H_2 \) accumulation in a similar mat when nitrogen fixation was inhibited. Both studies observed a production of other fermentation products as well as \( H_2 \), which support fermentation and not nitrogen fixation as the \( H_2 \) source. The immediate rise in \( H_2 \) after darkening observed in our study also points to fermentation as the major \( H_2 \) forming process, as *de novo* synthesis of the \( O_2 \) sensitive nitrogenase would require some time.

**CONCLUSION**

The analyzed microbial mat produced high concentrations of \( H_2 \) by fermentation after onset of darkness. The layers that had the highest photosynthetic activity in the light and became anoxic in the dark had the highest rate of \( H_2 \) production. Results from
addition of inhibitors suggest that sulfate reducing bacteria were the main hydrogenotrophs of the mat, but that methanogenic archaea also contributed to the disappearance of the H\textsubscript{2} peak. Both sulfate reducing bacteria and methanogens were apparently able to survive the high O\textsubscript{2} concentrations within the mat during illumination and become active again within less than 2 h of anoxia. Oxic conditions in the mat during illumination resulted in low H\textsubscript{2} consumption potential, and this low potential persisted at least 30 min after anoxia was reached during subsequent darkening. This indicates that the anaerobes in the mat could survive the oxic conditions during illumination, but in a dormant stage.

**AUTHOR CONTRIBUTIONS**

KM did all measurements and was leading in the writing of the manuscript. NR supervised the study, was active in the development of new sensors, and participated in the writing of the manuscript. LN co-supervised the study and participated in the writing of the manuscript.

**REFERENCES**

Anderson, K. L., Tayne, T. A., and Ward, D. M. (1987). Formation and fate of fermentation products in hot spring cyanobacterial mats. *Appl. Environ. Microbiol.* 53, 2343–2352.

Angel, R., Claus, P., and Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J.* 6, 847–862. doi: 10.1038/isme.2011.141

Appel, J., Phunpruch, S., Steinmüller, K., and Schulz, R. (2000). The bidirectional hydrogenase of *Synechocystis* sp. PCC 6803 works as an electron valve during photosynthesis. *Arch. Microbiol.* 173, 333–338. doi: 10.1007/s002030000139

Benemann, J. R., and Weare, N. M. (1974). Hydrogen evolution by nitrogen-fixing *Anabaena cylindrica* cultures. *Science* 184, 174–175. doi: 10.1126/science.184.4133.174

Berg, P., Risagaard-Petersen, N., and Rysgaard, S. (1998). Interpretation of measured concentration profiles in sediment pore water. *Limnol. Oceanogr.* 43, 1500–1510. doi: 10.4319/lo.1998.43.7.1500

Bolhuis, H., Cretoiu, M. S., and Stal, L. J. (2014). Molecular ecology of microbial mats. *FEMS Microbiol. Ecol.* 90, 335–350. doi: 10.1111/1574-6941.12408

Burow, L. C., Woebken, D., Bebout, B. M., McMurdie, P. J., Singer, S. W., Pett-Ridge, J., et al. (2012). Hydrogen production in photosynthetic microbial mats in the Elkhorn Slough estuary, Monterey Bay. *Appl. Environ. Microbiol.* 82, 1464–1471. doi: 10.1128/AEM.02240-12

Burow, L. C., Woebken, D., Marshall, I. P. G., Singer, S. W., Pett-Ridge, J., Prufer-Bebout, L., et al. (2014). Identification of Desulfobacterales as primary hydrogenotrophs in a complex microbial mat community. *Geomicrobiology* 12, 221–230. doi: 10.1111/gbi.12080

Canfield, D. E., and Des Marais, D. J. (1991). Aerobic sulfate reduction in microbial mats. *Science* 251, 1471–1473. doi: 10.1126/science.11538266

Crank, J. (1975). *The Mathematics of Diffusion*. Oxford: Oxford University Press.

Dolla, A., Fournier, M., and Dermon, Z. (2006). Oxygen defense in sulfate-reducing bacteria. *J. Biotechnol.* 126, 87–100. doi: 10.1016/j.jbiotec.2006.03.041

Drutschmann, M., and Klemme, J.-H. (1985). Sulfide-repressed, membrane-bound hydrogenase in the thermophilic facultative phototroph, *Chloroflexus aurantiacus*. *FEMS Microbiol. Lett.* 28, 231–235. doi: 10.1111/j.1574-6968.1985.tb00797.x

Fenchel, T. (1998). Formation of laminated cyanobacterial mats in the absence of benthic fauna. *Aquat. Microb. Ecol.* 14, 235–240. doi: 10.3354/ame14235

Finke, N., Vandeneken, V., and Jørgensen, B. B. (2007). Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. *FEMS Microbiol. Ecol.* 59, 10–22. doi: 10.1111/j.1574-6941.2006.00214.x

Fournier, M., Zhang, Y., Wildschut, J. D., Dolla, A., Voordouw, J. K., Schriemer, D. C., et al. (2003). Function of oxygen resistance proteins in the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *J. Bacteriol.* 185, 71–79. doi: 10.1128/JB.185.1.71-79.2003

Fründ, C., and Cohen, Y. (1992). Diurnal cycles of sulphate reduction under oxic conditions in cyanobacterial mats. *Appl. Environ. Microbiol.* 58, 70–77.

Gest, H., Ormerod, J. G., and Ormerod, K. S. (1962). Photometabolism of *Rhodospirillum rubrum*: light-dependent dissimilation of organic compounds to carbon dioxide and molecular hydrogen by an anaerobic citric acid cycle. *Arch. Biochem. Biophys.* 97, 21–33. doi: 10.1016/0003-9861(62)90039-5

Glud, R. N., Gundersen, J. K., Revsbech, N. P., and Jørgensen, B. B. (1994). Effects on the benthic diffusive boundary layer imposed by microelectrodes. *Limnol. Oceanogr.* 39, 462–467. doi: 10.4319/lo.1994.39.2.0462

Glud, R. N., Jensen, K., and Revsbech, N. P. (1995). Diffusivity in surficial sediments and benthic mats determined by use of a combined N\textsubscript{2}O-O\textsubscript{2} microsensor. *Geochim. Cosmochim. Acta* 59, 231–237. doi: 10.1016/0016-7037(94)00321-C

Hoehler, T. M., Albert, D. B., Alperin, M. I., Bebout, B. M., Martens, C. S., and Des Marais, D. J. (2002). Comparative ecology of H\textsubscript{2} cycling in sedimentary and phototrophic ecosystems. *Antonie Van Leeuwenhoek* 81, 575–585. doi: 10.1023/A:1020517924466

Hoehler, T. M., Alperin, M. I., Albert, D. B., and Martens, C. S. (1998). Thermodynamic control on hydrogen concentrations in anoxic sediments. *Geochim. Cosmochim. Acta* 62, 1745–1756. doi: 10.1016/S0016-7037(97)00106-9

Hoehler, T. M., Bebout, B. M., and Des Marais, D. J. (2001). The role of microbial mats in the production of reduced gases on the early Earth. *Nature* 412, 324–327. doi: 10.1038/35085554

Horne, A. J., and Lessner, D. J. (2013). Assessment of the oxidant tolerance of *Methanosarcina acetivorans*. *FEMS Microbiol. Lett.* 343, 13–19. doi: 10.1111/1574-6968.12115

Jasso-Chávez, R., Santiago-Martínez, M. G., Lira-Silva, E., Pineda, E., Zepeda-Rodiguez, A., Belmont-Díaz, J., et al. (2015). Air-adapted *Methanosarcina acetivorans* shows high methane production and develops resistance against oxygen stress. *PLOS ONE* 10:e0117331. doi: 10.1371/journal.pone.0117331

Jeroschewski, P., Steuckart, C., and Kühl, M. (1996). An amperometric microsensor for determination of H\textsubscript{2}S in aquatic environments. *Anal. Chem.* 24, 4351–4357. doi: 10.1021/ac96091b

Jo, J. H., and Kim, W. (2016). Carbon material distribution and flux analysis under varying glucose concentrations in hydrogen-producing *Clostridium tyrobutyricum* JMt. *J. Biotechnol.* 228, 103–111. doi: 10.1016/j.jbiotec.2016.04.051

Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed – The role of sulphate reduction. *Nature* 296, 643–645. doi: 10.1038/296643a0

Jørgensen, B. B. (2000). “Bacteria and marine biogeochemistry,” in *Marine Geochemistry*, eds H. D. Schulz and M. Zabel (Heidelberg: Springer), 169–206.

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Jørgensen, B. B., and Cohen, Y. (1977). Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. *Limnol. Oceanogr.* 22, 657–666. doi: 10.4319/lo.1977.22.4.0657

Jørgensen, B. B., and Revsbech, N. P. (1983). Colorless sulfur bacteria, *Beggiaota* spp. and *Thiothrix* spp., in O₂ and H₂S microenvironments. *Appl. Environ. Microbiol.* 45, 1261–1270.

Lee, H., Vermaas, W. F. J., and Rittmann, B. E. (2010). Biological hydrogen production: prospects and challenges. *Trends Biotechnol.* 28, 262–271. doi: 10.1016/j.tibtech.2010.01.007

Lee, J. Z., Burow, L. C., Woebken, D., Everroad, R. C., Kubo, M. C., Spormann, A. M., et al. (2014). Fermentation couples *Chloroflexi* and sulfate-reducing bacteria to *Cyanobacteria* in hypersaline microbial mats. *Front. Microbiol.* 5:61. doi: 10.3389/fmicb.2014.00061

Lovley, D. R., Dwyer, D. F., and Klug, M. J. (1982). Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Appl. Environ. Microbiol.* 43, 1373–1379.

Millero, F. J., Plese, T., and Fernandez, M. (1988). The dissociation of hydrogen-sulfide in seawater. *Limnol. Oceanogr.* 33, 269–274. doi: 10.1002/es402956r

Minz, D., Fishbain, S., Green, S. J., Muyzer, G., Cohen, Y., Rittmann, B. E., et al. (1999). Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. *Appl. Environ. Microbiol.* 65, 4659–4665.

Moexlara, R., and Stal, L. (1997). A comparison of fermentation in the cyanobacterium *Microcystis* PCC7806 grown under a light/dark cycle and continuous light. *Eur. J. Phycol.* 32, 373–378. doi: 10.1080/09670269701001737309

Newport, P. J., and Nedwell, D. B. (1988). The mechanisms of inhibition of *Desulfovibrio* and *Desulfotomaculum* species by selenate and molybdate. *J. Appl. Bacteriol.* 65, 419–423. doi: 10.1111/j.1365-2672.1988.tb01911.x

Nielsen, M., Revsbech, N. P., and Kühl, M. (2015). Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. *Front. Microbiol.* 6:276. doi: 10.3389/fmicb.2015.00276

Oremland, R. S., and Blackburn, T. H. (1983). Microelectrode studies of the photosynthesis and O₂, H₂S, and pH profiles of a microbial mat. *Limnol. Oceanogr.* 28, 1062–1074. doi: 10.4319/lo.1983.28.6.1062

Revsbech, N. P., Larsen, L. H., Gundersen, J., Dalgaard, T., Ulloa, O., and Thamdrup, B. (2009). Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. *Limnol. Oceanogr. Methods* 7, 371–381. doi: 10.4319/lo.2009.7.371

Revsbech, N. P., Nielsen, L. P., Christensen, P. B., and Sørensen, J. (1988). Combined oxygen and nitrous oxide microsensor for denitrification studies. *Appl. Environ. Microbiol.* 54, 2245–2249.

Revsbech, N. P., Trampe, E., Lichtenberg, M., Ward, D. M., and Kühl, M. (2016). In situ hydrogen dynamics in a hot spring microbial mat during a diel cycle. *Appl. Environ. Microbiol.* 82, 4209–4217. doi: 10.1128/AEM.00710-16

Scholten, J. C., Conrad, R., and Stams, A. J. (2000). Effect of 2-bromo-ethane sulfonate, molybdate and chloroform on acetate consumption by methanogenic and sulfate-reducing populations in freshwater sediment. *FEMS Microbiol. Ecol.* 32, 35–42. doi: 10.1111/j.1574-6941.2000.tb00696.x

Stal, L. (1995). Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytol.* 131, 1–32. doi: 10.1111/j.1469-8137.1995.tb03051.x

Stams, A. J. M., and Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat. Rev. Microbiol.* 7, 568–577. doi: 10.1038/nrmicro2166

Warthmann, R., Cypionka, H., and Pfennig, N. (1992). Photoproduction of H₂ from acetate by syntrophic cocultures of green sulfur bacteria and sulfur-reducing bacteria. *Arch. Microbiol.* 157, 342–348. doi: 10.1007/BF00248679

Wiesenburg, D. A., and Guinasso, N. L. (1979). Equilibrium solubilities of methane, carbon-monoxide, and hydrogen in water and sea-water. *J. Chem. Eng. Data* 24, 356–360. doi: 10.1021/je50083a006

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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