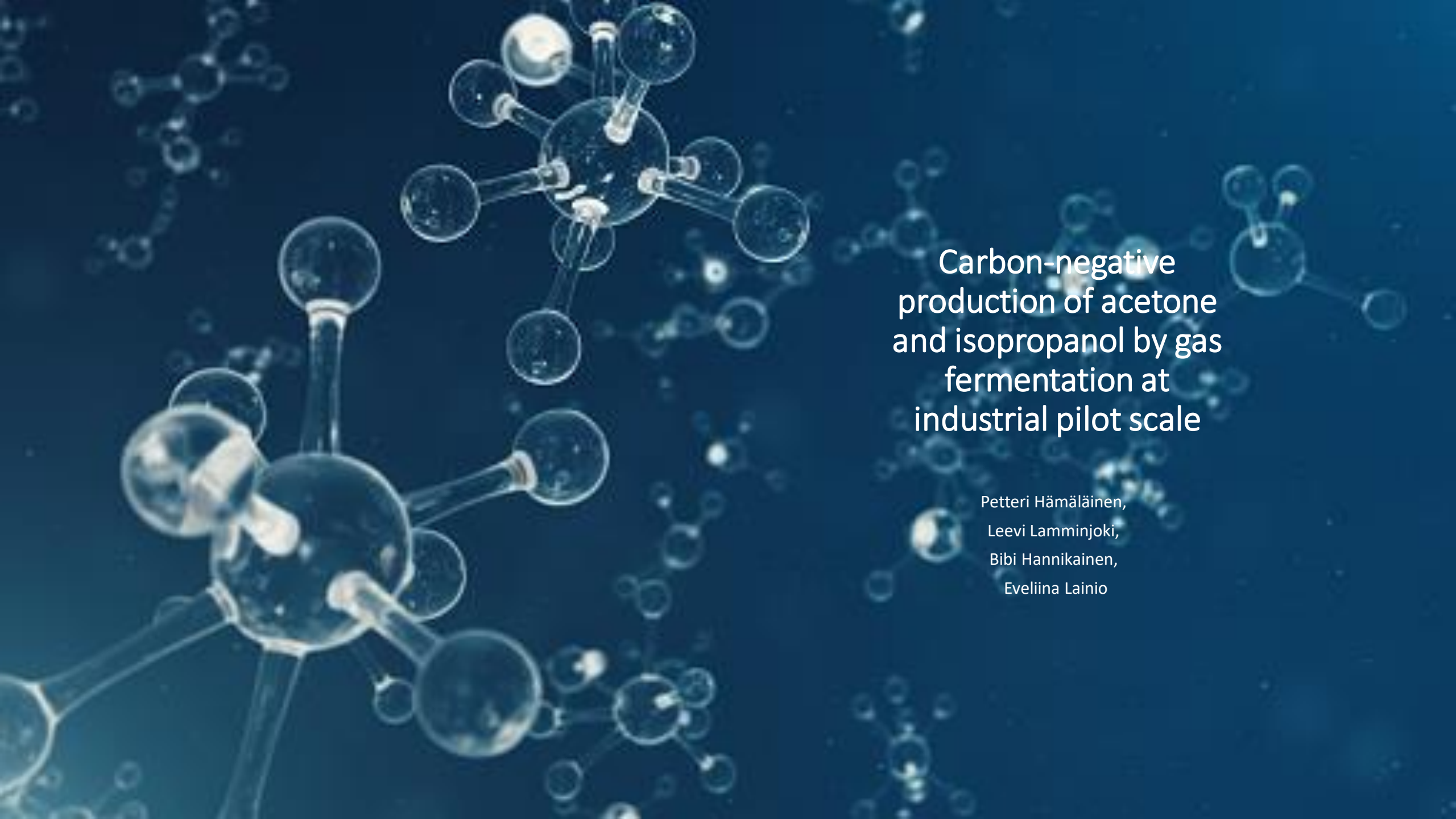

Carbon-negative production of acetone and isopropanol by gas fermentation at industrial pilot scale

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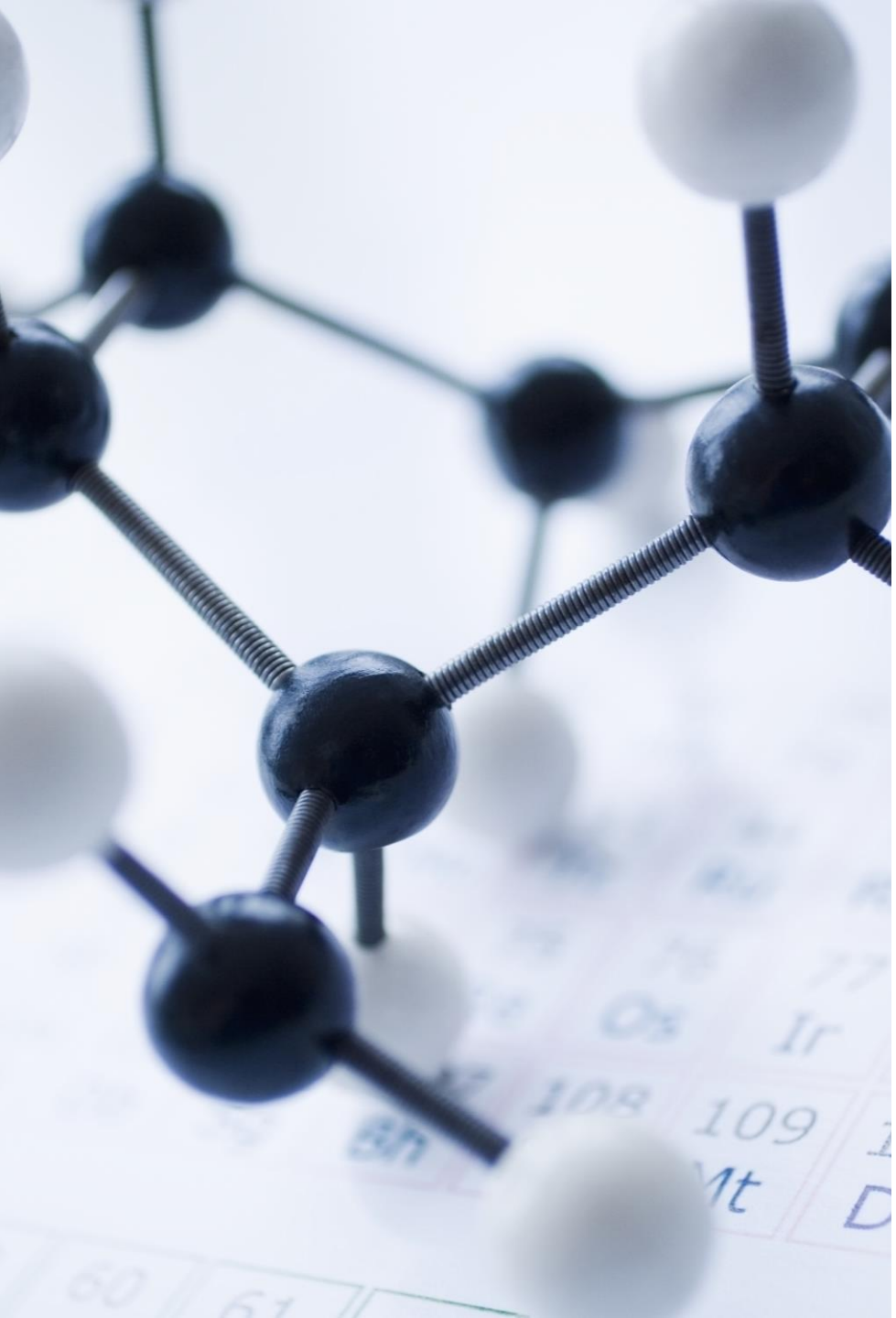


Introduction and aim

- Acetone and isopropanol (IPA) are commonly derived from fossil resources, such as oil, natural gas and coal
- The study discusses the development of a carbon-negative fermentation route to produce acetone and isopropanol from industrial emissions and syngas
- Development of a carbon-negative fermentation route to produce acetone and isopropanol from abundant, low-cost waste gas feedstocks
- The study aims to use gas fermentation as a way to produce acetone and isopropanol
- Utilizing autotrophic organisms, such as anaerobic acetogens to build products from carbon oxides
- The group chose to use an engineered strain of *Clostridium autoethanogenum*, an anaerobic bacterium that can naturally produce ethanol through gas fermentation in an industrial scale
- By capturing these gases before they enter the atmosphere, the approach could provide a way to produce acetone and isopropanol in a carbon-negative circular economy approach

Methods

- Anaerobic bacterium *Clostridium autoethanogenum*
  - Already used to produce ethanol through gas fermentation
- Make it a producer of acetone or IPA via enzyme engineering
- Started with pathway optimization to identify optimal sets of heterologous pathway enzymes
  - High-throughput strain engineering
  - Genome mining
- Optimization of strains for enhanced flux
- Process optimization and scale-up

Approaches

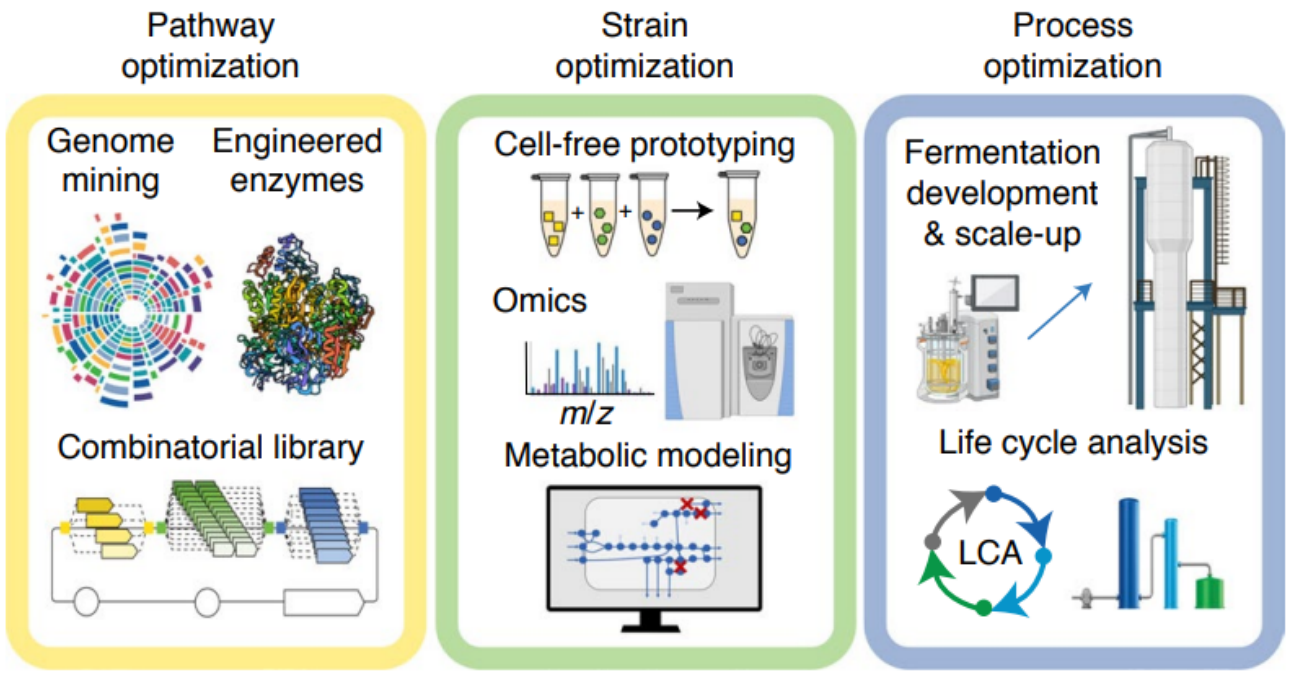
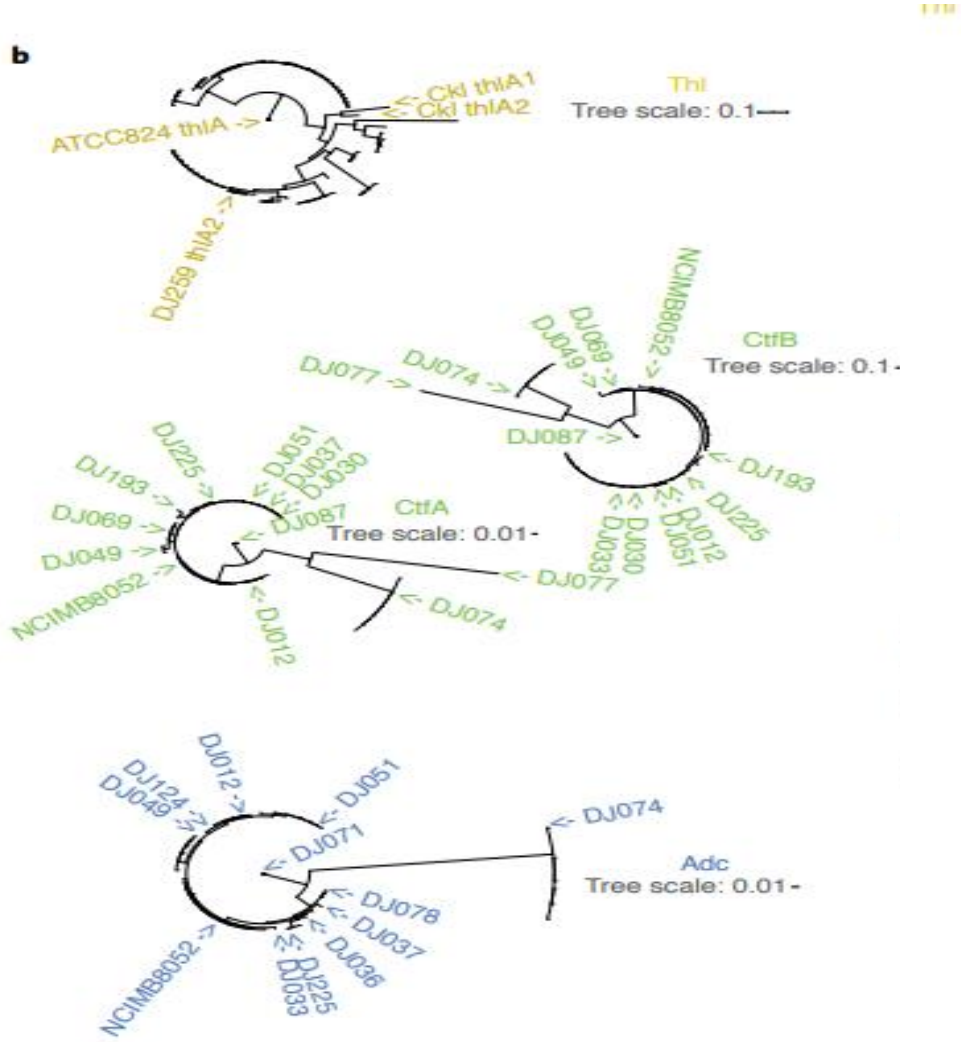


Fig. 1 | Overview of our three-pronged approach for pathway, strain and process optimization. Overview of applied tools and strategies to advance acetone and IPA production from waste gases from a proof of concept to industrial level.

- To achieve efficient acetone and IPA production, three-pronged approach was used
- High-throughput strain engineering workflows, omics analysis, cell-free systems, kinetic modeling, fermentation scale-up and life-cycle analysis (LCA) were integrated
- First, optimal sets of heterologous pathway enzymes to carry out the desired molecular transformations were identified
- Then, strains for enhanced flux to product were optimized
- Finally, process optimization, scale-up and LCA were carried out

Pathway optimization

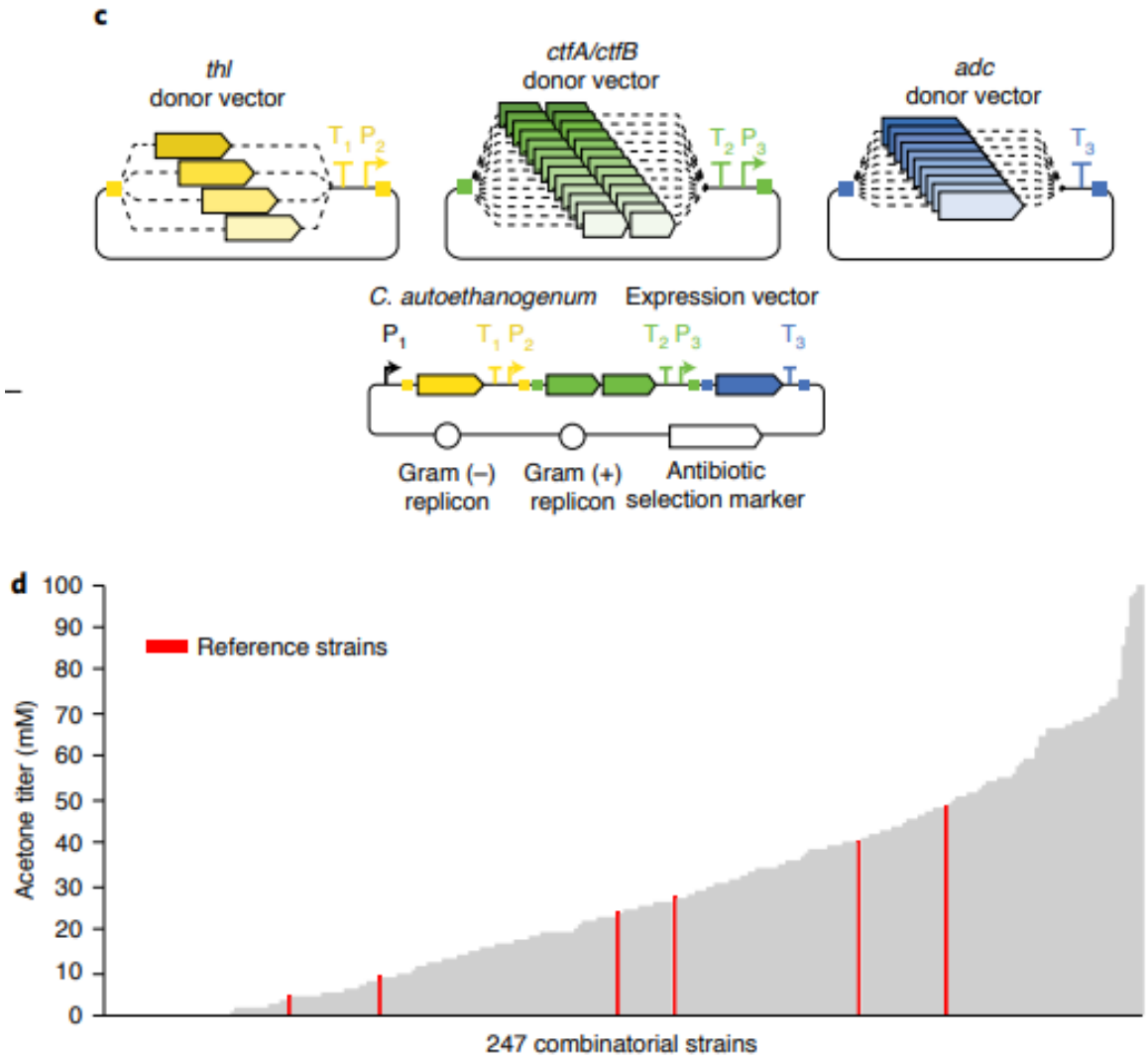


- Aim: identify pathway enzymes and pick the best designs

- sAdh knockout strain (Δ0553) was generated

- After transformation into Δ0553, a total of 247 strains harboring distinct acetone biosynthesis pathway designs were obtained & screened

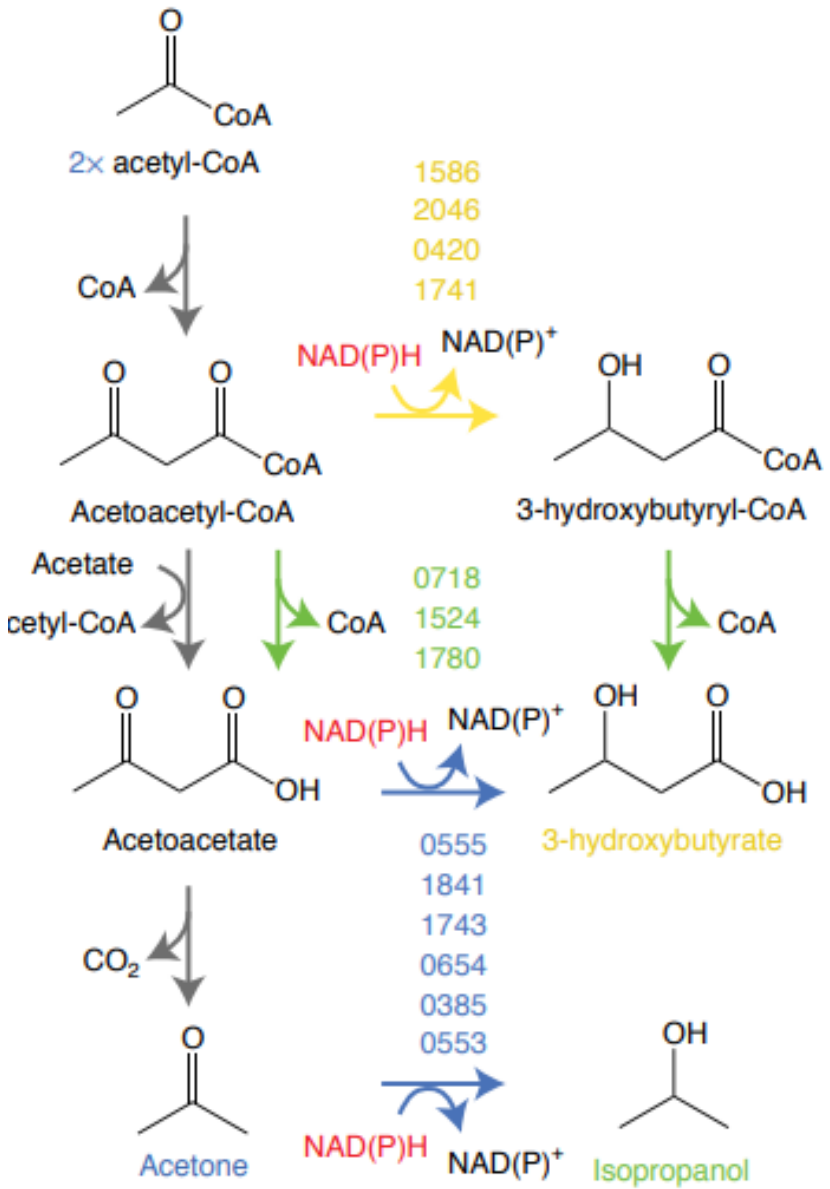
b) Sequence mining of the DJ collection led to identification of a large diversity of acetone biosynthesis enzyme sequences



C) Combinatorial library assembly strategy to refactor selected acetone biosynthesis genes.

D) Acetone end-point titers observed in screening 247 strains with unique acetone pathway designs; designs that use genes from wild-type reference strains are highlighted in red

- TOP5 designs were moved forward to continuous fermentation testing and genome integration



Candidate enzymes

Strain optimization

- Production strains were optimized to increase titers
- Genome-scale model and evolutionary algorithm were used to predict which knock-outs (KOs) increase flux to acetone and eliminate unwanted byproducts
- To prototype knock-out candidate targets, iPROBE approach was adapted
  - CFE was used to create an array of cell extracts individually enriched with acetone biosynthesis enzymes and effector candidate enzymes
  - Next, in vitro acetone production from glucose using native E. coli catabolism coupled with two different combinations of ThlA, CtfAB and Adc was established

-> the KO candidate enzymes were added to cell-free acetone biosynthesis reactions

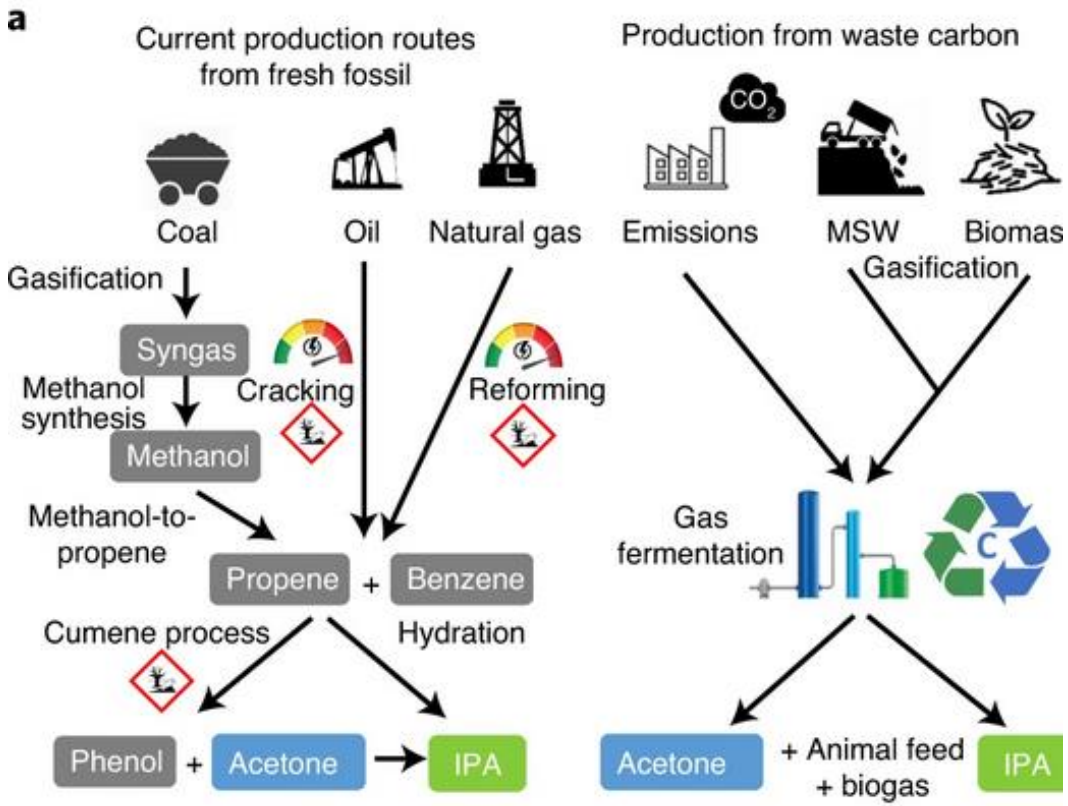
- The 2,3-BDO pathway was targeted, and the optimized acetone pathway operons were integrated
- To optimize the flux to acetone, select strains were analyzed by omics measurements and kinetic modeling for bottlenecks in the pathways and tuning of enzyme levels tested in the iPROBE system

Process optimization

- Continuous fermentation process for acetone in a benchtop continuous stirred-tank reactor (CSTR)
- Whole genome sequencing
- LCA

Continuous IPA production in lab-scale CSTRs

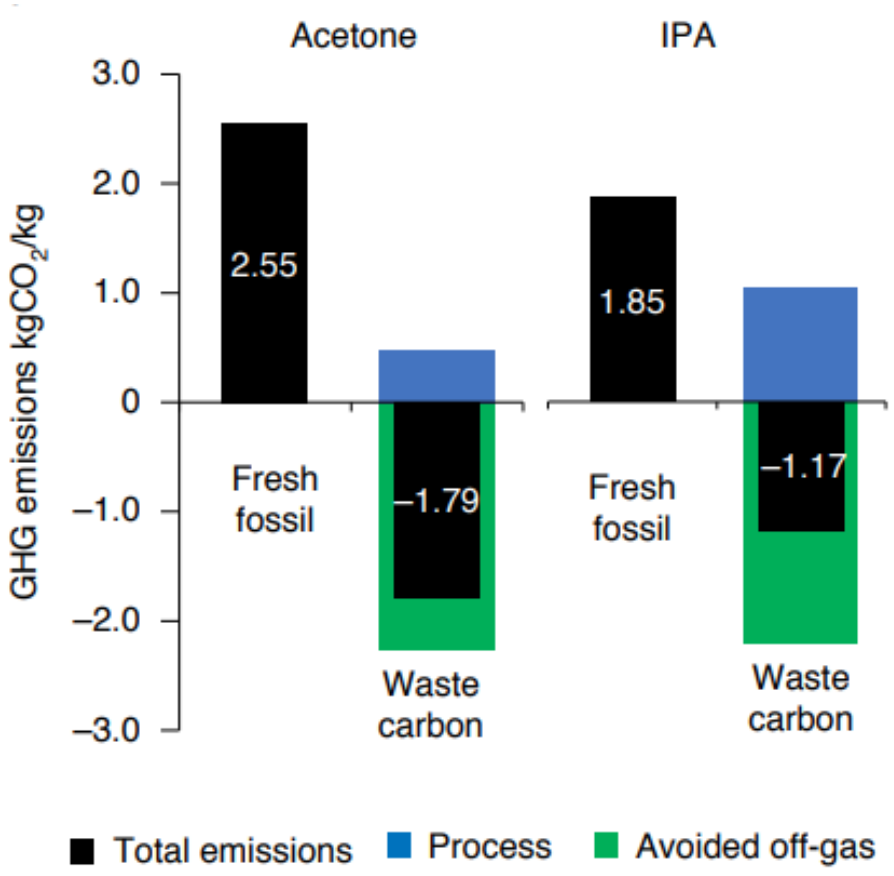
Achievements



- The engineered bioprocess was able to produce commercially viable outputs --> 3 g/L/h
- Engineered *C. Autoethanogenum strains* expressed high selectivity

  --> up to 90%
- Proved that acetogens can be engineered to produce complex molecules at high selectivity

Achievements



- Generally very emission heavy process was made to bind carbon instead of releasing it
- CO_2 emission for acetone and IPA using gas fermentation:

What did not work?

- A gene coding for a harmful side product could not be identified and therefore knocked out

- The possible gene KO´s were so numerous there wasn’t enough time/resources to try them all

Importance and path forward

The research showed that this kind of process is possible

The largest number of combined genome modifications in an autotroph or *Clostridium* strain

Shows path forward for next research

Conclusions

- Rewiring *C. autoethanogenum* from an ethanol producer to a producer of acetone or IPA
- Final production strain comprised multiple genome modifications, including pathway integration, four gene KOs and overexpression of two genes
- Carbon-negative production of acetone (-1,78 $kgCO_2e/kg$) and IPA (-1,17 kgCO2e/kg) with production rate of 3 g/L/h and 90% selectivity.
- Traditional acetone (2,55 kgCO2e/kg) and IPA (1,85 kgCO2e/kg) production emissions

Additional references

- Clifford, C. (2018). This start-up turns pollution from factories into fuel that powers cars – and one day planes, *Master class*, online article [referenced 26.4.22], available from: https://www.cnbc.com/2018/07/27/lanzatech-turns-carbon-waste-into-ethanol-to-one-day-power-planes-cars.html.
- Liew, F., Henstra, A.M., Köpke, M., Winzer, K., Simpson, S.D., Minton, N.P. (2017). Metabolic engineering of Clostridium autoethanogenum for selective alcohol production, *Metabolic Engineering*, *1*(40), 104-114, ISSN 1096-7176, https://doi.org/10.1016/j.ymben.2017.01.007.