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Supplemental information

Biological hydrogen storage and release through multiple cycles of bi-directional hydrogenation of CO_2 to formic acid in a single process unit

Fabian M. Schwarz, Jimyung Moon, Florian Oswald, and Volker Müller

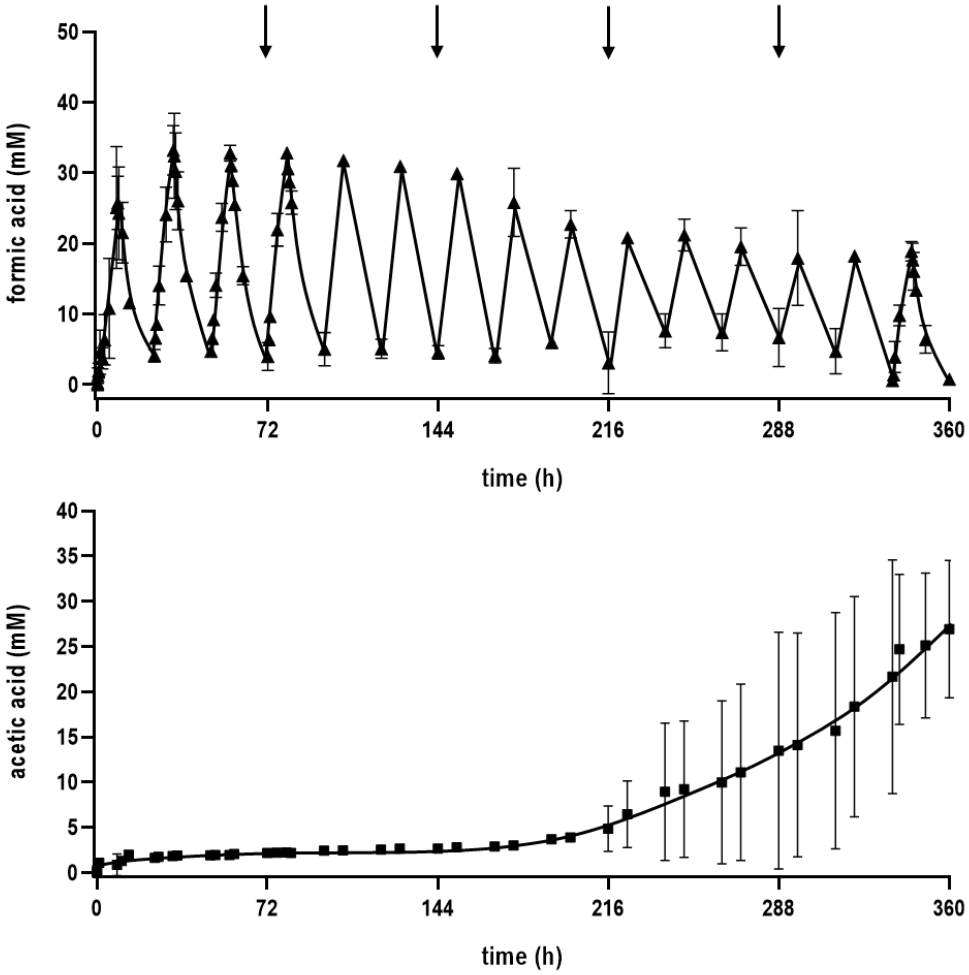
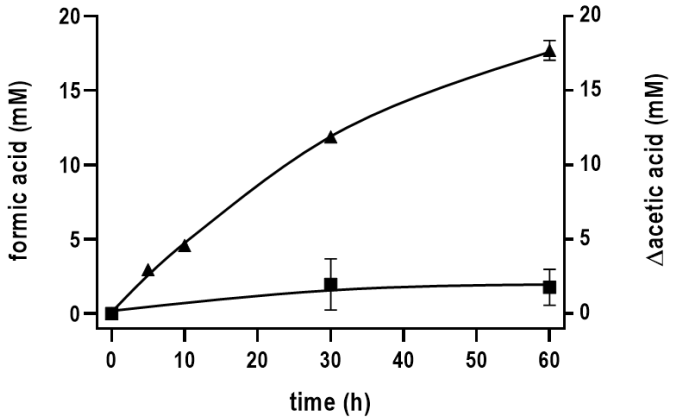


Figure S1. Long-term application of bi-directional hydrogenation of CO_2 to formic acid in a bioreactor with repetitive addition of monensin. Shown are A) 15 formic acid formation/oxidation cycles of the entire process (360 h) and B) corresponding side-product formation profile of acetic acid. Every 72 h, 15 µM of monensin was added to the bioreactor broth indicated by black arrows. All data points are mean ± SD, N = 3.



	serum bottle experiment (fresh buffer)	serum bottle experiment (re-used bioreactor buffer)
Specific formic acid production rate [mmol g^{-1} h^{-1}]	19	29

Figure S2. Uncoupling effect of used bioreactor buffer on fresh cell suspensions of *A. woodii*. Serum bottle experiments were performed using the bioreactor buffer (at t_{360}h) relieved from cells by centrifugation without the addition of new monensin. Freshly prepared *A. woodii* cells were transferred into the spent buffer to determine their ability to convert H_2 and CO_2 (80:20%, 1×10^5 Pa overpressure) to formic acid. Due to residual acetic acid in the spent buffer, the difference of formed acetic acid (Δ acetic acid) to the initial acetic acid is shown. Prior to the start of the experiment 27 mM of acetic acid was present. Triangles, formic acid; squares, acetic acid. All data points are mean ± SD, N = 2.

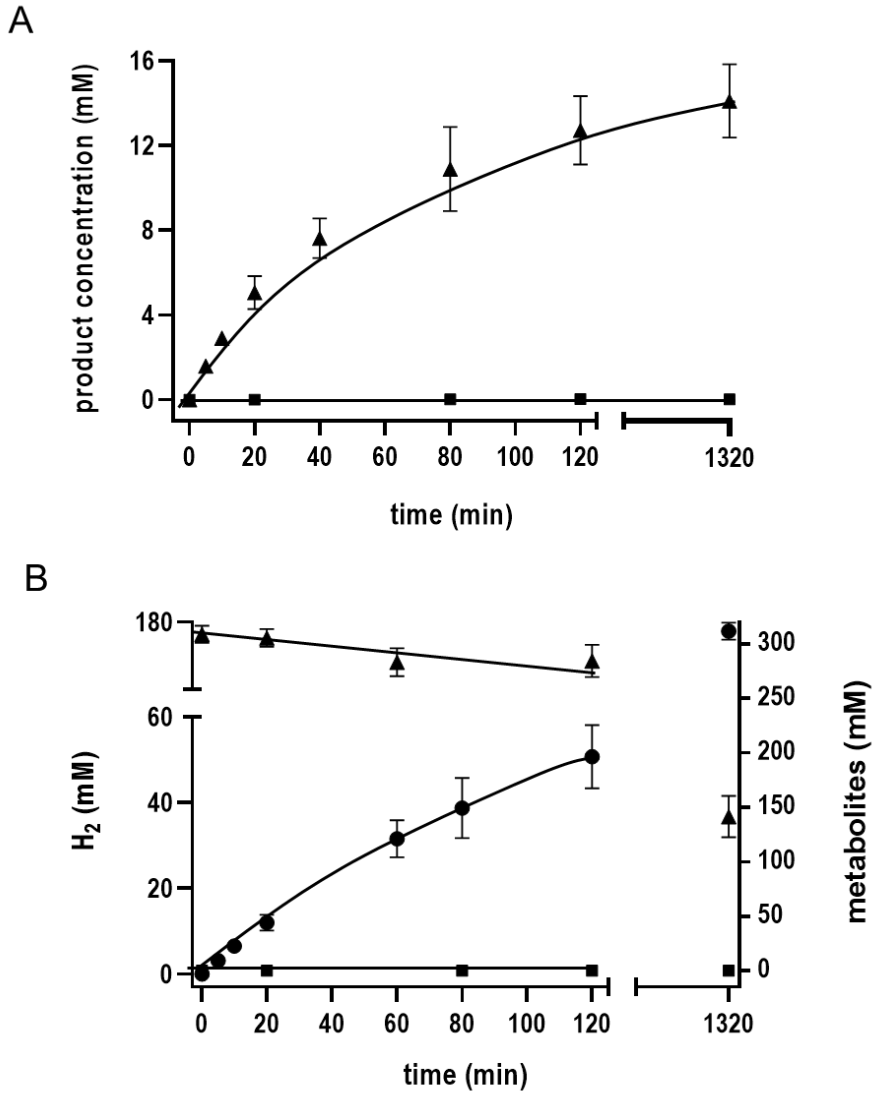


Figure S3. H_2-dependent CO_2 reduction and formate-driven H_2 production in resting cells of *A. woodii* Δ*metVF*. A) Resting cells (1 mg/mL) of *A. woodii* Δ*metVF* were resuspended in K-phosphate buffer (50 mM K-phosphate, 20 mM KCl, 2 mM DTE, pH 7.0) buffer with a H_2 + CO_2 (80:20%, 1 × 10^5 Pa overpressure) atmosphere or B) in the same K-phosphate buffer containing 300 mM sodium formate. The product and metabolite concentrations were determined. Triangles, formic acid; squares, acetic acid; circles, H_2. All data points are mean ± SD, N = 3.

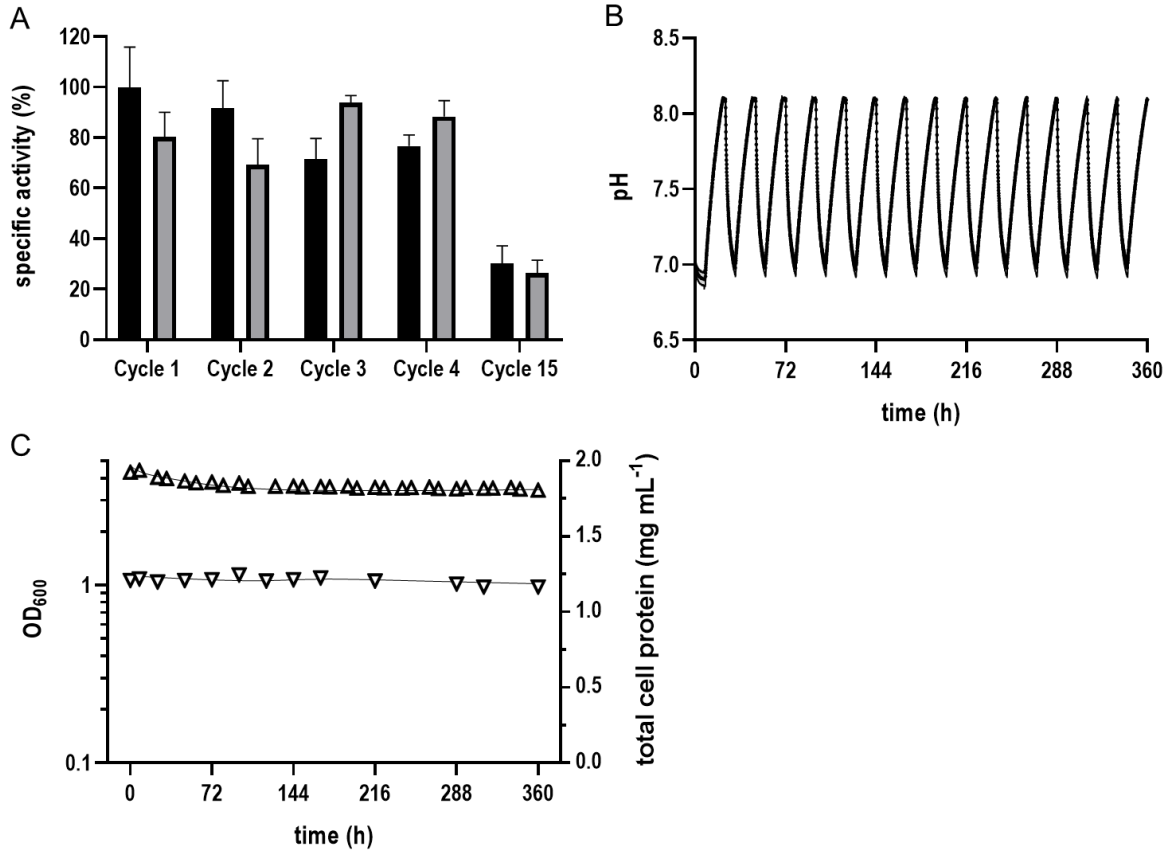


Figure S4. Overview over the catalytic activity, optical density and pH in a bioreactor with *A. woodii* Δ*metVF* cells performing multiple cycles of bi-directional hydrogenation of CO_2 to formic acid. A) Specific activity of formic acid production (black bars) and formic acid oxidation (grey bars). 100% of the activity corresponds to a formic acid production rate of 3.0 mmol g^{-1} h^{-1} and a formic acid oxidation rate of 1.7 mmol g^{-1} h^{-1}. B) pH profile of the entire fermentation. 17 mM of phosphoric acid and no base was needed as pH correcting agent in the entire process. C) Optical density at 600 nm and total cell protein concentration. Empty triangles up, optical density at 600 nm; empty triangles down, total cell protein concentration. All data points are mean ± SD, N = 3.