FEMS Microbiology Ecology 38 (1986) 347–351
Published by Elsevier

FEC 00090

Biological oxidation of hydrogen in soils flushed with a mixture of H_2, CO_2, O_2 and N_2

(Hydrogen-oxidising bacteria; landfill; hydrogen evolution; soil biofilters)

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Received 1 August 1986
Revision received 23 September 1986
Accepted 26 September 1986

1. SUMMARY

A stainless steel cylinder filled with soil was flushed upstream with a H_2/CO_2/air mixture. The consequence was a strong enrichment of the aerobic, autotrophic hydrogen-oxidising microflora, which reached densities enabling them to oxidize 84.5 ml $H_2 \cdot dm^{-2} \cdot h^{-1}$ in the first 25-cm layer. H_2 concentration profiles, hydrogen uptake activity and cell numbers correlated well with each other. Most of the organisms isolated were dinitrogen fixers. Thus, soils containing hydrogen-oxidising bacteria may act as a biological shield between H_2-rich environments and air, and may be utilized as biofilters, e.g., in the waste-processing industry.

2. INTRODUCTION

The autotrophic, aerobic hydrogen-oxidising bacteria are able to utilise the reducing power and oxidation energy of molecular hydrogen to perform carbon dioxide fixation [1,2]. Most of them also utilise organic compounds as carbon and energy sources. Hydrogen is taken up only above a threshold concentration [3]. This threshold lies in the range of 1–10 nM for most of the strains studied. It is higher than both the H_2 concentration in equilibrium with the atmosphere and the concentration generally found in steady-state systems such as soil and fresh water [4,5]. Thus, ecological niches where these bacteria may express their H_2-oxidising capabilities are restricted to oxic environments with hydrogen concentrations above this threshold.

A strong hydrogen production may arise from dumping grounds where organic matter is deposited under conditions of restricted oxygen diffusion [6–8]. Under such conditions, microbiologically catalysed events occur in a certain sequence (Fig. 1) beginning with oxygen exhaustion and climaxing with the full operation of the methanogenic syntrophy. Between these two events, a transitory fermentative phase takes place, accompanied by a first peak of gas evolution, the main components being H_2 (up to 30%) and CO_2. Then the gas production decreases dramatically, and H_2 is progressively replaced by methane.

This H_2-evolving phase is accompanied by the production of volatile organic compounds, and is

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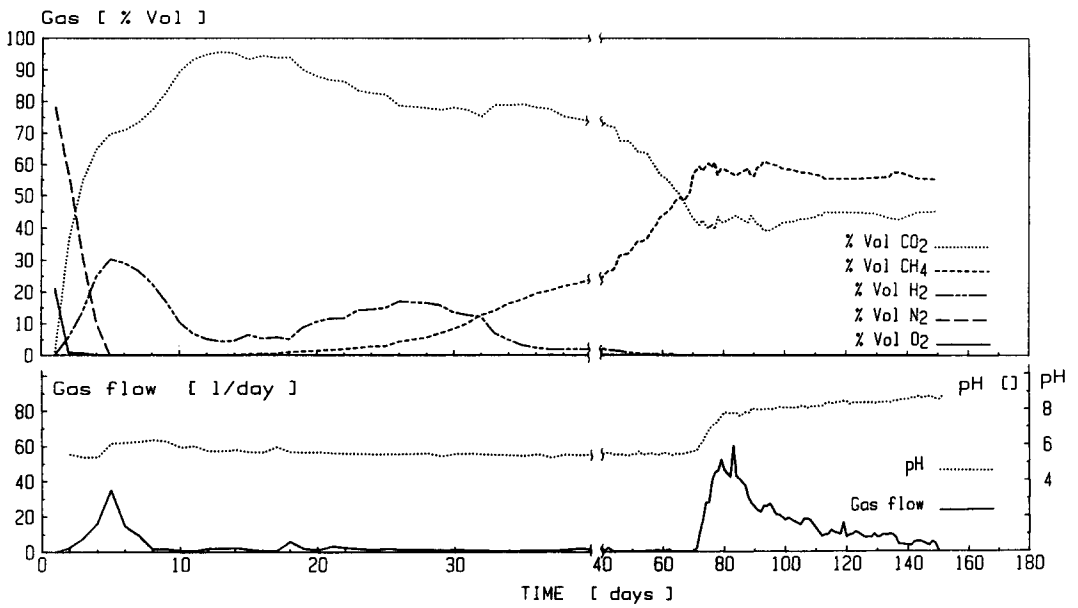

Fig. 1. Evolution of gases from a mixture of organic wastes deposited under anoxic conditions. A 1600-l hard polyethylene vessel without external air access was filled with a mixture of 443 kg urban waste (65% dry matter content) ground and sorted at diameter < 8 cm and 200 l digested sludge (5% dry matter content). The percolating liquid was recirculated from the bottom to the surface. The temperature was maintained at 33–35°C. Total gas production was recorded continuously and gas composition was analysed at regular intervals.

mainly responsible for the odours evolving from landfill disposals and garbage tanks.

Covering a compacted landfill with 30–50 cm of soil almost completely eliminates the emission of odours [9]. The soil acts here as a support for bacterial growth at the interface between the anaerobic garbage and air. There, an aerobic bacterial flora could oxidise the gases and other volatile compounds evolving from the decomposing waste mass. A similar process could occur in soil or compost biofilters (Fig. 2) used to depurate the mixture of air and gases evolving in an urban waste container, e.g., in an incineration plant.

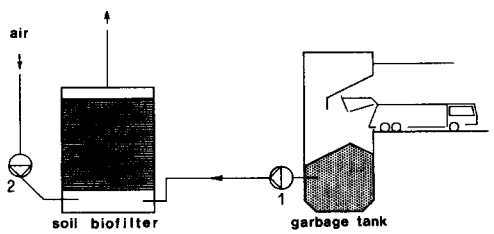

Fig. 2. Scheme of the possible use of a soil biofilter to depurate the gases evolving from a waste container. 1, waste gas pump; 2, air pump.

The aim of the research reported here was to test the effect of flushing a soil similar to that covering a dumping ground with an air : H_2 : CO_2 mixture, and to follow the kinetics and distribution of hydrogen-oxidising activity and the distribution and characteristics of the hydrogen-oxidising bacteria in such an environment.

3. MATERIALS AND METHODS

3.1. Soil column

The apparatus used was a 30-l stainless steel column, 28 cm in diameter (Fig. 3). It was filled with an alluvial siliceous sand with a moisture content of about 20%. The column was flushed upwards with a mixture of 1/3 artificial air (20% O_2 + 80% N_2) and 2/3 of a 20% H_2 + 80% CO_2 mixture. These proportions allowed complete oxidation of hydrogen to occur. The H_2 and CO_2 contents corresponded to the mean composition

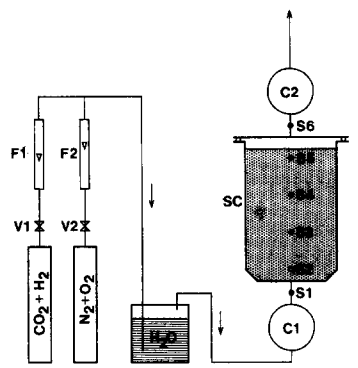

Fig. 3. Scheme of the experimental soil column. V1, V2, precision valves; F1, F2, flow meters; C1, C2, gas counters; SC, soil column; S1–S6, gas sampling points.

of the gases produced during the hydrogen-evolving phase of anaerobic waste decomposition.

The system was fitted with 6 gas-sampling points at different levels, 2 flow meters with precision valves to control the gas admission at the input of the fermenter, 2 gas meters to measure the input and output gas flows, and 3 temperature probes inside the soil column. It was run at ambient temperature.

3.2. Gas analysis

The gas was analysed using a two-column gas chromatograph (GOW-MAC) fitted with a molecular sieve (5Å, 60–80 mesh) and Carbosieve B (120–140 mesh) columns (Supelco, Bellefonte, PA, U.S.A.) and with a thermal conductivity detector. This set-up allowed us to separate and detect all the components in the gas mixture.

3.3. Hydrogen uptake measurements

Hydrogen uptake activity was measured in a Warburg respirometer, with oxygen as terminal electron acceptor, as described by Aragno and Schlegel [1].

3.4. Culture conditions

A basal mineral medium for hydrogen-oxidising bacteria [1] was used throughout this study. Most probable number (MPN) determinations were performed by enrichment culture in 18-mm-diameter tubes containing 4 ml of mineral medium, inoculated by a series of decimal dilutions of the sample (1 ml inoculum, 5 tubes/dilution). The tubes were incubated with slight agitation (80 rev./min) under a $H_2/O_2/CO_2$ mixture (85 : 5 : 10) at 27°C for 14 days.

The procedures for isolation and identification of hydrogen-oxidising bacteria have been described elsewhere [1].

4. RESULTS

The gas mixture was first provided at a rate of 285 $ml \cdot dm^{-2} \cdot h^{-1}$. The kinetics of gas composition measured at the output are shown in Fig. 4. After 450 h, H_2 completely disappeared while flowing through the column, provided that O_2 was present in excess. The input and output gas compositions are shown in Table 1. The subsequent reappearance of hydrogen was due to an accidental decrease in O_2 concentration in the input gas mixture. This confirms that oxygen was the main electron acceptor for hydrogen uptake as expected for an aerobic H_2-lithotrophic respiration.

Then, the total gas flow was increased to 640 $ml \cdot dm^{-2} \cdot h^{-1}$. This flow was of the same order of magnitude as the maximal flow expected from the anaerobic decomposition of a 1-m layer of garbage. Increasing the gas flow up to this value did not result in the reappearance of hydrogen at the output, indicating that the bacterial flora was undersaturated with respect to the amount of H_2 provided. Gas sampling at different levels in the

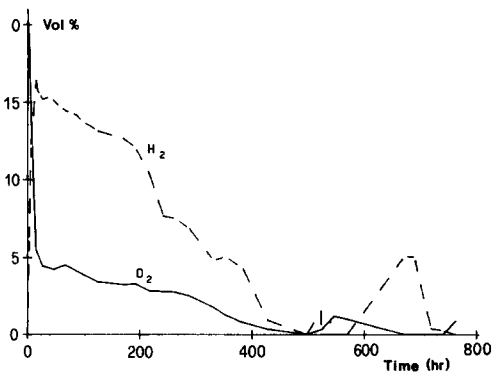

Fig. 4. Amounts of H_2 and O_2 in the gas at the output of the column with time.

Table 1

Composition of the gas mixture at the input and output of the soil column after 767 h of flushing

Gas	Input (%)	Output (%)
H_2	13.3	0
O_2	6.7	1.7
CO_2	53.3	65
N_2	26.7	33.3

cylinder provided profiles like the typical one shown in Fig. 5.

At the end of the experiment, after 767 h, H_2 had completely disappeared in the first 25 cm of soil. Samples were taken at different depths, respirometric hydrogen uptake activity was measured, and estimates of the cell numbers of aerobic hydrogen-oxidising bacteria were made (Table 2). The distributions of hydrogen-uptake activity and cell numbers correlate well with each other and with the gas concentration profiles. Based on an assumed dry weight of 0.2 μg per 10^6 cells and a protein content of 60% of the cell dry matter, the specific hydrogen uptake activity in the 5–15 cm level would be 2860 $\mu l\ H_2 \cdot h^{-1} \cdot mg^{-1}$ protein, which is in the same order as specific activities measured in pure autotrophic cultures of most hydrogen-oxidising bacteria.

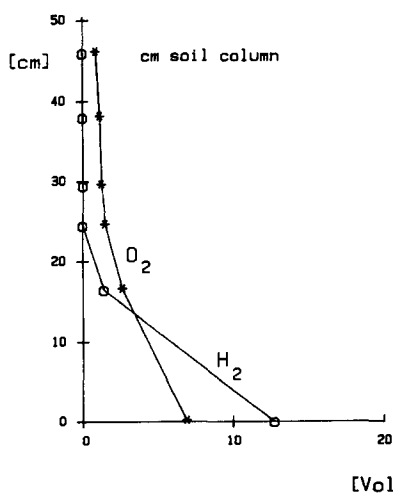

Fig. 5. A typical profile of H_2 and O_2 contents in the soil column. Time after the beginning of the experiment, 767 h; total gas flow, 640 $ml \cdot dm^{-2} \cdot h^{-1}$.

Table 2

Numbers of hydrogen-oxidising bacteria (determined by the most probable number method) and specific H_2-uptake activity of the soil column after 767 h of flushing

Total gas flow = 640 $ml \cdot dm^{-2} \cdot h^{-1}$

Level from the bottom (cm)	MPN (g^{-1} dry soil)	H_2 uptake ($\mu l\ H_2 \cdot min^{-1} \cdot g^{-1}$ dry soil)
0– 5	170×10^6	1.81
5–15	350×10^6	2.02
15–25	35×10^6	1.17
25–35	35×10^6	0.39
35–45	3.5×10^6	0.10

From the last positive dilutions in enrichment cultures, we isolated different types of hydrogen-oxidising bacteria. The most frequent ones were strains related to *Pseudomonas pseudoflava* and to *Xanthobacter autotrophicus,* as well as a third, not yet identified type. A taxonomic study of these different types is at present being undertaken in our laboratory. The bacteria share two properties of peculiar interest: (a) they are versatile, facultative heterotrophs, able not only to grow autotrophically with molecular hydrogen, but also to utilise a large variety of organic volatile compounds. Thus, such organisms, although they mainly grow lithotrophically, would be able to remove odorous volatile organic compounds as well. (b) most of our isolates are dinitrogen fixers. This allows them to overcome a limitation by shortage of combined nitrogen in the soil. This explains why cell numbers as high as 350×10^6 g^{-1} were reached, i.e., one order of magnitude higher than a normal 'total' soil flora. The distribution of dinitrogen fixing activity in similar experimental systems is at present under study in our laboratories.

5. DISCUSSION

These results show that autotrophic hydrogen-oxidising bacteria may play a major role in the aerobic oxidation of H_2 evolving from a landfill or other macrovolumes of anaerobically decaying organic matter. The question arises whether such a phenomenon, also a similar H_2 evolution from

decaying organic matter and its oxidation at the oxic–anoxic interface, occurs in microenvironments. Conrad and coworkers have pointed out that the kinetic characteristics of H_2 oxidation in normal soil [4] and in water bodies [3,5] do not meet those of 'classical' bacteria in pure cultures: the apparent K_m was much lower, and no threshold was noticed within the detection limits of their analytical system (0.1 nM), thus allowing H_2 uptake to occur even at atmospheric concentrations. This activity was supposed to be due to soil enzymes rather than to soil microorganisms [10]. Thus, H_2-oxidising bacteria would have a more indirect effect on the atmospheric hydrogen budget, preventing H_2 from reaching the atmosphere by forming a 'shield' between hydrogen-rich environments and air. Whether significant H_2 concentrations occur at the oxic–anoxic interface of freshwater sediments should be investigated further. We found relatively large numbers of hydrogen-oxidising bacteria in the thin, oxidised superficial layer of eutrophic freshwater sediments [11] (Aragno, unpublished results), but such an accumulation could also be the consequence of bacterial sedimentation from the water column (B. Schink, personal communication).

This confirms the general assumption that microorganisms are globally more important in removing volatile compounds produced in the biosphere before they reach the atmosphere, than in removing trace volatile compounds from the atmosphere at ppb or ppt concentration ranges. The use of hydrogen-oxidising bacteria in soil biofilters appears promising for the treatment of gases evolving from decaying wastes.

ACKNOWLEDGEMENTS

This work was supported by grant 3.517-83 from the Swiss National Science Foundation. The co-operation of the CER, Bioggio, and valuable technical help by G. Muschi were greatly appreciated.

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