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# Bioenergy Production Based on an Engineered Mixotrophic Consortium for Enhanced $CO_2$ Fixation


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# Combining the two biochemical $CO_2$ utilization routes: **Mixotrophy**

*Heterotrophy*

Atmospheric $CO_2$ + Light ► Sugars (biomass)

Sugars ► Chemicals and biofuels

**Relatively high productivity**

But

**Carbon loss during fermentation as $CO_2$ > 33%**

*Non-photosynthetic autotrophy (acetogen)*

Biogenic $CO_2$ + $H_2$ ► Acetate

(>70% energetic efficiency)

**$CO_2$ conversion at high efficiency**

But

**Relatively low productivity**

***Mixotrophy (Biological reactive carbon capture)***

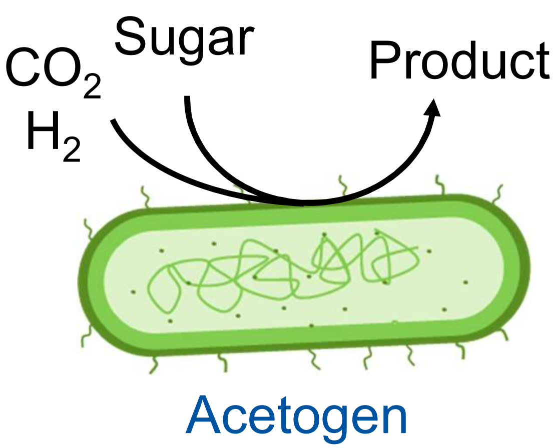
Sugars (biomass) + $CO_2$ + $H_2$ ► Chemicals and biofuels

**High productivity & carbon neutral/negative**

Goal: demonstrate the paradigm of $CO_2$ utilization as "stoichiometry extender"

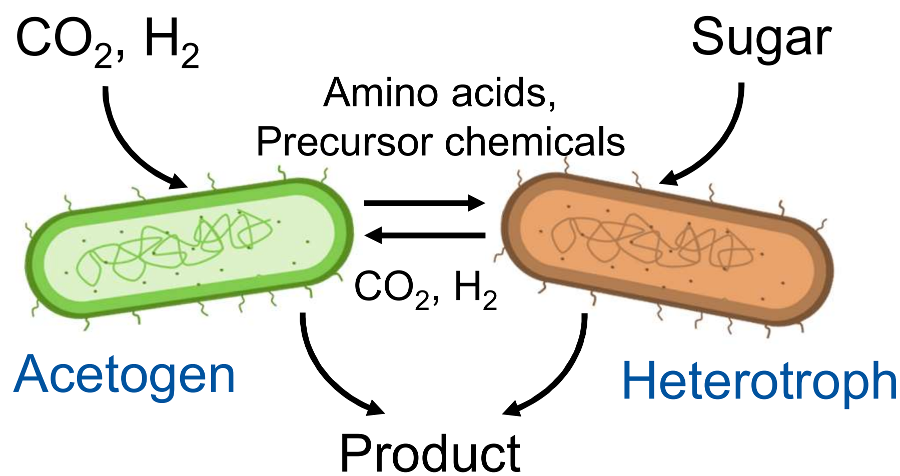
# Designing a mixotrophic biosystem using multiple species

## Mixotrophy by single species




Preference for sugars over $CO_2$
And
Extensive genetic engineering

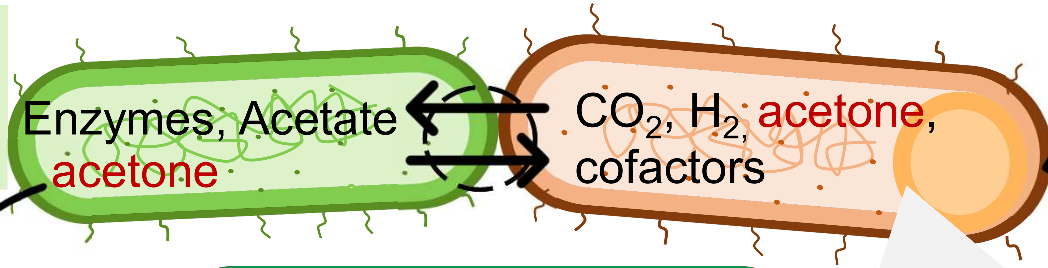
## Mixotrophy by multiple species




Modularity
And
Less genetic engineering (nature's way)

# Engineering a syntrophic consortium for mixotrophic isopropanol production

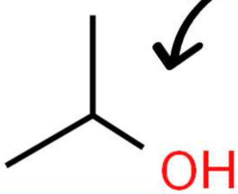
*C. ljungdahlii* (*Clj*)
***No growth on glucose***




Fermentable sugars

*C. acetobutylicum* (*Cac*)

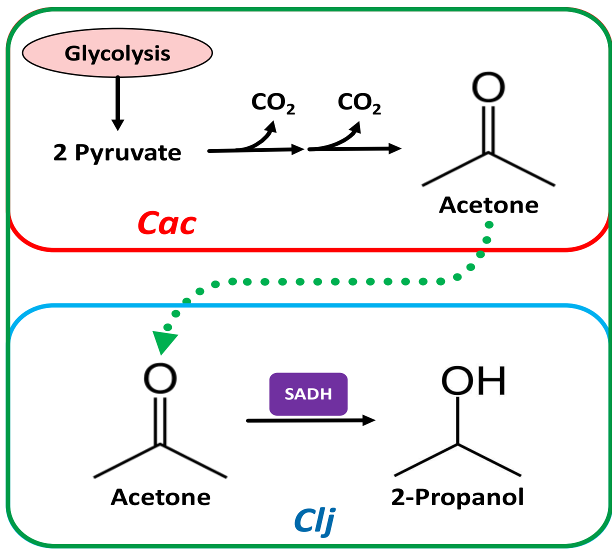
Cut off 4C metabolism

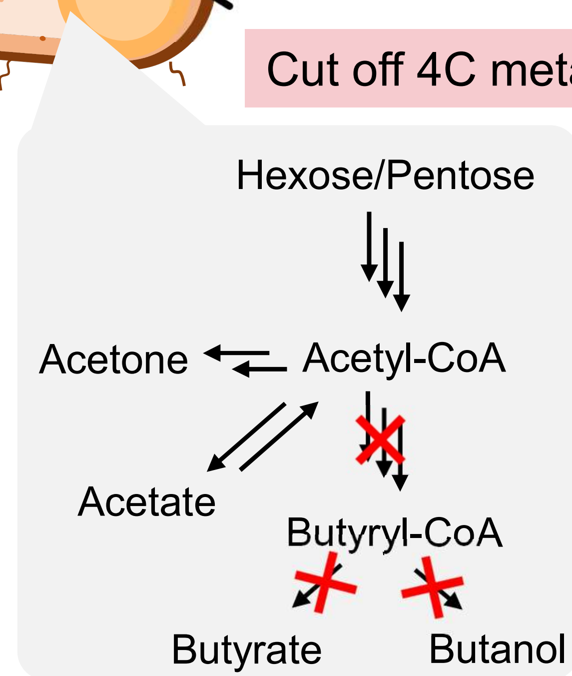



Isopropanol (IPA)

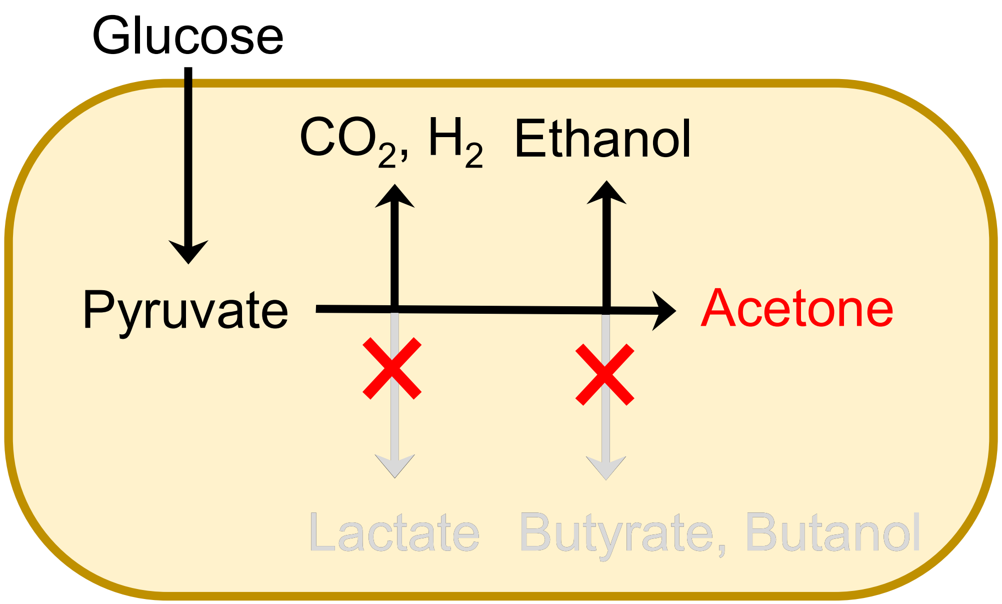
80% IPA & 20% EtOH

- $0.50-1.20/lb
- 3.0 billion $ (2022)
- CAGR of 6.9% to 2032



# Enhancing acetone/IPA selectivity through genome engineering




*C. acetobutylicum (Cac)*

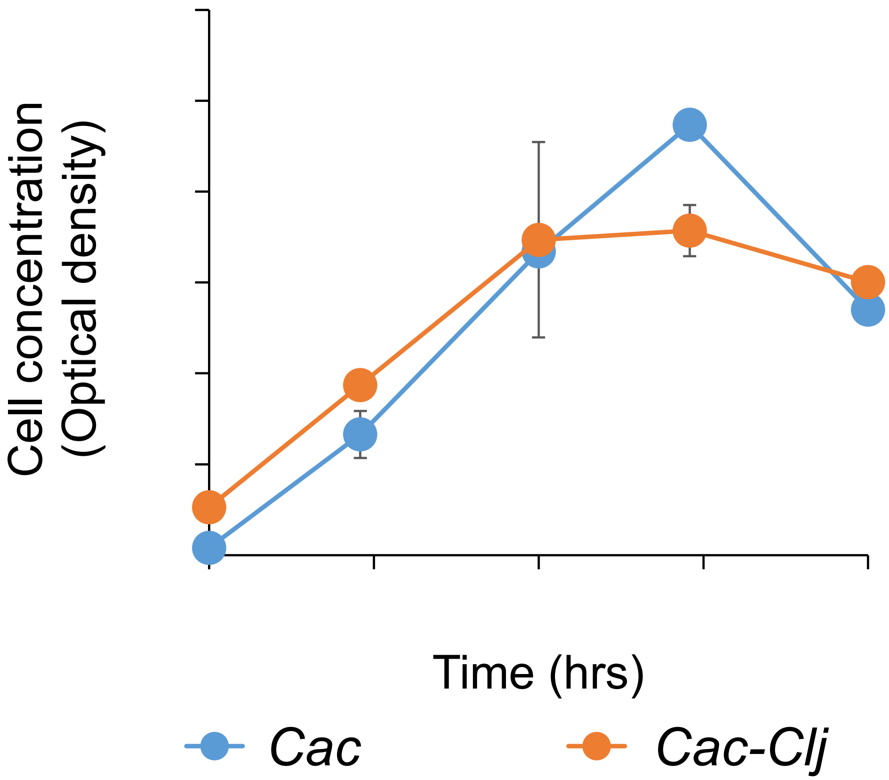
Cas9 genome editing of *Cac*

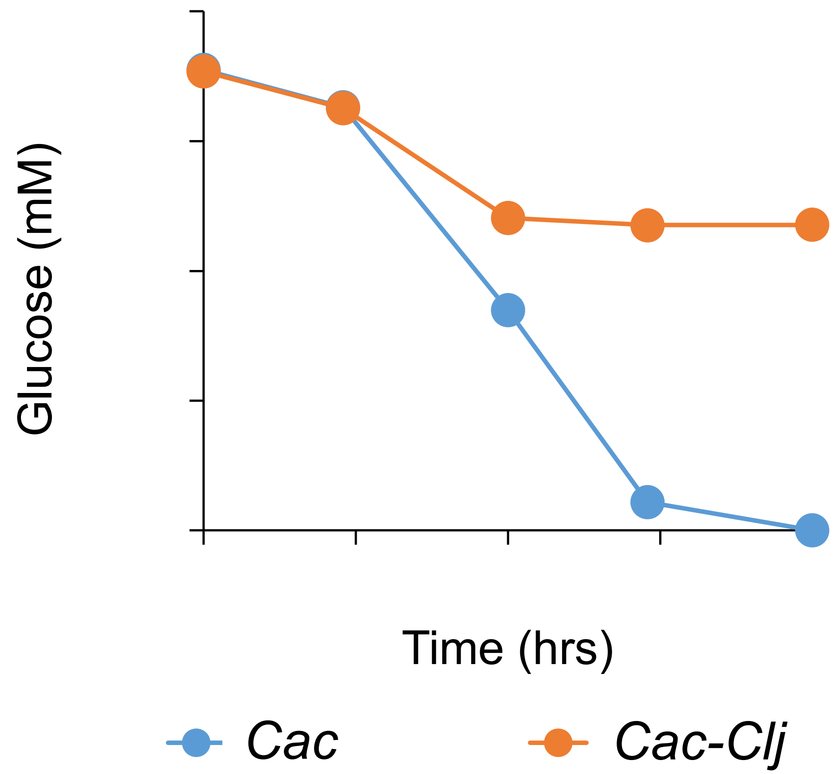
1. No butyrate, butanol formation, higher acetone selectivity

2. <10 mM lactate formation

3. Ethanol and $H_2$ are the two major electron sinks

# The engineered *Cac-Clj* showed fermentation cessation



# *Cac* cells became more oxidized when cultured with *Clj*

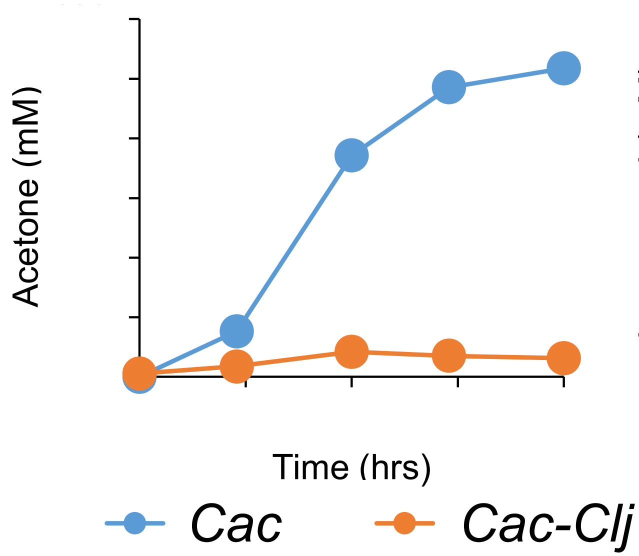
1 Glucose = 2 Acetyl-CoA + 2 NAD(P)H + 2 $CO_2$

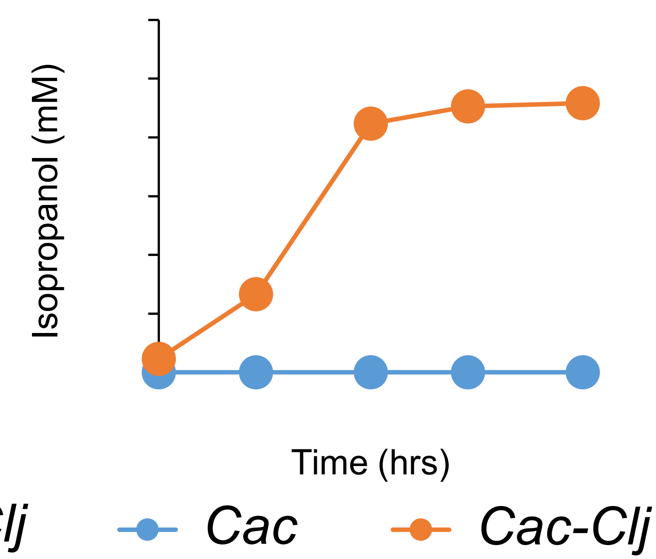
Oxidized product

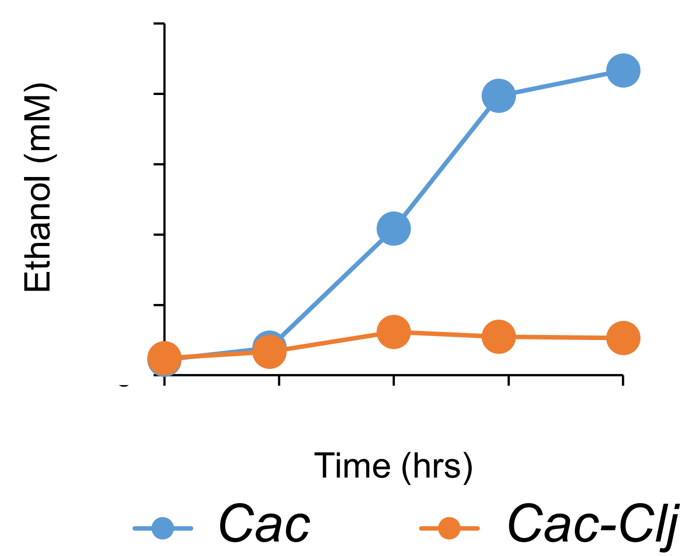
2 Acetyl-CoA = 1 Acetone + 1 $CO_2$

Reduced product

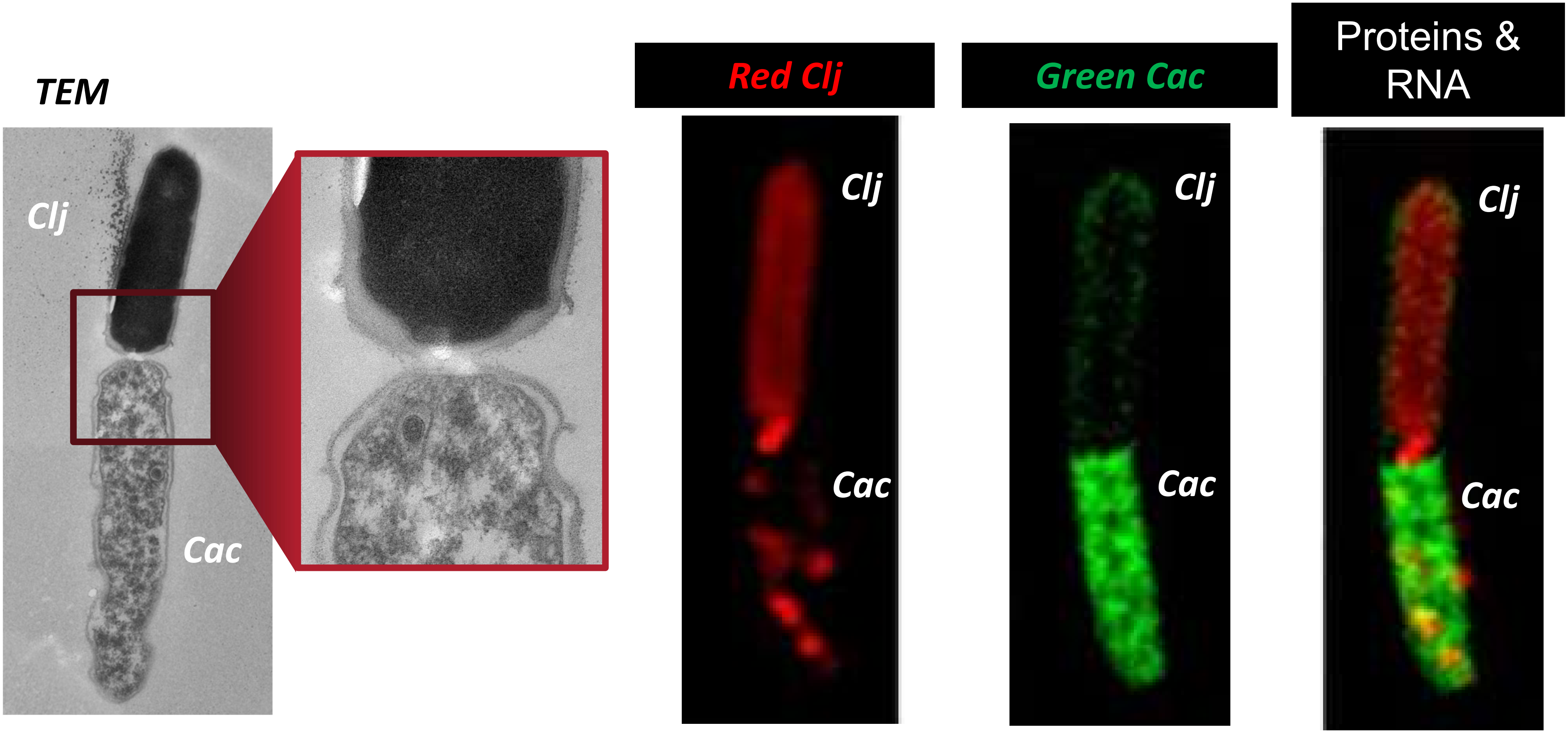
2 Acetyl-CoA + 2 NAD(P)H = 2 Ethanol



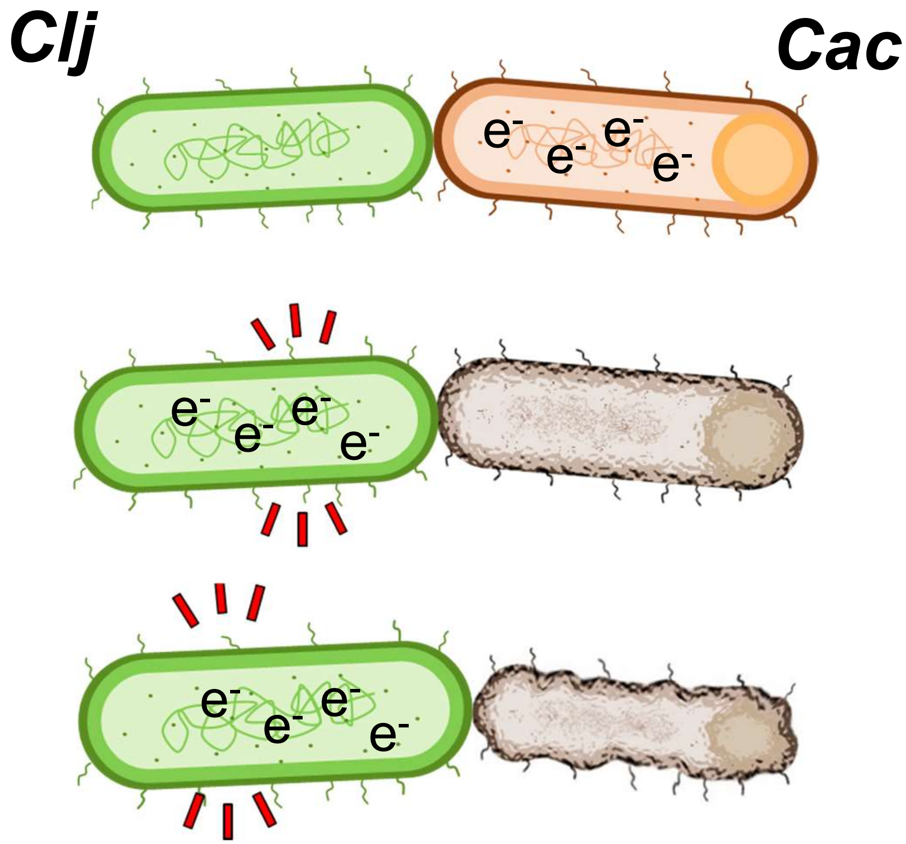



# Physical 'touch' between *Cac* and *Clj*

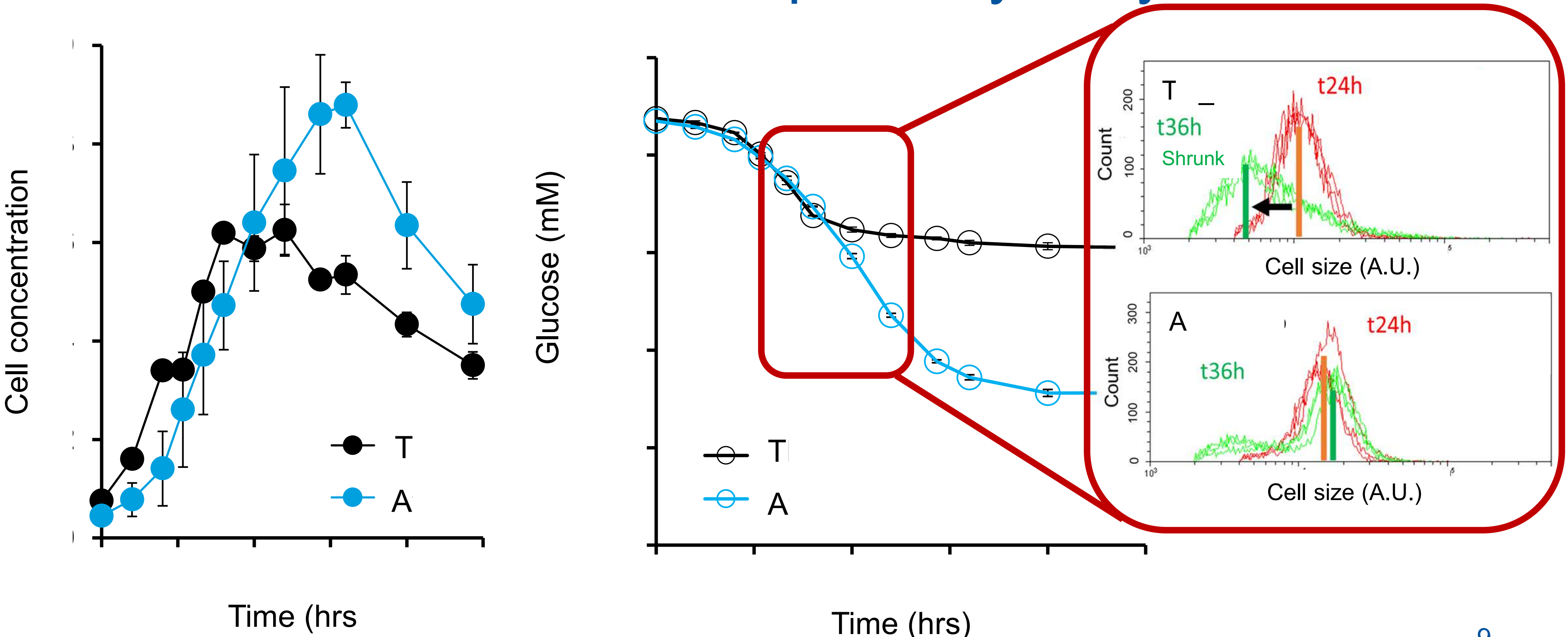



Charubin et al., mBio 11:e02030-20.(2020)

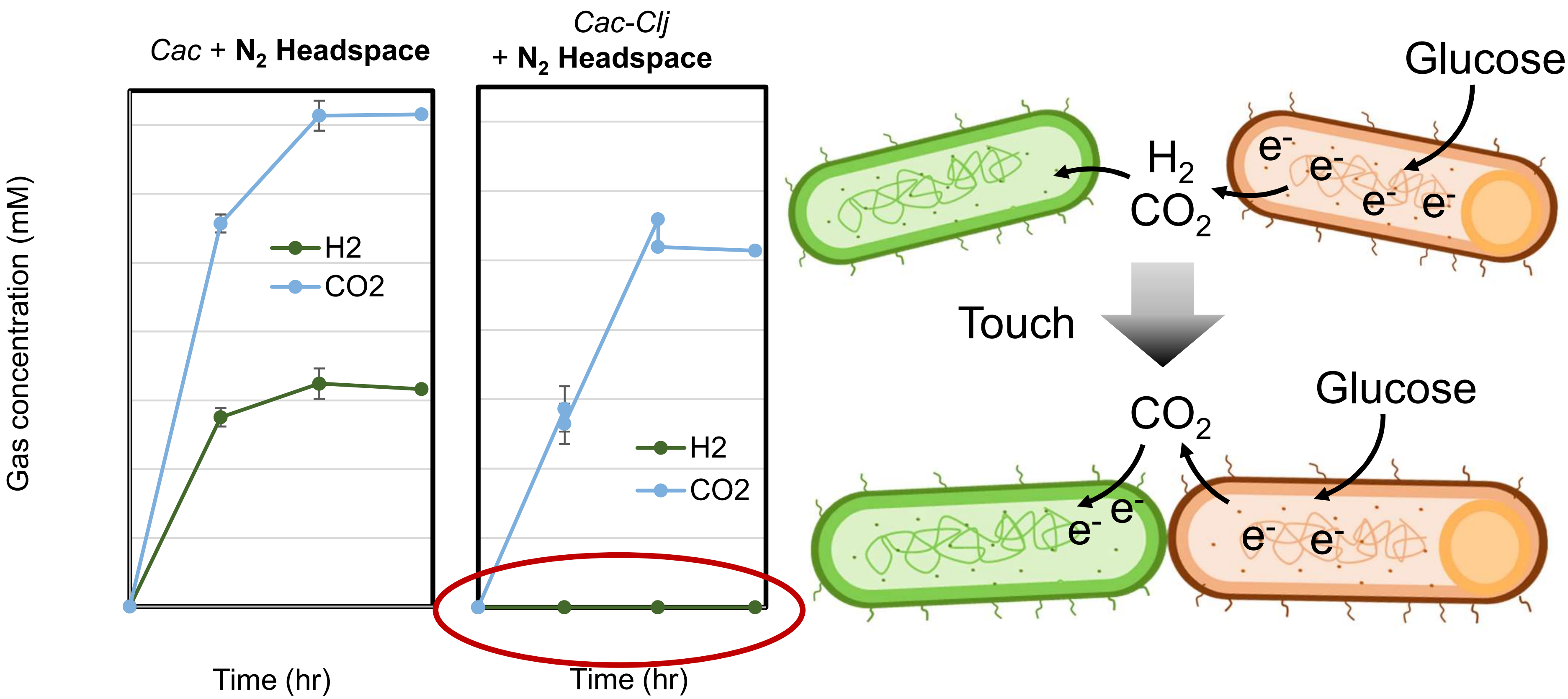
# Direct electron transfer from *Cac* to *Clj* made the *Cac* unhealthy




# Solving the growth cessation problem by expressing a redox insensitive pathway enzyme



# Improved electron management through ‘touch’



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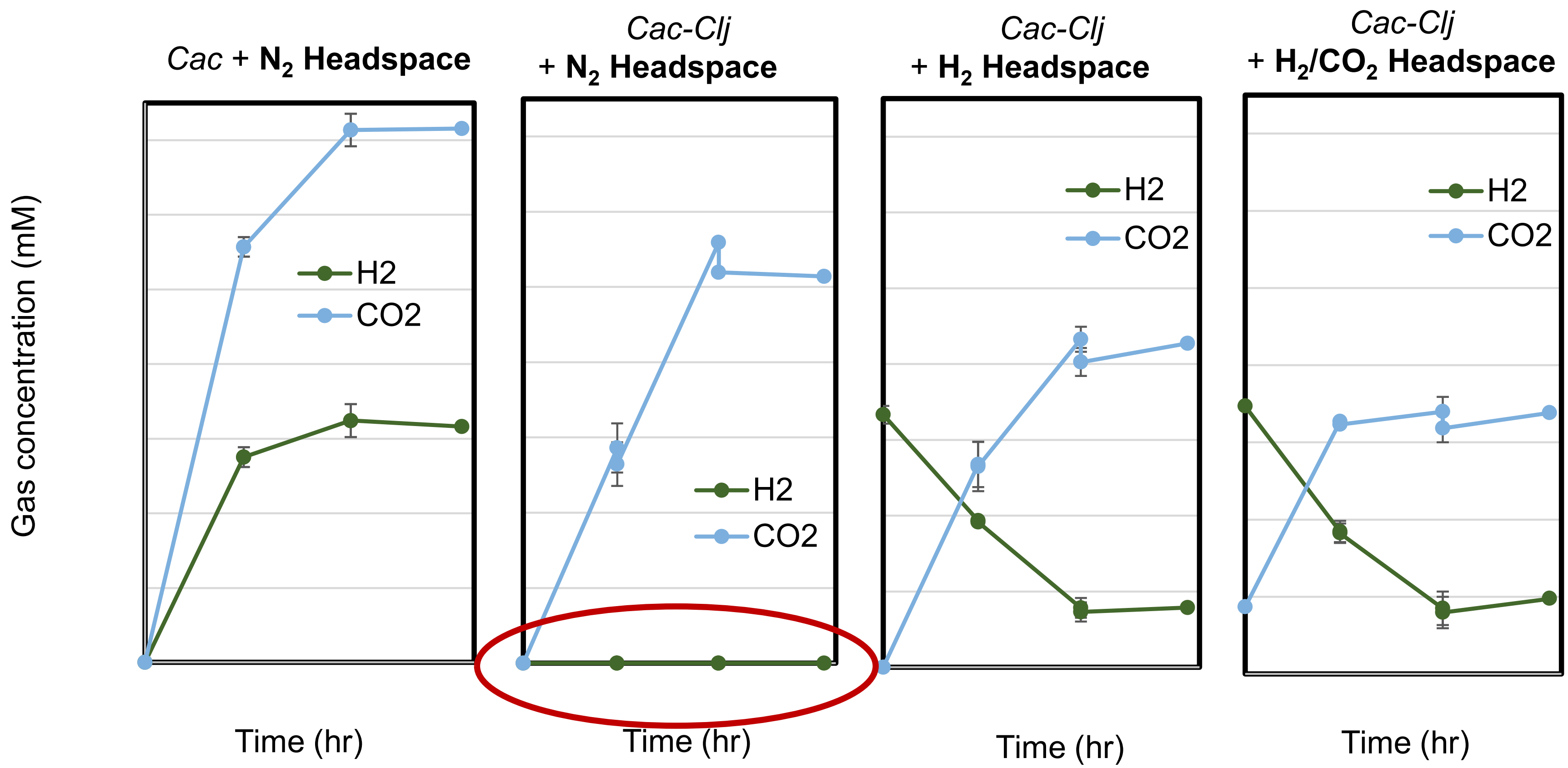
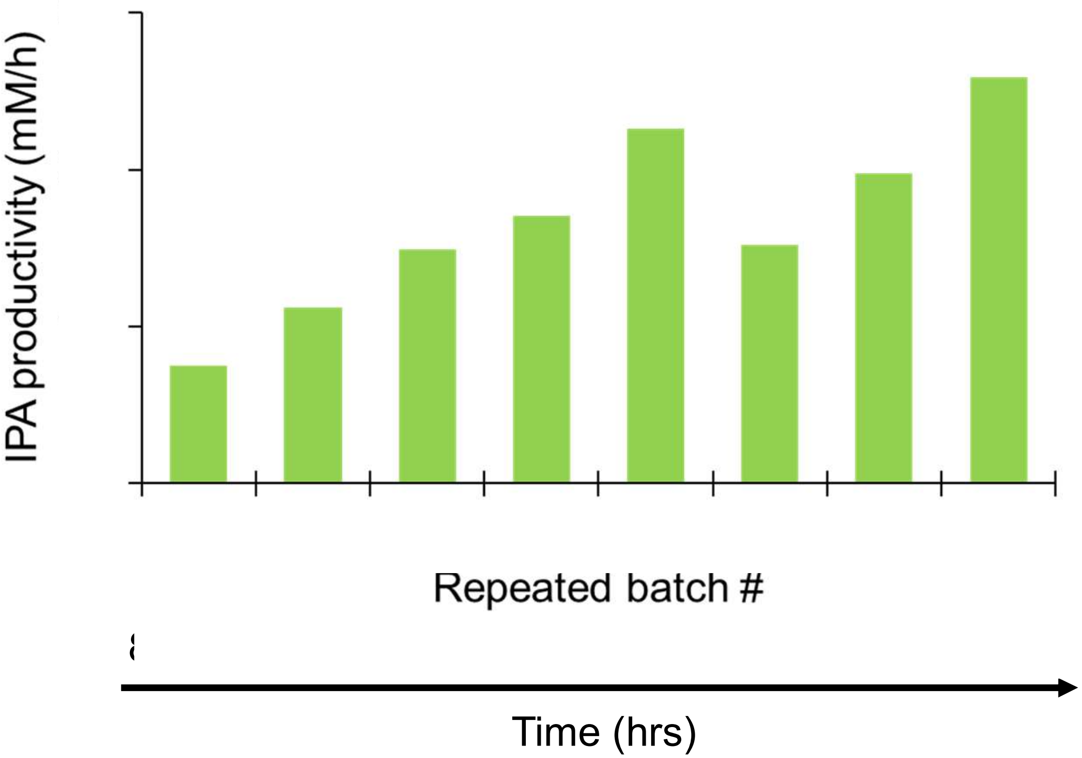
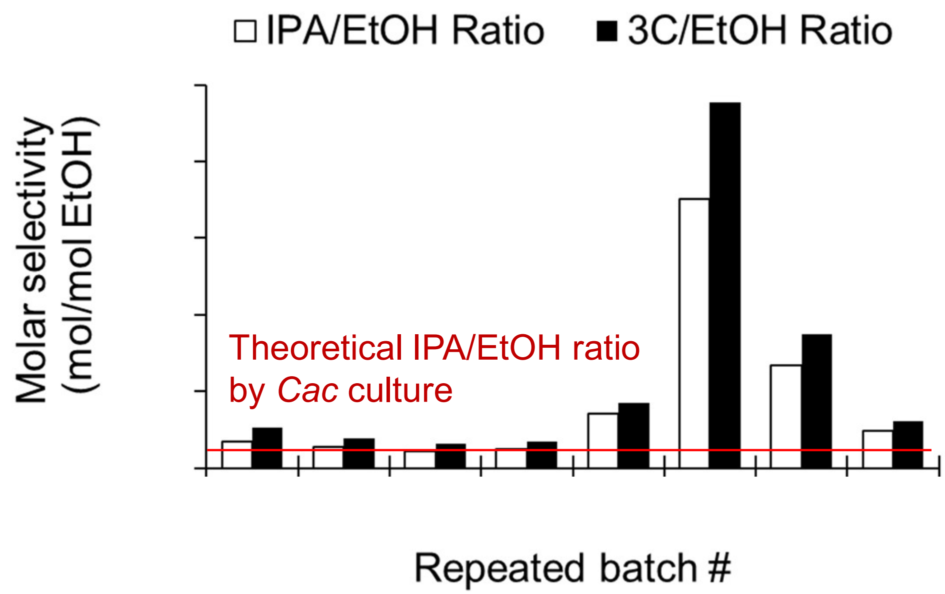
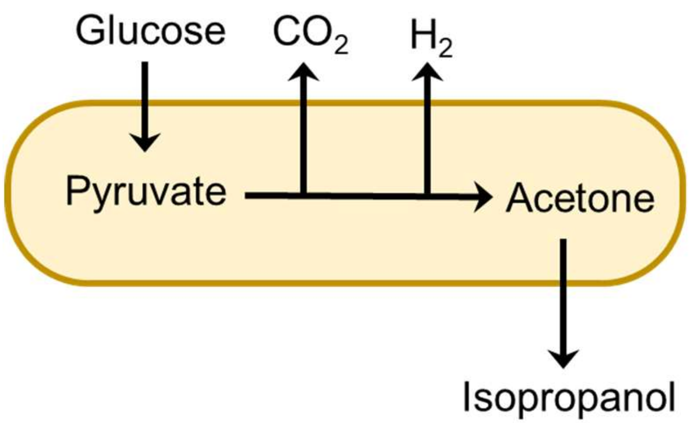


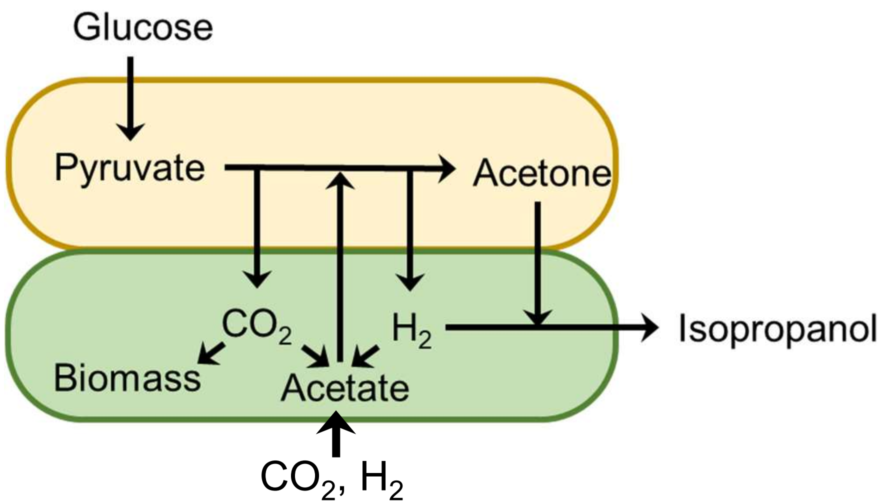
# Achieving a high IPA selectivity by the engineered *Cac-Clj* culture



# **Mixotrophy**: the paradigm of $CO_2$ utilization as "stoichiometry extender"




- Maximum theoretical IPA yields: 0.5 (Cmol/Cmol glu)
- Maximum theoretical carbon recovery: 50%

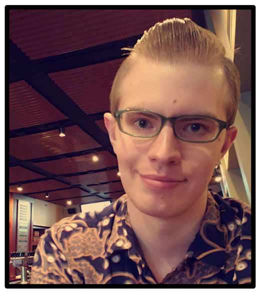
- Experimental IPA yields: **>0.8 (Cmol/Cmol glu)**
- Carbon recovery: **>100% (carbon negative)**
- IPA productivity > 20 mM/h (1.4 g/L/h)
- IPA/EtOH molar ratio > 10.5
- IPA titers >200 mM

# Acknowledgement

- Papoutsakis Lab (UD)


Eleftherios T. Papoutsakis (PI)


Hyeongmin Seo (Postdoc)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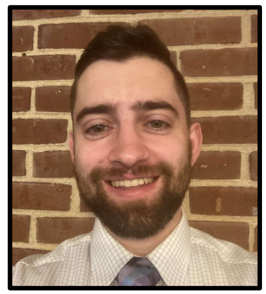
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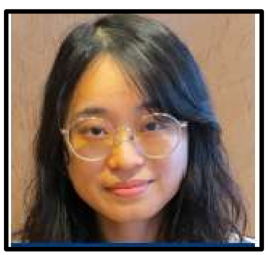
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