Desulfotomaculum carboxydivorans as biocatalyst for synthesis gas purification

Jan Sipma[1], Sofiya N. Parshina[2], Gatze Lettinga[1], Alfons J.M. Stams[2] and Piet N.L. Lens[1]

[1] Sub-Department of Environmental Technology, Wageningen University, Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, the Netherlands

[2] Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, the Netherlands

ABSTRACT. Anaerobic methanogenic sludges harbor moderately thermophilic bacteria capable of hydrogen formation from carbon monoxide (CO). Recently, our group isolated a moderately thermophilic, anaerobic, chemolithoheterotrophic, sulfate-reducing bacterium, designated as *Desulfotomaculum carboxydivorans* from an anaerobic sludge treating paper mill wastewater, capable of hydrogenogenic, i.e. hydrogen producing, growth on CO both in the presence and absence of sulfate. The isolate with (T_{opt} = 55ºC) grows with a generation time of 1.7 hours and is not inhibited by 200 kPa CO, producing equimolar amounts of H_2 from CO. In the presence of sulfate the formed H_2 is used for sulfate reduction. Therefore, *D. carboxydivorans* is interesting in both the biotechnological treatment of sulfate-rich inorganic wastewaters using synthesis gas as a cheap electron donor as well as in the purification of synthesis gas to a high purity hydrogen gas.

1 INTRODUCTION

Hydrogen gas attracts great interest as a potential clean future fuel for use in fuel cells and it is an excellent electron donor in biotechnological processes, especially in bio-desulfurization (Van Houten *et al*., 1994, 1997). Bulk production of H_2 relies on the conversion of fossil fuels, biomass or hydrocarbon rich wastes into synthesis gas by gasification, partial oxidation or steam reforming (Armor, 1999). These processes result in synthesis gas mainly composed of H_2, carbon monoxide (CO) and CO_2. The relative abundance of carbon monoxide restricts its applicability in energy production by fuel cells (Ledjeff-Hey *et al*., 2000) and in biotechnological reductive processes, such as biological sulfate reduction (Van Houten *et al*., 1996). CO is toxic to the catalyst in low temperature proton exchange membrane fuel cells (PEMFC) as well as to many hydrogenotrophic microorganisms (Ledjeff-Hey *et al*., 2000; Mörsdorf *et al*., 1992). Conversion of CO to H_2 results in a greater utilization potential of synthesis gas. Currently, this CO conversion is performed in chemical catalytic systems, which operate at high temperatures (300 – 650ºC).

H_2 can be produced biologically from CO via the water-gas-shift reaction (equation 1) performed by phototrophic (Mörsdorf *et al*., 1992) and anaerobic thermophilic carboxydotrophic microorganisms (Table 1), which all employ a similar CO metabolism

with concomitant H_2 production for which recently the term 'hydrogenogenesis' was proposed (Svetlichnyi *et al*., 2001).

$$CO_{(g)} + H_2O \rightarrow CO_{2(g)} + H_{2(g)} \qquad \Delta G^{\circ\prime} = -20\ kJ.mol^{-1} \qquad (1)^{\#}$$

Obligate anaerobic hydrogenogenic CO converting microorganisms were isolated exclusively from thermophilic hot environments related to volcanic activity, which have been found to contain small amounts of CO (Symonds *et al*., 1994). All these isolates showed fast growth on CO with generation times (t_d) between 1.1 and 8.3 hours at partial pressures of CO in the gas phase exceeding 100 kPa (Table 1).

Table 1. Obligate anaerobic hydrogenogenic CO converting microorganisms.

Microorganism	T_{Opt} (°C)	pH opt	t_d (h)	Max P_{CO} (kPa)	Ref.
Carboxydothermus hydrogenoformans	70-72	6.8-7.0	2	101	1
Carboxydothermus restrictus	70	7.0	8.3	101	2
Caldanaerobacter subterraneus subsp. *pacificus*	70	6.8-7.1	7.1	110	3
Carboxydocella thermoautotrophica	58	7.0	1.1	101	4
Thermosinus carboxydivorans	60	6.8-7.0	1.2	100	5
Thermococcus strain AM4	82	6.8	n.r.	101	6

n.r. not reported

Ref: 1. Svetlichny *et al*. (1991), 2. Svetlichny *et al*. (1994), 3. Sokolova *et al*. (2001), 4. Sokolova *et al*. (2002), 5. Sokolova *et al*. (2004a), 6. Sokolova *et al*. (2004b).

Since, the key-enzymes for H_2 production from CO, *i.e.* carbon monoxide dehydrogenases (Ferry, 1995) and hydrogenases (Vignais *et al*., 2001), are widespread in microorganisms it could be expected that anaerobic CO metabolism occurs in many habitats. In agreement, hydrogenogenic CO conversion was found in several anaerobic bioreactor sludges (Sipma *et al*., 2003). Batch experiments at elevated temperatures (55°C) with several mesophilic anaerobic sludges revealed the presence of viable populations of hydrogenogenic CO-oxidizing bacteria producing equimolar amounts of H_2 (Sipma *et al*., 2003). Application of H_2-evolving carboxydotrophic thermophiles in a biotechnological process allows more cost effective production of H_2 from synthesis gas than the current chemical catalytic purification method. In general, biological systems operate at far more moderate conditions and biological catalysts are highly efficient (Bredwell *et al*., 1999).

[#] The standard Gibbs' free energy change at neutral pH and standard conditions ($\Delta G^{\circ\prime}$) was calculated using thermodynamic data from Amend and Shock (2001). At 55°C, $\Delta G_{55}' = -22.3\ kJ.mol^{-1}$.

2 MATERIALS AND METHODS

2.1 Inocula

Anaerobic granular (methanogenic) sludge samples were obtained from a full-scale anaerobic reactor treating wastewater from several paper mills (Industriewater Eerbeek, Eerbeek, The Netherlands). This sludge was originally cultivated at 30-35°C. In a previous paper we have reported on laboratory experiments on CO conversion by this sludge (Sipma *et al*., 2004). Furthermore, nine full-scale grown anaerobic granular sludges were tested for hydrogenogenic CO conversion capacity.

2.2 Medium composition

The basal medium contained (in mM): NH_4Cl 5.6, $CaCl_2.2H_2O$ 0.7, $MgCl_2.6H_2O$ 0.5, NaCl 5.1, $Na_2S.9H_2O$ 0.3, 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 $Na_2HPO_4.2H_2O$.

2.3 Experimental design

Incubations were performed in serum bottles in a temperature controlled shaker-incubator type RFI-125 (Infors AG, Basel, Switzerland) at 200 rpm. All experiments were performed at 55ºC and an initial pH of 7.0.

2.4 Analysis

The headspace gas composition was measured on a gas chromatograph 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 5Å capillary column of 30 m (0.53 mm; 15µm; CP7544). The temperatures of the oven, injection port and FID detector were 90ºC, 100ºC and 250ºC, respectively.

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).

3 RESULTS AND DISCUSSION

3.1 Isolation and characterization of Desulfotomaculum carboxydivorans

Bottles with crushed Eerbeek sludge were incubated at 55°C in the presence of 160-180 kPa CO in the headspace. After a few series of dilution under an atmosphere of 100% CO (at 160-180 kPa) an enrichment culture was obtained that contained at least 3 morphologically different strains of bacteria. Further dilution series supplemented with 20 mM sodium sulfate under 160 kPa CO resulted in a suspension of morphologically identical cells. Roll-tubes with the same medium supplemented with 5% agar and pure CO in the gas phase were prepared to obtain separate colonies. Some of the obtained colonies were subsequently inoculated in the liquid medium. The purity of the obtained strain, designated as strain CO-1-SRB, was monitored by phase-contrast microscopy

after cultivation on CO with and without sulfate, cultivation on H_2/CO_2 with and without sulfate and product analysis.

Phylogenetic analysis of the 16S rRNA gene sequence placed CO-1-SRB to the genus *Desulfotomaculum* closely resembling *Desulfotomaculum nigrificans* DSM 574^T and *Desulfotomaculum* sp. RHT-3 (99 and 100% similarity respectively). Nevertheless, the latter strains were completely inhibited at high levels of CO and only metabolized CO in the presence of sulfate. Based on phylogenetic and physiological features strain CO-1-SRB represented a novel species within the genus *Desulfotomaculum*, with the type species *Desulfotomaculum carboxydivorans* (DSM 14880). The full description of *D. carboxydivorans* will be published shortly (Parshina *et al*., In Press).

Cells were gram-positive, motile, spore-forming rods. The temperature and pH range for growth were 30-68°C (T_{opt} = 55°C) and pH 6.0-8.0 (pH_{opt} = 7.0), respectively. The generation time of *D. carboxydivorans* is about 1.7 hours. The unique property of this isolate is that carbon monoxide (CO) could serve as a sole energy and carbon source both in the presence and absence of sulfate. CO was converted to H_2 and CO_2 and in the presence of sulfate the formed H_2 was used for sulfate reduction. Therefore, this bacterium holds a promise in both synthesis gas purification as well as in synthesis gas utilization in sulfate reduction processes at elevated temperatures as occurs in flue gas desulfurization processes.

3.2 Occurrence of hydrogenogenic CO converting microorganisms

From nine full-scale grown anaerobic sludges, eight were found capable of converting CO rapidly to H_2 when incubated at 55°C. Out of these, five were furthermore capable of reducing sulfate with the formed H_2, which may indicate the presence of *D. carboxydivorans*. Nevertheless, four enrichments were not capable of sulfate reduction and were morphologically distinct from *D. carboxydivorans* and at least 2 different bacteria could be distinguished between them. So far, these bacteria have not been characterized and described and therefore it can not be concluded whether they represent new species or rather undiscovered metabolic properties of know species, as CO has been generally overlooked in describing physiological characteristics. Nevertheless, together with our previous results on CO conversion by anaerobic granular sludges (Sipma *et al*., 2003), the present of fast growing hydrogenogenic CO converting organisms in readily available sources holds a promise as biocatalysts in synthesis gas purification. Some preliminary analysis of these moderately thermophilic hydrogenogens revealed generation times between 4 and 6 hours at partial CO pressures (P_{CO}) between 160 and 180 kPa.

3.3 Growth of D. carboxydivorans on CO

Figure 1 presents a typical growth curve of *D. carboxydivorans* in the absence of sulfate and at an initial P_{CO} of 160 kPa. Nearly stoichiometric amounts of H_2 and CO_2 are produced. Furthermore, the final concentration of CO is below the detection limit of the standard GC used to measure the gas composition, *i.e.* below 400 ppm. Despite CO conversion proceeds fast, the maximal biomass density is already reached when still 60 kPa CO was present in the gas phase. Due to the limited buffer capacity in these incubations (20 mM phosphate buffer) and the production of CO_2, the pH decreased rapidly (Figure 1B).

Analysis of trace CO concentration revealed that the minimal CO concentration that could be achieved was not limited by the affinity of the microorganism as values as low

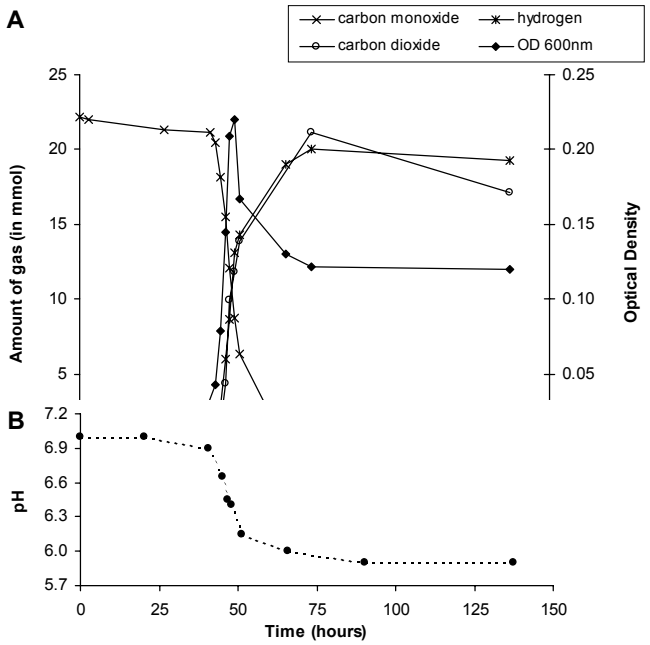

Figure 1. Growth curve of *D. carboxydivorans* grown on 160 kPa CO in the absence of sulfate (initial pH = 7.0; T = 55ºC) is shown in (A) and the evolution of the pH during CO conversion in (B).

as a few ppm could be achieved. Analysis of incubations started at different CO concentrations revealed that most likely the thermodynamic limitations determine the final CO concentration, whereas at high initial P_{CO} the pH decrease might become inhibitory to CO conversion. Due to the formation of H_2 and CO_2 the Gibbs free energy change decreases and approaches to zero. In this respect the selective removal of CO_2, e.g. by absorption in an alkaline NaOH solution, results in a lower ΔG value and consequently lower final CO concentrations as observed in batch incubations with another hydrogenogenic CO converting microorganism (A.M. Henstra, personal communications).

3.4 Design issues for biotechnological synthesis gas purification processes

The fundamental difference in biological conversion of waste gas compared with synthesis gas, which is rather a purification than pollution mitigation, is the concentration of the compound that requires conversion. Whereas, the concentration of the pollutants in biological waste gas treatment usually is in the lower ppm range, the concentration of CO in synthesis gas may exceed 50% in coal gasification (Perry *et al.*, 1997). In case of biological waste gas treatment a vast experience in reactor design already is available, from which a future commercial biological hydrogen production from synthesis gas may benefit. The use of biotrickling filters has received considerable attention in waste gas treatment as they are relatively easy to control, with respect to nutrient supply and pH (Cox and Deshusses, 2001). Ariga *et al*. (1986) already pointed out the potential advantages of using honeycomb-monolith bioreactors for

bioconversions of poorly soluble gaseous substrates due to their low pressure drop and thin liquid layers.

The application potential of biological synthesis gas purification depends first of all on the capabilities of the selected microorganism. When designing a synthesis gas purification system another crucial factor is the required product specification, especially with respect to the permitted remaining CO levels, which will depend on the application of the produced H_2 gas. For application in low temperature PEMFC, the purification requirement is to the level of maximally 100 ppm CO (Ledjeff-Hey *et al*., 2000), and preferably even less than 10 ppm (Otsuka *et al*., 2002; Ledjeff-Hey *et al*., 2000). For other applications, e.g. when H_2 is used in chemical or biological reductive processes, the requirements may be less stringent.

For a high rate biological CO converting reactor a high P_{CO} would be desirable to increase the CO flux from the gas to the liquid phase. *D. carboxydivorans* did not exhibit any toxic effects at P_{CO} levels of 200 kPa, whereas CO conversion increased with increasing pressures up to 200 kPa (data not shown). A drawback of operation at increased pressures is that the CO levels in the final product gas will increase due to thermodynamic limitations (equation 1). Calculation for a typical synthesis gas derived from coal gasification (starting composition H_2, CO and CO_2 is 30%,60% and 10%), revealed that at 101 kPa the thermodynamical minimal achievable exit CO concentrations can reach 120 ppm, whereas at 1 Mpa the theoretical minimal concentration exceeds 1% CO in the product gas.

The combination of a biological CO conversion process with a physico-chemical gas separation technique may overcome some of the difficulties in designing a biotechnological alternative for the chemical catalytic process. Selective recovery of H_2, e.g. by application of gas separation membranes, would result in a high purity grade product gas whereas the unconverted CO could be directed back to the reactor. Furthermore, the removal of CO_2 from the product gas, e.g. by physical absorption in cold methanol, as in the rectisol wash (Hochgesand, 1970), offers the possibility for selective CO_2 sequestration and geological storage (Gale, 2004), thus preventing its emission to the atmosphere. Liberation of high purity CO_2 could be even useful for reuse in chemical synthesis of e.g. methanol (Pruschek *et al*., 1997) or in greenhouse horticulture, which nowadays consumes large quantities of natural gas for the production of CO_2.

The fast generation times and high CO conversion rates of recently isolated anaerobic thermophilic microorganisms, such as *D. carboxydivorans* holds a promise for the development of a biological alternative for the chemical catalytic water gas shift reactors.

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