

## Research Article

# Deep Conversion of Carbon Monoxide to Hydrogen and Formation of Acetate by the Anaerobic Thermophile *Carboxydothemus hydrogenoformans*

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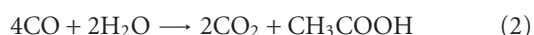
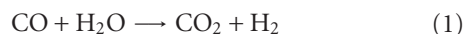
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*Carboxydothemus hydrogenoformans* is a thermophilic strictly anaerobic bacterium that catalyses the water gas shift reaction, the conversion of carbon monoxide with water to molecular hydrogen and carbon dioxide. The thermodynamically favorable growth temperature, compared to existing industrial catalytic processes, makes this organism an interesting alternative for production of cheap hydrogen gas suitable to fuel CO-sensitive fuel cells in a future hydrogen economy, provided sufficiently low levels of CO are reached. Here we study CO conversion and final CO levels in cultures of *C. hydrogenoformans* grown in batch cultures that were started with a 100% CO gas phase with and without removal of formed CO<sub>2</sub>. Final CO levels were 117 ppm without CO<sub>2</sub> removal and below 2 ppm with CO<sub>2</sub> removal. The Gibbs free energy change calculated with measured end concentrations and the detection of acetate suggest that *C. hydrogenoformans* shifted from a hydrogenogenic to an acetogenic metabolism.

## 1. Introduction

*Carboxydothemus hydrogenoformans* is a strictly anaerobic carboxydotrophic hydrogenogenic thermophilic bacterium [1] that conserves energy for growth by performing the water gas shift reaction, the conversion of carbon monoxide with water to hydrogen and carbon dioxide (reaction (1)). The standard Gibbs free energy change of the reaction per mol of CO is relatively small,  $-20 \text{ kJ mol}^{-1}$ . Production of acetate from CO (reaction (2)) or from H<sub>2</sub> and CO<sub>2</sub> (reaction (3)) is not reported for *C. hydrogenoformans*, despite the presence of the required genes in the genome of *C. hydrogenoformans* [2]



The water gas shift reaction is applied in industry for production of relative cheap hydrogen gas from synthesis gas. Synthesis gas is a mixture of mainly H<sub>2</sub>, CO, and CO<sub>2</sub> which is produced by partial oxidation or autothermal reforming of hydrocarbon-rich sources such as fossil fuels, domestic and agricultural wastes, and other biomass sources [3–5]. Water-gas shift catalysis is applied in successive high and low temperature steps at 400 and 200°C to convert the bulk of CO to final concentrations of not below 1000 ppm. Low-temperature fuel cells are sensitive to CO and require CO levels of below 10 ppm or of a few hundred ppm for novel types that apply improved membranes [6]. Hydrogen gas derived from synthesis gas, while relatively cheap, is therefore not suitable for these types of fuel cells. Highly desired is a water-gas shift process capable of removing CO from synthesis gas to levels below 10 ppm in a single step.

Synthesis gas is also of interest as source for tentative biotechnological processes. Several anaerobic microorganisms

TABLE 1: Observed partial pressures and the calculated Gibbs free energy change of CO conversion by *C. hydrogenoformans* in batch culture.

Culture	$t$ (h)	H <sub>2</sub> (Pa)	CO (Pa)	CO <sub>2</sub> (Pa)	Acetate (mM)	$\Delta G_{R1}^{(a)}$	$\Delta G_{R2}^{(a)}$
-CO <sub>2</sub> trap	0.5	$6.0 \cdot 10^2$	$2.4 \cdot 10^5$	$1.1 \cdot 10^3$	<u>0.2</u> <sup>(b)</sup>	-53	-52
	9.5	$2.0 \cdot 10^5$	$1.8 \cdot 10^4$	$1.3 \cdot 10^5$		-16	
	55	$2.0 \cdot 10^5$	$3.9 \cdot 10^1$	$1.3 \cdot 10^5$	3.8	+1	-18
+CO <sub>2</sub> trap	0.5	$2.2 \cdot 10^2$	$2.6 \cdot 10^5$	$8.4 \cdot 10^2$	<u>0.2</u>	-57	-52
	12.5	$2.0 \cdot 10^5$	$1.4 \cdot 10^4$	$1.2 \cdot 10^4$		-22	
	55	$2.1 \cdot 10^5$	<u><math>0.4 \cdot 10^0</math></u>	<u><math>3.5 \cdot 10^2</math></u>	<u>0.2</u>	-3	-16

<sup>(a)</sup> $\Delta G_{R1}$  and  $\Delta G_{R2}$ : Gibbs free energy change (kJ/mol CO) for reaction (1) and reaction (2), respectively, at 70°C [10].

<sup>(b)</sup>Underlined: detection limits that were used in calculation of free energy.

are known that can convert the synthesis gas constituents (CO and H<sub>2</sub> + CO<sub>2</sub>) into valuable products, for example, methane, ethanol, butanol, and polyhydroxyalkanoates, in what is called synthesis gas fermentation [7, 8]. More microorganisms exist that produce these compounds with only H<sub>2</sub> + CO<sub>2</sub>, but these are often sensitive to CO.

Biotechnological application of microorganisms like *C. hydrogenoformans* to produce a hydrogen gas with minimal CO is thus interesting. They bring the advantage that they operate at lower temperatures than existing water-gas shift catalyst, which is thermodynamically favorable for the removal of CO. Here, we describe the results of a study on CO conversion by and final CO thresholds of *C. hydrogenoformans* metabolism in batch culture. Removal of CO<sub>2</sub> is studied as it is a means to acquire lower final CO thresholds. The effect on metabolism in relation to minimal free energy change is discussed.

## 2. Materials and Methods

*C. hydrogenoformans* (DSM6008) was obtained from the German Culture Collection (DSMZ, Braunschweig) and cultured in 585 mL serum-stoppered bottles in a medium buffered with 200 mL MOPS [9] and a 100% CO gas phase at 65°C and 200 rpm (1" stroke Innova 44 incubator, New Brunswick Scientific). Bottles contained a glass reaction tube with either 5 mL water as control or 5 mL 10 M NaOH to serve as CO<sub>2</sub> trap. CO, H<sub>2</sub>, CO<sub>2</sub>, acetate, and growth of *C. hydrogenoformans* were analysed as described [9]. Gas and liquid samples were taken with intervals of 180 minutes until 15.5 h. Trace levels of CO ( $P_{CO} < 800$  Pa) were analysed on a GC2010 fitted with MTN-1 methanizer (Shimadzu, Japan) and FID by injecting gas samples of 100  $\mu$ L with a glass gastight syringe that were allowed to equilibrate with atmospheric pressure just before injection into the GC. The Gibbs free energy changes were calculated with observed gas partial pressures and tabulated data for 70°C [10]. Partial pressures of CO<sub>2</sub> and H<sub>2</sub> at 15.5 h were used for calculation of  $\Delta G$  of later samples where only trace CO was measured, unless stated otherwise.

## 3. Results and Discussion

The metabolic capacity of *C. hydrogenoformans* cultures to achieve low CO concentrations by CO conversion to H<sub>2</sub> and

the effect of CO<sub>2</sub> removal were studied. *C. hydrogenoformans* was grown in batch cultures with a CO gas phase. Incubations were done with and without a CO<sub>2</sub> trap. The optical density and CO, H<sub>2</sub>, and CO<sub>2</sub> concentrations were measured over time (Figure 1). The final pH and acetate concentrations were also measured. With measured concentrations, the Gibbs free energy changes for reaction (1) and (2) were calculated (Table 1).

The cultures with CO<sub>2</sub> trap showed very similar CO uptake rates and H<sub>2</sub> production rates compared to cultures without trap, however, with a clearly longer lag phase and lower final OD than cultures without trap. Likely, the removal of CO<sub>2</sub> affects growth, as it is an intermediate in the carbon assimilation by *C. hydrogenoformans* through the acetyl-CoA pathway. Additionally, the CO<sub>2</sub> trap, which is an alkaline solution, could trap the acid gas, H<sub>2</sub>S, which serves as source of sulfur and the low redox potential needed for growth of *C. hydrogenoformans*. Once CO conversion in cultures with CO<sub>2</sub> trap reached comparable rates to cultures without trap, CO<sub>2</sub> started to accumulate until most CO was consumed. Thereafter CO<sub>2</sub> dropped to end point concentrations below the detection limits (<350 kPa). Final CO levels in cultures without CO<sub>2</sub> trap were 117 ppm, while in cultures with CO<sub>2</sub> trap the CO levels had dropped below the detection limit of 2 ppm.

With measured concentrations, using detection limits where concentrations could not be measured, the Gibbs free energy change was calculated for reactions (1) and (2) (Table 1). For reaction (1), the conversion of CO with water to CO<sub>2</sub> and H<sub>2</sub>,  $\Delta G$  were +1.4 and -3 kJ/mol CO for cultures without and with CO<sub>2</sub> trap, respectively. These values are closer to thermodynamic equilibrium ( $\Delta G = 0$ ) than can be expected based on minimal biological energy quantum theory that takes the Gibbs free energy to translocate one proton over the cytoplasmic membrane as minimum, which was assumed -20 kJ mol<sup>-1</sup> [11], even when a degree of variability is allowed for the minimal biological energy quantum [12]. Instead of reaction (1), the conversion of CO to H<sub>2</sub>, another metabolic reaction that causes the removal of CO in later stages of culture. Acetate was detected in cultures without CO<sub>2</sub> trap, which suggests *C. hydrogenoformans* shifted from reaction (1) to reaction (2) during cultivation. The  $\Delta G$  for reaction (2) with average concentration of 3.8 mM acetate was -18 kJ mol<sup>-1</sup> for cultures without CO<sub>2</sub> trap, which is much closer to theoretical -20 kJ mol<sup>-1</sup> of the minimal biological energy quantum. However, in cultures with CO<sub>2</sub>

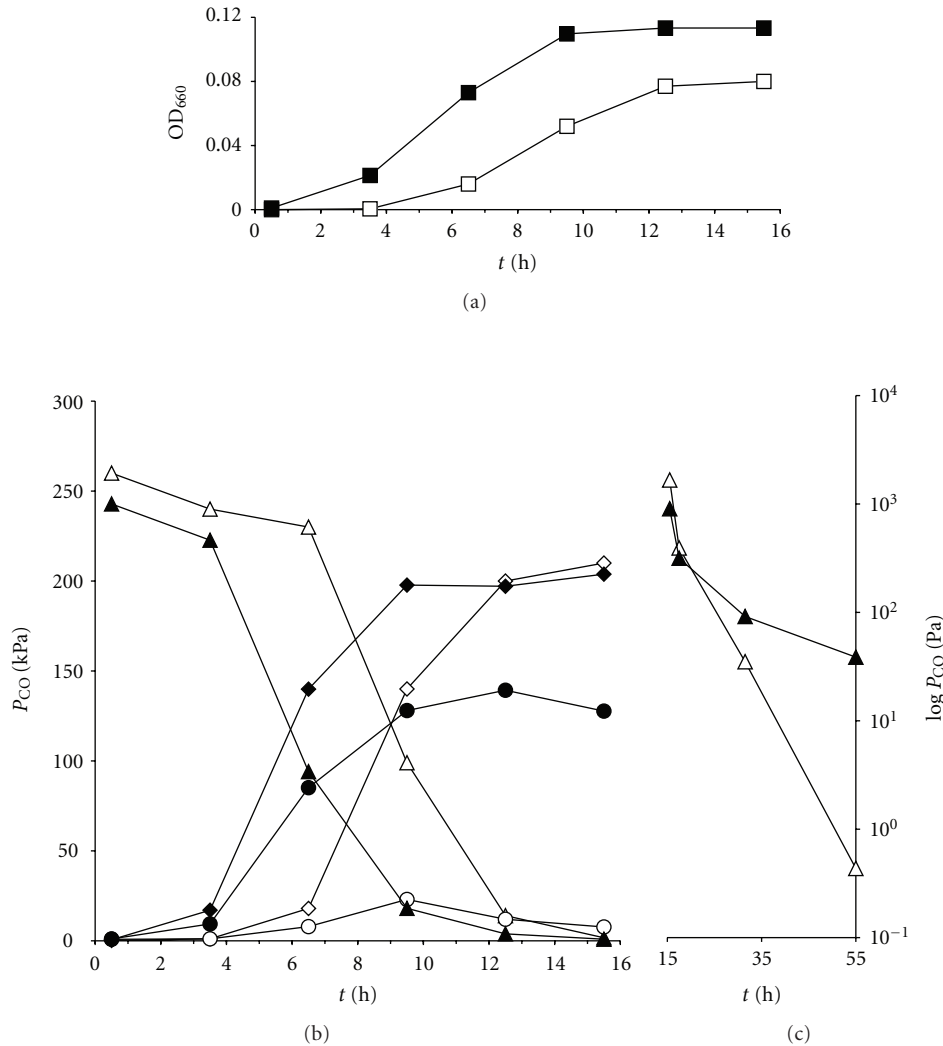


FIGURE 1: Final CO levels of the hydrogenogenic CO metabolism of *C. hydrogenoformans* was studied batch cultures, with CO<sub>2</sub> trap and without CO<sub>2</sub> trap (open versus closed symbols, resp.), in triplicate. Optical density of cultures ((a), square symbols), and partial pressures of CO ((b), triangles), H<sub>2</sub> (diamonds), and CO<sub>2</sub> (circles) were monitored over time and plotted on a linear axis. Trace levels of CO (c) were plotted on a logarithmic axis from 15.5 h onward.

trap, acetate was not detected. The detection limit for acetate was 0.2 mM. It is possible that enough CO or CO<sub>2</sub> was not available to produce more acetate than the detection limit in cultures with CO<sub>2</sub> trap. With 0.2 mM acetate, a  $\Delta G_{R2}$  of  $-16 \text{ kJ mol}^{-1}$  CO was calculated which is more in accordance with the minimal biological energy quantum.

#### 4. Conclusion

Batch cultivation of *C. hydrogenoformans* with CO resulted in final CO concentrations of 117 ppm. With removal of CO<sub>2</sub> during cultivation, even lower CO concentrations of below 2 ppm were reached. The low CO levels in the produced hydrogen-rich gas make the gas suitable for application in CO-sensitive processes. Low-temperature fuel cells require CO to be present below 10 ppm [8]. Improved fuel cell membranes allow several hundred ppm [13, 14]. While the bulk

of CO was converted to H<sub>2</sub> by *C. hydrogenoformans*, it is likely that metabolism shifted to production of acetate from CO in final stages of incubation. Production of acetate by *C. hydrogenoformans* was not shown before. To apply such a biological catalyst, further research is needed on kinetic aspects, mainly relating to mass transfer at the gas-liquid-microbe interfaces.

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