Fermentation of CO_2 and H_2 for formic acid production by *Thermoanaerobacter kivui*

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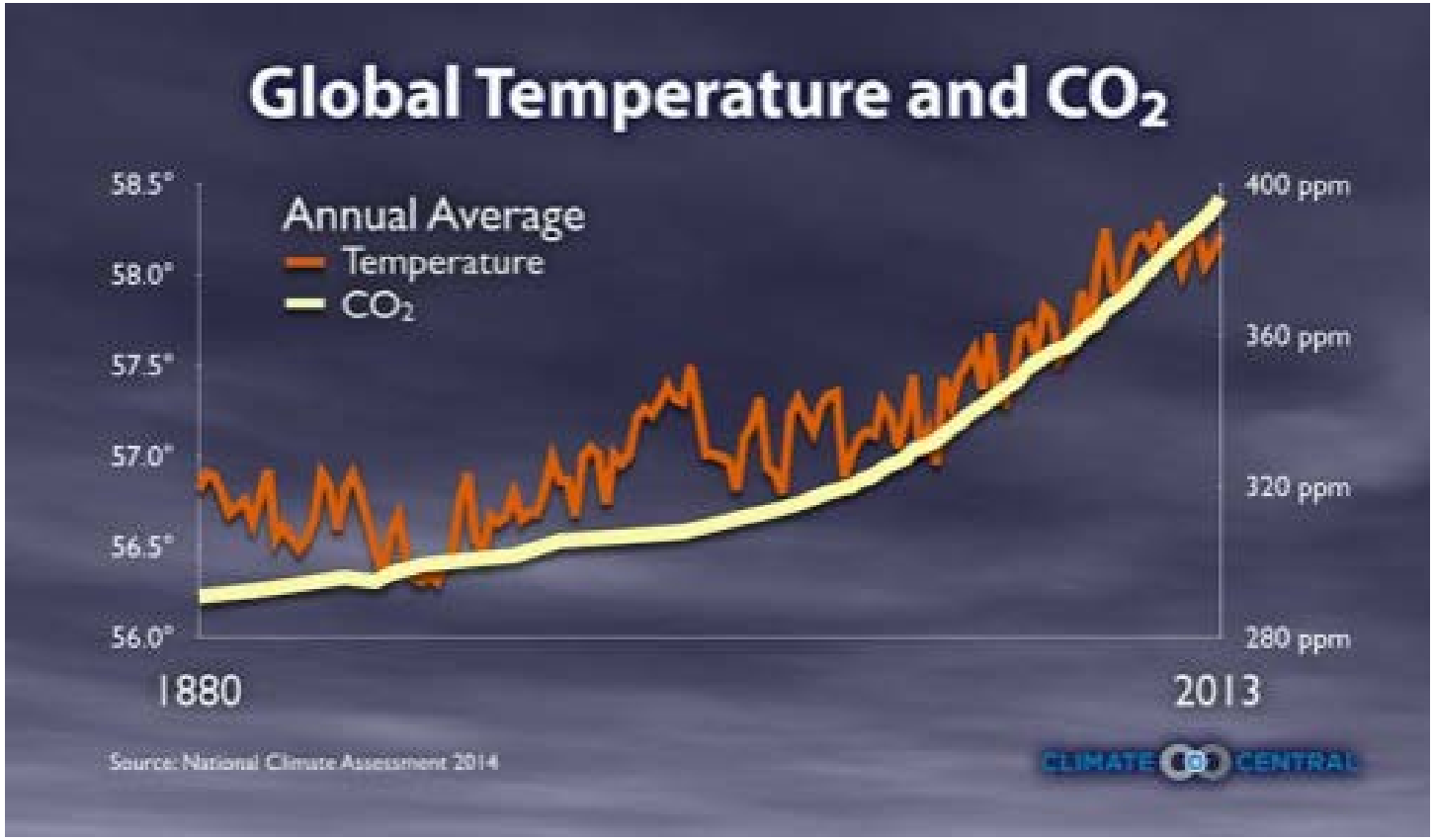
Carbon dioxide

- 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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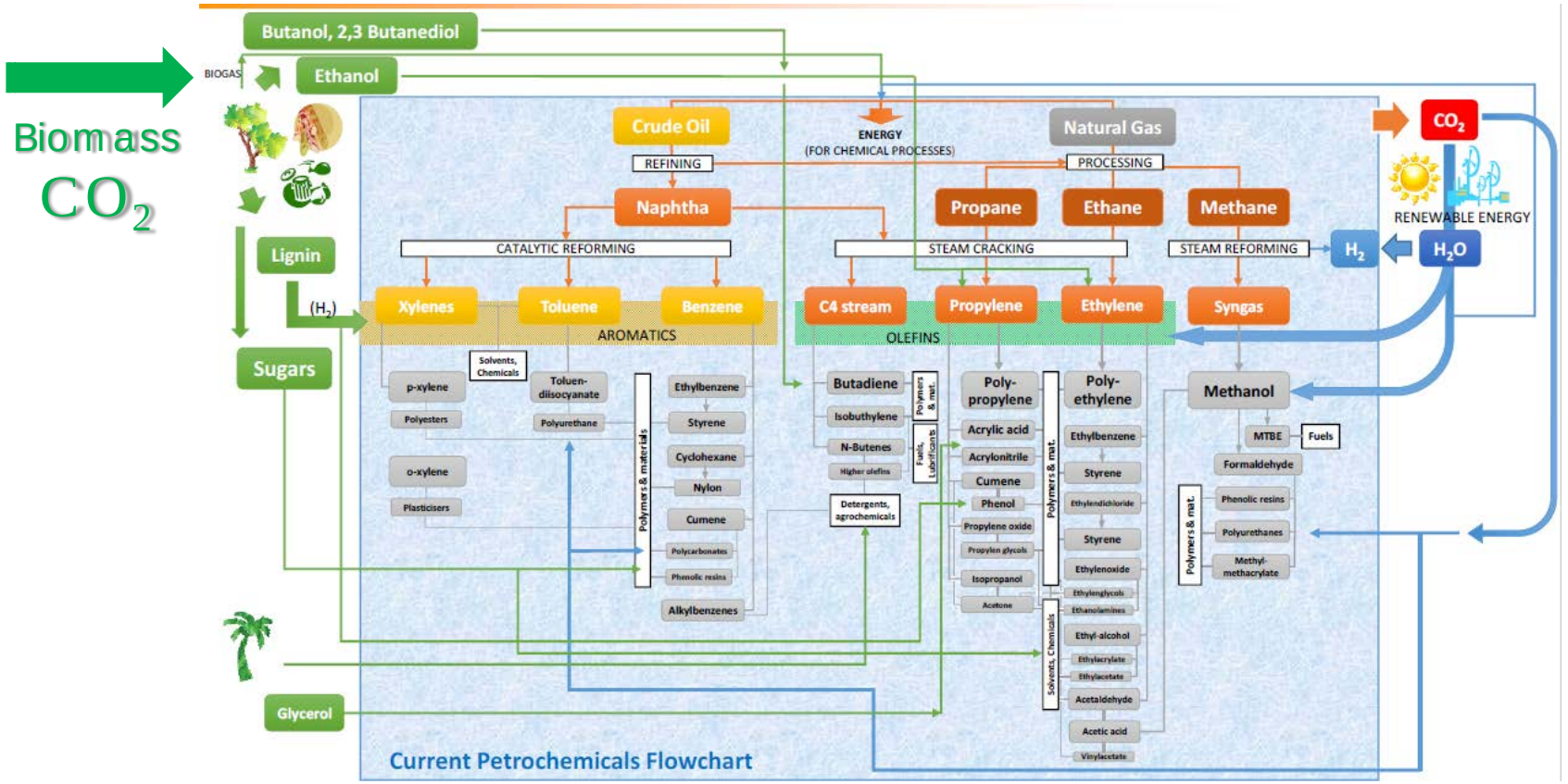
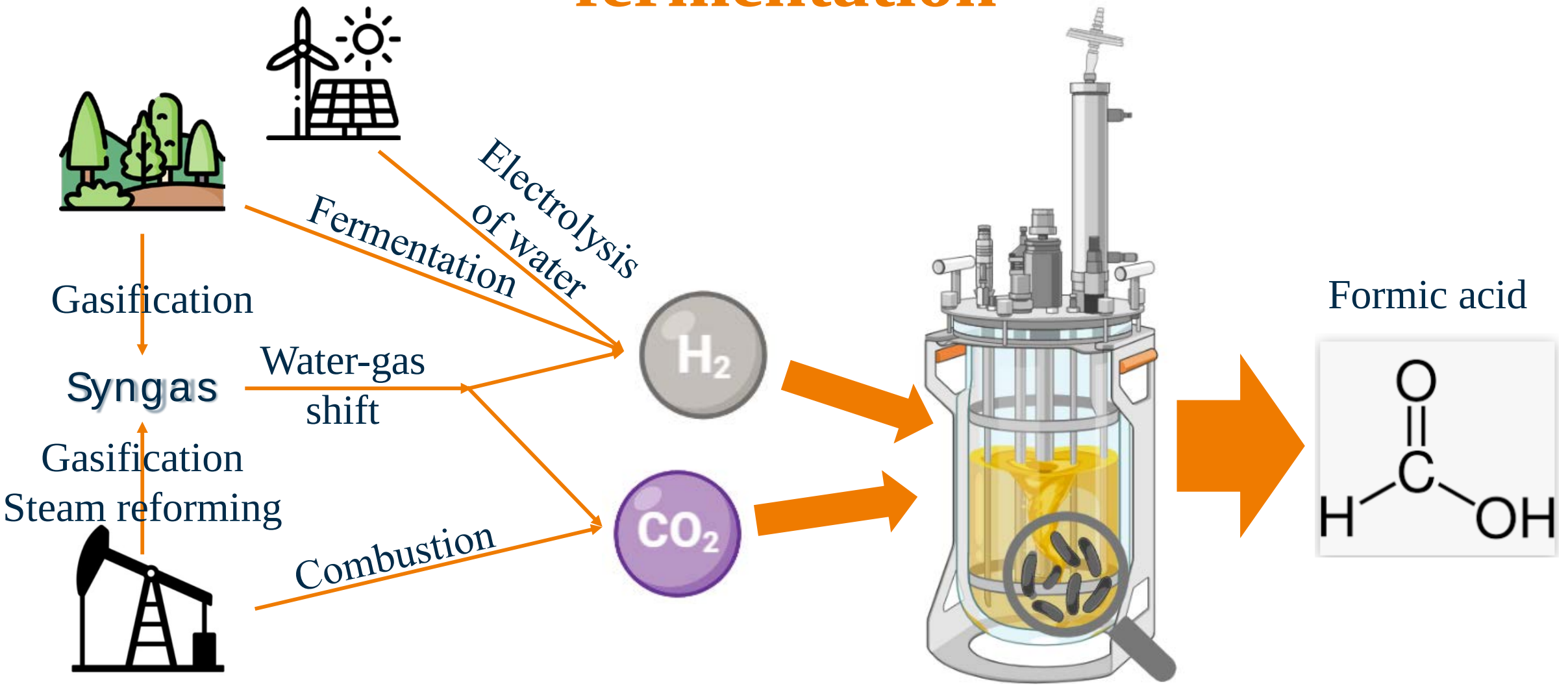
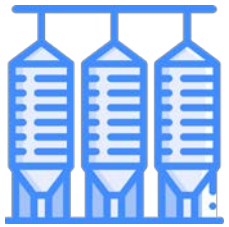
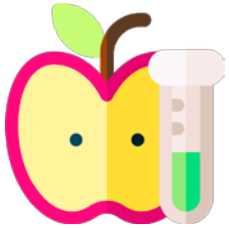
Biorefinery concept

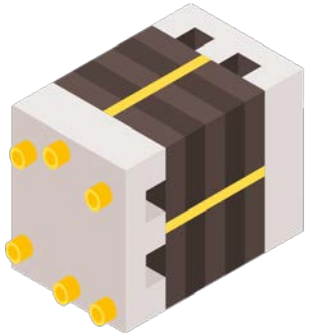


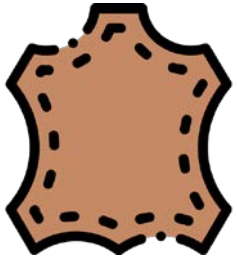
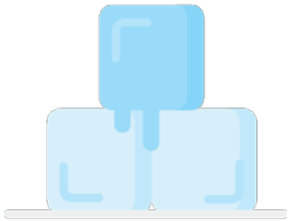
Formic acid production from carbon dioxide fermentation



Formic acid



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

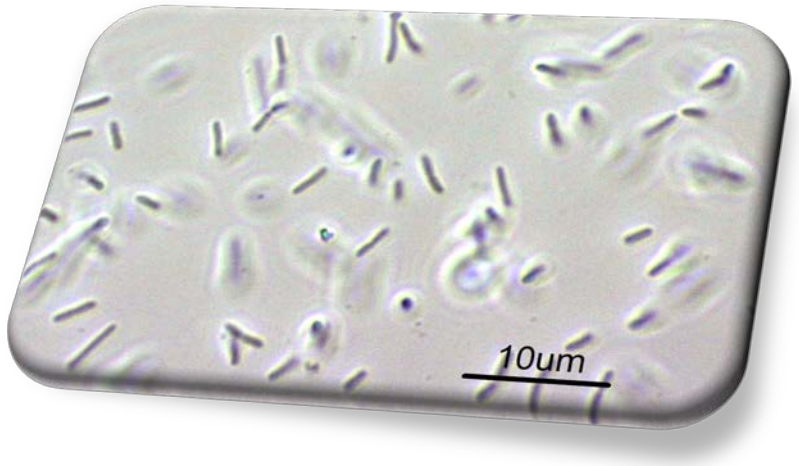
Low volumetric energy density

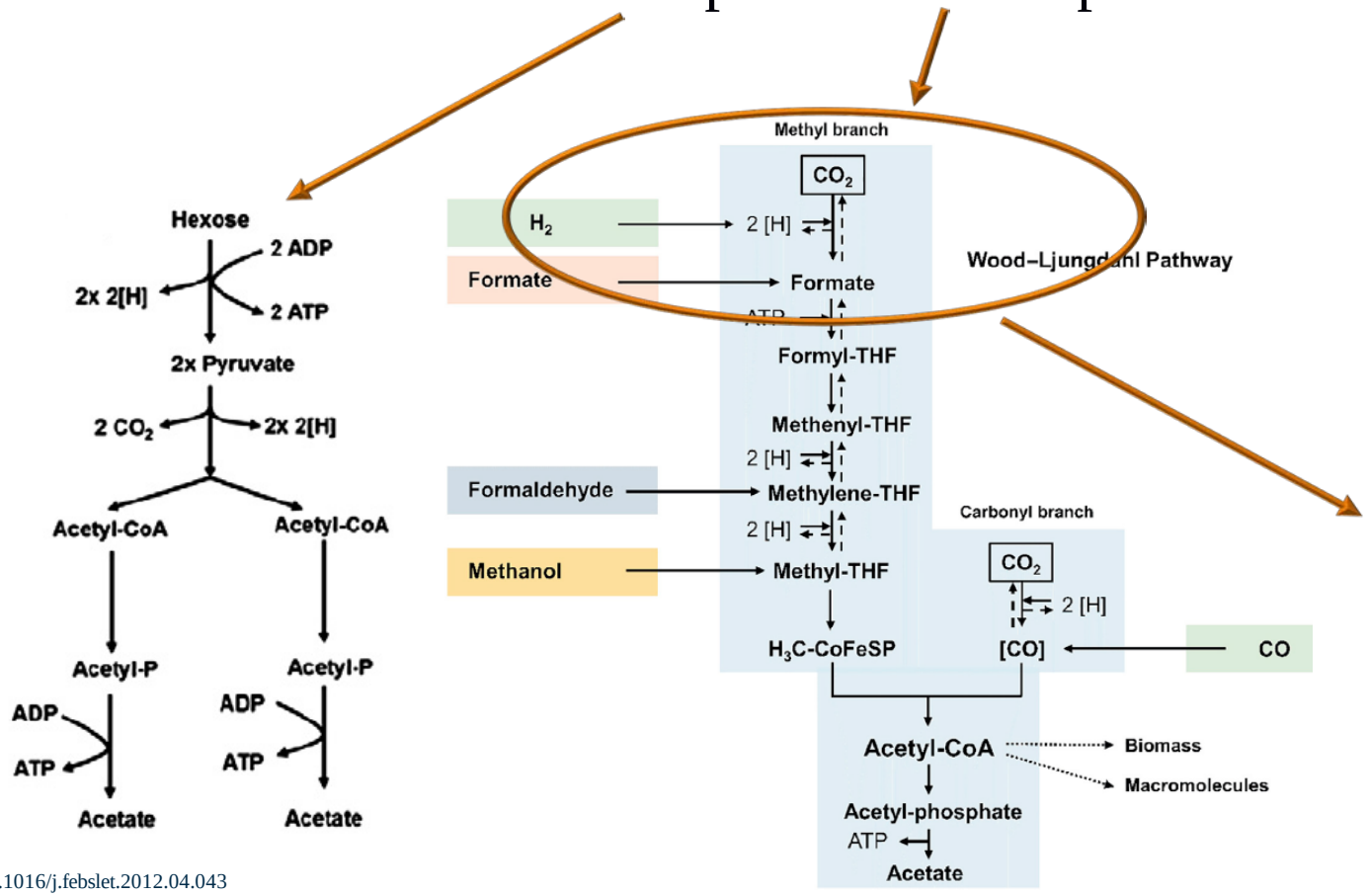
Explosion hazard when stored in large amounts

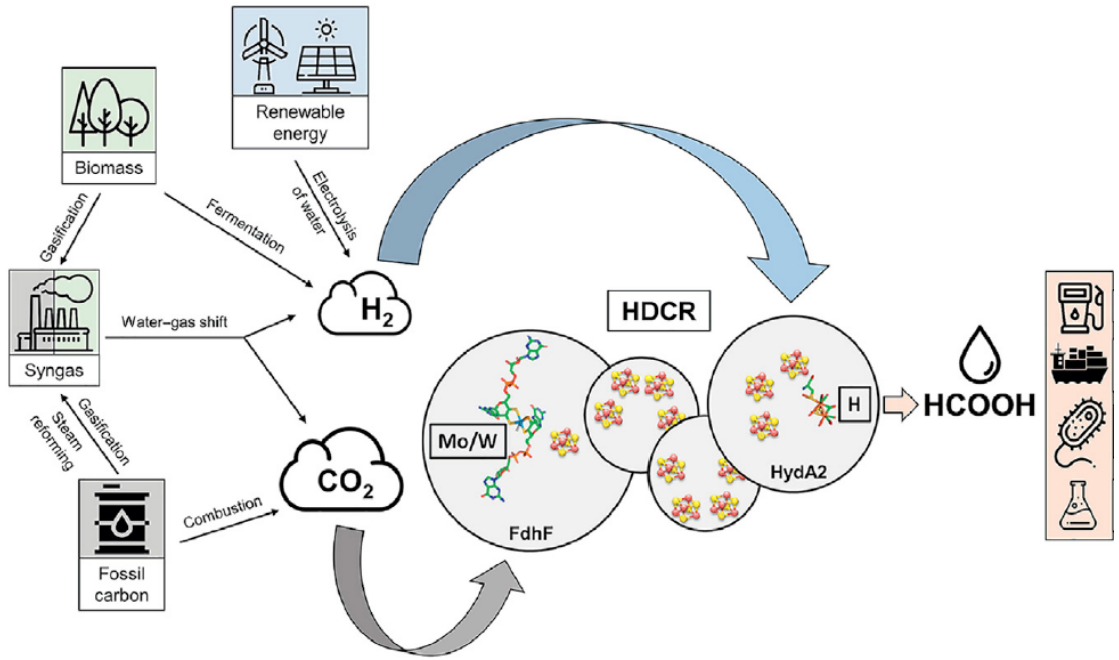
LOHC

Thermoanaerobacter kivui

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





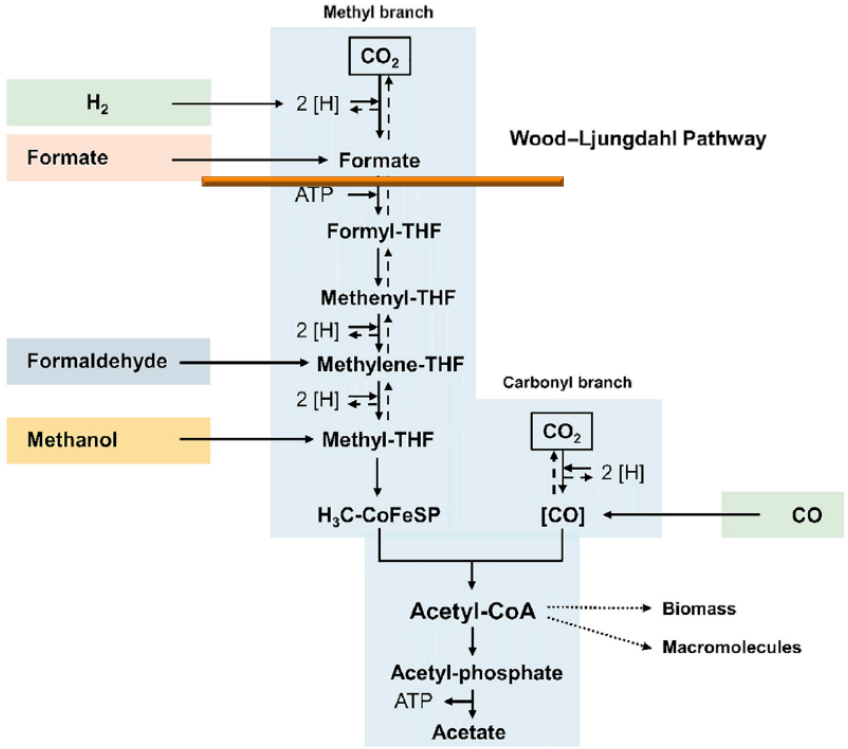


10.1016/j.febslet.2012.04.043

10.1016/j.tibtech.2019.05.008

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 CO_2 to formate.
- The addition of 300 mM of $KHCO_3$ switches the culture to the production of formate instead of acetate by inhibiting the further downstream processing of formate.



10.1186/s13068-020-1670-x

Goal of this study

Prove that the addition of 300 mM of $KHCO_3$ leads to the production of formate not only in the case of resting cells but also of replicating cells.

10.1186/s13068-020-1670-x

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

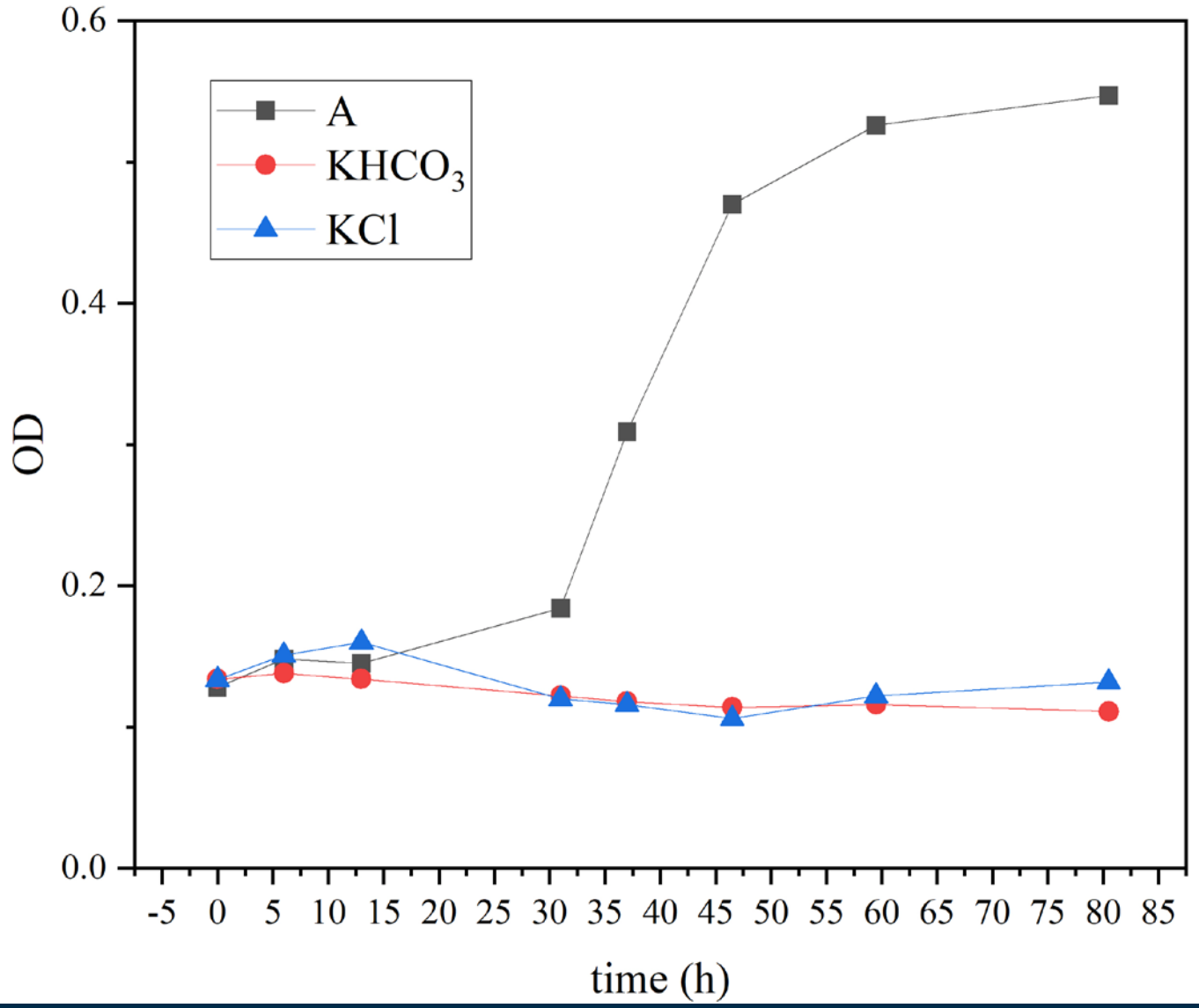
Conditions tested

Autotrophic (H_2:CO_2 = 80:20)

Autotrophic + 300m M $KHCO_3$

Autotrophic + 300m M KCl

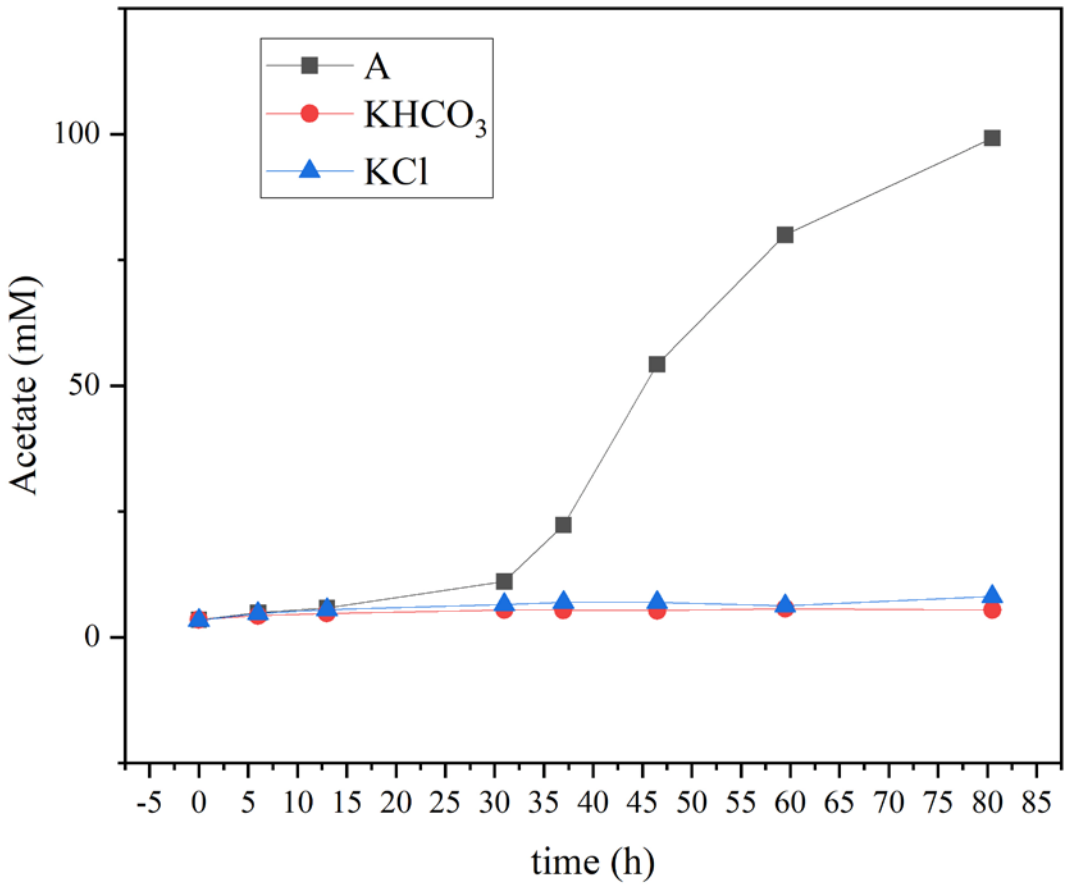
Effect of bicarbonate on the Optical Density

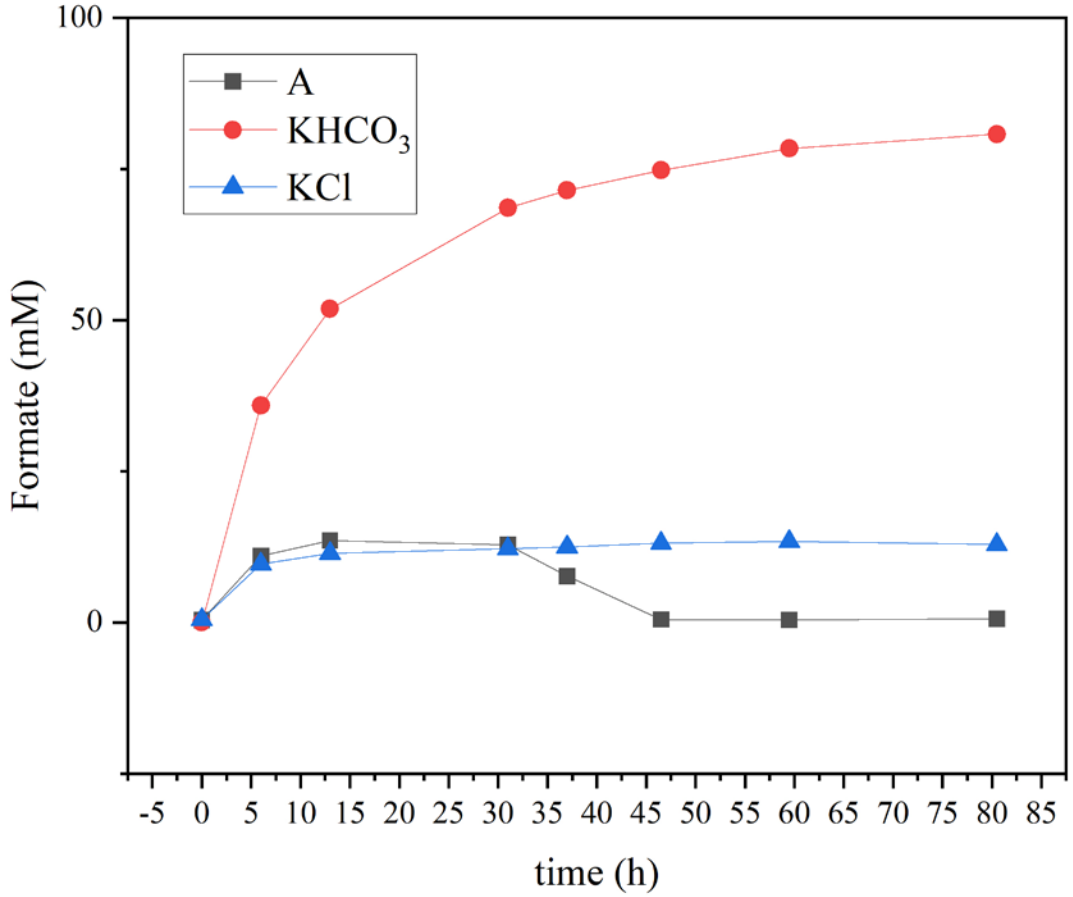


Adding $KHCO_3$ or KCl the optical density did non increase

Growth arrest

Effect of bicarbonate on acetate and formate production





Conclusions

T. kivui is able to convert H_2+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 CO_2 and H_2

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1859

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Thanks for the attention!

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Any questions?

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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 CO_2**
  - Not yet produced in large scale
  - require only a two-electron reduction of CO_2

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