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H_2 enrichment from synthesis gas by *Desulfotomaculum carboxydivorans* for potential applications in synthesis gas purification and biodesulfurization

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Abstract *Desulfotomaculum carboxydivorans*, recently isolated from a full-scale anaerobic wastewater treatment facility, is a sulfate reducer capable of hydrogenogenic growth on carbon monoxide (CO). In the presence of sulfate, the hydrogen formed is used for sulfate reduction. The organism grows rapidly at 200 kPa CO, pH 7.0, and 55°C, with a generation time of 100 min, producing nearly equimolar amounts of H_2 and CO_2 from CO and H_2O. The high specific CO conversion rates, exceeding 0.8 mol CO (g protein)$^{-1}$ h^{-1}, makes this bacterium an interesting candidate for a biological alternative of the currently employed chemical catalytic water–gas shift reaction to purify synthesis gas (contains mainly H_2, CO, and CO_2). Furthermore, as *D. carboxydivorans* is capable of hydrogenotrophic sulfate reduction at partial CO pressures exceeding 100 kPa, it is also a good candidate for biodesulfurization processes using synthesis gas as electron donor at elevated temperatures, e.g., in biological flue gas desulfurization. Although high maximal specific sulfate reduction rates (32 mmol (g protein)$^{-1}$ h^{-1}) can be obtained, its sulfide tolerance is rather low and pH dependent, i.e., maximally 9 and 5 mM sulfide at pH 7.2 and pH 6.5, respectively.

Keywords *Desulfotomaculum carboxydivorans* · Carbon monoxide · Hydrogenogen · Thermophilic · Sulfate reduction · Biohydrogen production · Biological flue gas desulfurization

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Introduction

Bulk production of hydrogen is done by gasification of fossil fuels, although in principle any organic matter can be gasified to a H_2-rich gas. Besides H_2, synthesis gas may contain large amounts of carbon monoxide (CO) depending on the organic matter source (Perry et al. 1997; Armor 1999). The CO present in synthesis gas represents a substantial amount of energy that should be recovered. Therefore, conversion of CO to H_2, according to the water–gas–shift reaction ($CO+H_2O \rightarrow CO_2+H_2$; $\Delta G^\circ = -20$ kJ mol^{-1}) improves the energetic efficiency of H_2 production processes. Currently, this shift reaction is performed at high temperatures and pressures using chemical catalysts (Czuppon et al. 1995). The discovery of bacteria capable of growth employing a biological analog of the water–gas shift reaction (as reviewed in Sipma et al. 2006) holds a promise for the design of a biological alternative for this chemical catalytic reaction. All these isolates showed fast growth on CO, with

generation times between 1.1 and 8.3 h at partial pressures of CO in the gas phase exceeding 100 kPa (Sipma et al. 2006).

Besides the use of H_2 as possible future energy carrier, H_2 has also great potential in biotechnological reductive processes, e.g., biodesulfurization of inorganic sulfite/sulfate containing wastewaters (Van Houten et al. 1994, 1997). Treatment of sulfate-rich inorganic wastewater requires addition of an electron donor. Direct use of synthesis gas without a H_2 purification step significantly reduces the operational costs (Van Houten and Lettinga 1996; Du Preez and Maree 1994), provided that the CO present in synthesis gas does not inhibit the biological sulfate reduction process. In general, sulfate reducing bacteria are highly sensitive to CO (Davidova et al. 1994). However, *Desulfotomaculum nigrificans*, *Desulfosporosinus orientis* (Klemps et al. 1985), and *Desulfovibrio desulfuricans* (Davidova et al. 1994) are capable of using CO up to 20 kPa as electron donor for sulfate reduction. Recently, two sulfate reducers, *Desulfotomaculum kuznetsovii* and *Desulfotomaculum thermobenzoicum* subsp. *thermosynthrophicum* were shown capable of CO utilization at CO levels in the gas phase up to 50 kPa (Parshina et al. 2005a). The recently isolated *Desulfotomaculum carboxydivorans* is the first sulfate reducer that shows uninhibited growth on 180 kPa CO at 55°C, both in the presence and absence of sulfate (Parshina et al. 2005b). In the absence of sulfate, it grows hydrogenogenically on CO producing nearly equimolar amounts of H_2 and CO_2 (Parshina et al. 2005b).

As a result of its unique properties to convert CO with H_2O as electron acceptor to H_2 and CO_2, according to the water–gas shift reaction, combined with sulfate reduction using the produced hydrogen, *D. carboxydivorans* may be applicable in both synthesis gas purification and biological sulfate reduction processes at elevated temperatures, e.g., flue gas desulfurization. To evaluate the potential of *D. carboxydivorans* in both processes, more detailed information on its physiological features is desired. In this paper, the substrate conversion kinetics of *D. carboxydivorans* in the presence and absence of sulfate are presented.

Materials and methods

Experimental set-up

Tests for assessing selected physiological features of *D. carboxydivorans* (DSM 14880, VKM B-2319; Parshina et al. 2005b) were all performed in serum bottles incubated in a temperature-controlled (55°C) shaker type RFI-125 (Infors AG, Basel, Switzerland) at 200 rpm. The basal medium contained (in mM): NaCl 5.1, NH_4Cl 5.6, $CaCl_2{\cdot}2H_2O$ 0.7, $MgCl_2{\cdot}6H_2O$ 0.5, $Na_2S{\cdot}9H_2O$ 0.6, yeast extract 500 mg l^{-1}, and 1 ml l^{-1} of an acid-and-alkaline trace element solution according to Stams et al. (1993). The medium was buffered at pH 7.0 using 8.2 mM KH_2PO_4 and 11.4 mM $Na_2HPO_4{\cdot}2H_2O$, when CO was the substrate. When H_2/CO_2 was supplied in the gas phase, the concentration of the phosphate buffer was decreased to 10% of the former and 23.8 mM sodium bicarbonate was supplemented to buffer the medium at pH 7.0 with 30 kPa of CO_2 in the gas phase at 55°C. Physiological tests were performed in serum bottles of 117, 310, or 570 ml containing 50, 100, and 200 ml of basal medium, respectively. The bottles were sealed with butyl rubber stoppers and the gas phase was changed to CO or H_2/CO_2 (80/20). Before inoculation with *D. carboxydivorans*, the bottles were autoclaved at 121°C for 30 min.

Experimental design

Experiments to determine the growth curves on CO as sole substrate in the absence or presence of sulfate for *D. carboxydivorans* were performed in 570-ml serum bottles. The gas phase contained about 150 kPa CO and 10 to 20 kPa N_2. Experiments to determine the yield of *D. carboxydivorans* when growing on CO were performed in serum bottles of 117, 310, and 570 ml with different P_{CO} levels. To determine the growth yield of *D. carboxydivorans* with pyruvate, ethanol and H_2/CO_2, as sole substrate, experiments (in triplicate) were performed in 117-ml bottles. Experiments with glucose and pyruvate (20 mM) were performed in the absence of sulfate, whereas experiments with ethanol (20 mM) and H_2/CO_2 (1.6 bar H_2/CO_2 80:20) were performed in the presence of 20 mM sulfate. Experiments to determine the threshold CO concentration in incubations with CO as sole substrate were performed in 117-ml bottles at P_{CO} varying from about 10 to 50 kPa and 80 to 100 kPa N_2. The maximal hydrogenotrophic sulfide production was determined in 117-ml bottles, with 200 kPa H_2/CO_2 in the presence of 20 mM sulfate and adjusted bicarbonate buffer to give a starting pH of 7.0. The effect of the pH on maximal sulfide accumulation was assessed with sulfate and H_2/CO_2 (200 kPa) and varying bicarbonate concentrations to obtain incubations at different initial pH. These values were compared with the maximum sulfide accumulation in batch incubations of *D. carboxydivorans* grown in the presence of CO as sole external substrate and 20 mM sulfate.

Analysis

The pressure in the bottles was determined using a membrane pressure unit, 0–4 bar absolute (Wal Mess- und Regelsysteme GmbH, Oldenburg, Germany). The headspace gas composition was measured on a gas chromatograph (GC) HP 5890 (Hewlett Packard, Palo Alto, USA). The detection limit for

CO with the used settings was 400 ppm. Trace concentrations of CO were determined on a Shimadzu GC 2010, equipped with a methanizer (Shimadzu MTN-1). This GC was further equipped with a Chrompack Molsieve 5-Å capillary column of 30 m (0.53 mm; 15 μm; CP7544). The temperatures of the oven, injection port, and FID detector were 90, 100, and 250°C, respectively. The CO detection limit was lower than 1 ppm.

Volatile fatty acids (VFA) and ethanol were analyzed on a HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, USA) according to Weijma et al. (2000). Sulfide was measured according to Trüper and Schlegel (1964). Sulfate was measured on a DX-600 IC system (Dionex Corporation, Salt Lake City, USA) as described previously (Sipma et al. 2004). Pyruvate was measured with a Spectrasystem high-pressure liquid chromatography system equipped with an autosampler and refractomonitor. The acids were separated on a Polyspher OAHY column (30 cm × 6.5 mm; Merck, Darmstadt, Germany) in 0.01 N H_2SO_4 at a flow rate of 0.6 ml min^{-1} and a column temperature of 60°C. Quantification was performed by differential refractometry. Biomass production was monitored by measuring the optical density at 600 nm (OD_{600}) using a Spectronic 601 spectrophotometer (Milton Roy, Ivyland, USA) and 1.5-ml Plastibrand PMMA cuvettes (Brand GmbH, Wertheim, Germany). The protein concentration was determined according to Bradford (1976). Biomass concentrations were converted into the corresponding amount of protein using the empirical relationship between the protein concentration and the measured OD_{600} determined for *D. carboxydivorans* as:

$$[\text{protein}] = 135 \cdot OD_{600}, \quad (1)$$

in which: [protein] = the protein concentration in mg l^{-1}.

Solubilities of CO, CO_2, and CH_4 were calculated using data from Lide (2001), solubility of H_2 was calculated according to Perry et al. (1997), and the amounts produced or consumed were calculated by taking into account both gas and liquid phases. All chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany). CO (purity 99.997%) was supplied by Hoek Loos (Rotterdam, The Netherlands).

Results

Hydrogenogenic CO conversion

Figure 1 presents a growth curve of *D. carboxydivorans* on CO as sole substrate at an initial P_{CO} of 160 kPa in the absence of sulfate. Fast growth on CO was observed with a generation time of about 100 min. Figure 1 shows that nearly stoichiometric amounts of H_2 and CO_2 are produced. Furthermore, the final concentration of CO was below

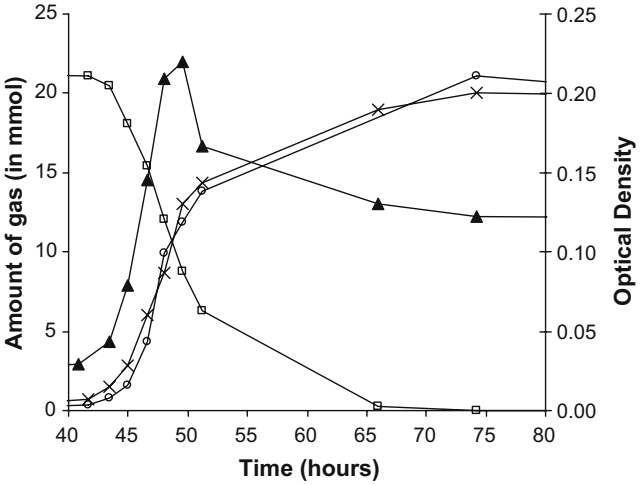

Fig. 1 Growth of *D. carboxydivorans* on CO as sole substrate in the absence of sulfate at 55°C, presenting a partial growth curve showing the evolution of carbon monoxide (□), carbon dioxide (○), hydrogen (×) and OD_{600} (▲)

400 ppm, i.e., the detection limit of the standard GC used. Although CO conversion proceeds fast, the maximal biomass density is already reached when still 60 kPa CO is present in the gas phase. Due to the limited buffer capacity (20 mM phosphate buffer) and the CO_2 production, the pH decreased rapidly as represented for a growth of *D. carboxydivorans* on CO in Fig. 2, which may affect the biomass activity. Growth stopped at pH 6.0, but CO conversion continued resulting in residual CO levels below 400 ppm.

From a growth curve of *D. carboxydivorans* on CO, CO conversion rates (mmol h^{-1}) were calculated between each sampling point (Fig. 2). Specific rates were obtained by considering the average biomass concentration in the corresponding time interval. Maximal specific CO conversion rates are reached early in the growth phase when the

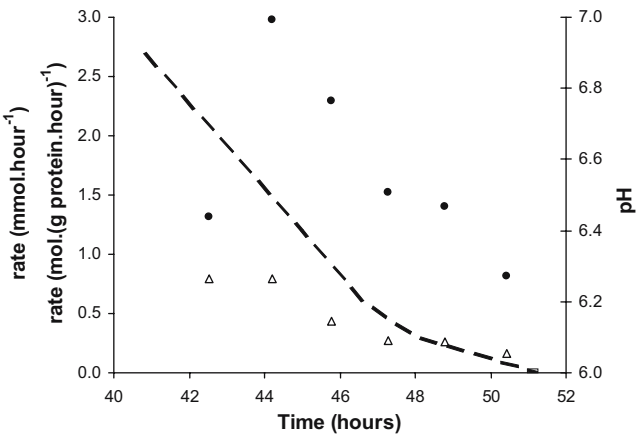

Fig. 2 CO conversion rates determined from a growth curve of *D. carboxydivorans* growing on CO in the absence of sulfate; presenting the overall CO conversion rates in mmol h^{-1} (●), the specific CO conversion rate in mol (g protein)$^{-1}$ h^{-1} (△) as well as the measured pH values during exponential growth (- - -)

biomass concentration is limited and pH values exceed 6.5. It reflects the high CO flux from the gas to the liquid phase and relative high CO concentrations in the liquid phase at the start. Due to consumption of CO and subsequent decrease of the pressure, the driving force for mass transfer decreases and the maximal CO flux decreases as well. From Fig. 2 the maximal specific CO conversion rate is estimated at 0.8 mol CO (g protein)$^{-1}$ h^{-1}, which decreased rapidly due to either the decreased CO flux from the gas to the liquid phase or the decreased pH.

Growth yield on CO and selected alternative substrates

From 12 different growth experiments with different amounts of CO in the gas phase, the yield of *D. carboxydivorans* biomass protein for growth on CO was determined as 0.42±0.07 mg protein produced per millimole CO converted (Fig. 3). Data were only used if *D. carboxydivorans* was growing exponentially as cell lysis occurring after the exponential growth phase results in yield underestimations.

For evaluation of the use of alternative substrates to induce growth of *D. carboxydivorans*, growth yields and growth rates with selected substrates were determined. Although, *D. carboxydivorans* has a short generation time on CO, the protein yield is not very high, which combined with the relative short period of growth during CO conversion (Fig. 1) could result in biomass limitations. Therefore, the use of alternative substrates for the growth of *D. carboxydivorans* could aid in the generation of high amounts of biomass, either for rapid start up or enhanced process stability. The growth yields with pyruvate (without sulfate), ethanol (with sulfate) and H_2/CO_2 (with sulfate) are estimated at 1.8±0.1, 2.1±0.2 and 1.3±0.1 mg protein (mmol substrate)$^{-1}$, respectively. The generation times were 5 (pyruvate), 19 (ethanol), and 8 h (H_2/CO_2). As both ethanol and H_2 are exclusively used in the presence of sulfate as electron acceptor, their potential use is restricted to those processes in which sulfate reduction is of interest.

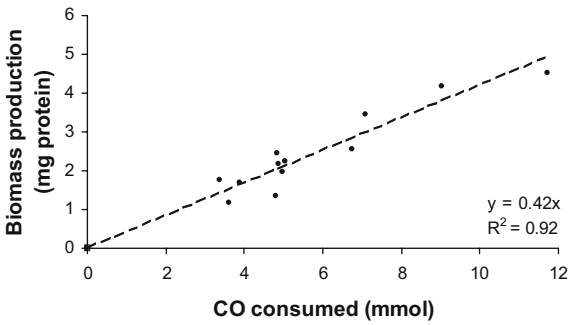

Fig. 3 Biomass yield of *D. carboxydivorans* growing on CO as sole substrate at 55°C and an initial pH of 7; the biomass protein concentration is given as a function of the amount of CO converted

The highest biomass density was observed in incubations with glucose (OD_{600}=0.6) at a relatively short generation time of 6 h, suggesting that glucose may be a suitable substrate for biomass production. Unfortunately, the growth yield of *D. carboxydivorans* on glucose could not be determined due to interference of medium components, i.e., high phosphate concentrations, in the glucose analysis.

CO threshold concentrations

In all incubations CO was converted to final headspace concentrations below 400 ppm, as CO was not detected with the standard GC. To determine the residual CO concentrations more accurately, headspace samples were analyzed for trace concentrations of CO. Results of analysis of samples from growth curves with an initial P_{CO} of about 150 kPa revealed that the final measured concentration was about 130 ppm at pH≤5.9. For an evaluation of the effect of thermodynamic limitations on the residual CO concentrations, the following equations were used:

$$\Delta G = \Delta G'_{55} + R \cdot 10^{-3} \cdot T \cdot \ln \mathrm{Kp} \quad (2)$$

in which: $\Delta G'_{55}$=−22.3 kJ mol^{-1}; R is the gas constant (8.3145 J mol^{-1} K^{-1}); T in Kelvin; and the equilibrium constant (Kp) is defined as:

$$\mathrm{Kp} = \left(\frac{P_{H_2} \cdot P_{CO_2}}{P_{CO}}\right) \times \frac{1}{101300} \quad (3)$$

in which P_{CO} is the partial pressure of CO in Pa.

Theoretically, the reaction proceeds at 55°C as long as ΔG is smaller than 0, and thus the value for ln Kp should be smaller than 8.17. The ΔG examined for four incubations, considering the final P_{CO} (30, 26, 32, and 25 Pa, respectively), P_{CO2} (96, 92, 95, and 98 kPa, respectively) and P_{H2} (123, 124, 125, and 81 kPa, respectively), resulted in values of 0.25, 0.55, 0.09, and −0.34 kJ mol^{-1}, respectively. This suggests that the thermodynamic limitations determine the residual CO concentrations. This is further supported by the results presented in Table 1, which show that the theoretical (assuming ΔG=0) and measured residual CO concentrations in incubations with different initial P_{CO} do not differ greatly. Furthermore, as the final concentrations are much lower than the 130 ppm measured previously, the affinity of *D. carboxydivorans* for CO is not limiting the removal of CO to residual CO concentrations as low as 5 ppm.

Sulfate reduction by *D. carboxydivorans*

A considerable higher biomass yield, as indicated by the higher OD_{600}, is reached with *D. carboxydivorans* growing

Table 1 Thermodynamic calculations of the residual CO concentration in the gas phase (assuming ΔG=0; ln Kp=8.17) compared with measurements of the final CO concentration after 14 days of incubation (initial pH=7.0; T=55°C; no sulfate added)

Initial P_{CO}[a] kPa	Initial P_{N2} KPa	Maximal P_{CO2}[b] kPa	Maximal P_{H2}[b] kPa	Calculated residual CO[c] Pa	Calculated residual CO[c] ppm	Measured residual CO ppm
10	113	7	9	0.18	1	5
17	120	12	15	0.50	3	9
19	125	13	17	0.62	4	6
25	132	17	22	1.04	6	17
28	132	19	25	1.32	8	16
32	136	22	29	1.78	9	17
45	146	30	40	3.34	15	19
51	152	34	46	4.36	19	22

The final pH ranged from 5.9 to 6.3 at the lowest initial P_{CO}.

[a] Initial P_{CO} was measured.

[b] In these calculations the maximal amount of CO_2 and H_2 in the gas phase was calculated, assuming equimolar CO conversion into H_2 and CO_2. The total amount of CO_2 and H_2 were recalculated into the amount of each gas in the gas phase using the following relations:

$$\frac{[\text{gas}]_{(\text{liq})}}{[\text{gas}]_{(\text{gas})}} = K_H \cdot RT \quad \text{and} \quad n_{\text{gas}} = \frac{n_{\text{tot}}}{\left(1+K_H \cdot RT \cdot \frac{V_{\text{liq}}}{V_{\text{gas}}}\right)}$$

in which: K_H=Henry constant 55°C (CO_2: 1.61×10^{-4} mol m^{-3} Pa^{-1}; H_2: 7.65×10^{-6} mol m^{-3} Pa^{-1}); R=8.3145 J mol^{-1} K^{-1}; T in Kelvin; n_{liq}=moles in the liquid phase; V_{liq}=50 mL and V_{gas}=67 mL.

[c] The residual CO was calculated as minimal $P_{CO} = \frac{\left[\left(P_{CO_2}/101,300\right)\cdot\left(P_{H_2}/101,300\right)\right]}{3{,}544.948} \times 101,300$ (pressures in Pa), which was divided by the total pressure at the end of the incubation and multiplied by 1.10^6 to obtain the residual CO values in ppm.

on CO as sole external substrate in the presence of sulfate compared to the hydrogenogenic growth on CO in the absence of sulfate (Fig. 4 vs Fig. 1). Analysis of the growth curves in the presence of sulfate reveals that the sulfide production coincides with CO conversion: the sulfide concentration starts to increase when the P_{CO} still exceeds 100 kPa. The maximal obtained sulfide concentration was 4.9 mM at a final pH of 6.5 with CO as external electron donor. The maximal sulfate reduction rate amounted 0.3 mmol h^{-1}, corresponding to 32 mmol SO_4^{2-} (g protein)$^{-1}$ h^{-1} (Fig. 4).

The biomass yield with CO in the presence of sulfate was estimated using the previously assessed yield on CO. The calculations were performed using the changes measured between the start and the end of the exponential phase, i.e., 13.57 mmol CO converted, 0.6 mmol sulfate reduced, and an OD_{600} change of 0.345 units (liquid volume of 200 ml). The amount of CO converted led to the synthesis of 5.7 mg protein, and a protein concentration of 28.5 mg l^{-1}, which corresponds to an OD_{600} change of 0.211. The remainder of the OD increase (0.134 units) is assumed to be the result of hydrogenotrophic sulfate reduction. This OD increase of 0.134 units corresponds to a protein concentration of 18.1 mg l^{-1} or an absolute amount of 3.6 mg protein. The yield for hydrogenotrophic sulfate-dependent growth then amounts to 6 mg protein per millimole sulfate reduced, or 1.5 mg protein per millimole H_2 consumed, which is in good agreement with the value calculated previously for growth on H_2/CO_2 in the presence of sulfate, i.e., 1.3±0.1 mg protein per millimole substrate.

Sulfide inhibition

The results of the incubations fed with CO and sulfate reveal that the maximal sulfide concentration produced is about 5 mM, despite that the CO-derived H_2 was not depleted. This indicates that inhibition due to sulfide prevented the further reduction of sulfate. Incubations with

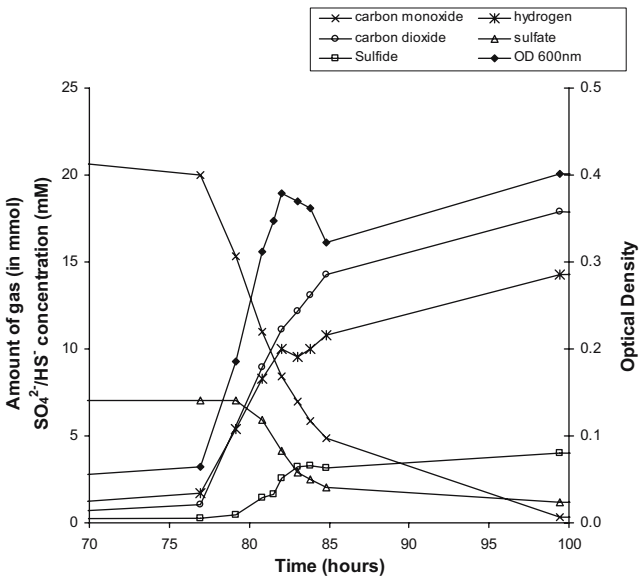

Fig. 4 Partial growth curve of *D. carboxydivorans* grown on 160-kPa CO added as sole substrate in the presence of sulfate (initial pH=7.0; T=55°C) focused on the exponential growth phase

H_2 and CO_2 as electron donor and carbon source in the presence of sulfate resulted in the accumulation of sulfide up to 10.5 mM. The difference in maximum sulfide concentration in incubations with CO compared to those with H_2/CO_2 can likely be attributed to an effect of the pH. Hydrogenogenic conversion of CO leads to a drop in pH due to CO_2 formation, whereas in incubations with H_2/CO_2 the pH increases due to CO_2 consumption for growth.

The latter obviously also occurs in incubations with CO; however, CO conversion of 160 kPa CO results theoretically in the production of 160 kPa CO_2, discarding for simplicity differences in solubility and consumption for biomass synthesis. Therefore, the acidifying potential is much higher than what can be compensated due to growth in the subsequent hydrogentrophic sulfate reduction process. The effect of the final pH on the maximal sulfide concentration is shown in Fig. 5. At the end of the experiments, there were still sufficient amounts of H_2 (>60 kPa) and CO_2 (>100 kPa) present, which confirms that sulfide production did not cease due to a limitation of electron donor or carbon source, but rather due to sulfide inhibition. Figure 5 shows a clear increase in sulfide tolerance with increased pH values.

The sulfide inhibition at pH 6.5 is reached at a total sulfide concentration less than half of the inhibiting concentration at pH 7.8. This can be due to the relatively large contribution of undissociated H_2S at low pH. However, not only undissociated H_2S contributes to the inhibition, as the H_2S concentration at pH 7.8 is less than 20% of the H_2S concentration at pH 6.5. This suggests that at high pH values the dissociated form of sulfide (HS^-) contributes to the overall sulfide inhibition as well.

In incubations under an initial gas phase of CO in the presence of sulfate, the maximal sulfide concentration measured was in the range of 3.6–4.9 mM at pH values of 6.4–6.5. When combining these values in Fig. 5, it can be seen that these values fit well with those determined in incubations supplemented with H_2/CO_2 at similar pH values.

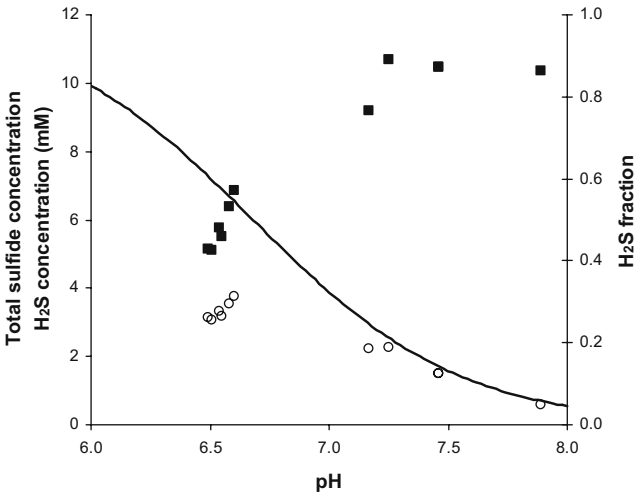

Fig. 5 Maximal produced total sulfide (■) and calculated H_2S concentration (○) in incubations with H_2/CO_2 and 20 mM sulfate with a final pH between 6.5 and 7.9. The *line* represents the fraction of undissociated H_2S $\left(\text{fraction}_{H_2S} = \frac{1}{10^{(\text{pH}-\text{pK}_a)}+1}\right)$

Discussion

Desulfotomaculum carboxydivorans in synthesis gas purification processes

The fast growth (generation time 100 min) and high specific conversion rates (about 0.8 mol CO (g protein)$^{-1}$ h^{-1}) of *D. carboxydivorans* on CO with nearly equimolar production of H_2 makes it an excellent biological alternative for the chemical water–gas shift reaction to purify synthesis gas. The CO conversion rates assessed in batch experiments reveal that the highest specific conversion rates are already obtained in the early exponential phase when the biomass protein concentration is still relatively low, suggesting that the conversion is mass-transfer limited. In this case, the maximal possible flux of CO from the gas phase to the liquid phase will determine the overall volumetric CO conversion rates. The flow from the gas to the liquid phase can be described by Eq. 4:

$$\Phi_{m,CO} = k_L a\left(C^*_{CO} - C_{CO,L}\right), \tag{4}$$

in which: $\Phi_{m,CO}$ is the molar flow rate (mol m^{-3} s^{-1}); $k_L a$ the gas-liquid mass transfer coefficient (s^{-1}); C_{CO}* the equilibrium concentration for CO in water (mol m^{-3}) and $C_{CO,L}$ the CO concentration in the liquid phase. When the conversion proceeds with high efficiency and mass transfer limits the biological activity the CO concentration in the liquid phase ($C_{CO,L}$) can be assumed to be zero and consequently Eq. 4 reduces to:

$$\Phi_{m,CO} = k_L a \cdot C^*_{CO}. \tag{5}$$

C^*_{CO} can be calculated by means of Henry's Law according to:

$$C^*_{CO} = P_{CO} \cdot k_H \tag{6}$$

in which: P_{CO} is the partial CO pressure (Pa) in the gas phase and k_H is Henry's constant for CO, i.e., 6.3×10^{-6} mol m^{-3} Pa^{-1} at 55°C (Lide 2001). This results in:

$$\Phi_{m,CO} = k_L a \cdot (P_{CO} \cdot k_H) \tag{7}$$

Thus, the partial pressure of CO (P_{CO}) greatly affects the mass transfer rates of CO to the liquid phase. Bioreactors operated at 1 MPa CO would increase the mass transfer by a factor 10 compared to operation at 100 kPa of CO in the gas phase. Operation of a biological hydrogenogenic CO converting reactor at elevated pressures is not only beneficial

for mass transfer rates, but is also desired for the product gas to minimize transportation costs. In the experiments conducted with both *D. carboxydivorans* (Fig. 1) and Eerbeek sludge (Sipma et al. 2004) CO toxicity did not manifest at a P_{CO} as high as 160 to 180 kPa.

Synthesis gas from steam reforming of light hydrocarbon feeds is produced at pressures between 2.16 and 2.51 MPa and temperatures in the range of 800–870°C (Czuppon et al. 1995). To minimize the size of the bioreactor it would be beneficial to operate at elevated pressures. Nevertheless, as the synthesis gas needs to be cooled to a temperature of around 55°C, the pressure will decrease from more than 2 MPa to values of around 0.7 MPa. Gasification of coal is performed at temperatures exceeding 1,300°C and pressures higher than 2 MPa (Czuppon et al. 1995), which corresponds to maximal gas pressures of about 0.4 MPa at 55°C. The affinity of *D. carboxydivorans* for CO is sufficiently high to remove CO to values as low as 5 ppm (Table 1), but thermodynamic limitations as a result of high concentrations of H_2 and CO_2 will result in higher exit CO levels.

Posttreatment of the product gas is therefore most likely needed and could be performed with selective gas membranes (Perry et al. 1997; Koros and Mahajan 2000). The use of gas membranes, furthermore, allows a constant recovery of H_2 from the system, enabling the recycling of unconverted CO and maintaining a relatively high P_{CO} to facilitate mass transfer of CO. Selective H_2 recovery combined with CO_2 removal from the process gas phase results in more favorable thermodynamics for CO conversion and could enable CO_2 storage into the deep subsurface (Gale 2004) or its reuse in, e.g., chemical synthesis of methanol (Pruschek et al. 1997) or green house horticulture.

Although a pressure of 0.7 MPa does not seem extreme for bacteria, viz. barotolerant microorganisms have been reported to grow at pressures up to 40–50 MPa (Madigan et al. 1997), the effect of such pressures as well as high P_{CO} still needs to be evaluated. Assuming a synthesis gas with 12% CO at a pressure of 0.7 MPa at 55°C, typical for methane steam reforming (Czuppon et al. 1995), the maximal P_{CO} in the reactor will be only 84 kPa, which will not inhibit *D. carboxydivorans*. Similarly, no inhibiting effect of the P_{CO} is expected for coal gas, as its total pressure after decreasing the temperature to 55°C is about 0.4 MPa, with a CO content of around 50% (Czuppon et al. 1995). Nevertheless, the presence of potential inhibitors in the feed gas at higher partial pressures may require their removal from the feed synthesis gas, e.g., H_2S or mercaptans may have to be removed, e.g., using alkaline scrubbers.

In a study that addressed CO conversion in three types of reactors, it was found that a biotrickling filter gave higher efficiencies than CSTR and bubble column reactors (Klasson et al. 1992). This was attributed to the operational conditions, which approach plug flow. Furthermore, according to Bredwell et al. (1999) in biotrickling filters, the k_La is relatively independent of the gas flow rate for sparingly soluble gasses. In general, increased liquid and gas velocities result in higher mass transfer, resulting in k_La values of up to 0.24 s^{-1} for bubble columns (Bredwell et al. 1999), although such measures are accompanied with increased power demands, which is economically/commercially unattractive. Differences in treatment costs as evaluated by Zuber et al. (1997) revealed a 50% cheaper treatment with a biotrickling filter compared to a gas lift reactor (Zuber et al. 1997). When selecting a biotrickling filter, the maximal flux of CO could be determined assuming a k_La of 0.015 s^{-1} as determined for this reactor type in CO conversions at 37°C (Klasson et al. 1992).

Although a modest temperature change, which is the case in the range of 37–55°C, has a negligible effect on the interfacial area (a), they slightly affect k_La due to changes of the medium viscosity (Perry et al. 1997). Nevertheless, taking the value determined by Klasson et al. (1992), the maximal flux of CO to the biomass in a biotrickling filter can be determined as a function of the P_{CO} according to Eq. 7, i.e., at a P_{CO} of 200 kPa (synthesis gas from coal) the maximal CO flux is 1,600 mol m^{-3} day^{-1}. This equals a H_2 production capacity of almost 40 m^3 m^{-3} reactor volume under standard conditions (25°C and 101.325 kPa). The conversion of 1,600 mol(CO) m^{-3} day^{-1} would result in a biomass yield of about 690 g protein m^{-3} day^{-1}. This results in a required CO conversion activity of the biomass of about 0.1 mol CO (g protein)$^{-1}$ h^{-1}, which is eight times lower than the maximal CO conversion rates estimated for *D. carboxydivorans* (Fig. 2).

Desulfotomaculum carboxydivorans in biological desulfurization processes

The extremely high CO conversion rates (0.8 mol CO (g protein)$^{-1}$ h^{-1}) of *D. carboxydivorans*, combined with its high sulfate reduction rates (32 mmol SO_4^{2-} (g protein)$^{-1}$ h^{-1}) and its ability to use both CO and H_2, the major constituents of synthesis gas, makes its application for flue gas desulfurization very promising. Van Houten et al. (1997) determined a k_La value of 0.030 s^{-1} for H_2 for a gas lift reactor operated at 55°C. This k_La (H_2) can be converted to estimate the k_La for CO, using the following equation:

$$k_La_{(CO)} = k_La_{(H_2)} \cdot \frac{D_{co}}{D_{H_2}}. \quad (8)$$

The diffusivity (D) of both CO and H_2 was estimated at 55°C using the Wilke–Chang equation (Perry et al. 1997), estimating a water viscosity at 55°C (0.5 mPa s) from Lide (2001) and values for molecular volumes from Treybal (1985). The calculated diffusivity of H_2 at 55°C amounted

to 6.28×10^{-9} m^2 s^{-1} and that of CO to 3.97×10^{-9} m^2 s^{-1}. The resulting value of k_La for CO (0.019 s^{-1}) used in Eq. 8 gives a maximal CO flux exceeding 1,000 mol CO m^{-3} day^{-1}. As four moles of CO are needed to form the required 4 mol of H_2 for the reduction of 1 mol of sulfate, the maximal sulfate reduction rate based on the maximal rate of CO supply exceeds 250 mol sulfate m^{-3} day^{-1} or 25 kg sulfate m^{-3} day^{-1}. When using synthesis gas, the maximal theoretical sulfate reduction rate is even higher due to the presence of H_2 in the feed gas and the higher k_La for H_2.

However, *D. carboxydivorans* suffers from a pH dependent sensitivity for sulfide inhibition, i.e., 9 mM sulfide at pH 7.2 and 5 mM at pH 6.5 causes complete inhibition (Fig. 5). Thus, most likely not toxicity for CO, but for sulfide determines the sulfate reduction capacity of *D. carboxydivorans*, as sulfide production occurred even at CO levels exceeding 100 kPa, which in practice likely will not be reached when using synthesis gas as electron donor. Therefore, to develop a high-rate sulfate-reducing bioreactor employing *D. carboxydivorans*, additional features to maintain the sulfide concentration below the inhibiting levels are required. To maintain sulfide concentrations sufficiently low, operation at slightly elevated pH values could be considered, or application of an alkaline H_2S absorber through which the recycle gas is led. The H_2S absorbed from the gas phase could be fed to a second micro-aerobic biological reactor, preferably operated at high pH to minimize consumption of chemicals, in which the sulfide is partially oxidized to elemental sulfur (Janssen et al. 2000). Another attractive option is the use of H_2S extractive membranes (Chuichulcherm et al. 2001; De Smul and Verstraete 1999) placed inside the bioreactor mixed liquor, which could result in direct recovery of elemental sulfur when combining with an Fe^{3+}-containing extraction solution.

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