APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1996, p. 1583–1588 Vol. 62, No. 5
0099-2240/96/$04.00+0

The Importance of Hydrogen in Landfill Fermentations

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Received 20 November 1995/Accepted 26 February 1996

Forty-two samples taken from two landfills were monitored for CH_4 production and apparent steady-state H_2 concentration. The rates of methanogenesis in these samples ranged from below the detection limit to 1,900 μmol kg (dry weight)$^{-1}$ day^{-1}, and the median steady-state hydrogen concentration was 1.4 μM in one landfill and 5.2 μM in the other. To further investigate the relationship between hydrogen concentration and methanogenesis, a subset of seven landfill samples was selected on basis of their rates of CH_4 production, H_2 concentrations, sample pHs, and moisture contents. Samples with H_2 concentrations of <20 nM had relatively small amounts of volatile fatty acids (VFAs) (undetectable to 18.6 mmol of VFA kg [dry weight]$^{-1}$), while samples with H_2 concentrations of >100 nM had relatively high VFA levels (133 to 389 mmol of VFA kg [dry weight]$^{-1}$). Samples with high H_2 and VFA contents had relatively low pH values (≤6.3). However, methanogenic and syntrophic bacteria were present in all samples, so the lack of methanogenesis in some samples was not due to a lack of suitable inocula. The low rates of methanogenesis in these samples were probably due to inhibitory effects of low pH and VFA accumulation, resulting from a thermodynamic uncoupling of fatty acid oxidation. As in other anaerobic ecosystems, H_2 is a critical intermediate that may be used to monitor the status of landfill fermentations.

The use of landfills is the major route of garbage disposal in the United States (39). The anaerobic mineralization of organic matter placed in landfills is generally assumed, but usually less than 10% of the theoretically expected amount of methane is ever recovered (5). Recognizing and understanding the limitations of methanogenesis in landfills are increasingly important for improving refuse decomposition. Several studies have examined the effects of moisture (15, 19, 21), temperature (19, 40), pH extremes (20), and high sulfate levels (15) on landfill methanogenesis. However, few methods are available for predicting the impending failure of methanogenesis in landfill cells, as there are for digester systems. Candidate procedures include monitoring alkalinity (17, 22, 32, 37), trace gas concentrations (18, 22, 23, 28, 29, 31, 37), or volatile fatty acids (VFAs) (1, 32, 37) or bacterial enumeration (37).

Of these possibilities, measurement of the hydrogen concentration appears to be a particularly promising approach. Hydrogen is relatively easy to measure and is a critical intermediate in anaerobic fermentations. Hydrogen has to be maintained at a level low enough to allow exergonic H_2 production from the biodegradation of alcohols and VFAs while high enough to allow exergonic H_2 consumption (24). The steady-state concentration of hydrogen has also been suggested as an indicator of the dominant terminal electron-accepting process occurring in anaerobic environments (26). Hydrogen accumulations in landfill samples could be indicative of uncoupled fermentations. Further support for this hypothesis requires linking elevated H_2 concentrations with depressions in the sample pH and decreased rates of refuse methanogenesis. To our knowledge, there is no baseline survey of the H_2 levels in landfills, especially in relation to the endogenous rates of landfill methanogenesis.

Therefore, methanogenesis, hydrogen concentrations, and pHs were measured in 42 samples taken from two different landfills. The microbiology and chemistry of the refuse were then evaluated for selected subsamples. We found that the steady-state concentration of H_2 in landfills was comparable to that in other electron donor-rich anaerobic environments. Samples that had low rates of methanogenesis and accumulated H_2 did not lack a suitable microflora. Rather, the H_2 accumulation was indicative of the buildup of VFAs, which lead to the depression of the sample pH and subsequently to depression of methanogenesis.

MATERIALS AND METHODS

Sample collection, incubation, and enumeration of bacteria. Refuse samples were collected from the Fresh Kills Landfill, Staten Island, N.Y., in October 1989 and from the Collier County Landfill, Naples, Fla., in March 1990, shipped to the laboratory, and stored as previously reported (15). Upon arrival, 200 to 300 g of each sample was placed in plastic anaerobic vessels as previously described (15). Additionally, seven landfill samples with differing rates of methanogenesis but similar moisture contents (approximately 25% by weight) were chosen for more intensive study. Duplicate incubations of these samples were prepared by placing 100 g of each sample in 250-ml wide-mouth bottles fitted with no. 9 rubber stoppers. The stoppers had been pierced with severed Balch tubes sealed with 1-cm-thick butyl rubber stoppers and aluminum crimp seals. Hydrogen and methane concentrations in the headspace and vessel pressures were monitored over time.

The samples were incubated at room temperature before they were analyzed for pH values, numbers of methanogenic and proton-reducing bacteria, and organic acid concentrations. To that end, the refuse contents were subdivided, and 10 g of each replicate was used for dry weight determinations (100°C for 24 h). Another 10-g portion of each sample was extracted for 1 min with 50 ml of oxygen-free deionized water to determine the sample pH and the concentration of organic acids. The pH values were measured immediately following the extraction by using a glass electrode, while a 1-ml portion of each extract was stored frozen for subsequent organic acid analysis.

The methanogenic and proton-reducing bacteria were enumerated by blending, in an anaerobic glove bag, 20-g portions of the refuse samples with 100 ml of sterile, anoxic phosphate buffer (23.7 mM, pH 7.2) for 1 min to dislodge cells (4). The resulting slurry was squeezed to remove the solids, and the liquid portion was assayed by using a three-tube most-probable-number procedure. Autotrophic and acetoclastic methanogens were enumerated in a mineral medium with H_2-CO_2 (4:1, 138 kPa) or acetate (20 mM) as an electron donor, respectively. The medium contained the following, in grams liter^{-1}: NaCl, 8; NH_4Cl, 10; KCl, 1; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 2; $CaCl_2 \cdot 2H_2O$, 0.4; resazurin, 0.0001; $NaHCO_3$, 3.5; and $Na_2S \cdot 9H_2O$, 0.05. Trace metal and vitamin solutions (43) were added at 10 and 5 ml/liter, respectively. The final pH of the medium

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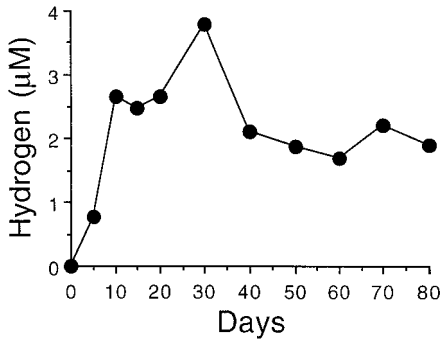

FIG. 1. Hydrogen concentration over time in a landfill sample that achieved an apparent steady-state H_2 concentration. Datum points are the average values for duplicate incubations.

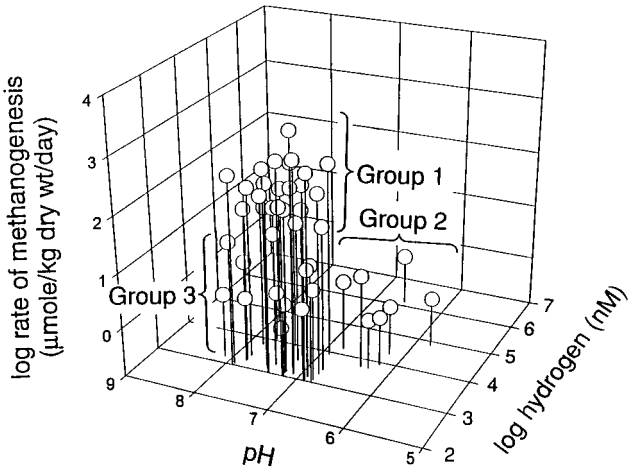

FIG. 2. Log-transformed CH_4 production rates and apparent H_2 concentrations plotted with pH values for 42 refuse samples taken from two landfill sites.

was 7.2. To enumerate the propionate- and butyrate-oxidizing bacteria, the medium was amended separately with propionate and butyrate to give a final fatty acid concentration of 20 mM. A hydrogen-scavanging organism is required for the exergonic degradation of fatty acids, so the most-probable-number dilution tubes were amended with a culture of *Methanospirillium hungateii* JF1 that had been grown overnight at 37°C on a rotary shaker as previously described (11). Tubes scored as positive had at least twice the amount of methane in the headspace as uninoculated tubes.

Analytical methods. The pressure generated in the incubation vessels was monitored with a hand-held pressure transducer (9). Headspace CH_4 concentrations were measured by flame ionization gas chromatography (6), and headspace H_2 concentrations were measured with a gas chromatograph equipped with a reducing gas detector and a Spherocarb 60/80 column (1 m by 0.32 cm) (Trace Analytical, Menlo Park, Calif.). The carrier gas was N_2 with a flow rate of 20 ml/min. The column and detector temperatures were 90 and 265°C, respectively. The hydrogen concentrations for the landfill samples were calculated with an Ostwald coefficient of 0.01941 for H_2 (42) and with the assumption that the "solvent" was pure water. The concentrations of organic acids in the water extracts were analyzed by high-pressure liquid chromatography (HPLC) as previously described (13).

The changes in Gibbs free energies ($\Delta G'$ values) for reactions that occur in the landfill samples were calculated by using ΔG_0 values (38) and the concentrations or partial pressures of the products and reactants measured at the end of the incubation period at prevailing temperatures and pH conditions (8). The concentration of the undissociated form of each organic acid was calculated from the total concentration of the acid (determined by HPLC analysis) and the pH of the sample by using the Henderson-Hasselbach equation (45).

RESULTS

For most landfill samples, the hydrogen concentration fluctuated but eventually reached an apparent steady-state value (<5 μM) (Fig. 1). However, some samples did not approach a steady state but continued to accumulate H_2 over time. For the latter samples, the H_2 concentration at the end of the incubation period was used in the comparisons described below. When the H_2 concentrations and rates of methanogenesis were log transformed and plotted against pH, they tended to cluster into three groups (Fig. 2). Samples with high rates of methanogenesis had low hydrogen concentrations and neutral to slightly alkaline pH values (group 1), while some samples with low rates of methane production had acidic pH values and high H_2 concentrations (group 2). A third group of samples had low methane production rates, low hydrogen concentrations, and neutral pH values (group 3). Methanogenesis in the samples in group 3 was limited by moisture content, since experimentally manipulated increases in this parameter increased methanogenesis (15). The median steady-state hydrogen concentrations of the Fresh Kills and the Collier County landfill samples were 1.4 and 5.2 μM, respectively.

This survey suggested that the hydrogen concentration was a reasonably good indicator of whether landfill refuse fermentations were coupled to methanogenesis. To further examine this possibility, subsamples with similar moisture contents were selected for further analysis based on their endogenous rates of methanogenesis. Figure 3 contrasts a refuse sample with a high rate of methanogenesis with one that produced little methane. The methanogenic sample reached an apparent steady-state hydrogen concentration of about 10 nM, while no comparable steady-state hydrogen concentration was evident in the nonmethanogenic sample. For the latter sample, hydrogen accumulated over the course of the incubation without the production of methane. Similar trends were observed in the other subsamples selected for study (Fig. 4). Samples with low rates of methanogenesis, i.e., 1.8 to 2.4 μmol of CH_4 kg (dry weight)$^{-1}$ day^{-1}, had hydrogen concentrations that ranged from 4.4×10^2 to 1.0×10^5 nM and pH values of between 5.5 and 6.5. Samples with high rates of methanogenesis, i.e., 64.9 to 191.0 μmol kg (dry weight)$^{-1}$ day^{-1}, had much lower hydrogen concentrations (7.1 to 17.2 nM) and neutral to slightly alkaline pH values. These findings allowed the samples to be characterized as producing either small or large amounts of methane (low- and high-methane producing samples, respectively) for subsequent comparisons.

The presence and survival of the requisite microorganisms could have limited refuse methanogenesis. Lower numbers of methanogens were measured in the low-methane-producing samples than in the high-methane-producing samples. However, with one exception, acetoclastic and autotrophic methanogenic bacterial numbers did not differ significantly from each other in the refuse subsamples, regardless of the abilities of the samples to make methane (Fig. 5). Differences between fatty acid-oxidizing bacterial numbers were not as obvious. The butyrate-oxidizing bacteria were generally equally numerous as or more numerous than the propionate-oxidizing bacteria (Fig. 6). There were more butyrate-oxidizing bacteria in the high-methane-producing samples, but this trend was not as evident when the propionate-oxidizing bacteria were similarly compared.

While large variations in microbial numbers were not evident, the same was not true of refuse organic acid concentrations (Fig. 7). The high-methane-producing samples had organic acid concentrations ranging from undetectable to 18.6 mmol g (dry weight)$^{-1}$, while the low-methane-producing samples accumulated high concentrations of both straight- and branched-chain organic acids. The latter samples had from 133

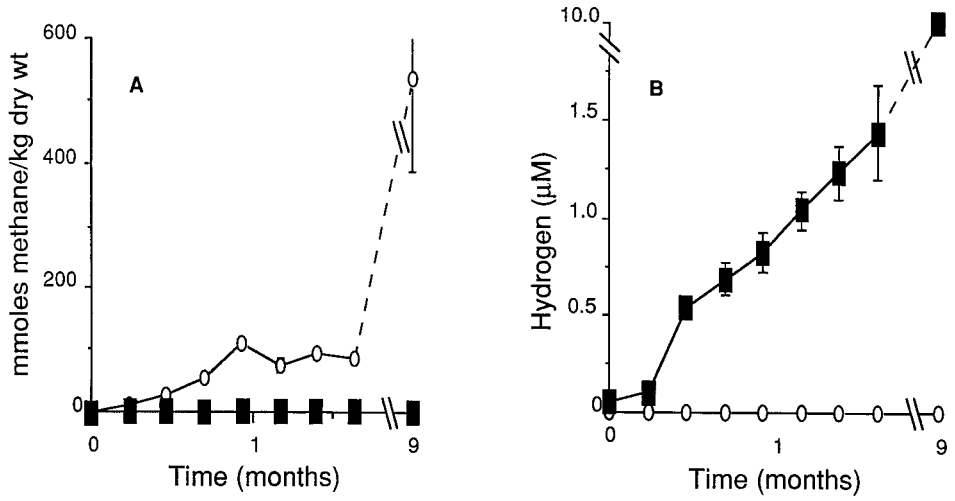

FIG. 3. Comparison of methane (A) and hydrogen (B) production in a methanogenic (○) and a nonmethanogenic (▮) refuse sample. The error bars represent the standard deviations for duplicate incubations.

to 389 mmol of organic acids g (dry weight)$^{-1}$. This accumulation suggests why the pH values of these refuse samples were below 6.5 and also likely explains why methanogen numbers and activity are depressed in these samples.

The accumulation of organic acids in the low-methane-producing samples could be the result of thermodynamic constraints imposed by the concentration of H_2 formed during the initial fermentation reactions of refuse. The $\Delta G'$ values for selected reactions that occurred in landfill samples were calculated with data collected at the end of the incubation period (Table 1). The oxidation of the fatty acids, propionate, butyrate, valerate, and hexanoate, in the methane-producing samples was exergonic ($\Delta G' < -61$ kJ/mol). In the low-methane-producing samples, the oxidation of these compounds was either not as favorable ($\Delta G' > -28$ kJ/mol) or endergonic. The exception was hexanoate oxidation in sample 5 (−58.7 kJ/mol). However, this value is less exergonic than the values for the same reaction in the high-methane-producing samples. These data suggest that as the VFAs were formed during the initial fermentation reactions, the proton-reducing bacteria in the methanogenic samples were able to metabolize these compounds, while the activity of these organisms in the low methane-producing samples was hindered. The oxidation of lactate was very favorable ($\Delta G' < -139$ kJ) in all of the samples.

The $\Delta G'$ values for autotrophic methanogenesis in all assayed samples were highly negative (Table 1). The $\Delta G'$ values for acetoclastic methanogenesis were also negative. Interestingly, these values were less favorable for the high-methane-producing samples than for the low-methane-producing samples. These data suggest that the lack of methanogenic activity in some samples was not due to thermodynamic constraints on this process. Additionally, it appears unlikely that acetogenesis from H_2 and CO_2 occurred to any appreciable degree, since the $\Delta G'$ values for this process were endergonic in all but one sample.

Another possible explanation for the lack of methanogenic activity in the low-methane-producing samples was inhibition due to the undissociated forms of organic acids at low pH values (12). The total concentrations of the undissociated forms of the organic acids in samples 5 through 7 were 3.0, 19.3, and 19.6 mmol kg (dry weight)$^{-1}$, respectively. These

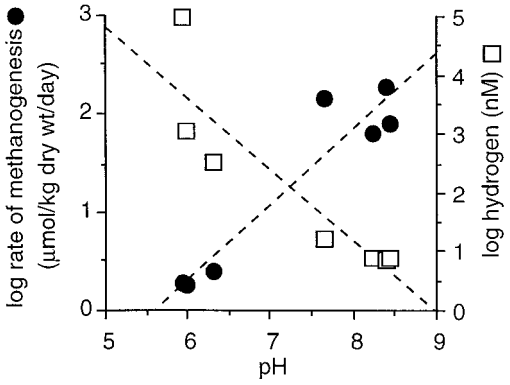

FIG. 4. Comparison of methane production, pH, and H_2 accumulation in selected landfill samples. The dashed lines are the best-fit lines between the datum points.

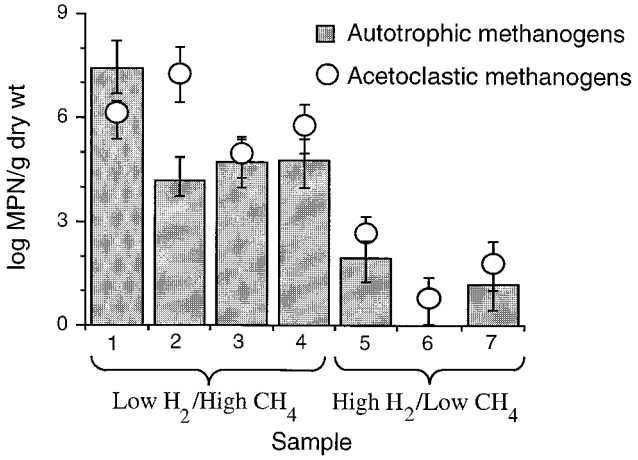

FIG. 5. Enumeration of methanogenic bacteria in high- and low-CH_4-producing refuse samples. Error bars represent the 95% confidence limits. MPN, most probable number.

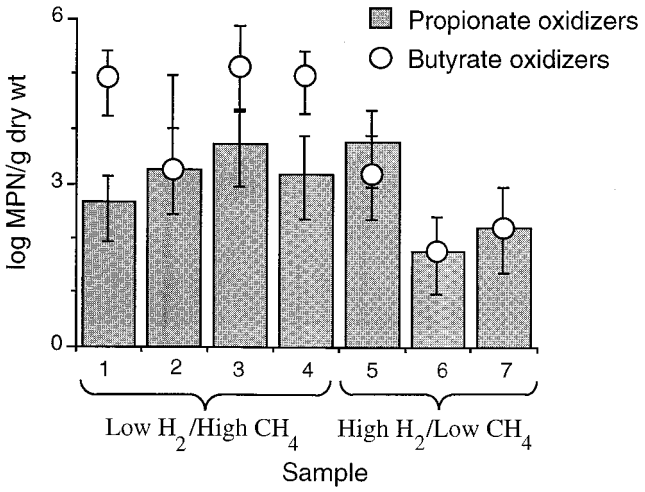

FIG. 6. Enumeration of fatty acid-oxidizing bacteria in high- and low-CH_4-producing refuse samples. Error bars represent the 95% confidence limits. MPN, most probable number.

values were well above the value of 0.5 mM (as acetic acid) shown to cause unstable operating conditions in anaerobic wastewater digesters (3).

DISCUSSION

The levels of H_2 occurring in refuse fermentations have not been systematically evaluated previously. While the range of H_2 concentrations was large (Fig. 2), the median value in the samples from both landfills (1.9 μM) was similar to those reported for sewage digesters (1.2 μM) (33) and the bovine rumen (1.4 μM) (35). The latter environments tend to be electron donor rich and electron acceptor poor, as landfill environments also appear to be. The landfill H_2 concentrations likely reflect the balance, or lack thereof, between production and consumption reactions. Initial fermentative reactions are generally energetically favorable even when the concentration

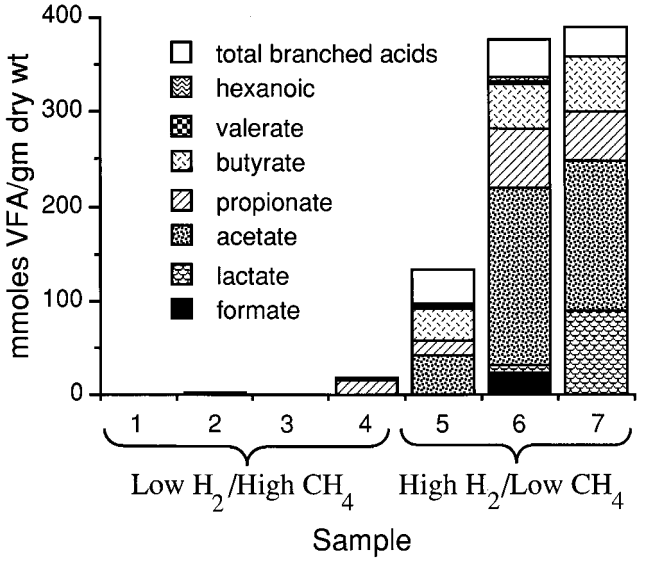

FIG. 7. Concentrations of VFAs in high- and low-CH_4-producing refuse samples.

of H_2 reaches 1 atm (ca. 100 kPa) (41, 44). In a balanced fermentation, H_2 is consumed by methanogens or other organisms, and an apparent steady state was achieved (Fig. 1). With continued incubation, the apparent steady-state H_2 concentration of the high-methane-producing samples declined to levels typical for electron donor-limited methanogenic environments (Fig. 3 and 4) (26). The decline in the steady-state H_2 concentration presumably reflects a large reduction in readily fermentable organic matter in these samples over time. On the other hand, the nonmethanogenic subsamples exhibited the signs of organic overloading, including an accumulation of H_2 and VFAs along with low pH values and a lack of methanogenic activity (Fig. 3, 4, and 7). Organic overloading in anaerobic digester systems results from an uncoupling of degradative reactions (16). Organic overloading is possible in landfills, since they are typically composed of about 47% potentially fermentable materials, such as paper, yard, and food wastes (36).

The inhibition of methanogenesis in some landfill samples can be due to a variety of reasons, including the lack of adequate moisture and suitable inocula. While the former is undoubtedly important (15), it is not a major issue in our current study since we purposely chose samples with comparable moisture contents. The latter reason also does not account for the failure to observe methane production. While absolute cell numbers varied, the appropriate syntrophic and methanogenic populations were evident in each of the samples examined, regardless of its ability to produce methane.

A more likely explanation for the failure to find methanogenesis in some samples includes the accumulation of VFAs and the attendant reduction in pH (14, 27). A potential mechanism for the inhibition at low pH is the toxicity of the undissociated forms of the acids (12). The total concentrations of the undissociated forms of the organic acids were much higher than those found to inhibit propionate degradation in acclimated sludge (12) or to cause unstable operating conditions in sludge digesters (3). However, more recent studies showed that short-chain VFAs (C_2 to C_5) were not inhibitory to methanogenesis in manure slurries at concentrations of ≤50 mM (1).

The H_2 accumulation in the low-methane-producing samples could also adversely affect methanogens. Ahring et al. (2) found neither growth nor acetate utilization by the acetoclastic methanogen *Methanosarcina thermophila* when H_2 levels were ≥1.99 μM. Additionally, *Methanosarcina barkeri* 227 did not consume acetate until the H_2 present was depleted (10). The accumulation of acetate in the low-methane-producing samples (Fig. 7) may be in part a result of an adverse effect of H_2 on the acetoclastic methanogens (Fig. 4). The subsequent accumulation of acetate in these samples would further inhibit other methanogens by decreasing pH values.

The accumulation of VFAs in the low-methane-producing samples is the result of the thermodynamic limitations imposed by the high levels of acetate and H_2 present. As expected, the free energies available for VFA oxidation in these samples were much lower than those in the high-methane-producing samples (Table 1). In both fluidized-bed reactors and lake sediments, VFA oxidations have been thermodynamically inhibited by the accumulation of these two methanogenic precursors (25, 34). In these systems, the oxidation of the VFAs ceased when a critical ΔG value was reached for each reaction even though these values were still negative (25, 34). This might also occur for the oxidative reactions in the low-methane-producing samples (Table 1).

Our results argue that H_2 can be used as an easily measured and sensitive indicator of the status of refuse fermentations. Pauss and Guiot (31) found dissolved H_2 concentrations in

TABLE 1. Changes in Gibbs free energies for selected reactions in landfill samples

Reaction	$\Delta G'$ (kJ/reaction)[a] for:						
	Samples with high CH_4 production				Samples with low CH_4 production		
	1	2	3	4	5	6	7
H_2 production from:							
Propionate[b]	−74.2	−71.8	−64.1	−66.4	−28.7	8.6	−23.7
Butyrate[c]	−118.7	−61.4	−84.3	−118.2	−23.3	6.0	−12.2
Valerate[d]	−92.2	−64.3	−83.6	−83.5	−13.4	24.6	−8.8
Hexanoate[e]	−165.2	−128.8	−167.7	−167.5	−58.7	14.6	−25.1
H_2 consumption							
Methanogenesis[f]	−77.8	−81.2	−77.4	−83.4	−124.0	−80.8	−170.0
Acetogenesis[g]	42.9	31.8	44.0	48.5	30.4	−8.9	36.9
Acetate consumption							
Methanogenesis[h]	−11.6	−11.0	−11.0	−16.1	−49.5	−71.9	−79.3

[a] Calculated by using $\Delta G'_0$ values from reference 38 and concentrations or partial pressures of the products and reactants at the end of the incubation period. A value of 10×10^{-6} M was used for the products and reactants when their concentrations were below detection limits.
[b] $CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$.
[c] $CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$.
[d] $CH_3(CH_2)_3COO^- + 2H_2O \rightarrow CH_3CH_2COO^- + CH_3COO^- + H^+ + 2H_2$.
[e] $CH_3(CH_2)_4COO^- + 4H_2O \rightarrow 3CH_3COO^- + 2H^+ + 4H_2$.
[f] $4H_2 + H^+ + HCO_3^- \rightarrow 3H_2O + CH_4$.
[g] $4H_2 + H^+ + 2HCO_3^- \rightarrow 4H_2O + CH_3COO^-$.
[h] $CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$.

sewage sludge reactors to be positively correlated with the organic matter loading rate and negatively correlated with the methane production rate. The suggestion to monitor H_2 as an indicator of anaerobic fermentations is not new (18, 22, 28, 29). However, the tools to measure the low concentrations of H_2 typically found in anaerobic environments are relatively recently developed (7) and not routinely applied to landfill samples. It should be noted that a number of studies have indicated that measurements of the concentrations of H_2 in the gas phase can underestimate the amount dissolved in the aqueous phase of anaerobic digesters (30, 31). Even if this occurs in landfills, if H_2 is found to accumulate over time and not to reach an apparent steady state, a fermentation imbalance would be indicated and corrective measures could be initiated.

ACKNOWLEDGMENTS

We thank Veena Warikoo for helpful discussions and Neil Wofford for the culture of *M. hungateii* JF1.

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