Plant and Soil 87, 417–422 (1985). Ms. 6001
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Hydrogen consumption in soil

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Received 12 July 1984. Revised February 1985

Key words Consumption Hydrogen Microbiological Soil

Summary Gas chromatographic analysis of hydrogen in non-sterile soil incubated aerobically and anaerobically revealed that consumption of the gaseous hydrogen correlated very highly with the initial hydrogen substrate concentration. Hydrogen consumption was not observed in sterile soil. In anaerobically incubated soil, methane evolution was not related to H_2 consumption. The optimum temperature range for H_2 consumption in both aerobic and anaerobically incubated soil was between 20 and 30°C. Activity rapidly decreased at soil temperatures above and below this optimum temperature range.

Introduction

The hydrogen-oxidizing bacteria are taxonomically a diverse group of 28 species belonging to 15 genera (Pseudomonas, Alcaligenes, Azospirillium, Derxia, Flavobacterium, Microcyclus, Paracoccus, Renobacter, Rhizobium, Xanthobacter, Arthrobacter, Bacillus, Mycobacterium, Nocardia and Hydrogenomonas[1,6]. As a group, the hydrogen bacteria are very metabolically diverse bacteria. Their mixotrophic metabolism allows them to use both inorganic and organic compounds as energy and/or carbon sources. A recent publication reported the kinetics of soil hydrogenases catalyzing the oxidation of atmospheric hydrogen. Two types of hydrogenase activity were described; one active under aerobic soil conditions and the other under anaerobic conditions[2]. In another study[3], it was suggested that the ecological niches of hydrogen bacteria could only be those soil microsites which are aerobic yet adjacent to anaerobic sites where high mixing ratios of H_2 are made available during the degradation of organic compounds. Since hydrogen is an important intermediate in the mineralization of decomposing organic matter under anaerobic conditions, the hydrogen can then be used as an electron donor, allowing the formation of methane and hydrogen sulfide. It has also been suggested that high H_2-mixing ratios could be created by the root nodules of leguminous plants, producing H_2 while fixing dinitrogen[3].

The influence of various environmental conditions on hydrogen-consumption in soil still requires additional research as a complete

understanding of this activity is not known[6]. In the present study, a gas chromatographic method for measuring hydrogen-consumption in soil was used to investigate the effect of temperature, aerobic and anaerobic gas phase conditions, and substrate concentrations on H_2 consumption in soil.

Materials and methods

Soil samples

Sandy loam was collected from the surface 10 cm of an agricultural field at Floradale, Ontario, Canada. The soil was passed through a 2 mm sieve and used in H_2 consumption experiments. Some characteristics of the soil were measured as previously described[7] and are presented in Table 1.

Table 1. Some characteristics of the soil

Soil texture (sandy loam)	
Sand	56%
Silt	34%
Clay	10%
pH (water)	6.5
Water holding capacity	0.43 ml/g
No. heterotrophs	1.3×10^7/g dry weight

H_2 consumption in soil

A 10-g sample of freshly sieved soil was placed in a 50-ml Erlenmeyer flask and adjusted to 60% of its water holding capacity with sterile distilled water. All flasks were capped with serum stoppers (Suba-Seal, Barnsley, England) and the appropriate volume of pure H_2 was injected into the flask with a Hamilton gas-tight syringe to give a range of H_2 concentrations from 178 to 893 nmoles/flask. Anaerobic flasks were prepared by evacuating for 5 min and refilling with pure helium to 1 atm prior to addition of the hydrogen substrate. This was repeated 3 times to ensure anaerobic conditions. This method of establishing an anaerobic environment has been previously used for studying anaerobic nitrogen fixation[7], and leaves only a trace of O_2 which is barely detectable using gas chromatographic analysis. Sterile control soils were prepared by autoclaving flasks containing soil for 1.5 h at 121°C on two consecutive days. All incubations were carried out statically at the desired temperature in the dark. Hydrogen consumption was measured using a thermal conductivity gas chromatograph equipped with a 183 cm × 6 mm stainless steel column packed with 80/100 mesh molecular sieve 5A support. The gas chromatograph was operated at a bridge current of 170 mA and the oven temperature was maintained at 50°C. At the appropriate time, 1.0 ml of the gas phase in the serum-stoppered flasks was removed using a gas-tight syringe, and injected into the gas chromatograph. All experiments were carried out in triplicate. Hydrogen concentrations were determined from internal standards of pure H_2 gas. All gas concentrations were corrected for temperature and gas phase volumes within the flasks.

The effect of temperature on H_2 consumption in soil was studied using the same method as previously described. Pure H_2 was added to either aerobic or anaerobically incubated soil at a concentration of 892 nmoles/flask. Flasks containing soil and substrate were then incubated in the dark for 24 h, at which time H_2 analysis was carried out using the gas chromatograph.

Methane which could be evolved in anaerobically incubated soil, was monitored with an Antek 300 gas chromatograph equipped with a flame ionization detector and a 183 cm × 3 mm, 80/100 mesh Porapak N column operated at 100°C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. The flow rates for H_2 and air were 30 and 300 ml/min, respectively.

Pure methane standards were used to prepare a standard curve for the determination of unknown CH_4 concentrations.

Statistical analysis

Lines of best fit and correlation coefficients were estimated using an Apple II plus microcomputer and a CMA statistics program (Charles Mann and Associates, Santa Fe Trail, California, USA).

Results and discussion

Hydrogen consumption in aerobically incubated soil supplied with a range of initial H_2 concentrations was measured after 24, 48, and 72 h (Fig. 1). Hydrogen consumption correlated very highly with the initial H_2 substrate concentration. For example, the lowest correlation coefficient calculated was 0.83, indicating a high correlation between activity and substrate (Table 2). However, the increases in H_2 consumption were very gradual, especially during the first 48 h. The 24–48 h lag period suggested that an adaptation period may be necessary for enzyme synthesis, and/or time is required for the hydrogen-oxidizing bacteria to increase in numbers.

As seen in Fig. 2, H_2 consumption in anaerobically incubated soil

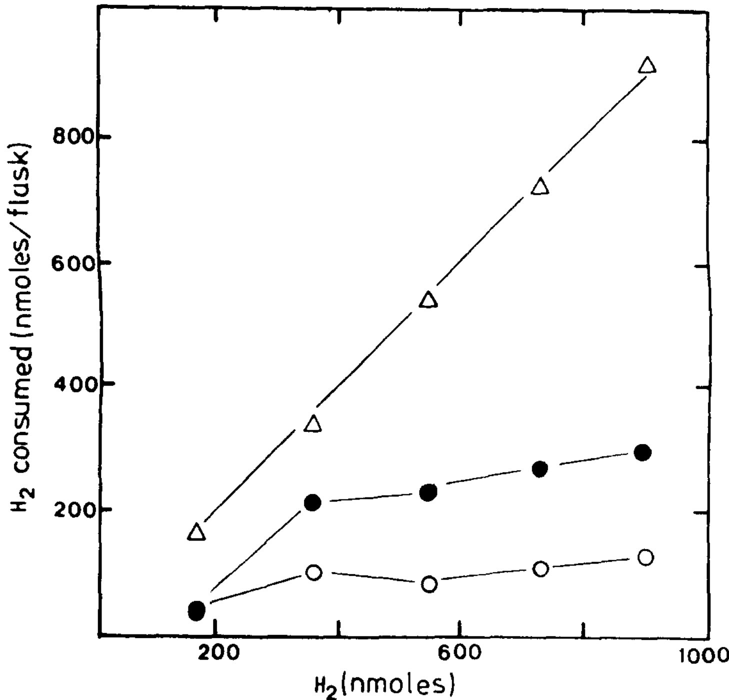

Fig. 1. H_2 consumption in soil as a function of the initial H_2 substrate concentration when incubated aerobically at 20°C for 24 h (○); 48 h (•) and 72 h (△). Sterile controls displayed no activity.

Table 2. Relationship between H_2 consumption and initial H_2 substrate concentration in soil. Lines of best fit and correlation coefficients were calculated from the data presented in Figs. 1 and 2, where X (nmoles H_2) and Y (nmoles H_2 consumed/flask)

Incubation (hours)	Line of best fit	Correlation coefficient (r^2)
Aerobic		
24	$Y = 0.39 \cdot X^{0.86}$	0.83
48	$Y = -711.87 + 147.35 \cdot \log X$	0.91
72	$Y = 1X + 1$	1.0
Anaerobic		
24	$Y = 13.67 \cdot X^{0.42}$	0.92
48	$Y = -966.76 + 218.26 \cdot \log (X)$	0.94
72	$Y = 1.72 \cdot X^{0.89}$	0.99

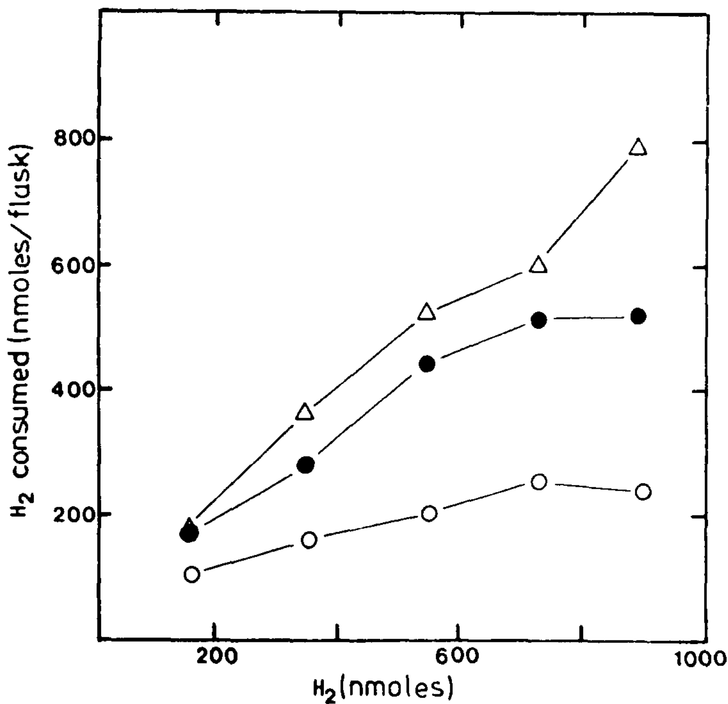

Fig. 2. H_2 consumption in soil as a function of the initial H_2 substrate concentration when incubated anaerobically at 20°C for 24 h (○); 48 h (●) and 72 h (△). Sterile controls displayed no activity.

was slightly higher during the 72 h period, as compared to H_2 consumption in the aerobic soil. Under anaerobic conditions, most H_2 is assumed to be utilized in the production of methane. However, gas samples taken from the anaerobic gas phase, and analysed using a flame ionization chromatograph, indicated that methane was not evolved

during the 72 h anaerobic incubation period. This suggested that H_2 consumption during this time was not related to methanogenesis. It has been reported that hydrogen can act as an electron donor for sulphate-reducing bacteria and hydrogen-metabolizing methanogenic bacteria[5]. Competition for H_2 could be one of the major causes for the apparent ecological separation between methanogenesis and sulfate reduction[5]. In the present study, it is also possible that methanogenic bacteria were not present, or only present in very insignificant numbers in the soil. This would suggest that anaerobic bacteria other than methanogens were responsible for H_2 consumption in the anaerobically incubated soil.

Figure 3 shows the significant effect of temperature on H_2 consumption in soil over a range of temperatures from 4 to 37°C. The optimum temperature range for activity was observed to be between 20 and 30°C, with activity sharply declining above and below this range. Hydrogen consumption in anaerobically incubated soil was higher at 4, 15 and 37°C, than in the aerobic soil incubated at the same temperatures. A temperature of 30°C for maximum activity has been reported for pure cultures of H_2-oxidizing bacteria in sterile soil[2]. This observation is in reasonable agreement with the results of this experiment.

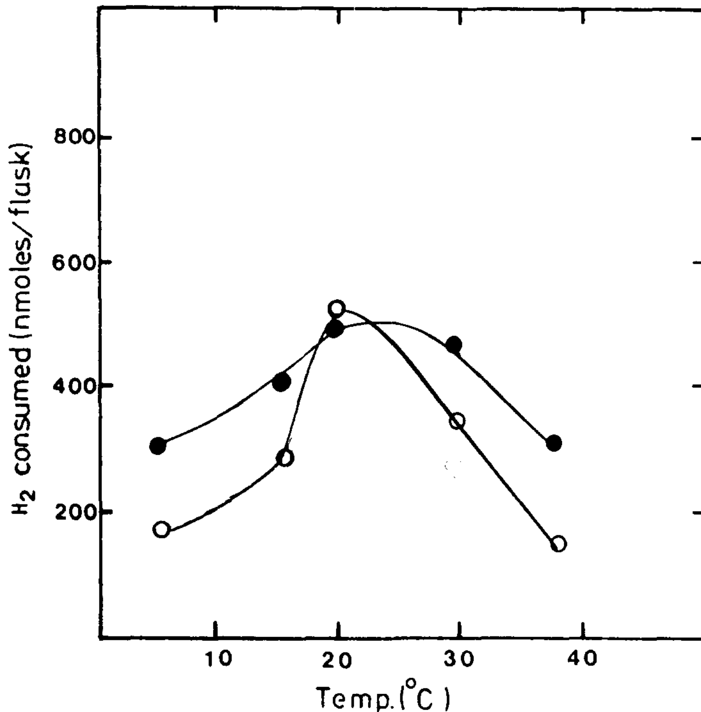

Fig. 3. H_2 consumption rate as a function of temperature in aerobically incubated soil (o) and anaerobically incubated soil (•) after 48 h.

Most H_2-oxidizing bacteria are similar to other heterotrophic aerobic bacteria. However, they are able to obtain energy by the oxidation of atmospheric H_2 using their electron transport system. Hydrogen is an intermediate in the decomposition of organic matter, and plays a central role in methanogenesis in anaerobic environments. Also, soil is the predominant sink for H_2 in the biosphere. Hydrogen-utilizing dinitrogen fixing bacteria (Xanthobacter) are capable of coupling the oxidation of H_2 with the fixation of N_2 and CO_2. It is possible that these organisms were responsible for some of the hydrogen consumption in soil. However, they generally prefer microaerophilic environments with O_2 concentrations ranging from 3 to 5%. From an ecological perspective, it is important to understand the consumption of H_2 in soil and the manner in which environmental factors influence the catalytic activity of soil hydrogenases. This is especially important if micro-organisms synthesize different types and amounts of hydrogenases under similar or different conditions. The activity of soil hydrogenases is most likely controlled by environmental conditions[3]. It is not known whether hydrogenases are actually one enzyme or a group of enzymes with very specific catalytic activity or very diverse activity[3]. This problem may be solved when it is possible to extract active hydrogenases from soil. Presently, this is not possible.

The present study does indicate that aerobic and anaerobic H_2 consumption occurs over the same temperature range. Also, good correlations were found between H_2-consumption activity and the H_2 substrate concentration provided.

Acknowledgements This research was supported by a Natural Sciences and Engineering Research Council of Canada operating grant.

References

1 Bowien B and Schlegel H G 1981 Physiology and biochemistry of aerobic hydrogen-oxidizing bacteria. Annu. Rev. Microbiol. 35, 405–452.

2 Conrad R and Seiler W 1979 The role of hydrogen bacteria during the decomposition of hydrogen by soil. FEMS Microbiol. Letts. 6, 143–145.

3 Conrad R, Weber M and Seiler W 1983 Kinetics and electron transport of soil hydrogenases catalyzing the oxidation of atmospheric hydrogen. Soil Biol. Biochem. 15, 167–173.

4 Lynch J M and Poole N J 1979 Microbial Ecology, A Conceptual Approach. John Wiley and Sons, New York.

5 Nedwell D B and Banat J M 1981 Hydrogen as an electron donor for sulfate-reducing bacteria in slurries of salt marsh sediment. Microb. Ecol. 7, 305–313.

6 Seiler W 1978 The influence of the biosphere on the atmospheric CO and H_2 cycles. *In* Environmental Biogeochemistry and Geomicrobiology, Vol. 3 Methods, Metals and Assessment, Ed. W E Krumbein. Ann Arbor, Michigan, pp 773–810.

7 Tam T-Y and Trevors J T 1981 Effects of pentachlorophenol on asymbiotic nitrogen fixation in soil. Water Air Soil Pollut. 16, 409–414.