Fermentation of CO$_2$ and H$_2$ for formic acid production by *Thermoanaerobacter kivui*

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Carbon dioxide

- Most abundant anthropogenically produced greenhouse gas
- Rapid increase in CO$_2$ concentration
- Direct correlation between the increase in the CO$_2$ concentration and the rise in surface earth temperature

CO$_2$ can be converted into fuels or chemicals
"Microbial cell factory (MCF) is a production facility of organisms that aims at achieving a high yield of metabolites by rewiring and optimizing the production process with the help of engineering and biology."

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Formic acid production from carbon dioxide fermentation

Syngas

- Gasification
- Gasification shift
- Steam reforming
- Water-gas shift
- Combustion
- Electrolysis of water
- Fermentation

H₂

CO₂

Formic acid
Formic acid

- Stable at ambient pressure and temperature for concentration < 98% wt.
- Not flammable
- Low toxicity
- Liquid, easy to handle

Low volumetric energy density

Explosion hazard when stored in large amounts
Thermoanaerobacter kivui

- thermophilic anaerobe acetogenic organism
- can sustain heterotrophic or autotrophic metabolism
Literature background

- Resting cells of *T. kivui* were proven by F.M. Schwarz and V. Muller as highly efficient whole-cell biocatalysts for the direct hydrogenation of CO₂ to formate.
- The addition of 300 mM of KHCO₃ switches the culture to the production of formate instead of acetate by inhibiting the further downstream processing of formate.

Goal of this study

Prove that the addition of 300 mM of KHCO₃ leads to the production of formate not only in the case of resting cells but also of replicating cells.
Experimental set-up

Bacteria in autotrophic conditions in serum bottles placed in an incubator

Spectrophotometer used to measure optical density

High-performance liquid chromatography (HPLC) used to quantify acetic acid and formic acid

Conditions tested

- Autotrophic
  \((\text{H}_2: \text{CO}_2 = 80:20)\)

- Autotrophic + 300mM KHCO\(_3\)

- Autotrophic + 300mM KCl
Effect of bicarbonate on the Optical Density

Adding KHCO₃ or KCl the optical density did non increase

Growth arrest
Effect of bicarbonate on acetate and formate production

![Graph showing the effect of bicarbonate on acetate and formate production.](image-url)
Conclusions

*T. kivui* is able to convert $\text{H}_2+\text{CO}_2$ to formic acid in presence of bicarbonate

Future prospects

- Test in laboratory scale bioreactor
- Investigation of the production of formate varying the pressure of $\text{CO}_2$ and $\text{H}_2$
Thanks for the attention!
Any questions?
Appendix
Formic acid production methods

- **acidolysis of formate salts, which are in turn by-products of other processes**
  - Important in the 1990s
  - The disadvantage is the unavoidable production of sodium sulfate.

- **coproduct with acetic acid in the liquid-phase oxidation of hydrocarbons**
  - Important before 1980s.
  - Between 0.05 and 0.25 tons of formic acid are produced for every ton of acetic acid. The reaction product is a highly complex mixture, and a number of distillation steps are required to isolate the products and to recycle the intermediates.

- **carbonylation of methanol to methyl formate, followed either by direct hydrolysis of the ester or by the intermediacy of formamide**
  - practiced industrially

- **electro-chemical reduction of CO₂**
  - Not yet produced in large scale
  - require only a two-electron reduction of CO₂
Resting cell

- For the preparation of resting cells, *T. kivui* was cultivated in 1L flasks in the growth media to the late exponential growth phase.
- Glucose and fructose grown cells were harvested at an OD600 of 1.7–2.0, CO-grown cells were harvested at OD600 of 0.6.
- The culture was centrifuged under anoxic conditions at 11,500 g and 4 °C for 10 min and was washed twice in imidazole buffer (50 mM imidazole–HCl, 20 mM MgSO4, 20 mM KCl, 2 mM DTE, 4 μM resazurin, pH 7.0). Afterwards, the cells were resuspended in the same buffer to a protein concentration of 1 mg/mL and kept in gas-tight Hungate tubes.
- All preparation steps were performed under strictly anoxic conditions at room temperature in an anaerobic chamber.
- The cells were directly used for the subsequent cell suspension experiments.
KHCO$_3$ effects

- ATP hydrolysis
- Inhibition of ATP synthesis

The bicarbonate modulate the relative affinities of the catalytic site for ATP and ADP