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BIOCON-CO_2

CATCO_2NVERS

Biocatalytic conversion of industrial off-gas carbon dioxide to commodity chemicals

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Background

Fossil-based industries emit off-gases rich in carbon dioxide (CO_2), the main greenhouse gas. The transition to a circular economy requires these off-gases to instead be used as carbon feedstock for sustainable production of commodity chemicals. An example is the one-carbon compound formate and its conjugate acid **formic acid**, which have applications in animal feed, leather and textile dyeing industries.

Objective

We aimed for biocatalytic conversion of concentrated CO_2 gases into the C1-compound formate. Biocatalytic conversion of CO_2 to formate can be catalyzed by the enzyme **formate dehydrogenase (FDH)**. Cost-effectiveness requires an FDH with high activity and high stability. FDHs from some anaerobic bacteria are known to be highly active, but lack oxygen tolerance. Vice-versa, oxygen-tolerant FDHs often lack CO_2-reducing activity.

Selecting an oxygen-tolerant, CO_2-reducing, robust FDH

We selected an oxygen-tolerant, NADH-dependent, *Thiobacillus* FDH (TsFDH) reported to have high CO_2-reducing activity [1]. Optimal conditions were 37°C and pH 5.5, at which **CO_2-reducing activity was 13 mU/mg**, more than threefold higher than that of commercial *Candida boidinii* FDH (CbFDH; Figure 1A). TsFDH was stable for 50 h, and was resistant to potential flue gas impurities benzene, toluene or xylene (Figure 1B)

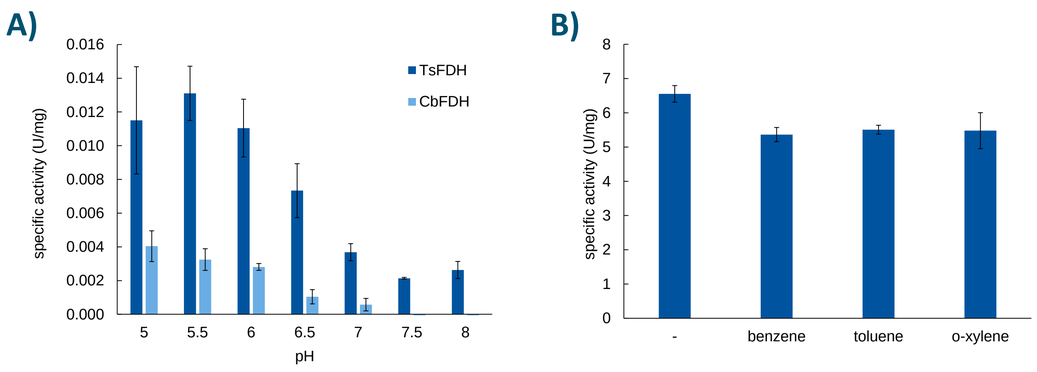

Figure 1. A) CO_2-reducing activity of heterologously produced and purified TsFDH and commercial CbFDH at 37°C. **B)** Formate-oxidizing activity of 22 µg/mL TsFDH after 8 h of incubation at 37°C in sodium citrate buffer pH 5.5 saturated with benzene, toluene, or o-xylene.

NADH regeneration

Regeneration of cofactor NADH was achieved with a **glucose dehydrogenase (GDH)**, which oxidizes D-glucose to D-gluconic acid and reduces NAD^+ back to NADH (Figure 2).

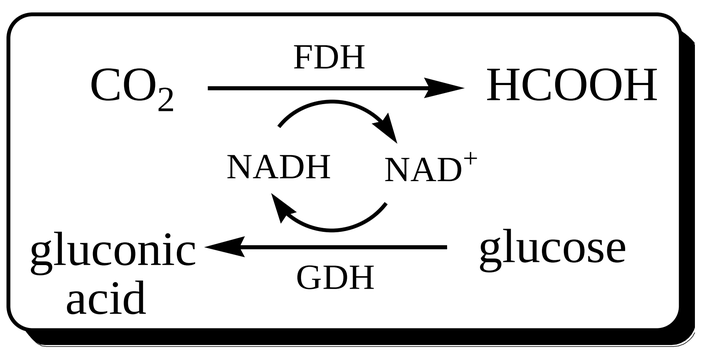

Figure 2. The overall reaction scheme catalyzed by FDH and GDH.

Biocatalytic reduction of CO_2 to formate

Higher formate titers were obtained at pH 6.5 than at pH 5.5 (Figure 3A), possibly due to NADH instability at lower pH [2]. In prolonged reactions at pH 6.5, **formate titers of 14-15 mM** could be obtained (Figure 3B).

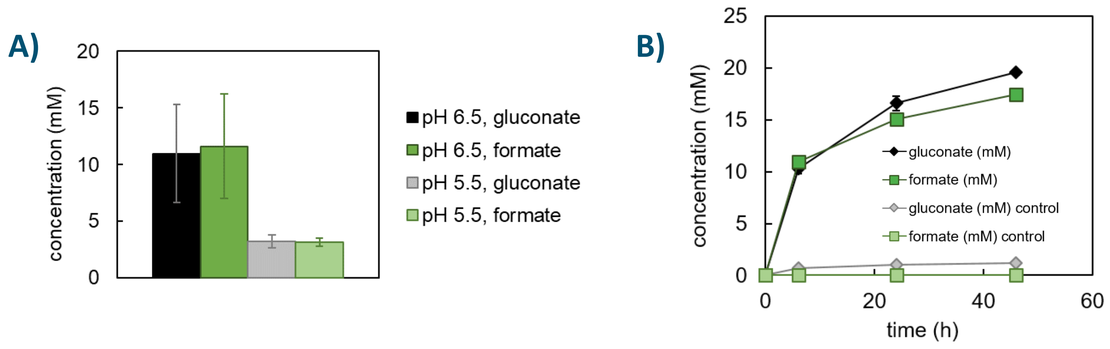

Figure 3. A) Formate and gluconate titers at pH 5.5 and 6.5 after 6 hours at 37°C. Reactions contained CO_2-saturated 0.2 M citrate-phosphate buffer, 5 mg/mL TsFDH, 1 mM NADH, 100 mM D-glucose, and a >2.5-fold excess of GDH activity, under a 1 atm CO_2 gas phase. **B)** Formate and gluconate production at pH 6.5 and 37°C. The titers in this graph are not corrected for 15-20% v/v reaction liquid loss to evaporation.

Realistic gas composition

The use of gas mixtures resembling **real off-gases** resulted in lower formate titers than achieved with pure CO_2 (Figure 4A). TsFDH lost 40% activity after incubation with H_2 and CO (Figure 4B), probably due to CO toxicity and reactivity.

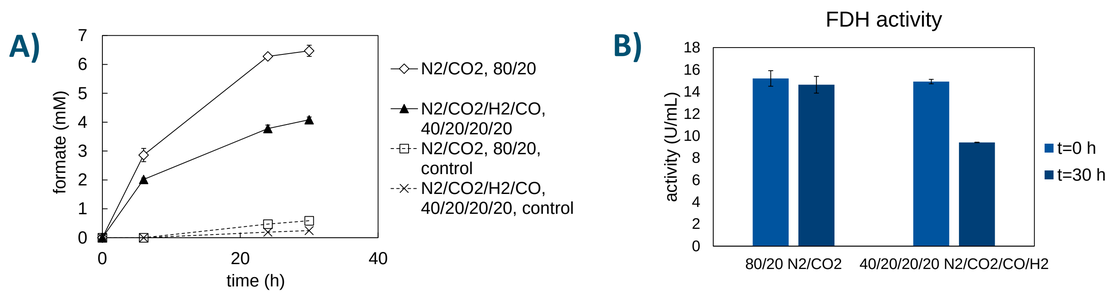

Figure 4. A) Formate production at 6.5 and 37°C with different gas phases containing N_2, H_2 and CO besides CO_2. **B)** Formate-oxidizing activity at the start and end of reactions with two different gas phases.

CO_2 utilization through biocatalytic carboxylation

CO_2 can be biocatalytically utilized through direct reduction into formate as shown here, but also through **carboxylation**, i.e. non-reductive incorporation into organic molecules (Figure 5). To circumvent the lack of known carboxylase enzymes, we are exploring the use of decarboxylase enzymes as novel carboxylation catalysts.

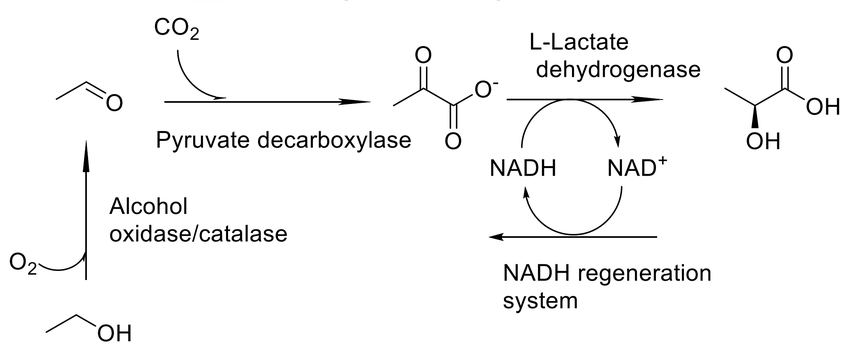

Figure 5. A reaction cascade for production of L-lactic acid from ethanol and CO_2, envisioning the use of pyruvate decarboxylase as an acetaldehyde carboxylase. For more information, visit the CATCO$_2$NVERS website: https://catco2nvers.eu/

Conclusions

This work illustrates the ongoing expansion and improvement of biocatalysts for conversion of CO_2 into commodity chemicals. We achieved a formate titer of 14-15 mM with 1 atm CO_2, and of 4-7 mM using realistic off-gas compositions with only 0.2 atm CO_2.

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Horizon 2020
European Union Funding for Research & Innovation

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreements no. 761042 (BIOCON-CO2) and 101000580 (CATCO2NVERS).

References

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