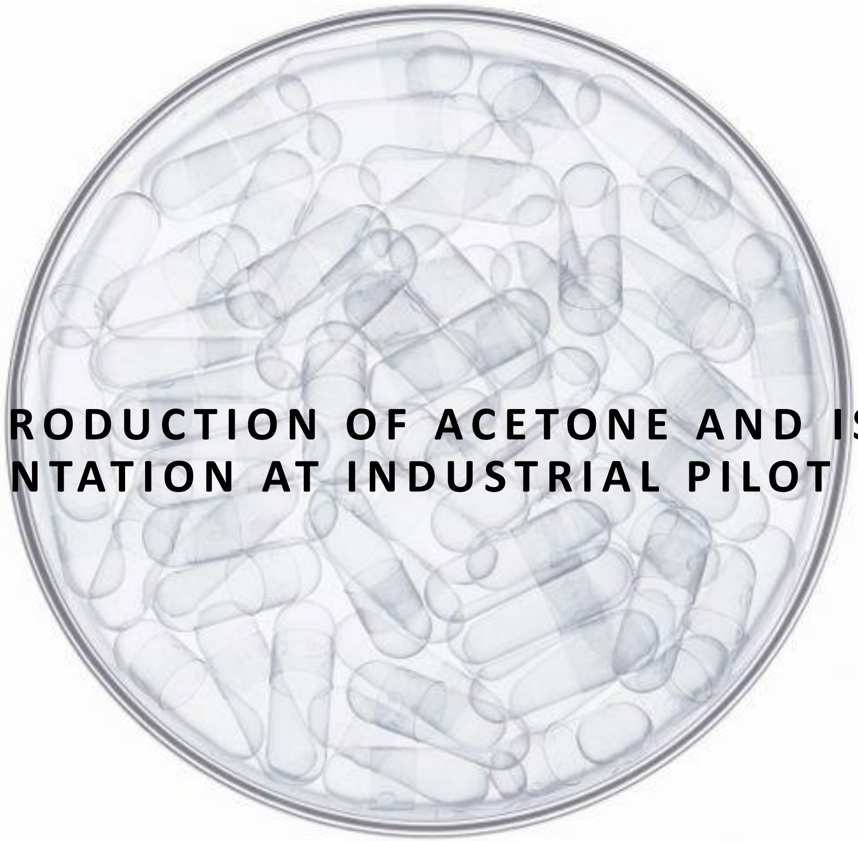

# CARBON-NEGATIVE PRODUCTION OF ACETONE AND ISOPROPANOL BY GAS FERMENTATION AT INDUSTRIAL PILOT SCALE

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# STRUCTURE

INTRODUCTION AND AIM

METHODS

ACHIEVEMENTS

CHALLENGES

WHY IS THIS IMPORTANT?

WHATS NEXT?

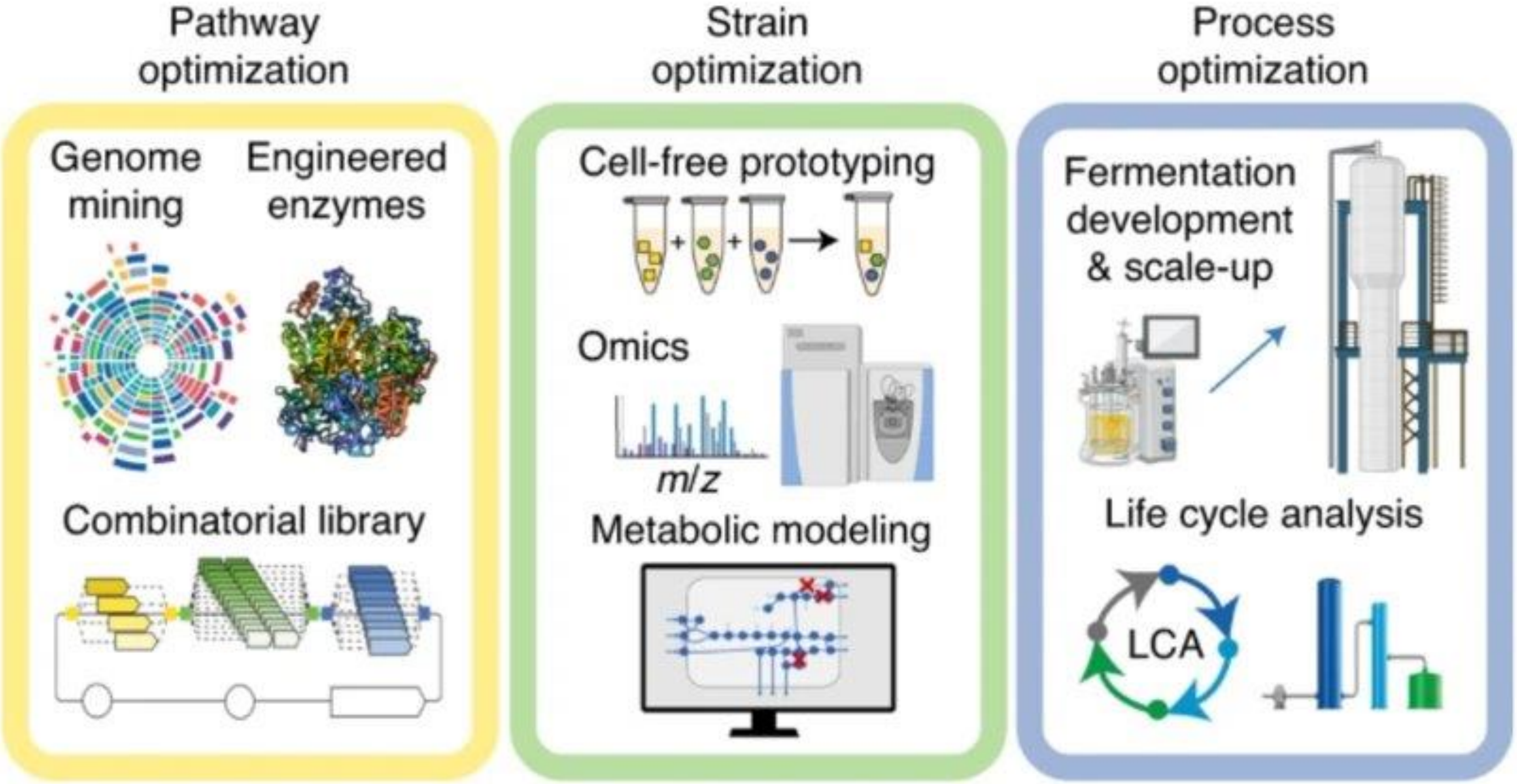
# INTRODUCTION

- Demand for sustainable production of chemicals due to climate change
- Acetone and isopropanol (IPA) are two industrially important chemicals that are traditionally produced from fossil fuels
- Biomanufacturing of these chemicals from waste gas by fermentation would enable sustainable and carbon negative production due to CO2 fixation
- However, no native autotroph produces acetone and IPA
- Autotrophic anaerobic acetogens are an attractive alternative as hosts for engineering efforts, due to them not needing light and using the reductive acetyl-CoA pathway

# AIM

- The aim of the research is to engineer the acetogen *Clostridium autoethanogenum* to be capable of producing acetone and isopropanol through gas fermentation
- This would enable the usage of low-cost waste gas streams containing C1 compounds, such as industrial emissions and syngas, thus paving the way for green chemistry alternatives for production of industrial chemicals
- If successful, the engineered strain would be the first viable alternative for industry scale production of the chemicals from "above-ground" carbon resources
- The approach used in the research could also be adapted for production of other important chemicals
- The strategy used comprises of three distinct steps

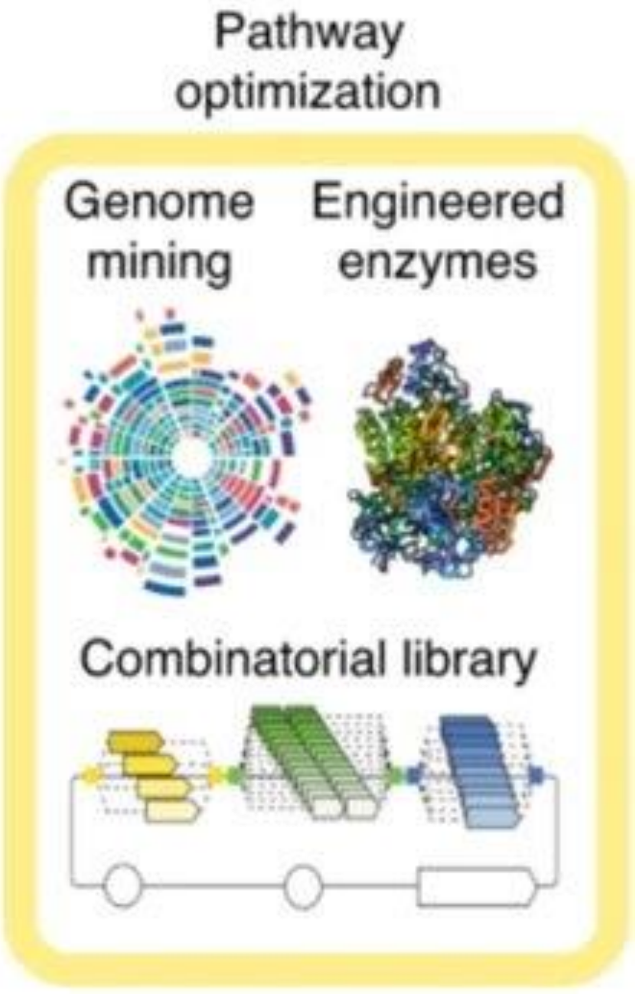
# METHODS AND APPROACHES



# PATHWAY OPTIMIZATION

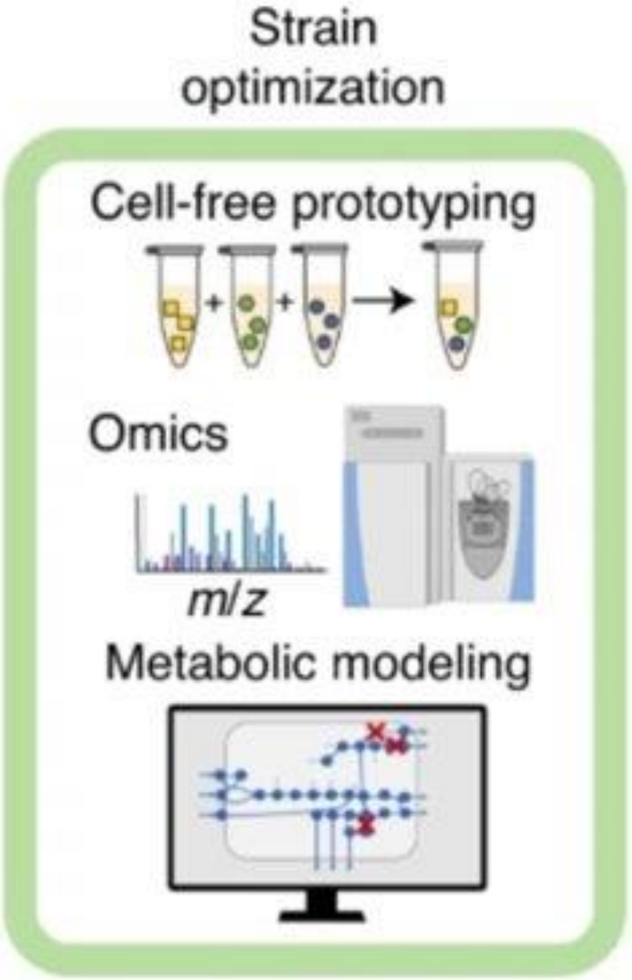
- First they went through 272 optimized industrial ABE (aceton-butanol - ethanol ) *Clostridium* strains and found 41 new acetone bio synthesis enzymes of which they used 30 to create 247 new combinatorial *C. autoethanogenum* strai ns.

→five best were selected for the next step



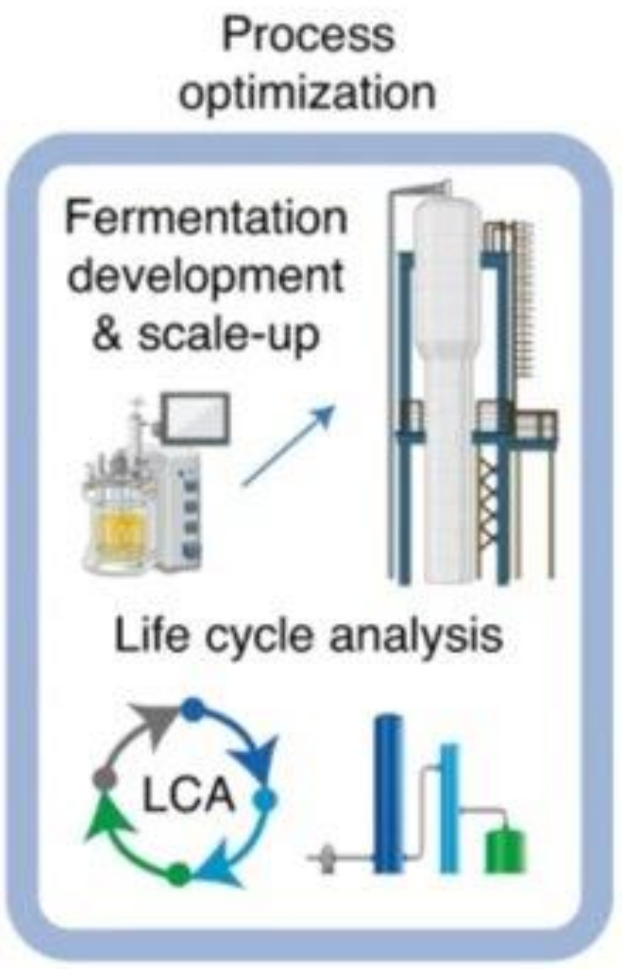
# STRAIN OPTIMIZATION

•Genome-scale model and evolutionary algorithm were used to find gene knockout (KO) candidates to increase flux to acetone and eliminate unwanted by-products. → 21 candidate gene KOs were found.
•Cell-free gene expression was used to evaluate their function: acetone biosynthesis enzymes and gene KO candidate enzymes in different combinations.
•Omics measurements and kinetic modelling was used to tune the expression levels and find bottlenecks of the production. Especially CtfAB expression needed to be increased



# PROCESS OPTIMIZATION

•Finally, they established a continuous fermentation process for acetone and scaled it up to a 120-litre pilot plant.
•LCA (life-cycle-analysis) was used to compare GHG (greenhouse gas) emissions of their production to fossil-based production.

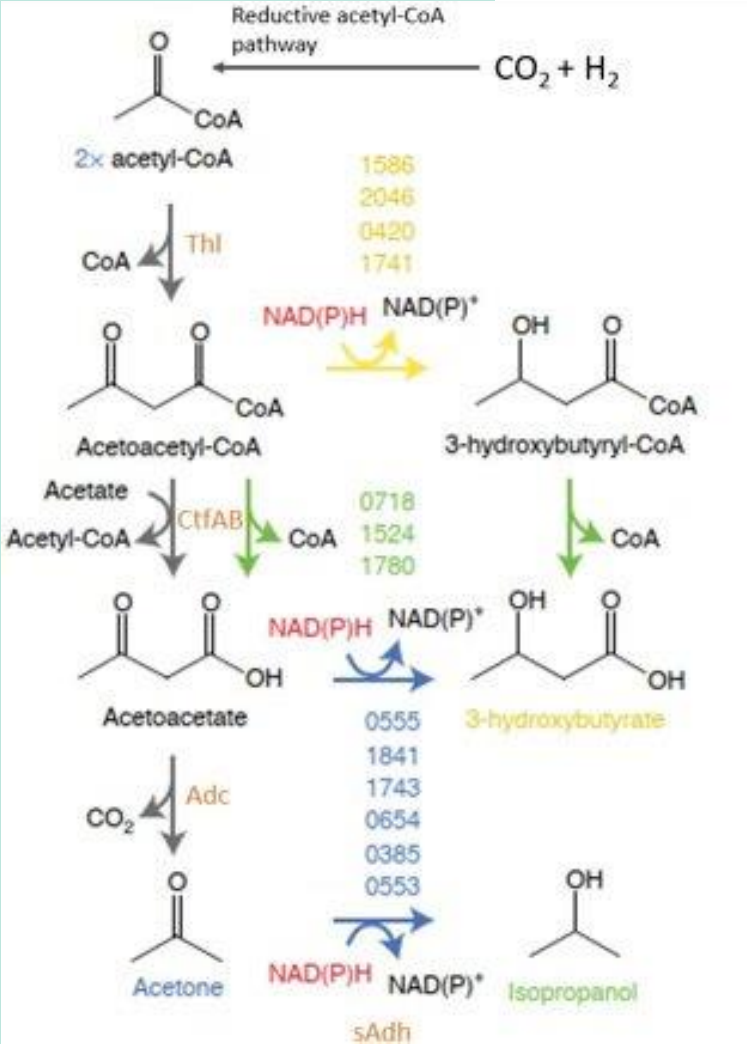


# WHAT WAS ACHIEVED

1. Identified the necessary enzyme combinations and the most productive strains were chosen to produce the products

2. Successful pathway knockout and the identification of bottlenecks

3. The aim was met successfully, Greener way to produce IPA and acetone




•Enzymes identified for the acetone pathway: Thiolase (ThlA), CoA transferase (CtfAB) and acetoacetate decarboxylase (Adc).

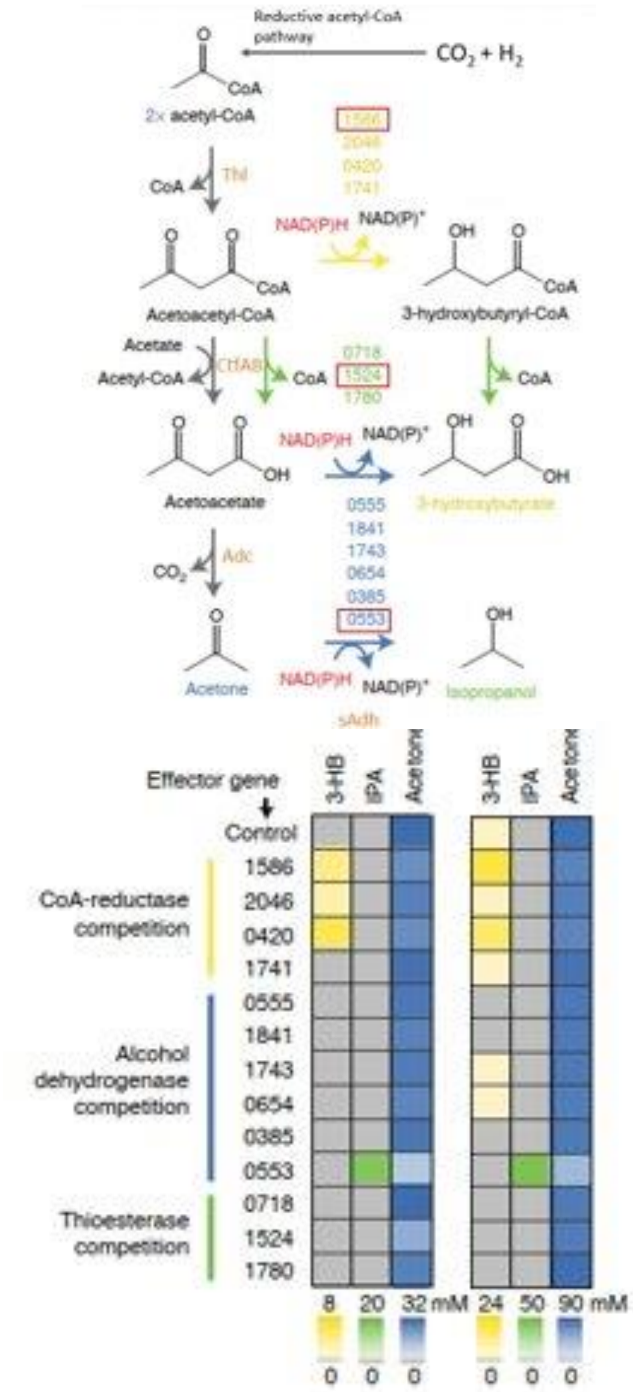
•Primary-secondary alcohol dehydrogenase (sAdh) eliminated for acetone producing strains, but kept when IPA is the end-product

•Most significant by-products identified were 3-hydroxybutyrate (3-HB) and 2,3-butanediol (2,3-BDO)

# WHICH KOS ARE THE MOST BENEFICIAL?

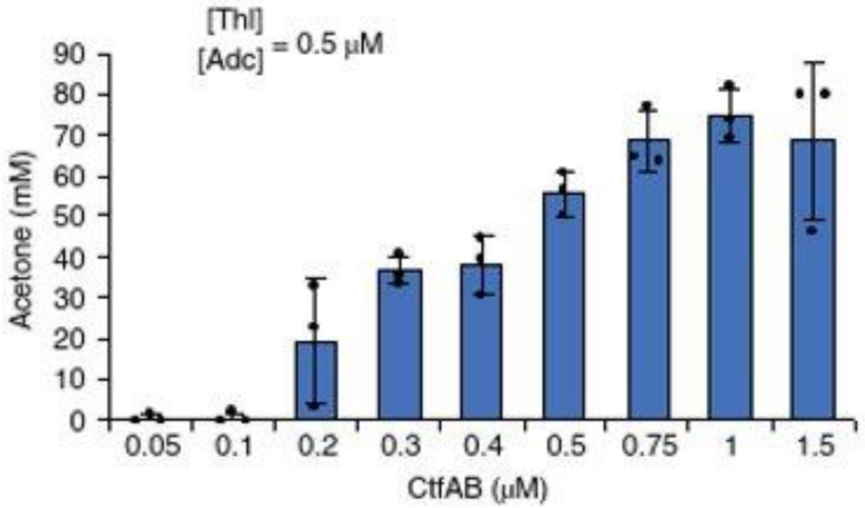
•ENZYMES MOST HEAVILY DECREASING ACETONE TITERS WERE IDENTIFIED TO BE 0553 (SADH), 1524 AND 1586

•ENZYME 2932 IN THE 2,3-BDO PATHWAY WERE ALSO DELETED.

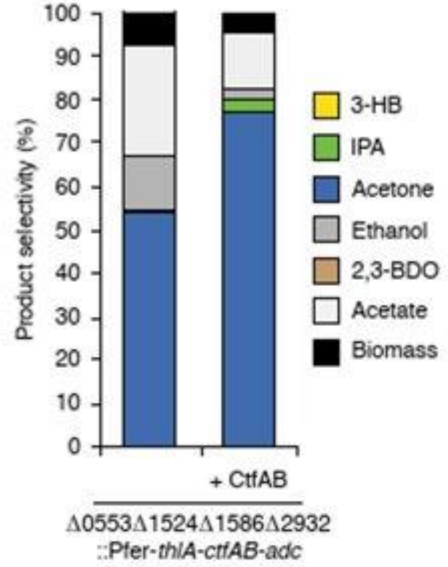


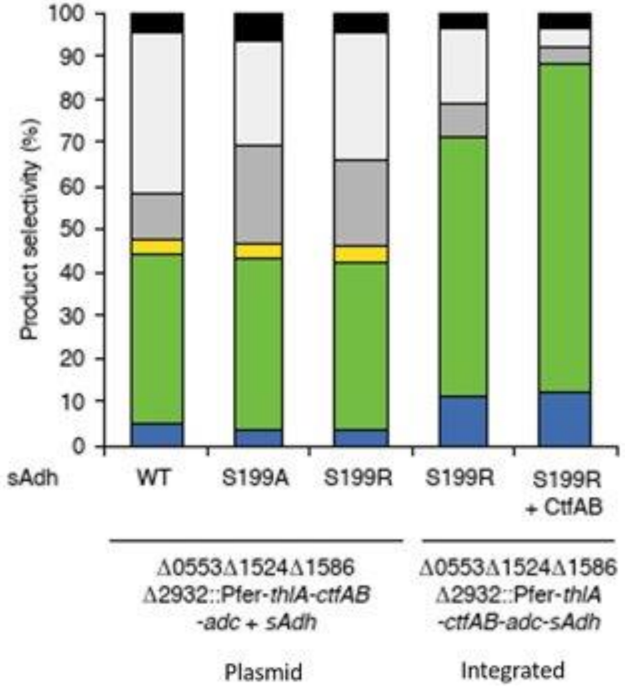
•A SECOND COPY OF CTFAB WAS ALSO INTRODUCED AS IT WAS IDENTIFIED AS A BOTTLENECK IN THE PATHWAY

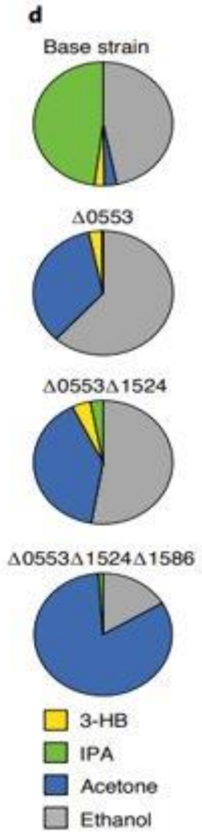
•PROTEOMIC MEASUREMENTS CONFIRMED THAT THE ACETONE PATHWAY ENZYMES WERE AMONG THE MOST ABUNDANT PROTEINS

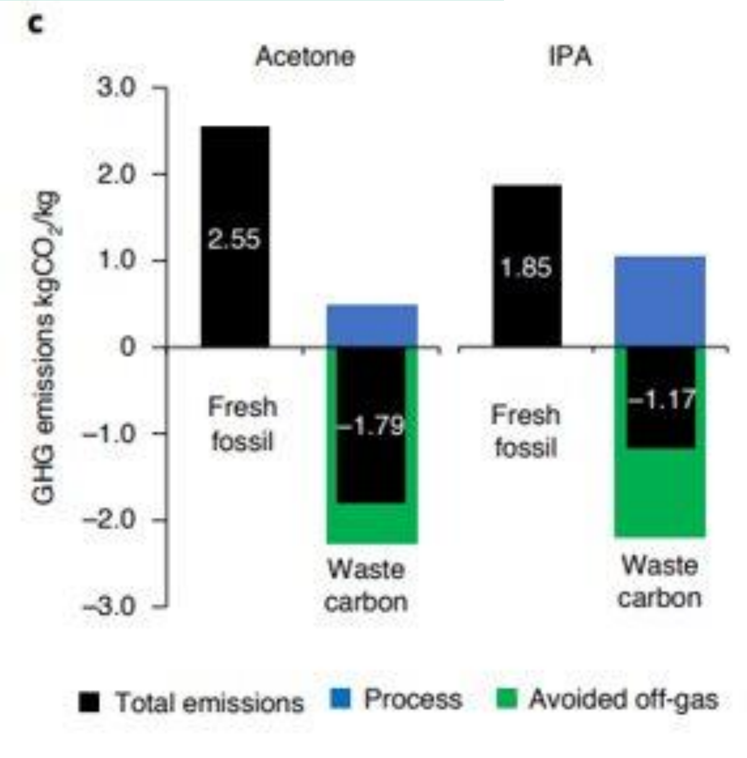


# PRODUCT SELECTIVITY FOR FINAL STRAINS AND AFTER DIFFERENT KNOCKOUTS






Gas fermentation fixes CO2 and has a negative carbon footprint, whereas traditional production methods of acetone and IPA result in net GHG emissions

# CHALLENGES

- The study was successful, however some challenges occurred
- Scaling up to industrial level:
- increasing heterogeneity of the bioreactor environment with scale
- Cost: low cost feedstock but the cost of the process could vary
- Process Optimization
- By-product elimination (already done in silico homology search)
- Product separation requirements
- Product purity
- Requires control of environmental conditions to prevent contamination

# WHY AND HOW IS THIS IMPORTANT

- Enables the production of industrially important products, IPA and acetone, from waste gas feedstocks
- Environmentally friendly
- A green way to produce IPA and acetone
- Previous production methods: high energy consumption, utilized fossil fuels and produced GHG
- Life cycle analysis: the carbon footprint of the products is negative
  → the process fixes carbon
- The productivity measures up to industrial processes

# WHATS NEXT?

- Scaling up the process for industrial purposes
- Process optimization
- Cost reduction through further research
- Developing process monitoring and control strategies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# THANK YOU!