APPLIED MICROBIAL AND CELL PHYSIOLOGY

S. N. Parshina · S. Kijlstra · A. M. Henstra · J. Sipma ·
C. M. Plugge · A. J. M. Stams

Carbon monoxide conversion by thermophilic sulfate-reducing bacteria in pure culture and in co-culture with *Carboxydothermus hydrogenoformans*

Abstract Biological sulfate (SO_4) reduction with carbon monoxide (CO) as electron donor was investigated. Four thermophilic SO_4-reducing bacteria, *Desulfotomaculum thermoacetoxidans* (DSM 5813), *Thermodesulfovibrio yellowstonii* (ATCC 51303), *Desulfotomaculum kuznetsovii* (DSM 6115; VKM B-1805), and *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* (DSM 14055), were studied in pure culture and in co-culture with the thermophilic carboxydotrophic bacterium *Carboxydothermus hydrogenoformans* (DSM 6008). *D. thermoacetoxidans* and *T. yellowstonii* were extremely sensitive to CO: their growth on pyruvate was completely inhibited at CO concentrations above 2% in the gas phase. *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* were less sensitive to CO. In pure culture, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* were able to grow on CO as the only electron donor and, in particular in the presence of hydrogen/carbon dioxide, at CO concentrations as high as 50–70%. The latter SO_4 reducers coupled CO oxidation to SO_4 reduction, but a large part of the CO was converted to acetate. In co-culture with *C. hydrogenoformans*, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* could even grow with 100% CO (P_{CO}=120 kPa).

S. N. Parshina (✉)
Laboratory of Microbiology of Anthropogenic Environments,
Winogradsky Institute of Microbiology,
Russian Academy of Sciences,
Prosp. 60 let Oktyabrya, 7-2,
117811 Moscow, Russia
e-mail: Sonjaparshina@mail.ru
Tel.: +7-095-1356553
Fax: +7-095-1356530

S. Kijlstra
Shell Global Solutions International,
B.V. P.O. Box 38000, 1030 BN Amsterdam,
The Netherlands

A. M. Henstra · C. M. Plugge · A. J. M. Stams
Laboratory of Microbiology,
Wageningen University,
Hesselink van Suchtelenweg 4,
6703 CT Wageningen,
The Netherlands

J. Sipma
Sub-Department of Environmental Technology,
Wageningen University,
Bomenweg 2,
P.O. Box 8129, 6700 EV Wageningen,
The Netherlands

Introduction

A mesophilic process that applies biological reduction of sulfate (SO_4)/sulfite (SO_3) to hydrogensulfide (H_2S), and subsequent biological conversion of the produced H_2S to elemental sulfur (S), is suggested as a cost-effective method for the removal of S compounds from waste streams (Maree et al. 1987; Lens et al. 1998). Thermophilic treatment of SO_4/SO_3 rich wastewater is an attractive alternative for the currently employed mesophilic treatment of hot wastewater of paper and pulp industries or for the conventional process of flue gas desulfurization.

Many SO_4-rich wastewaters are poor in organic matter. Therefore, a supply of an appropriate electron donor is essential to reduce SO_4. Hydrogen (H_2) is an excellent electron donor for SO_4 reduction (Widdel and Hansen 1992; van Houten et al. 1994, 1997). Synthesis gas is a cheap source of H_2-rich gas. It is produced by steam reforming of natural gas or by thermal gasification of coal, oil, biomass, or other organic matter (Graboski 1984). Synthesis gas, depending on its origin, typically contains H_2 (30–76 vol%), carbon monoxide [(CO) 15–59 vol%], carbon dioxide [(CO_2) 8–27 vol%], and traces of methane, nitrogen (N_2), and hydrogen sulfide (H_2S) (Perry et al. 1997). The major restriction of synthesis gas utilization for biological S removal is the relative high percentage of CO.

Some SO_4-reducing bacteria can use CO as an electron donor (Mörsdorf et al. 1992; Davidova et al. 1994). Nevertheless, they are also strongly inhibited by CO (Davidova et al. 1994). *Desulfotomaculum orientis* and *Desulfotomaculum nigrificans* grow slowly on CO up to 20% in the gas phase (Klemps et al. 1985), as does *Desulfovibrio desulfuricans* (Karpilova et al. 1983). *Desulfovibrio vul-*

garis oxidizes CO (maximum 4.5%) to CO_2 coupled to H_2 formation, which is subsequently used as an electron donor for SO_4 reduction (Lupton et al. 1984). Biological SO_4 reduction with a H_2/CO mixture as electron donor was studied in mesophilic lab-scale gas-lift reactors (van Houten et al. 1996). SO_4 reduction was observed with 20% of CO in the feed gas. However, 5% CO already resulted in lower rates of SO_4 reduction. The microbial population of the CO-fed reactor mainly consisted of *Desulfovibrio* and *Acetobacterium* species. The authors speculated that a main part of the CO was converted by homoacetogens, preventing CO toxicity for SO_4-reducing bacteria. Several anaerobic bioreactor sludges at 55°C were able to convert 100% CO in SO_4-free media to H_2 or to methane via H_2 as intermediate (Sipma et al. 2003). Recently, it was demonstrated that in anaerobic bioreactor sludges, both CO and H_2 were used by SO_4-reducing bacteria that tolerated and used high CO (P_{co}>1.6 bar) concentrations (Sipma et al. 2004).

Thus, current knowledge indicates that synthesis gas is poorly suitable as electron donor for SO_4 reduction under mesophilic conditions due to the sensitivity of SO_4-reducing bacteria towards CO. The mechanism of CO inhibition of SO_4 reduction is poorly understood. Here, we aim to avoid or reduce the effect of CO inhibition on SO_4 reduction, starting from two perspectives. Recent findings indicate that CO is better tolerated under thermophilic conditions, as illustrated above. We selected four thermophilic SO_4-reducing bacteria capable of chemolithoautotrophic growth with SO_4: *Desulfotomaculum thermoacetoxidans* (Min and Zinder 1990), *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* (Plugge et al. 2002), *Desulfotomaculum kuznetsovii* (Nazina et al. 1988), and *Thermodesulfovibrio yellowstonii* (Henry et al. 1994). *D. kuznetsovii* (Nazina et al. 1988) and *D. thermoacetoxidans* (Min and Zinder 1990) were characterized as a SO_4 reducers able to convert organic substrates completely to CO_2 coupled to SO_4 reduction. *D. thermobenzoicum* subsp. *thermosyntrophicum* (Plugge et al. 2002) and *T. yellowstonii* (Henry et al. 1994) oxidize organic substrates incompletely to acetate coupled to SO_4 reduction. *D. thermobenzoicum* subsp. *thermosyntrophicum* (Plugge et al. 2002) and *D. thermoacetoxidans* (Min and Zinder 1990) produce actetate and SO_4 during growth on H_2/CO_2 plus SO_4. These four strains have not been tested previously with CO. Conversion of CO to H_2 by thermophilic anaerobes may provide additional means to avoid CO inhibition. An increasing number of anaerobes grow by the conversion of CO to H_2 but do not reduce SO_4 (Fardeau 2004; Sokolova et al. 2001, 2002, 2004a, b; Svetlichnyi et al. 1991, 1994). Formation of H_2 and removal of CO by these bacteria may remove inhibition and thus stimulate SO_4 reduction. For this purpose, *Carboxydothermus hydrogenoformans* was selected and used in co-culture with the selected SO_4-reducing bacteria. Our results demonstrate the potential of thermophilic SO_4 reduction with synthesis gas. Further insight in CO inhibition on SO_4-reducing bacteria is provided as well.

Materials and methods

Bacterial strains and growth conditions

The following bacterial strains were used in the experiments: *C. hydrogenoformans* (DSM 6008) (Svetlichniy et al. 1991), *D. thermoacetoxidans* (DSM 5813) (Min and Zinder 1990), *D. thermobenzoicum* subsp. *thermosyntrophicum* (DSM 14055) (Plugge et al. 2002), *D. kuznetsovii* (DSM 6115; VKM B-1805) (Nazina et al. 1988), and *T. yellowstonii* (ATCC 51303) (Henry et al. 1994). Bacteria were grown anaerobically in a basal mineral bicarbonate-phosphate buffered medium that contained (in g/l of demineralized water) KH_2PO_4 (0.38), Na_2HPO_4 (0.54), NH_4Cl (0.3), NaCl (0.3), $CaCl_2·2H_2O$ (0.11), $MgCl_2·6H_2O$ (0.1), $NaHCO_3$ (2.4), $Na_2S·9H_2O$ (0.29), resazurin (0.0005), yeast extract (0.5 g/l), trace elements (1 ml), and vitamins (1 ml). Trace elements and vitamins were prepared as described by Stams et al. (1993). Sodium sulfate (20 mM) was added for the cultivation of SO_4-reducing bacteria. When indicated, 10 mM sodium pyruvate and 10 mM sodium sulfate were supplied. Bacteria were grown in 250-ml or 120-ml serum bottles that contained 50 ml medium and were sealed with butyl rubber stoppers and aluminum caps. The precultures were grown on H_2/CO_2 plus SO_4, for experiments with pure CO precultures were grown on 20% CO plus SO_4. In all experiments, the inoculum size was 5% of each species of bacteria. The headspace was flushed with H_2/CO_2 (80:20), CO, or different ratios of CO/N_2, CO/H_2, and $CO/H_2/CO_2$. The protocol for making the gas phase was as follows: 120-ml bottles with 50 ml of medium were flushed with N_2. Vacuum (0.2 bar) was created in the bottles, and CO was added to give a volume percentage (vol%) in the gas phase of 5, 20, 50 or 70 vol%. Then, N_2, H_2, or H_2/CO_2 was added to a pressure of 120 kPa (100 kPa=1 bar). For cultivation of *T. yellowstonii* with H_2/CO_2, 2 mM acetate was supplied as additional carbon source. When CO_2 was not present in the gas phase, bicarbonate was omitted from the medium and a two- to threefold higher concentration of phosphate buffer was added. Media were maintained at a pH of about 7.0. Bacteria were incubated at 60°C standing or shaken (150 rpm). For growth on CO, 2–100% of CO was initially used.

Substrates and products analyses

H_2 and CO were analyzed by a Chrompack gas chromatograph (CP9001) equipped with a TCD-detector. The capillary column was filled with fused silica (Molsieve 5A, 30 m × 0.53 mm). The oven temperature was 50°C, and the temperature of the TCD-detector was 100°C; argon was the carrier gas. Volatile free fatty acids were analyzed by HPLC as described by Stams et al. (1993). H_2S was analyzed according to Trüper and Schlegel (1964). Concentration of gaseous and liquid compounds after the analyses was expressed in mmol per liter of medium.

Results

Growth of pure cultures on pyruvate in the presence of CO

The thermophilic SO_4-reducing bacteria used in this research have different temperature optima and temperature limits. *T. yellowstonii* grows up to 70°C, and *D. kuznetsovii* is able to grow up to 85°C, while the other tested SO_4-reducing bacteria cannot grow above 65°C. *C. hydrogenoformans* has an optimum growth temperature of 70°C, but it grows well at 60°C. Therefore, all experiments were performed at 60°C. All selected SO_4-reducing bacteria can grow on H_2/CO_2 with SO_4 as electron acceptor. In standing cultures at 60°C all H_2 was converted within 12 days (data not shown).

Some mesophilic SO_4-reducing bacteria can grow with CO (or their growth is improved) only in the presence of organic carbon sources (Karpilova et al. 1983; Lupton et al. 1984). The CO sensitivity of the selected thermophilic SO_4-reducing bacteria was tested by cultivation (without shaking) in a medium that contained pyruvate (10 mM) and SO_4 (10 mM) under an atmosphere of 0, 2, 5, 20, or 50% CO. All tested bacteria were able to ferment pyruvate without a CO-containing gas phase. Nevertheless, no vis-

Fig. 1 Conversion of 100% carbon monoxide (*CO*) by binary culture of *Carboxydothermus hydrogenoformans* plus SO_4-reducing bacteria at standing (*open symbols*) and shaking (*closed symbols*) conditions. *Squares* carbon monoxide (*CO*), *rhombuses* hydrogen (*H₂*), *triangles* hydrogen sulfide (*H₂S*). **a** *C. hydrogenoformans* plus *Desulfotomaculum kuznetsovii*. **b** *C. hydrogenoformans* plus *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum*

ible growth of *D. thermoacetoxidans* and *T. yellowstonii* was observed when 2% CO was added. *D. kuznetsovii and D. thermobenzoicum* subsp. *thermosyntrophicum* grew with pyruvate under all tested CO concentrations.

Chemolithoautotrophic CO conversion by co-cultures

C. hydrogenoformans converts CO to H_2 and CO_2 and may, thus, relieve the CO toxicity for thermophilic SO_4-reducing bacteria in the binary cultures. The two SO_4-reducing bacteria, that were the most sensitive for CO, *D. thermoacetoxidans* and *T. yellowstonii*, were incubated with 20% and 50% CO as the only electron donor in co-culture with *C. hydrogenoformans*. CO was consumed, but no SO_4 reduction was observed (data not shown).

The co-cultures of *C. hydrogenoformans* and *D. kuznetsovii* or *D. thermobenzoicum* subsp. *thermosyntrophicum*, grown with 100% CO as sole carbon and energy source in standing cultures, converted CO and reduced SO_4 (Fig. 1a, b). When shaken, CO conversion and formation of H_2 by both co-cultures (Fig. 1a, b) occurred faster, but the fate of H_2 was different. When *C. hydrogenoformans* was cultivated with *D. kuznetsovii* without shaking (Fig. 1a), H_2 was formed gradually, and it was also consumed gradually. Overall, the H_2 concentration remained low. H_2 consumption coincided with H_2S formation. At the end of the experiment, 4.3 mM acetate was formed. Under shaken conditions, H_2 accumulated rapidly, and its further conversion occurred slowly (Fig. 1a). SO_4 reduction was inhibited (only 0.4 mM H_2S was formed) and H_2 was not consumed completely. More acetate (6.6 mM) was formed compared with the standing cultures. When *C. hydrogenoformans* was cultivated with *D. thermobenzoicum* subsp. *thermosyntrophicum* in standing cultures (Fig. 1b), the rates of H_2 formation and SO_4 reduction were similar to the rates in the co-culture with *D. kuznetsovii* (Fig. 1a). Under shaken conditions, H_2 was formed fast, but only after all CO was converted, the H_2 concentration decreased and SO_4 was reduced (Fig. 1b). At the end of the exeriment, the acetate concentration was 4 mM in standing cultures and 7.5 mM in shaken cultures.
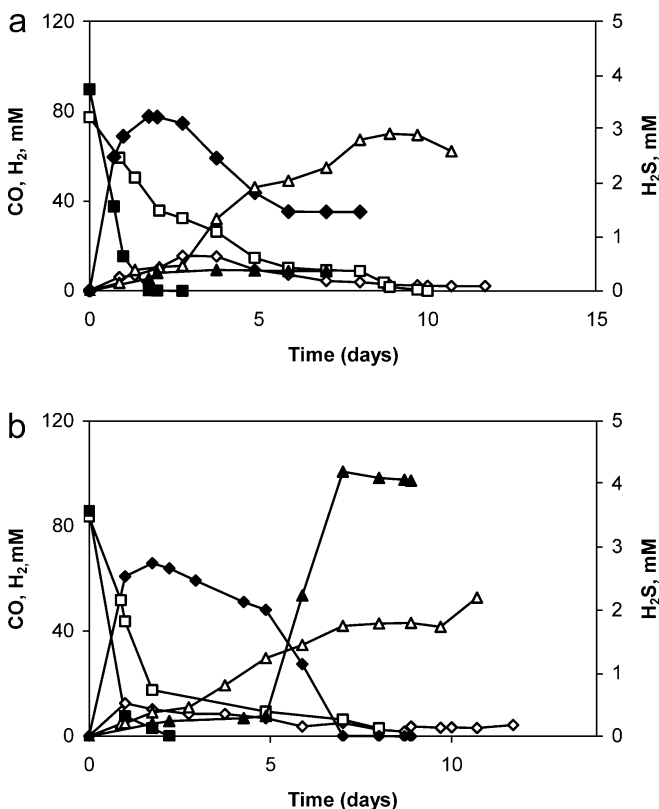
Conversion of mixtures of H_2 and CO by pure cultures and co-cultures

D. kuznetsovii and *D. thermobenzoicum* subsp. *thermosyntrophicum* were cultivated in the presence of SO_4, shaken with 0, 5, 20, 50 and 70% CO in the H_2/CO_2 gas phase (Table 1). Both *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* were able to convert CO, H_2, and CO_2 and reduce SO_4 (Table 1). Conversion of CO and a mixture of CO and H_2/CO_2 by *D. kuznetsovii* (Table 1) occurred slower than by *D. thermobenzoicum* subsp. *thermosyntrophicum* (Table 1). Data on 50% and 70% of CO in the H_2/CO_2 gas phase conversion by *D. kuznetsovii* are not shown. In our experiments, both SO_4-reducing bacteria formed acetate. At higher CO concentra-

Table 1 Conversion of different concentrations of carbon monoxide (*CO*) plus hydrogen/ carbon dioxide (*H$_2$/CO$_2$*) plus sulfate (*SO$_4$*) by *Desulfotomaculum kuznetsovii* and *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum*. *H$_2$S* Hydrogen sulfide

Gas phase	Time needed to complete degradation (days)		CO consumed (mmol/l)	H$_2$ consumed (mmol/l)	H$_2$S formed (mmol/l)	Acetate formed (mmol/l)
	CO	H$_2$				
Desulfotomaculum kuznetsovii						
0 CO + H$_2$/CO$_2$	–	9	–	52.8	9.5	0.7
5% CO + H$_2$/CO$_2$	8	14	5.0	51.2	7.5	3.6
20% CO + H$_2$/CO$_2$	30	34	17.6	41.6	2.9	4.5
Desulfotomaculum thermobenzoicum subsp. *thermosyntrophicum*						
0 CO + H$_2$/CO$_2$	–	5	–	53.1	8.3	3.5
20% CO + H$_2$/CO$_2$	7	18	16.1	43.0	7.0	7.8
50% CO + H$_2$/CO$_2$	10	27	39.0	49.3	8.0	7.7
70% CO + H$_2$/CO$_2$	14	18	47.0	25.0	2.6	5.0

tions, more CO was used for acetate production and less for SO$_4$ reduction (Table 1).

Substrate conversion by *C. hydrogenoformans* alone, *C. hydrogenoformans* in co-culture with *D. thermobenzoicum* subsp. *thermosyntrophicum* grown with 50% CO (Fig. 2a,

b; Table 2) and *D. thermobenzoicum* subsp. *thermosyntrophicum* alone with N$_2$ plus H$_2$/CO$_2$, CO plus N$_2$, or CO plus H$_2$ in the presence of SO$_4$ is shown (Fig. 2c, d, e; Table 2). The co-culture converted 50% CO in the same time as a pure culture of *C. hydrogenoformans* (Fig. 2a, b).

Fig. 2 Conversion of gases by *C. hydrogenoformans* and *D. thermobenzoicum* subsp. *thermosyntrophicum* at shaking conditions. **a** *C. hydrogenoformans* on 50% CO [plus nitrogen (N$_2$) plus SO$_4$]. **b** *C. hydrogenoformans* plus *D. thermobenzoicum* subsp. *thermosyntrophicum* on 50% CO (plus N$_2$ plus SO$_4$). **c** *D. thermobenzoicum* subsp. *thermosyntrophicum* on 50% N$_2$ (plus H$_2$/CO$_2$ plus SO$_4$). **d** *D. thermobenzoicum* subsp. *thermosyntrophicum* on 50% CO (plus N$_2$ plus SO$_4$). **e** *D. thermobenzoicum* subsp. *thermosyntrophicum* on 50% CO (plus H$_2$ plus SO$_4$). *Squares* CO, *rhombuses* H$_2$
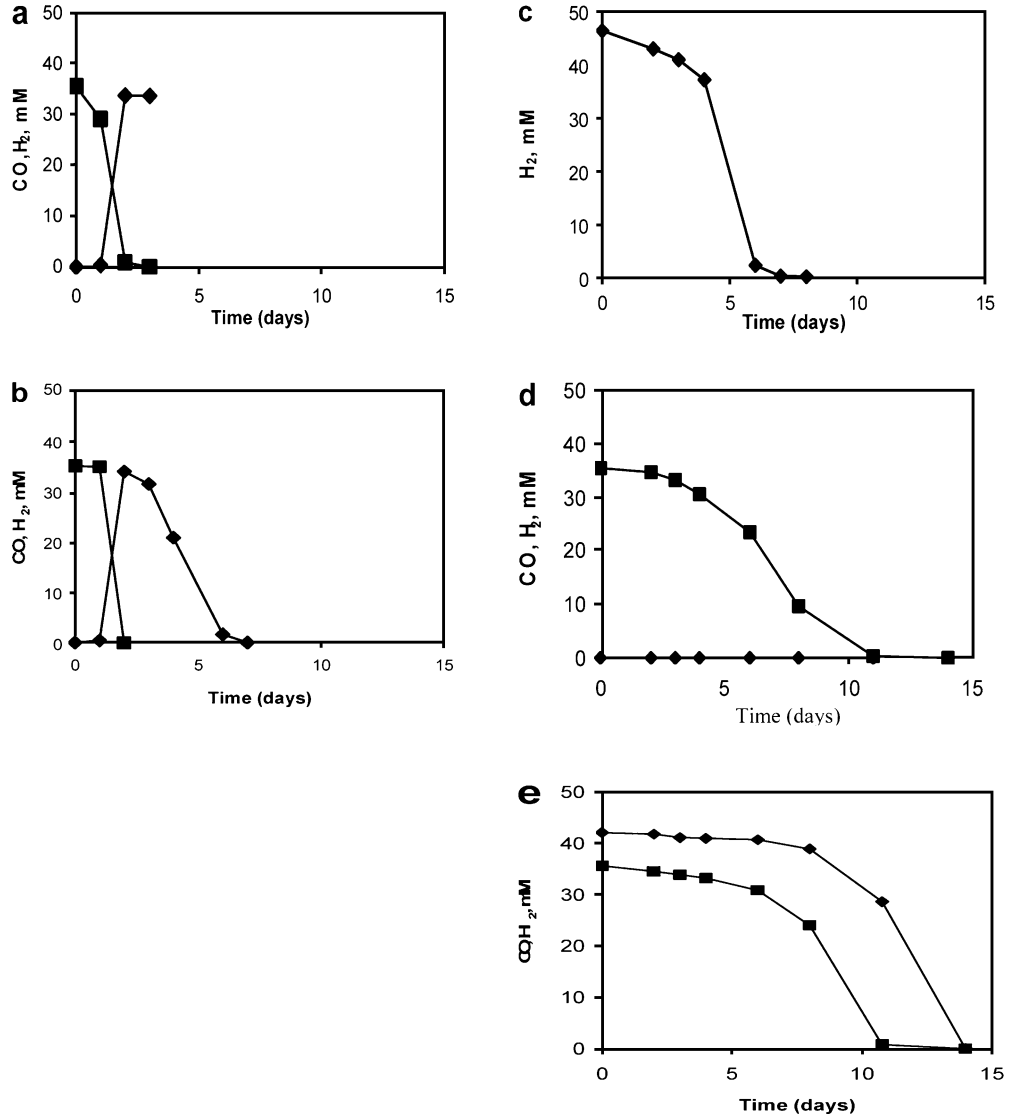
Table 2 Growth of *Carboxy-dothermus hydrogenoformans* and *D. thermobenzoicum* subsp. *thermosyntrophicum* in the atmosphere of different gases and products formation (supplement to Fig. 2)

Bacteria	Gases	Start concentration (mmol/l)	OD^a end	H_2S end (mmol/l)	Acetate formed (mmol/l)
C. hydrogenoformans	50% CO + N_2 SO_4	CO-37	nd^b	0	0
C. hydrogenoformans + *D. thermobenzoicum* subsp. *thermosyntrophicum*	50% CO + N_2 + SO_4	CO-36	nd^b	4.7	3.3
D. thermobenzoicum subsp. *thermosyntrophicum*	50% N_2 + H_2/ CO_2 + SO_4	H_2-48	0.19	9.2	2.5
D. thermobenzoicum subsp. *thermosyntrophicum*	50% CO + N_2 + SO_4	CO-37	0.21	5.0	4.2
D. thermobenzoicum subsp. *thermosyntrophicum*	50% CO + H_2 + SO_4	CO-36 H_2-42	0.31	8.2	9.5

[a] *OD* Optical density (wavelength 660 nm)
[b] *nd* Not determined

Products of CO and H_2 conversion are listed in Table 2. H_2S and a low amount of acetate (Table 2) were detected after complete conversion of H_2. When *D. thermobenzoicum* subsp. *thermosyntrophicum* was cultivated under a N_2 /H_2/CO_2 gas phase (Fig. 2c), H_2S and a small amount of acetate were detected at the end of the experiment (Table 2). *D. thermobenzoicum* subsp. *thermosyntrophicum* could also grow with 50% CO and formed H_2S and acetate (Fig. 2d; Table 2). When grown on CO/H_2, more acetate was formed (Table 2). In this experiment H_2 consumption followed CO conversion (Fig. 2e) similar to the experiment with CO/H_2/CO_2 (Table 1).

Discussion

CO tolerance of selected SO_4-reducing bacteria

Experiments with *D. thermoacetoxidans* and *T. yellowstonii* with pyruvate/CO and co-cultivation of these strains with *C. hydrogenoformans* on 100% CO indicate that these bacteria are highly sensitive towards CO and are not applicable for biological SO_4 reduction with synthesis gas. The two other bacteria, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum*, were remarkably tolerant to high CO concentrations. In pure culture, both bacteria tolerated 70% of CO during chemolithotrophic growth in the presence of SO_4. In co-culture, at standing conditions (with 100% CO), both *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermopropionicum* were able to reduce SO_4 with the H_2 formed by *C. hydrogenoformans*. However, when the co-cultures were shaken, SO_4 reduction of *D. kuznetsovii* was inhibited and only acetate was formed. Improved gas-to-liquid mass transfer of CO during shaking most likely resulted in inhibiting CO concentration in the liquid phase. In most cases, H_2 was used for acetate formation in addition to SO_4 reduction. Only one of the four investigated bacteria, *D. thermobenzoicum* subsp. *thermopropionicum,* was capable of SO_4 reduction under 100% CO in shaken co-culture with *C. hydrogenoformans.*

Effect of CO on the ratio of sulfidogenesis and acetogenesis

D. thermobenzoicum subsp. *thermosyntrophicum* coupled the oxidation of organic substrates to acetate formation and to SO_4 reduction. Acetate was formed during growth on H_2/CO_2 as well (Plugge et al. 2002). In our experiments, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* formed acetate during growth on H_2/CO_2 and different CO concentrations (Table 1). Initial amounts of H_2 were less in the bottles with higher CO concentrations. Nevertheless, more acetate was formed at high CO concentrations. At CO concentrations higher than 20% for *D. kuznetsovii* (data not shown) and higher than 50–70% for *D. thermobenzoicum* subsp. *thermosyntrophicum*), SO_4 reduction was partially inhibited. Thus, more CO was used for acetate formation than for SO_4 reduction. In our experiments the electron recovery was not complete in the cultures with CO/H_2/CO_2 mixtures. We can not exclude that other organic products could have been formed.

It has been postulated that *D. vulgaris* and *D. desulfuricans* first convert CO with H_2O to H_2 and CO_2, and then use H_2 for SO_4 reduction (Karpilova et al. 1983; Lupton et al. 1984). In our experiments with pure cultures of *D. kuznetsovii* (data not shown) and *D. thermobenzoicum* subsp. *thermosyntrophicum* grown on CO (Fig. 2d), H_2 was never detected as an intermediate of CO conversion. Instead, CO_2, acetate, and H_2S were formed as products of CO conversion. When SO_4-reducing bacteria were co-cultivated with *C. hydrogenoformans,* formation of H_2 from CO by *C. hydrogenoformans* was faster than the subsequent consumption of H_2 by SO_4-reducing bacteria (Fig. 1a, b). The absence of H_2 in the gas phase (Fig. 2d) suggests a direct conversion of CO coupled to SO_4 reduction by SO_4-reducing bacteria.
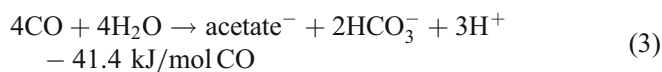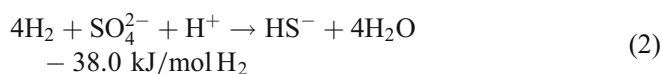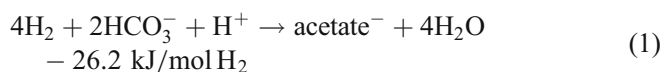
Inhibition of H_2 utilization and sulfidogenesis by CO

A typical curve of CO and H_2 conversion by *D. thermobenzoicum* subsp. *thermosyntrophicum* is shown in Fig. 2e.

Remarkably, H_2 consumption started later than CO consumption. In another experiment, the time needed to consume all H_2 became longer at higher CO concentrations (Table 1). It is generally reasoned that hydrogenase is inhibited by CO. Observations supporting this fact are omnipresent. When the acetogenic bacterium *Eubacterium limosum* was grown with a mixture of CO and H_2 (Sharak Genthner and Bryant 1982), H_2 consumption started after the CO concentration in the gas phase had decreased to values below 5%. This observation was explained by the possible inhibition of hydrogenase by CO. A similar hydrogenase inhibition was found for other anaerobic bacteria cultivated on CO (Daniels et al. 1977; Pankhania et al. 1986; Berlier et al. 1987; Adams 1990; Bennett et al. 2000).

SO_4-reducing bacteria are generally more sensitive towards CO than acetogens. CO partial pressures of 20% are the maximum tolerated by SO_4-reducing bacteria reported so far (Klemps et al. 1985; Karpilova et al. 1983). It is unlikely that inhibition of hydrogenase by CO is the sole reason of the sensitivity of SO_4-reducing bacteria towards CO. As discussed above, a shift towards acetogenesis occurs with increasing CO concentrations. Hydrogenase plays a central role in acetogenesis as well. Recently, Rother and Metcalf (2004) demonstrated growth of a *Methanosarcina acetivorans* strain on CO. Methanogenesis was largely inhibited by high CO concentrations; acetate was formed instead. It is unclear which step of methanogenesis is inhibited. We support the authors in their statement that further investigation on the physiological mechanism of inhibition by CO is necessary and like to extend this to the SO_4-reducing bacteria.

From our data we conclude that besides SO_4 reduction with H_2 and acetogenesis from H_2 and CO_2, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum* can convert CO to acetate and can couple CO oxidation directly to SO_4 reduction. Thus, we assume that these strains can perform the following reactions:

$$4H_2 + 2HCO_3^- + H^+ \rightarrow acetate^- + 4H_2O \\ - 26.2 \text{ kJ/mol } H_2 \tag{1}$$

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \\ - 38.0 \text{ kJ/mol } H_2 \tag{2}$$

$$4CO + 4H_2O \rightarrow acetate^- + 2HCO_3^- + 3H^+ \\ - 41.4 \text{ kJ/mol CO} \tag{3}$$

$$4CO + SO_4^{2-} + 4H_2O \rightarrow 4HCO_3^- + HS^- + 3H^- \\ - 53.2 \text{ kJ/mol CO} \tag{4}$$

This is the first report that provides evidence that thermophilic SO_4-reducing bacteria can grow at a high concentration (50–70%) of CO. In co-culture with *C. hydrogenoformans* growth and SO_4 reduction of *D. thermobenzoicum* subsp. *thermosyntrophicum* is even possible with 100% CO. In the latter case, CO is first converted to H_2 and CO_2, which is subsequently used by the SO_4 reducer. Our results show clearly that under moderately thermophilic conditions synthesis gas with high amounts of CO is an excellent electron donor for biotechnological SO_4 reduction at a high temperature. In particular, when the SO_4 reducers are co-cultivated with carboxydotrophic bacteria, high CO concentrations are tolerated. Thus, purification of the gas to reduce the CO content is not needed. It is not certain that in the bioreactor sludges the tested bacteria are dominant. However, as moderately thermophilic carboxydotrophic bacteria and SO_4-reducing bacteria can be easily enriched from different sources, it is not unlikely that they occur in bioreactors operated with CO as well. We show here unknown capacities of SO_4-reducing bacteria and possible pathways of CO conversion in bioreactors.

Our observations are important for the application of synthesis gas for biological S removal from flue gas or wastewater discharged at a high temperature.

References

Adams MWW (1990) The structure and mechanism of iron-hydrogenases. Biochim Biophys Acta 1020:115–145

Bennett B, Lemon BJ, Peters W (2000) Reversible carbon monoxide binding and inhibition at the active site of Fe-only hydrogenase. Biochemistry 39:7455–7460

Berlier Y, Fauque GD, LeGall J, Choi ES, Peck HD Jr, Lespinat PA (1987) Inhibition studies of three classes of *Desulfovibrio* hydrogenases: application to the further characterization of the multiple hydrogenases found in *Desulfovibrio vulgaris* Hilderborough. Biochem Biophys Res Commun 146:147–153

Daniels LG, Fuchs G, Thauer RK, Zeikus JG (1977) Carbon monoxide oxidation by methanogenic bacteria. J Bacteriol 132:118–126

Davidova MN, Tarasova NB, Mukhitova FK, Karpilova IU (1994) Carbon monoxide in metabolism of anaerobic bacteria. Can J Microbiol 40:417–425

Fardeau M-L, Salinas MB, L'Haridon S, Jeanthon Ch, Verhe F, Cayol J-L, Patel BKC, Garcia J-L, Ollivier B (2004) Isolation from oil reservoirs of novel thermophilic anaerobes phylogenetically related to *Thermoanaerobacter subterraneus:* reassignment *T. subterraneus, Thermoanaerobacter yonseiensis, Thermoanaerobacter tengcongensis* and *Carboxydibrachium pacificum*, to *Caldanaerobacter subterraneus* gen. nov., sp. nov., comb. nov. as four novel subspecies. Int J Syst Evol Microbiol 54:467–474

Graboski MS (1984) The production of synthesis gas from methane, coal and biomass. In: Herman RG (ed) Catalytic conversion of synthesis gas and alcohols to chemicals. Plenum, New York, pp 37–52

Henry EA, Devereux R, Maki JS, Gilmour CC, Woese CR, Mandelco L, Schauder R, Remsen CC, Mitchell R (1994) Characterization of a new thermophilic sulfate-reducing bacterium *Thermodesulfovibrio yellowstonii*, gen. nov. and sp. nov.: its phylogenetic relationship to *Thermodesulfobacterium commune* and their origins deep within the bacterial domain. Arch Microbiol 161:62–69

Karpilova IYu, Davidova MN, Belyaeva MI (1983) The effect of carbon monoxide on the growth and CO oxidation in sulfate-reducing bacteria (in Russian). Biol Nauki 1:85–88

Klemps R, Cypionka H, Widdel F, Pfennig N (1985) Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. Arch Microbiol 143:203–208

Lens PNL, Visser A, Janssen AJH, Hulshoff Pol LW, Lettinga G (1998) Biotechnological treatment of sulfate-rich wastewaters. Crit Rev Environ Sci Technol 28:41–88

Lupton FS, Conrad R, Zeikus JG (1984) CO metabolism of *Desulfovibrio vulgaris* strain Madison: physiological function in the absence or presence of exogenous substrates. FEMS Microbiol Lett 23:263–268

Maree JP, Gerber A, Hill E (1987) An integrated process for biological treatment of sulphate-containing industrial influents. J Water Pollut Control Fed 59:1069–1074

Min H, Zinder SH (1990) Isolation and characterization of a thermophilic sulfate-reducing bacterium *Desulfotomaculum thermoacetoxidans* sp. nov. Arch Microbiol 153:399–404

Mörsdorf G, Frunzke K, Gadkari D, Meyer O (1992) Microbial growth on carbon monoxide. Biodegradation 3:61–82

Nazina TN, Ivanova AE, Kunchaveli LP, Rozanova EP (1988) A new sporeforming thermophilic methylotrophic sulfate- reducing bacterium, *Desulfotomaculum kuznetsovii* sp nov. Mikrobiologiya 57:823–827

Pankhania IP, Gow LA, Hamilton WA (1986) The effect of hydrogen on the growth of *Desulfovibrio vulgaris* (Hildenborough) on lactate. J Gen Microbiol 132:3349–3356

Perry RH, Green DW, Maloney JO (1997) Perry's chemical engineers' handbook, 7th edn. McGraw-Hill, New York, pp 13–25

Plugge C, Balk M, Stams AJM (2002) *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov., a thermophilic syntrophic propionate-oxidizing spore-forming bacterium. Int J Syst Evol Microbiol 52:391–399

Rother M, Metcalf W (2004) Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. PNAS 101:16929–16934

Sharak Genthner BR, Bryant MP (1982) Growth of *Eubacterium limosum* with carbon monoxide as the energy source. Appl Environ Microbiol 43:70–74

Sipma J, Lens PNL, Stams AJM, Lettinga G (2003) Carbon monoxide conversion by anaerobic bioreactor sludges. FEMS Microbiol Ecol 44:271–277

Sipma J, Meulepas RJW, Parshina SN, Stams AJM, Lettinga G, Lens PNL (2004) Effect of carbon monoxide, hydrogen and sulfate on thermophilic (55°C) hydrogenic carbon monoxide conversion in two anaerobic bioreactor sludges. Appl Microbiol Biotechnol 64:421–428

Sokolova TG, Gonzalez JM, Kostrikina NA, Chernyh NA, Tourova TP, Kato C, Bonch-Osmolovskaya EA, Robb FT (2001) *Carboxydibrachium pacificum* gen.nov., sp. nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough. Int J Syst Evol Microbiol 51:141–149

Sokolova TG, Kostrikina NA, Chernyh NA, Tourova TP, Kolganova TV, Bonch-Osmolovskaya EA (2002) *Carboxydocella thermoautotrophica* gen. nov. sp. nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring. Int J Syst Evol Microbiol 52:1–6

Sokolova TG, Jeanthon Ch, Kostrikina NA, Chernyh NA, Lebedinsky AV, Stackebrandt E, Bonch-Osmolovskaya EA (2004a) The first evidence of anaerobic CO oxidation coupled with H$_2$ production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. Extremophiles 8:317–323

Sokolova TG, Gonzalez JM, Kostrikina NA, Chernyh NA, Slepova TV, Bonch-Osmolovskaya EA, Robb FT (2004b) *Thermosinus carboxydivorans* gen. nov., sp. nov., a new anaerobic, thermophilic, carbon-monoxide-oxidizing, hydrogenogenic bacterium from a hot pool of Yellowstone National Park. Int J Syst Evol Microbiol 54:2353–2359

Stams AJM, van Dijk JB, Dijkema C, Plugge CM (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. Appl Environ Microbiol 59:1114–1119

Svetlichniy VA, Sokolova TA, Gerhardt M, Ringpfel M, Kostrikina NA, Zavarzin GA (1991) *Carboxydothermus hydrogenoformans* gen. nov., sp. nov. a CO utilizing thermophilic anaerobic bacterium from hydrothermal environments of Kunashir island. Syst Appl Microbiol 14:254–260

Svetlichnyi VA, Sokolova TG, Kostrikina NA, Lysenko AM (1994) A new thermophilic anaerobic carboxydotrophic bacterium *Carboxydothermus restrictus* sp. nov. Microbiology 63:294–297

Trüper HG, Schlegel HG (1964) Sulfur metabolism in *Thiorhodaceae*. 1.Quantitative measurement of growing cells of *Chromatium okenii*. Antonie Van Leeuwenhoek 30:225–238

Houten RT van, HulshoffPol LW, Lettinga G (1994) Biological sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. Biotechnol Bioeng 44:586–594

Houten RT van, van der Spoel H, van Aelst AC, Hulshoff Pol LW, Lettinga G (1996) Biological sulphate reduction using synthesis gas as energy and carbon source. Biotechnol Bioeng 50:136–144

Houten RT van, Yu Yun S, Lettinga G (1997) Thermophilic sulphate and sulphite reduction in lab-scale gas-lift reactors using H$_2$/CO$_2$ as energy and carbon source. Biotechnol Bioeng 55:807–814

Widdel F, Hansen TA (1992) The dissimilatory sulfate- and sulfur-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, Berlin Heidelberg New York, pp 583–624