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Production and Consumption of Hydrogen in a Eutrophic Lake

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The vertical distribution of hydrogen was measured in the Loclat, a eutrophic and holomictic lake near Neuchâtel, Switzerland, before and during summer stratification. H_2 concentrations decreased with depth in the anaerobic hypolimnion and were often below the detection limit (2.5 nl of H_2 liter^{-1}) in the water adjacent to the lake sediment. H_2 was apparently not released from the lake sediment. The highest H_2 concentrations (>4 μl of H_2 liter^{-1}) were observed in the aerobic water of the epilimnion and metalimnion. There, the H_2 concentrations changed with time, indicating a turnover of H_2. The H_2 production processes could not be studied in the laboratory since incubation of water samples in light or darkness did not result in H_2 production but rather always in H_2 consumption. The possible role of cyanobacteria and algae for H_2 production is discussed. Aerobic or anaerobic H_2 consumption activities were observed at all depths of the water column, with highest activities in the hypolimnion. Aerobic H_2 consumption activity was insensitive to azide inhibition, but sensitive to heat, mercuric chloride, or cyanide. It was restricted to a particle fraction of 0.2 to 3.0 μm in size, so that it must be due to single bacterial cells. Aerobic hydrogen bacteria, on the other hand, occurred in clusters of >3.0 μm. Therefore, the hydrogen bacteria could not have caused the H_2 consumption in lake water. The aerobic H_2 consumption activity followed Michaelis-Menten kinetics, with a K_m of 67 nM H_2. This is an exceptionally low value compared with K_m values of hydrogenases in hydrogen bacteria and other species, but is similar to that for H_2-decomposing abiontic soil hydrogenases.

Hydrogen is an important intermediate in the mineralization process of organic matter under anaerobic conditions. Protons are used as an electron sink in the metabolism of many anaerobic bacteria releasing H_2 (14). However, H_2 partial pressures higher than 10 Pa usually do not establish in balanced systems for thermodynamic reasons (43), since otherwise the catabolism of the obligate proton-reducing microorganisms (5, 22, 23) would be inhibited. This would break off the complete mineralization of organic matter in anaerobic lake sediment, which is deficient in alternative electron acceptors such as nitrate or sulfate. H_2 partial pressure is usually kept below 10 Pa by the action of methanogenic or sulfate-reducing bacteria (21, 41–43) which utilize the H_2 as electron donor and produce methane and hydrogen sulfide. These gases diffuse from the lake sediment upwards until reaching conditions where they can be oxidized. In the case of H_2S, this is either the water depth to which light can penetrate, enabling the oxidation of H_2S by anoxygenic phototrophic green or purple sulfur bacteria (24–26), or the water depth were dissolved oxygen is available for the chemical oxidation of H_2S or the development of H_2S-oxidizing chemolithoautotrophic bacteria (13). In the case of CH_4, this is the water depth where enough oxygen is available for the oxidation of CH_4 by aerobic methanotrophic bacteria (16, 29, 30). It is believed that the fate of hydrogen eventually left over by methanogens and sulfate reducers is analogous to that of methane: it is generally assumed that H_2 diffuses from the lake sediment to the aerobic metalimnion, where it is consumed by aerobic hydrogen bacteria (knallgas bacteria) (2, 7). This theory of H_2 distribution in lakes has been presented in textbooks (3, 19) and was supported indirectly by the observations of Schweizer and Aragno (35), who found relatively high numbers of hydrogen bacteria in the metalimnion of a eutrophic lake. Up to date, however, this view of H_2 distribution in lakes has not been confirmed by direct measurements of H_2 concentrations in the water column because the expected H_2 concentrations are below the detection limit of generally available gas

chromatographic techniques that use hot-wire detectors.

We report on measurements of dissolved H_2 in the Loclat, a small eutrophic lake, by using a technique that allows the detection of H_2 concentrations as low as 2.5 nl of H_2 liter of water^{-1} (0.1 nM H_2), which is equivalent to an H_2 partial pressure of ca. 0.01 Pa.

MATERIALS AND METHODS

H_2 concentrations were measured in the Loclat or Lac de Saint-Blaise, a small eutrophic lake situated northeast of Lac de Neuchâtel, Neuchâtel, Switzerland, with a surface of ca. 4.5 ha and a maximum depth of 9.7 m. The Loclat has been studied before as to its population of aerobic hydrogen bacteria (35).

Water samples were taken in the middle of the lake at its deepest point. The water was sampled from different depths by means of a Niskin sampler and immediately transferred into 100-ml glass flasks which were completely filled. Metabolic activities were stopped by adding $HgCl_2$ solution to a final concentration of 50 μg ml^{-1}, which turned out to be sufficient for complete inhibition of H_2 consumption even in the presence of H_2S. The flasks were stored in darkness and transported to the laboratory for analysis of H_2 concentration within 2 h. The procedure for H_2 analysis was the same as for the analysis of dissolved CO and has been described in detail by Conrad and Seiler (12). Briefly, about half of the water within a glass flask was replaced by H_2-free synthetic air. The flask was then vigorously shaken to establish an equilibrium between the H_2 in the gas and liquid phases. Due to the low solubility of H_2 in water, more than 97% of the dissolved H_2 was recovered in the gas phase. The H_2 mixing ratio in the gas phase was then analyzed in an H_2 analyzer based on the HgO-to-Hg vapor conversion technique (36, 37), and the concentration of H_2 in the water was calculated from the H_2 mixing ratio in the gas phase and the volumes of the water and gas phases. The described method allowed the detection of 2.5 nl of H_2 liter of water^{-1} (0.1 nM H_2).

Incubation experiments were carried out in completely filled glass flasks (100 ml). $HgCl_2$ solution was added at the end of the incubation period. The flasks were incubated at 8 to 12°C in March and at 18 to 23°C in July. The water samples were either incubated in darkness or exposed to ambient daylight intensities of ca. 10 to 40 klx. In one experiment they were incubated in a light incubator with Gryolux fluorescent tubes (Memmert, Schwalbach, FRG) at 750 lx. Light intensity was measured with a Profisix exposure meter (Gossen, Erlangen, FRG). Water samples were filtered through Nuclepore filters with a pore size of 3.0 μm and subsequently through filters with a pore size of 0.2 μm. The filtered water samples were flushed with H_2-free synthetic air and distributed into sterilized glass flasks (100 ml) which were completely filled. The H_2 concentration in the flasks was adjusted by injecting 0.1 to 1.0 ml of sterile water, which had been equilibrated with a gas atmosphere containing 0.1 to 10% H_2. NaN_3, NaCN, or $HgCl_2$ was added by injecting 1 ml of a solution to give a final concentration of 50 μg ml^{-1}.

Temperature profiles in the lake were measured from the boat by using a TTM 72 temperature probe (Züllig, Rheineck, Switzerland). Oxygen was measured by the standard Winkler method. The redox potential of the water was immediately measured after sampling by means of an Eh electrode (Knick, Berlin, FRG). H_2S was qualitatively detected by the precipitation of HgS after addition of an $HgCl_2$ solution to the water sample. CH_4 was detected as an additional peak in the chromatogram from the H_2 analysis. The detection limit of CH_4 was ca. 1.5 μM CH_4.

Aerobic hydrogen bacteria were counted in water samples from different depths after transport of the samples to the laboratory under aseptic conditions. Aliquots, 2 and 10 ml, were filtered through 0.45-μm cellulose nitrate filters (Millipore Corp., Bedford, Mass.), which were then placed on agar plates with mineral medium (32) and incubated in jars containing 5 kPa of O_2, 10 kPa of CO_2, and 75 kPa of H_2 at 27°C for 2 weeks. The filters with visible colonies were then placed on filter paper disks, which were soaked with a solution of 0.1% triphenyltetrazolium chloride and incubated under pure H_2 for 30 min. Colonies which were hydrogenase positive turned red (2). The red colonies were discriminated into "normal" hydrogen bacteria giving colonies larger than 1 mm in diameter and "pinpoint" bacteria giving smaller colonies. The counting procedure was carried out in a parallel set by filtering 10-ml aliquots of water samples through 3.0-μm filters.

Aerobic hydrogen bacteria were also counted by means of a most-probable-number technique. Culture tubes with 3 ml of mineral medium were inoculated in five parallel sets with 1-ml aliquots of serial dilutions of the water samples. The tubes were incubated in jars containing 5 kPa of O_2, 10 kPa of CO_2, and 75 kPa of H_2 at 27°C for 2 weeks. Tubes with a visible turbidity as compared with an uninoculated control were counted as positive. The most probable number was calculated by using McCrady tables (27).

Other microorganisms were counted by filtering water samples through 0.45-μm Millipore filters. The filters were dried at room temperature and then bedded in immersion oil to make the filters translucent. The microorganisms retained on the filter were counted under a microscope.

The synthetic air (80% N_2 + 20% O_2) was obtained from Carba (Liebefeld, Switzerland) and was purified from traces of H_2 by passing the gas stream through columns filled with Hopkalit (Drägerwerke, Lübeck, FRG). The other chemicals were of the same origin described by Conrad and Seiler (12).

RESULTS

Vertical distributions of hydrogen and hydrogen bacteria. The vertical distributions of H_2 and aerobic hydrogen bacteria were determined in spring and summer. Typical results are shown in Fig. 1 and 2.

In March, a stable thermocline and chemocline had not yet been established; the water was oxygenated throughout with concentrations of 5 to 10 ml of O_2 liter^{-1}. The H_2 concentration varied from 20 to 225 nl of H_2 liter^{-1}, with concentration maxima at between 2- and 6-m

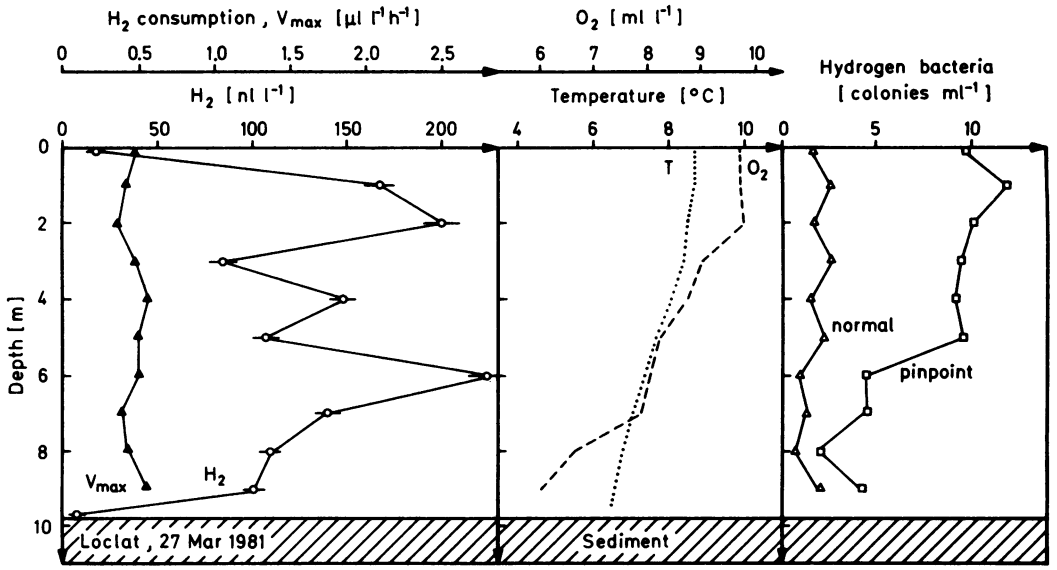

FIG. 1. Vertical distribution of hydrogen (○), H_2 consumption activity (▲), and hydrogen bacteria (△, □) in the Loclat during spring. Black triangles indicate the depths at which CH_4 and H_2S were first detected.

depths (Fig. 1). (On 27 March 1981, maxima were observed at 2-, 4-, and 6-m depths [Fig. 1].) Apparently, H_2 evolution occurred at these depths. Near the lake sediment, however, the H_2 concentration was very low, ca. 8 nl of H_2 liter^{-1}.

The counts of hydrogen bacteria with a normal colony size (diameter, ≥1 mm) varied between 1 and 2 colony-forming units (CFU) ml^{-1}, increasing slightly towards the water surface (Fig. 1). The same result was obtained when hydrogen bacteria were counted by the most-probable-number technique. Hydrogenase-positive pinpoint bacteria (diameter, ≤1 mm) were found in numbers of 9 to 12 CFU ml^{-1} in the upper 5-m water column and in numbers of 2 to 5 CFU ml^{-1} below.

H_2 consumption activity was measured after injecting water with a high H_2 concentration into the water samples to adjust the H_2 concentration to ca. 300 nl of H_2 liter^{-1}. The H_2 concentrations of the water samples were then measured immediately and 3 h after incubation at 10°C in darkness. The temporal decrease of the H_2 concentration was used to calculate V_{max} by using the integrated Michaelis-Menten equation and a K_m value of 1.5 µl of H_2 liter^{-1}. The H_2 consumption activity was relatively constant within the entire water column, with V^{max} values of 0.35 to 0.55 µl of H_2 liter^{-1} h^{-1} (Fig. 1).

In July, the lake was stratified with temperatures of ca. 8°C in the bottom and 21°C in the surface water. From 7 m below, the water was completely deficient of O_2 and had redox potentials lower than −175 mV. H_2S and CH_4 were detectable at depths below 6 to 7 m. H_2 concentrations reached values of 4,700 nl of H_2 liter^{-1}, more than 1 order of magnitude higher than in the spring. Interestingly, the highest H_2 concentrations were found at 1- to 4-m depths in the aerobic epilimnion and metalimnion, where the water was often supersaturated with O_2 originating from oxygenic photosynthesis by cyanobacteria (predominantly *Oscillatoria rubescens*) and algae. On 17 July 1981 (10 a.m.), maximum dissolved H_2 was observed at a 2-m depth (Fig. 2). In the hypolimnion, H_2 concentrations declined steadily towards the lake bottom, where they were often below the detection limit of our analysis technique (2.5 nl of H_2 liter^{-1}).

The counts of normal hydrogen bacteria were 4 to 7 CFU ml^{-1} in the epilimnion, decreased to 1 to 2 CFU ml^{-1} in the metalimnion, and increased again to 3 CFU ml^{-1} in the hypolimnion (Fig. 2). In the meta- and hypolimnions, about half of the normal hydrogen bacteria were yellow pigmented, characteristic for the genus *Xanthobacter*, whereas their population was smaller in the epilimnion. The distribution of hydrogenase-positive pinpoint bacteria was similar to that of normal hydrogen bacteria, but the pinpoints reached higher counts (6 to 21 CFU ml^{-1}). There was a distinct maximum of pinpoint colonies, especially at 1-m depth.

H_2 consumption activity in the water was measured in the same way as in the spring. The H_2 concentration in the water samples was adjusted to ca. 2 µl of H_2 liter^{-1} and measured

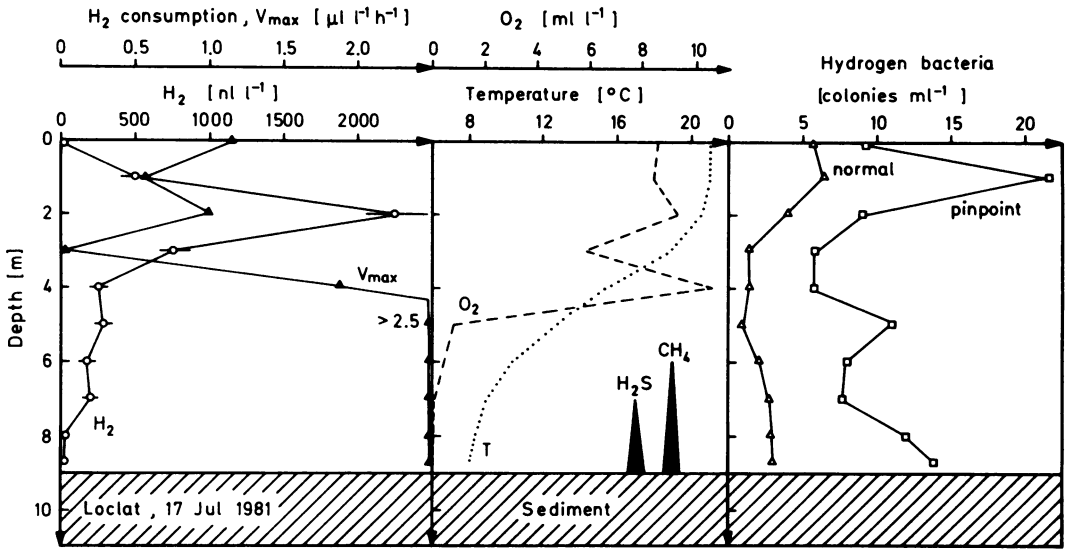

FIG. 2. Vertical distribution of hydrogen (○), H_2 consumption activity (▲), and hydrogen bacteria (△, □) in the Loclat during summer stratification. Black triangles indicate the depths at which CH_4 and H_2S were first detected.

immediately and 1 h after incubation at 20°C in darkness. H_2 consumption activity decreased from 1.2 µl of H_2 liter^{-1} h^{-1} at the lake surface to zero at a depth of 3 m. In the metalimnion, H_2 consumption decreased to more than 2.5 µl of H_2 liter^{-1} h^{-1}. In the hypolimnion, where the water contained no oxygen, the H_2 consumption was so rapid that the H_2 initially present was completely consumed within a 1-h incubation time, so that H_2 consumption rates must have been considerably higher than 2.5 µl of H_2 liter^{-1} h^{-1}.

In situ production and consumption of H_2. The H_2 concentration in the lake water changed with time. The highest concentrations, with values >4 µl of H_2 liter^{-1}, were always observed in the morning. Figure 3 shows an increase of dissolved H_2 in the epi- and metalimnions from the afternoon of 14 July to the morning of 15 July 1981, as well as a decrease of H_2 concentrations from the morning to the evening of 19 July. The turnover of H_2 was not correlated with a change in the distribution of the most conspicuous microorganisms. We counted the following under the microscope: total bacterial cells which increased in the hypolimnion with depth, up to 2 × 10^6 cells ml^{-1}; *Thiopedia* sp., which formed a layer in the upper part of the hypolimnion with up to 4 × 10^4 plates ml^{-1}; green algae in all parts of the lake with <400 cells ml^{-1}; diatoms in the aerobic lake water with 2,000 to 3,000 cells ml^{-1}; and *O. rubescens*, which formed a layer at 3- to 5-m depth with up to 4,000 filaments ml^{-1}.

In vitro H_2 consumption activity. Water samples from different depths were incubated either in daylight (10 to 40 klx) or in darkness. In both cases, H_2 decreased to <10% of the initial value within an incubation period of 6 h, indicating significant H_2 consumption activity. H_2 was consumed in aerobic as well as in anaerobic water samples. H_2 production was not observed. The same result was obtained when the samples were incubated in a light incubator at 750 lx. The H_2 consumption activity in the aerobic parts of the lake was further characterized. It was resistant to treatment with azide, but was inactivated with $HgCl_2$ or cyanide (Fig. 4). H_2 consumption activity was also inactivated by boiling the water sample, indicating that H_2 consumption activity was biologically mediated. When the water was filtered through 0.2-µm filters, H_2 was no longer consumed (Fig. 5). However, H_2 consumption was not affected by filtration through 3-µm filters, demonstrating that the H_2 consumption activity was bound to particles of 0.2 to 3.0 µm in size. The aerobic hydrogen bacteria, on the other hand, occurred mainly in clusters larger than 3 µm in size, since the units which formed normal-sized colonies were almost completely retained on 3-µm filters. The hydrogenase-positive pinpoint bacteria were also partially retained on 3-µm filters, but only by ca. 50%. Many of them apparently occurred as single cells. The ratios of CFU retained on membrane filters (3.0/0.45 µm) for normal hydrogen bacteria (colonies >1 mm) and pinpoint bacteria (colonies <1 mm), respectively, were as follows: 27 March 1981—1.00 ± 0.16 (standard deviation) and 0.43 ± 0.12; 17 July 1981—0.90 ± 0.22 and 0.53 ± 0.27. These are average values from 10

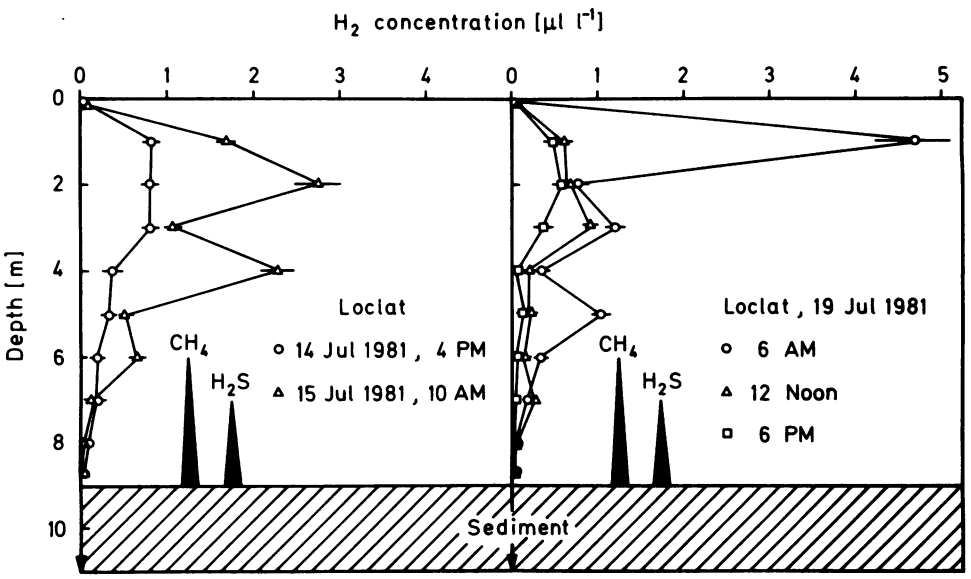

FIG. 3. Vertical distribution of H_2 at different times of day. Black triangles indicate the depths at which CH_4 and H_2S were first detected.

determinations throughout the water column (0- to 8.7-m depth). The aerobic H_2 consumption activity followed Michaelis-Menten kinetics. With a combined water sample from 4- to 8-m depths, a K_m value of 1.5 µl of H_2 liter^{-1} (67 nM H_2) was determined (Fig. 6).

DISCUSSION

The Loclat is a small eutrophic and holomictic lake. Every year, between May and October, it stratifies and shows a bloom of the oxygenic cyanobacterium *O. rubescens* in the metalimnion and a bloom of the anoxygenic phototrophic purple bacterium *Thiopedia* sp. in the upper hypolimnion (Aragno, unpublished observations). The Loclat is certainly representative of a typical eutrophic lake. It is generally accepted (3, 19) that H_2 is produced in anaerobic lake sediment and then diffuses into the water, where it is oxidized by aerobic hydrogen bacteria as soon as it reaches the aerobic zone. With this theory, the H_2 concentrations in the water column should show a vertical gradient, with the highest values near the lake sediment and the lowest values in the aerobic water zone. By contrast, however, all vertical profiles measured in the Loclat showed the highest H_2 concentrations in the aerobic epilimnion and metalimnion. In the anaerobic hypolimnion, H_2 concentrations were very low. Just above the lake sediment, H_2 could no longer be detected. These observations clearly indicate that H_2 production and consumption in a lake must differ substantially from those presently assumed in the literature.

The H_2 maxima in the aerobic water zone leave no doubt that H_2 must have been produced in this zone. Schink and Zeikus (personal communication) studied H_2 in Lake Mendota (Wis-

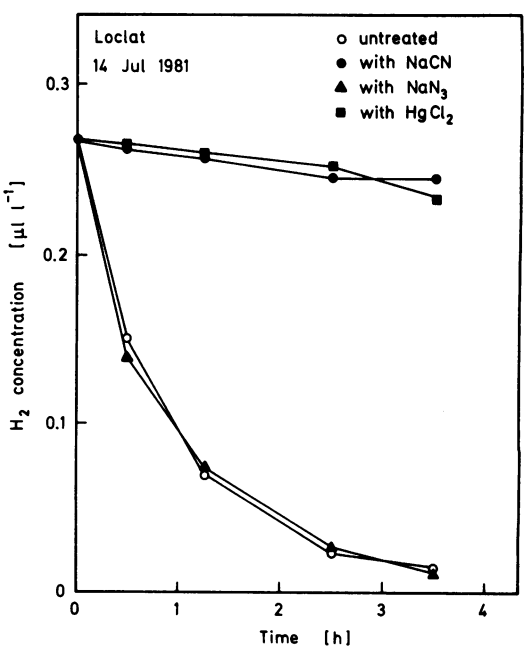

FIG. 4. H_2 consumption in aerobic surface water treated with inhibitors.

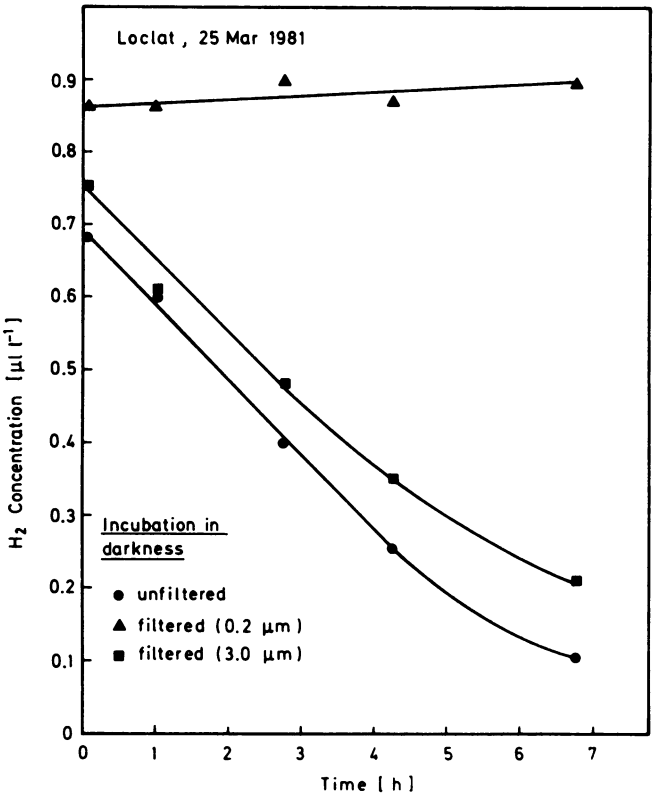

FIG. 5. H_2 consumption in filtered and unfiltered aerobic water. The water was a combined sample from 4-, 6-, and 8-m depths.

consin) and in Knaack Lake (Wisconsin) by means of a relatively insensitive H_2 detection technique (detection limit, ≥100 µl of H_2 liter^{-1}). Sometimes, they found up to 4.5 ml of H_2 liter^{-1} in the epilimnion of the lakes. Supersaturation of water with H_2 was also observed in the ocean (10, 38), so H_2 production in aerobic water may be a general feature. Presently, two processes are known which may produce H_2 under aerobic conditions: H_2 evolution by N_2-fixing cyanobacteria and H_2 evolution by eucaryotic algae (for review, see 1, 4, 6, 15).

Our observations did not show a coincidence of high H_2 concentrations in the water with high numbers of potential H_2 producers such as diatoms, green algae, or *Oscillatoria* sp. However, H_2 evolution may be less dependent on the number of cells than on the regulation of their activity. Presently we know little about regulation of H_2 production in vivo and even less about such regulation in the natural environment. Our observations indicate that the H_2 concentrations in the aerobic zone of the lake change with time of day. This change must be due to a change in the rate of H_2 production or H_2 consumption or both. The data base is too small as yet to derive a particular rhythm of H_2 turnover. Unfortunately, we were not able to see H_2 production in water samples in vitro. Therefore, the H_2 production process could not be studied experimentally. Either we did not meet the correct incubation conditions, or the responsible organisms became inactivated during sampling. Thus, the H_2 production process in the aerobic zone of lakes as well as the responsible microorganisms remain unknown.

The vertical H_2 distribution, as well as the laboratory experiments, demonstrated that H_2 was consumed in all parts of the lake. H_2 was consumed under aerobic and anaerobic conditions. The high H_2 consumption activities observed in the anaerobic hypolimnion are most likely due to anaerobic microorganisms which keep the H_2 concentrations very low. Near the lake sediment, H_2 concentrations were below 2.5 nl of H_2 liter^{-1}. This concentration is equivalent to an H_2 partial pressure of ca. 0.01 Pa, which is 3 to 4 orders of magnitude lower than that thermodynamically required for proton-reducing fatty acid oxidation (43). The anaerobic H_2-consuming bacteria must either have a high affinity for H_2 or a high total activity. K_m values

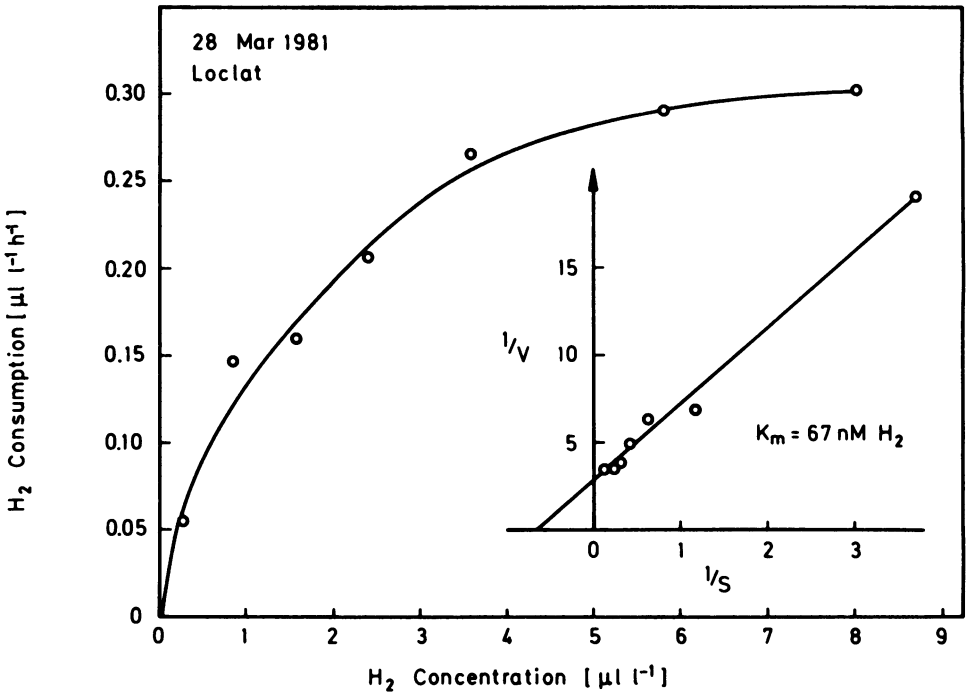

FIG. 6. H_2 consumption rate as a function of initial H_2 concentration. The water was aerobic and was sampled from 4-, 6-, and 8-m depths.

of 56 to 67 µl of H_2 liter^{-1} are reported for H_2 consumption in anaerobic lake sediments and H_2 conversion to CH_4 (17, 40). Compared with the low in situ concentrations of H_2, the K_m values are rather high. Therefore, the anaerobic H_2-consuming bacteria must have a high total activity. This assumption is in agreement with our observation of very high numbers of bacteria in the hypolimnion. Likely candidates for anaerobic H_2 consumption are sulfate reducers and methanogenic bacteria, which are able to grow on H_2 (21, 41, 44) and may even live in a synthrophic relationship with H_2-producing anaerobic microorganisms (5, 22, 23). Sulfate reducers and methanogens were active during summer stratification, as indicated by the observation of H_2S and CH_4 in the entire hypolimnion. Additional types of anaerobic H_2 metabolism, e.g., reduction of CO_2 to acetic acid (8), may also be of importance (for review, see 33).

Aerobic H_2 consumption in nature is usually ascribed to the activity of the hydrogen bacteria (2, 19, 31, 35). This theory has recently been questioned by Conrad and Seiler (9), who showed that hydrogen bacteria do not account for the rapid decomposition of atmospheric H_2 by soil (20). We must take into account, however, that the H_2 partial pressure in the atmosphere is very low (0.005 Pa; 34), resulting in an H_2 concentration in the soil aqueous phase of ca. 10 nl of H_2 liter^{-1}. In the water of the Loclat, the H_2 concentrations were 10 to 400 times higher, and one might argue that these concentrations are sufficient for the metabolic consumption by hydrogen bacteria. In fact, sometimes the vertical distribution of H_2 consumption activity was very similar to that of hydrogen bacteria. This was especially true in the spring, when the whole water body was aerobic and well mixed, and the H_2 consumption activity and hydrogen bacteria were relatively homogeneously distributed. In summer, however, numbers of hydrogen bacteria were relatively low, but H_2 consumption activities were high, although the water was highly oxygenated due to oxygenic photosyntheses. A correlation between H_2 consumption activity and pinpoint bacteria was not observed. These bacteria grew very poorly on hydrogen, but contained hydrogenase activity. They did not grow in the liquid culture medium of the most-probable-number test, whereas the hydrogen bacteria did grow and resulted in titers similar to those determined by counting the normal-sized colonies on agar plates. Therefore, the pinpoint bacteria are not necessarily true chemolithoautotrophic hydrogen bacteria; they possibly use H_2 as an additional energy or electron source while growing on organic impurities of the agar (mixotrophy or chemolithoheterotrophy).

Incubation experiments showed that H_2 was consumed in lake water by metabolic processes, bound to particles of 0.2 to 3 μm in size. Interestingly, the normal hydrogen bacteria were almost quantitatively retained on 3-μm filters and, therefore, cannot account for the observed H_2 consumption activity. The pinpoint bacteria, on the other hand, passed the 3-μm filters in relatively large amounts, so these bacteria could play a role in the consumption of H_2.

Aerobic H_2 consumption processes in the lake water had an extremely high affinity for H_2. The K_m for H_2 was 67 nM, which is almost 2 orders of magnitude lower than the lowest K_m value reported for hydrogen in the literature (for review, see 1, 7, 28). Experiments in our laboratory show that normal hydrogen bacteria as well as pinpoint bacteria have K_m values higher than 1 μM H_2 (unpublished data). This observation questions a major role of hydrogen or pinpoint bacteria in the consumption of H_2 in aerobic lake water. It is of interest that the K_m value of the H_2 consumption activity in lake water was similar to those (11 to 83 nM H_2) reported for H_2 decomposition in soils, which is probably due to the activity of abiontic soil hydrogenases (11; R. Conrad, M. Weber, and W. Seiler, Soil Biol. Biochem., in press). Abiontic enzymes (39) can be associated with dead or non-proliferating microbial cells or attached to cell fragments which are retained on 0.2-μm filters. Abiontic hydrogenases may be inactivated by boiling or by treatment with mercuric chloride or cyanide, in agreement with our incubation experiments. H_2 consumption in aerobic lake water may therefore be due to abiontic hydrogenases rather than to chemolithoautotrophic, chemolithoheterotrophic, or mixotrophic hydrogen-utilizing bacteria.

Aerobic hydrogen-oxidizing bacteria may play a role in the control of H_2 escaping from particles into the free lake water. Whereas the free water usually contained less than 4 μl of H_2 $liter^{-1}$, much higher H_2 concentrations may locally arise on particles large enough to constitute microsites for anaerobic decomposition of organic matter. This hypothesis would explain the localization of hydrogen bacteria on particles larger than 3 μm, whereas heterotrophic bacteria are usually attached to particles smaller than 3 μm (18). H_2-producing algae or cyanobacteria, being larger than 3 μm, may also constitute an ecological niche for hydrogen bacteria.

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