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The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor *

Ralf Cord-Ruwisch [1], Hans-Jürgen Seitz [2], and Ralf Conrad [2]

[1] Laboratoire de Microbiologie, ORSTOM, Université de Provence, 3 Place Victor-Hugo, F-13331 Marseille Cedex 3, France
[2] Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, Federal Republic of Germany

Abstract. The effect of different electron acceptors on substrate degradation was studied in pure and mixed cultures of various hydrogenotrophic homoacetogenic, methanogenic, sulfate-reducing, fumarate-reducing and nitrate-ammonifying bacteria. Two different species of these bacteria which during organic substrate degradation produce and consume hydrogen, were cocultured on a substrate which was utilized only by one of them. Hydrogen, which was excreted as intermediate by the first strain (and reoxidized in pure culture), could, depending on the hydrogen acceptor present, also be used by the second organism, resulting in interspecies hydrogen transfer. The efficiency of H_2 transfer was similar when methanol, lactate or fructose were used as organic substrate, although the free energy changes of fermentative H_2 formation of these substrates are considerably different. In coculture experiments nitrate or fumarate > sulfate > CO_2/CH_4 > sulfur or CO_2/acetate were the preferred electron acceptors, and an increasing percentage of H_2 was transferred to that bacterium which was able to utilize the preferred electron acceptor. In pure culture the threshold values for hydrogen oxidation decreased in the same order from $\leq 1{,}100$ ppm for homoacetogenic bacteria to about 0.03 ppm for nitrate or fumarate reducing bacteria. The determined H_2-threshold values as well as the percentage of H_2 transfer in cocultures were related to the Gibbs free energy change of the respective hydrogen oxidizing reaction.

Key words: Interspecies H_2-transfer – H_2-threshold – Sulfate-reduction – Nitrate-reduction – Methanogenesis – Competition for H_2 – Gibbs free energy change – Thermodynamics – Electron acceptor

Hydrogen is a key intermediate during degradation of organic matter in anaerobic biotic environments (Hungate 1967; Zehnder 1978; Bryant 1979; Wolin 1982). H_2 is consumed by methanogenic, sulfate-reducing, and homoacetogenic bacteria. Members of these bacterial groups are able to grow chemolithotrophically with H_2 as sole energy source. Thereby they compete for H_2 that has been excreted by fermentative bacteria. Since H_2 concentrations are usually extremely low in anaerobic environments (Strayer and Tiedje 1978; Robinson et al. 1981; Lovley et al. 1982; Conrad et al. 1985, 1986; Novelli et al. 1987) microorganisms are outcompeted for H_2 by others which more effectively utilize traces of H_2. Competition for H_2 between sulfate-reducing and methanogenic bacteria has been thoroughly investigated in the past. It has been shown that sulfate reducing bacteria are able to outcompete the CO_2 reducing methanogenic bacteria for hydrogen because of their higher affinity and higher growth yield (Winfrey and Zeikus 1977; Abram and Nedwell 1978a, b; Kristiansson et al. 1982; Lovley et al. 1982; Lovley and Klug 1983; Lupton and Zeikus 1984; Robinson and Tiedje 1984). However, the question remained why sulfate-reducing bacteria are enabled to exhibit higher affinities for H_2 than methanogenic bacteria (Kristiansson et al. 1982). Recently the threshold concentration for H_2 rather than kinetic parameters has been discussed as the factor determining the outcome of competition for traces of hydrogen (Lovley 1985; Ward and Winfrey 1986). According to this model, the successful organism keeps the H_2 partial pressure below the level that is necessary to allow H_2 oxidation by competitors. Sulfate-reducing bacteria should therefore have lower H_2 threshold levels than methanogenic bacteria. In fact, it has been demonstrated that thresholds of hydrogen oxidation were about one order of magnitude lower in sediments which contained sulfate besides bicarbonate as electron acceptor (Lovley et al. 1982).

However, presently it is unknown which factors affect the magnitude of H_2 threshold concentrations in pure or defined mixed cultures. Beside the catalytic properties of the hydrogenases which catalyze hydrogen oxidation, the Gibbs free energy change of the total reaction, including reduction of the terminal electron acceptors may also be of importance.

Hydrogenotrophic anaerobes, such as chemolithotrophic homoacetogens and sulfate reducers do not only consume H_2 but also produce H_2 (Hatchikian et al. 1976; Tsuji and Yagi 1980; Traoré et al. 1981; R. Cord-Ruwisch, M. Rousset, R. Conrad, unpublished work) when metabolizing organic substrates. Methanogens produce H_2 when growing on CO (O'Brien et al. 1984) or formate (Tzeng et al. 1975) and just recently it has been demonstrated that H_2 is even produced during acetate cleavage by *Methanosarcina barkeri* (Lovley and Ferry 1984; Phelps et al. 1985; Krzycki et al. 1987; Boone et al 1987) and during homoacetogenesis on methanol (Cord-Ruwisch and Ollivier 1986; Heijthuijsen and Hansen 1986). By testing different representatives of homoacetogenic, sulfidogenic, and methanogenic bacteria (Cord-Ruwisch 1986; R. Cord-Ruwisch, M. Rousset, R. Conrad, unpublished work) we have recently shown that H_2 is in general a minor but significant product during

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Offprint requests to: R. Cord-Ruwisch

metabolism of various organic substrates. This indicates that facultatively hydrogenotrophic anaerobes maintain a certain H_2 partial pressure during metabolism. Maintenance of a certain H_2 partial pressure, however, would allow the utilization of this H_2 by other bacteria provided they exhibit a H_2 threshold below that level.

Here we show that the threshold concentration of hydrogen is predominantly dependent on the redox potential of the terminal electron acceptor, and that H_2 is transferred from one facultatively hydrogenophilic bacterium to another one, if the latter is able to more effectively utilize the traces of H_2 due to the thermodynamically more favourable electron acceptor.

Materials and methods

Bacteria. The following bacterial strains were obtained from the German Collection of Microorganisms (DSM); *Desulfovibrio fructosovorans* strain JJ (DSM 3604), *Desulfovibrio desulfuricans* strain ESSEX (DSM 642), *Desulfovibrio vulgaris* strain G6 (as isolated from the defined syntrophic association with *Syntrophus buswellii*, DSM 2612TB), *Methanobrevibacter arboriphilus* (DSM 744), *Methanobrevibacter smithii* (DSM 861), *Methanococcus vannielii* (DSM 1224), *Methanobacterium formicicum* (DSM 1535), *Methanospirillum hungatei* (DSM 864), *Sporomusa acidovorans* (DSM 3132), *Sporomusa sphaeroides* (DSM 2875), *Wolinella succinogenes* (DSM 1740). *Desulfobulbus elongatus* and *Methanospirillum* SK 6 were kindly donated by Dr. Samain, INRA, Lille, France and Dr. F. Widdel, University of Urbana, Illinois, USA, respectively. *Desulfovibrio vulgaris* strain Hildenborough was obtained from Dr. C. Hatchikian, CNRS-Marseille, France. *Acetobacterium carbinolicum* and *Acetobacterium woodii* strain NZ Va 16 were kindly provided by B. Eichler and R. Bache, University of Konstanz, FRG, respectively. *Sporomusa termitida* isolated from termite hindguts, was kindly donated by Dr. Breznak, Michigan State University, E-Lansing, USA.

Medium and growth conditions. The anaerobic, bicarbonate buffered, sulfide-reduced freshwater medium, as described for fatty acid degrading sulfate-reducing bacteria (Widdel and Pfennig 1984) was modified by adding 1–3 mM acetate (as carbon source) and by omitting sulfate. For coculture experiments and for pure cultures with *Acetobacterium woodii*, *Wolinella succinogenes* and *Sporomusa* species 0.05% yeast extract was added. Electron donors and acceptors were added from sterile anaerobic stock solutions. If not otherwise stated, their final concentrations were (in mmol/l): fructose 10; lactate 20; methanol 20; formate 40; sulfate 20; nitrate 10; fumarate 60; caffeate 5; sulfite 10; thiosulfate 20; sulfur as powder in excess. All chemicals used were of reagent quality.

Threshold experiments. H_2-thresholds were determined in cultures grown heterotrophically on organic electron donors or chemolithotrophically on H_2. In heterotrophically grown cultures H_2 was produced and consumed during organic substrate degradation until a nearly constant H_2 concentration, i.e. the threshold, was reached after depletion of the organic substrate. In H_2-grown cultures, the gas phase was replaced by H_2-free N_2/CO_2 (80/20) and pressurized with N_2 to 1 bar overpressure. A limiting amount of H_2 (giving about 10,000 ppm) was added and the oxidation of H_2 was followed until a threshold was reached. The H_2-threshold measurements were carried out at 28–34° C using 120 ml serum bottles with 80% gas phase of H_2- and O_2-free N_2/CO_2 (80/200) mixtures. Throughout the experiments the cultures were rapidly stirred or shaken to ensure equilibrium of gas concentrations between the liquid phase and the gas phase. Hydrogen partial pressures were determined by analyzing gas samples taken from the headspace and correcting for the headspace pressure measured with a needle manometer.

Analytical methods. Methane and alcohols were determined by gas chromatography as described by Cord-Ruwisch et al. (1986). Sulfide was determined spectrophotometrically as colloidal CuS (Cord-Ruwisch 1985). Organic acids and fructose were analyzed by HPLC (Cord-Ruwisch et al. 1986). Hydrogen was measured with a H_2 analyzer based on the HgO-Hg conversion technique (Seiler 1978; Seiler et al. 1980), and with the commercial H_2 analyzer (RGD2 & RGA2) of Trace Analytical, Stanford, CA, USA. The detection limit for H_2 was 0.01 ppm.

Competition experiments. All coculture experiments were carried out at 34° C with 10% freshly grown inoculum taken during the exponential growth phase of the respective pure cultures. Bacteria were grown on methanol lactate or fructose with or without a second bacterial strain which was unable to utilize the organic substrate but was able to use H_2 as electron donor which was eventually excreted by the first strain. The second bacterial strain was able to utilize electron acceptors which were added but could not be used by the first strain. The H_2-producing and H_2 consuming reactions studied are summarized together with the standard Gibbs free energies per mol of H_2 formed or utilized and the redox potentials under standard conditions and pH 7 (Table 1). The fermentation balances of the cocultures were determined after the organic substrate was utilized by measuring the degradation products (acetate, ethanol) and the reduced electron acceptors. The stoichiometry of the H_2-producing reactions listed in Table 1 allowed to calculate the amount of reducing equivalents transferred (presumably as H_2) from one strain to the other.

Results

Coculture experiments

In pure culture, the homoacetogenic bacterium *Sporomusa acidovorans* produced 0.75 mol acetate from oxidation of 1 mol of methanol by using CO_2 as electron acceptor (Cord-Ruwisch and Ollivier 1986). However, when cocultured with hydrogen consuming anaerobes, *S. acidovorans* reduced much less CO_2 to acetate and instead allowed growth of the hydrogenotrophes present, by transfer of reducing equivalents (Table 2).

This transfer occurred with more than 90% efficiency when *S. acidovorans* was cocultured with fumarate, sulfate or nitrate-reducing H_2 oxidizers (Table 2). In this case *S. acidovorans* oxidized methanol, presumably to CO_2, but did not form acetate, while the cocultured bacterium reduced sulfate to sulfite (*Desulfovibrio* sp.), nitrate to ammonium (*Wolinella succinogenes*), or fumarate to succinate (*W. succinogenes*) in almost stoichiometric amounts. Since the cocultured hydrogenotrophic bacteria were unable to oxidize

Table 1. Overview of reactions studied and of their Gibbs free energies and redox potentials under standard conditions

Reactions			$\Delta G^{o\prime}$ [kJ/mol H_2]	$E^{o\prime}$ [mV]
H_2-producing reactions				
$C_6H_{12}O_6 + 4\,H_2O$	↔	$2\,CH_3-COO^- + 2\,HCO_3^- + 4\,H^+ + 4\,H_2$	−51.5	−681
$CH_3-CHOH-COO^- + 2\,H_2O$	↔	$CH_3-COO^- + HCO_3^- + H^+ + 2\,H_2$	−2.0	−424
$CH_3OH + 2\,H_2O$	↔	$HCO_3^- + H^+ + 3\,H_2$	+7.7	−374
H_2-consuming reactions				
$4\,H_2 + 2\,HCO_3^- + H^+$	↔	$CH_3-COO^- + 4\,H_2O$	−26.1	−279
$H_2 + S^o$	↔	$HS^- + H^+$	−27.8	−270
$4\,H_2 + HCO_3^- + H^+$	↔	$CH_4 + 3\,H_2O$	−33.9	−238
$4\,H_2 + SO_4^{2-} + H^+$	↔	$4\,H_2O + HS^-$	−38.0	−217
$4\,H_2 + S_2O_3^{2-}$	↔	$2\,HS^- + 3\,H_2O$	−43.5	−189
$3\,H_2 + SO_3^{2-}$	↔	$HS^- + 3\,H_2O$	−57.2	−118
H_2 + caffeate	↔	Hydrocaffeate	−85.5[a]	+29
H_2 + fumarate	↔	Succinate	−86.2	+32
$4\,H_2 + NO_3^- + 2\,H^+$	↔	$NH_4^+ + 3\,H_2O$	−149.9	+363

Values calculated from Thauer et al. (1977). $E^{o\prime}$ of the couples accepting electrons from H_2 was calculated relative to the redox potential of H_2 (−414 mV)
[a] Estimated from Grbić-Galić (1985)

Table 2. The effect of different hydrogen consuming bacteria on CO_2 reduction to acetate by *Sporomusa* species degrading methanol, fructose or lactate

Organic substrate	Strain I	Strain II	Electron accept. II	Electron accept. II reduced per mole substrate oxidized	Acetate produced from CO_2-reduction	Transfer of H_2 [%] to strain II
Methanol	*Sporomusa*	*Methanosprill.*	CO_2	0.27 methane	0.41	36— 55
Methanol	*acidovorans*	*Desulfovibrio*	sulfate	0.69 sulfide	0.00	92—100
Methanol		*Wolinella*	nitrate	0.73 ammonium	0.00	97—100
Fructose		*Desulfovibrio*	sulfate	0.94 sulfide	0.00[a]	94—100
Methanol	*Sporomusa*	*Desulfovibrio*	sulfur	0.24 sulfide	0.57	8— 24
Methanol	*sphaeroides*	*Wolinella*	fumarate	2.75 succinate	0.00	92—100
Lactate		*Wolinella*	fumarate	1.73 succinate	0.00[b]	86—100

All values given in mol per mol substrate degraded. Transfer of hydrogen was calculated from the relative amount of electron acceptor II reduced as well as from the deficit of acetate produced from CO_2
[a] 1.88 mol acetate produced/mol fructose were considered to originate from fructose degradation
[b] 0.95 mol acetate produced/mol lactate were considered to originate from lactate degradation

methanol they apparenty were able to "pull" reducing equivalents presumably as H_2, from the methanol-degrading *Sporomusa*. The same observation was made when fructose served as electron donor or with *Sporomusa sphaeroides*, degrading lactate, in the presence of the mentioned hydrogenotrophs. CO_2-reducing methanogens or sulfur reducing *Desulfovibrio* only "pulled" 8—40% of the total reducing equivalents produced as H_2 by the *Sporomusa* species. Similar results were obtained when *Acetobacterium woodii* served as H_2 producing bacterium.

Growing on lactate plus sulfate, *Desulfovibrio vulgaris* allowed 24—30% transfer of reducing equivalents (as H_2) to *W. succinogenes* reducing NO_3^- which is the energetically more favourable electron acceptor than sulfate. However, no H_2 was transferred to *Methanospirillum hungatei* or *S. sphaeroides* using an electron acceptor which is energetically less favourable than sulfate (Table 3). When replacing sulfate by elemental sulfur, which is the energetically less favourable electron acceptor *Desulfovibrio vulgaris* no longer produced sulfide but transferred all reducing equivalents to *W. succinogenes* (Table 3). Cocultures of sulfur-reducing *D. vulgaris* with CO_2 reducing *S. acidovorans* or *M. hungatei* resulted in reduction of both electron acceptors (Table 3).

In the absence of external electron acceptors *Desulfovibrio fructosovorans* strain JJ ferments 2 mol fructose to 1 mol succinate, 1 mol acetate, and 1 mol ethanol (Cord-Ruwisch et al. 1986). Only traces of hydrogen (7,600 ppm) were produced during this fermentation. In the presence of sulfur or sulfate *D. fructosovorans* also used these external electron acceptors and produced sulfide instead of ethanol or succinate. External electron acceptors such as sulfate or sulfur apparently were competing for reducing equivalents with the internal electron acceptors such as acetylaldehyde or fumarate. The same shift of electron flow away from the formation of the fermentation products ethanol and succinate was observed when the activity of hydrogen consuming bacteria allowed the use of protons as external electron sink by coupling by oxidation of fructose via interspecies H_2 transfer to CO_2 or NO_3^- reduction (Table 4). The amount of H_2 transferred to the accepting systems was correlated to the free energy change of the oxidation of H_2 with the electron acceptor used. Virtually all reducing

Table 3. The effect of different hydrogen consuming bacteria on sulfide production by *Desulfovibrio vulgaris* strain G6 during lactate degradation

Electron acceptor I	Strain II	Electron acceptors reduced$_2$			H_2 transferred [%] to strain II
		Electron acceptor II	Strain I (sulfide)	Strain II	
Sulfate	*Methanospirillum*	CO_2	0.47	0.0	0
Sulfate	*Sporomusa sphaeroides*	CO_2	0.51	0.0	0
Sulfate	*Wolinella*	nitrate	0.35	0.12 ammonia	24– 30
Sulfur	*Methanospirillum*	CO_2	0.73	0.30 methane	60– 64
Sulfur	*Sporomusa acidovorans*	CO_2	0.34	0.37 acetate[a]	74– 83
Sulfur	*Wolinella*	nitrate	0.0	0.46 ammonia	92–100

Values given in mol per mol lactate degraded
[a] Corrected by considering of 1 mol acetate produced per mol of lactate

Table 4. Effect of different external hydrogen acceptors on the reduction of internal electron acceptors (ethanol and succinate production) by *Desulfovibrio fructosovorans* during the growth on fructose. Values are given in mol per mol fructose degraded

External H_2-sink		Acetate	Ethanol	Succinate	External hydrogen acceptor reduced	H_2 transfer to external H_2-sink
Without		0.4	0.3	0.8	–	0.0
Sulfur		1.1	0.2	1.0	1.0 H_2S	1.0
Sulfate		1.9	0.0	0.0	1.0 H_2S	4.0
Sporomusa sphaeroides	(CO_2)	2.4	0.1	1.0	0.4 acetate	1.6
Methanospirillum hungatei	(CO_2)	1.2	0.0	0.3	0.7 CH_4	2.8
Wolinella succinogenes	(NO_3^-)	1.7	0.0	0.1	1.2 NH_4^+	4.0

equivalents of fructose were used to reduce sulfate or nitrate if present as external electron acceptor, whereas only part of the electrons were used to reduce CO_2 or sulfur as external H_2 sink. In the latter case, internal electron acceptors generated during fermentation of fructose apparently were preferred and succinate and ethanol were excreted as reduced products of fructose oxidation.

Pure culture experiments

The results of the coculture experiments suggested that the efficiency of competition between the strains depended on their capacity to oxidize low concentrations of hydrogen. Therefore, the threshold values of hydrogen oxidation were measured in pure cultures of the individual strains used for the coculture experiments and compared to the threshold values of other strains of homoacetogenic, methanogenic and sulfate-reducing bacteria. Figure 1 shows the partial pressures of H_2 in cultures of *Desulfovibrio desulfuricans* growing on lactate with different electron acceptors. H_2 was intermediarily formed during lactate degradation similar as shown by previous experiments (Hatchikian et al. 1976; Tsuji and Yagi 1980; Traoré et al. 1981; Lupton et al. 1984) but was degraded until a certain threshold value was reached. These threshold H_2-mixing ratios were about 5–25 ppm when sulfate, thiosulfate, sulfite or sulfur served as the only electron acceptor (Fig. 1). With nitrate, however, which is an energetically much more favourable electron acceptor than sulfate (see Table 1) hydrogen was oxidized down to mixing ratios 100-fold lower (0.02–0.03 ppm, Fig. 1).

Figure 2 shows similar experiments with *A. woodii* growing on hydrogen as electron donor and with or without

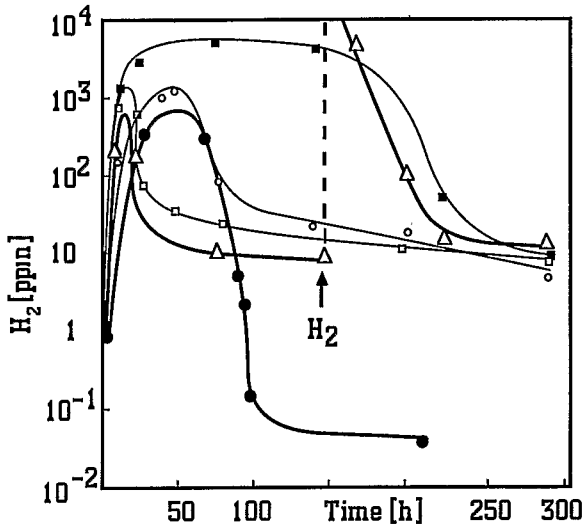

Fig. 1. Time course of hydrogen production and consumption during and after lactate degradation by *Desulfovibrio desulfuricans* Essex with different external electron acceptors. Concentrations are given in the Materials and methods section: ● Nitrate, △ sulfate, □ thiosulfate, ○ sulfite, ■ sulfur

caffeate as additional electron acceptor (Bache and Pfennig 1981; Tschech and Pfennig 1984). The final H_2 threshold was much lower with caffeate than with CO_2 as electron acceptor, again indicating that the energetically more favourable electron acceptor allows the utilization of lower H_2 concentrations. Similar H_2 threshold concentrations

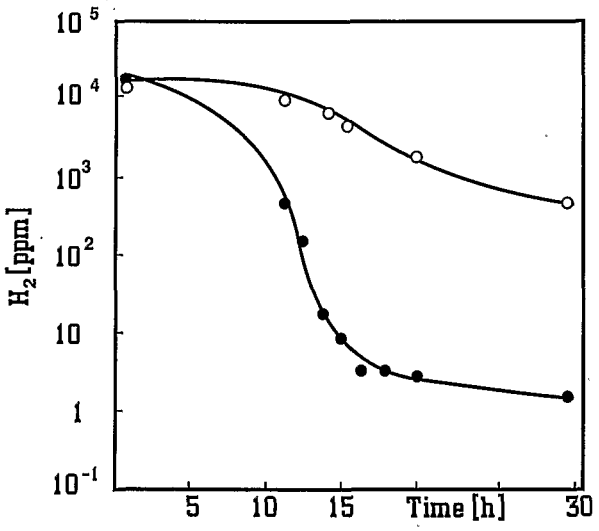

Fig. 2. Time course of hydrogen oxidation by *Acetobacterium woodii* with CO_2 and CO_2 + caffeate as terminal electron acceptor. Two cultures were grown on H_2/CO_2 (80/20) and on H_2/ CO_2 + caffeate [5 mM], respectively. After the end of growth H_2 (ca. 10,000 ppm) was added to both cultures and caffeate [1 mM] to one of them. ○ CO_2, ● CO_2 + caffeate

were reached, however, with *W. succinogenes* and *Desulfovibrio desulfuricans* which both are able to oxidize H_2 by the reduction of fumarate to succinate or nitrate to ammonia (Table 5).

Table 5 summarizes the H_2-thresholds that were measured for the individual H_2-oxidizing reactions by using various strains of bacteria. The results indicate that there is a systematic trend from relatively high (ca. 1,000 ppm H_2) to very low (ca. 0.03 ppm H_2) threshold concentrations with increasing Gibbs free energy changes of the H_2-consuming reaction.

Discussion

Our results show that two bacterial species could compete in coculture for an organic substrate although one of them was unable to utilize the substrate in pure culture. This is most probably due to the fact that small amounts of H_2 are produced during the degradation of the substrate by the first species and that both species compete for the excreted H_2. This results in an interspecies H_2 transfer as soon as the second bacterium has a similar or higher H_2 utilizing efficiency than the first one. The efficiency of transfer of reducing equivalents was paralleled by the efficiency to utilize H_2 down to low threshold concentrations and dependent on the energetics of the H_2-utilizing reaction. Hence, electron acceptors with increasing redox potentials resulted in decreasing H_2 thresholds and increasing H_2 transfer to those bacterial species which were able to utilize the energetically more favourable electron acceptor. This interspecies communication via H_2 appears to be independent of the H_2 producing reaction, as similar results were obtained with methanol, lactate and fructose as organic substrates. These substrates are distinguished by the Gibbs free energy and thus by the ease of H_2 liberation during substrate degradation.

The degradation of a substrate can be accomplished in syntrophy by one bacterium oxidizing the substrate by reducing protons and the other bacterium oxidizing H_2 by reducing a suitable electron acceptor (McInerney and Bryant 1980; Wolin 1982; Mah 1982; Schink 1987). Recently it has been shown that methanol-utilizing homoacetogens completely transferred the reducing equivalents generated during substrate oxidation to H_2-utilizing sulfate reducers (Cord-Ruwisch and Ollivier 1986; Heijthuijsen and Hansen 1986). The interspecies H_2-transfer took place although the homoacetogen was not able to conserve energy for growth during oxidation of methanol. Interspecies H_2-transfer was even observed in cocultures of sulfate-reducing *Desulfovibrio* with the acetate utilizing *Methanosarcina* as H_2 donor (Phelps et al. 1985). *Methanosarcina* apparently produced less CH_4 from the methyl group of acetate when an H_2-accepting bacterium was present that was able to "pull" reducing equivalents by energetically favourable H_2-uptake reaction. In this case the two electron acceptors in competition for reducing equivalents were apparently the methyl group of acetate and sulfate (via proton reduction to H_2). Our results are in line with these observations, indicating that reducing equivalents are utilized by those metabolic reactions which allow the higher yield of energy.

Our results further indicate that H_2-transfer may be brought about by reaching a lower H_2-threshold concentration when the H_2-utilizing reaction is thermodynamically more efficient. This supports our view (R. Cord-Ruwisch, M. Rousset, R. Conrad, unpublished work) that facultatively hydrogenotrophic bacteria communicate by means of a particular range of H_2 concentration (H_2-buffer) that is maintained within the ecosystem as long as the availability of electron donors and acceptors is maintained, i.e., the thermodynamic conditions of H_2 utilization are constant.

The thermodynamic conditions do not only depend on the nature of the redox couple but also on the concentrations of the electron donor H_2 and of the different electron acceptors. Hence, the low solubility of elemental S^0 as electron acceptor may explain why sulfur-reducing bacteria competed less efficiently with homoacetogenic and methanogenic bacteria for reducing equivalents, so that the latter species were able to pull 60–80% of the reducing equivalents from sulfur-reducing but not from sulfate-reducing *Desulfovibrio*. Similarly, the fermentative disproportionation of fructose by *Desulfovibrio fructosovorans* was partially replaced by reduction of external sulfur or sulfate, or by transfer to H_2-utilizing homoacetogens and methanogens, although the reduction of intracellularly generated fumarate to succinate should be energetically more favorable. The intracellular fumarate concentrations were most probably too low to compete for reducing equivalents with the high concentrations of external electron acceptors. This is in accordance with the observation that fumarate was used in addition to sulfate as electron acceptor when the electron donor H_2 was saturating, but were used instead of sulfate, when H_2 concentrations were low (unpublished work).

Nitrate is used as electron acceptor for respiratory nitrate ammonification by *Wolinella succinogenes* (Bokranz et al. 1983) and *Desulfovibrio desulfuricans* (Seitz and Cypionka 1986). Both bacteria showed the highest efficiency to use low concentrations of H_2 and to pull reducing equivalents from other facultative hydrogenotrophic bacteria in the

Table 5. Effect on electron acceptor on the threshold of H_2 in different hydrogenophilic bacteria growing on organic substrates or H_2. Values given are means of at least duplicate experiments. Reproducibility was about ± 50%

Electron acceptor oxidized/reduced	Microorganism	Substrate	H_2 [ppm]
CO_2/acetate	*Sporomusa acidovorans*	methanol	430
	Sporomusa termitida	H_2	830
	Acetobacterium woodii	H_2	520
	Acetobacterium carbinolicum	H_2	950
Sulfur/sulfide	*Desulfovibrio fructosovorans* JJ	lactate	24
	Desulfovibrio desulfuricans Essex	lactate	10
	Wolinella succinogenes	H_2	5
CO_2/CH_4	*Methanospirillum hungatei*	formate	25
	Methanospirillum hungatei	H_2	30
	Methanobrevibacter smithii	H_2	100
	Methanobrevibacter arboriphilus	H_2	90
	Methanobacterium formicum	H_2	28
	Methanococcus vannielii	H_2	75
Sulfate/sulfide	*Desulfovibrio fructosovorans* JJ	lactate	12
	Desulfovibrio vulgaris Hildenborough	lactate	19
	Desulfovibrio vulgaris G6	lactate	16
	Desulfovibrio desulfuricans Essex	lactate	8
	Desulfovibrio desulfuricans Essex	H_2	9
	Desulfobulbus elongatus	lactate	13
Sulfite/sulfide	*Desulfovibrio desulfuricans* Essex	lactate	6
Thiosulfate/sulfide	*Desulfovibrio desulfuricans* Essex	lactate	7
Caffeate/hydrocaffeate	*Acetobacterium woodii*	H_2	3
Fumarate/succinate	*Wolinella succinogenes*	H_2	0.02
	Desulfovibrio fructosovorans JJ	fumarate	0.9[a]
Nitrate/ammonia	*Desulfovibrio desulfuricans*	lactate	0.03
	Desulfovibrio desulfuricans	H_2	0.03
	Wolinella succinogenes	H_2	0.02

[a] *D. fructosovorans* disproportionates fumarate to acetate and succinate, so that fumarate as electron acceptor may become limiting at the end of growth rather than H_2

presence of an organic electron donor. Even sulfate reduction was partially outcompeted by nitrate ammonification. It has earlier been shown that sulfate reduction in sediments may be inhibited upon addition of nitrate. This effect has been explained by the toxicity of eventually produced intermediates (nitrite) (Hukelikian 1943; Jenneman et al. 1986). However, the inhibitory effect of nitrate may as well be explained by successful competition for H_2 as important intermediate of organic matter degradation similar as the inhibitory effect of sulfate on methane production was explained (Winfrey and Zeikus 1977; Abram and Nedwell 1978a; Kristiansson et al. 1982).

Our results confirm the general validity of the concept that H_2-consuming reactions with better energetics dominate in mixed cultures and presumably also in the environment. Our results further indicate that the level of the H_2 threshold concentration apparently is not limited by the first step, the oxidation of H_2 catalyzed by hydrogenases but by the redox potential of the terminal electron acceptor: Whereas H_2-threshold concentrations were markedly different for different electron acceptors, they appear rather similar for the same electron acceptor even if different bacterial strains with different hydrogenases and other enzyme activities were tested.

It is unknown, whether the terminal electron acceptor may affect the kinetic properties (K_m, μ_{max}) of the H_2 consumption reaction. It has been calculated that the rate of organic substrate degradation in H_2-syntrophic cocultures is dependent on the efficiency of the H_2 consumer to use low concentrations of H_2 (Powell 1984, 1985; Archer and Powell 1985). In light of these calculations our observations suggest that the terminal electron acceptor may be the limiting factor for the rate of substrate degradation. This is in accordance with the observation that syntrophic cocultures grow more rapidly with sulfate reducers than with methanogens as H_2 scavengers (Boone and Bryant 1980; Mountfort and Bryant 1982; McInerney et al. 1981). The relation of rates of substrate degradation to the presence of different electron acceptors and for different H_2 scavenging bacteria is presently studied in our laboratory.

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