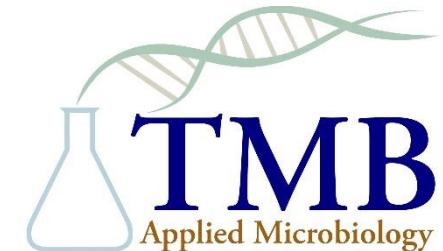


# BioREFINE-2G Workshop

**Bioplastics from  
2<sup>nd</sup> generation Biorefineries**



**Strain development for  
diacid production**

**Lisa Wasserstrom**  
**Anders Sandström**  
**Marie Gorwa-Grauslund**  
**Applied Microbiology,**  
**Lund University**

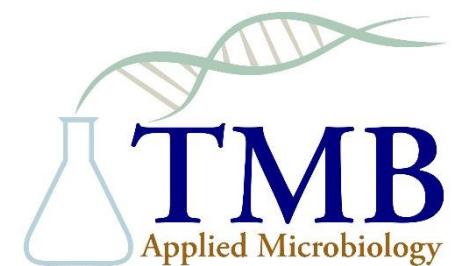


# Applied Microbiology, Lund University

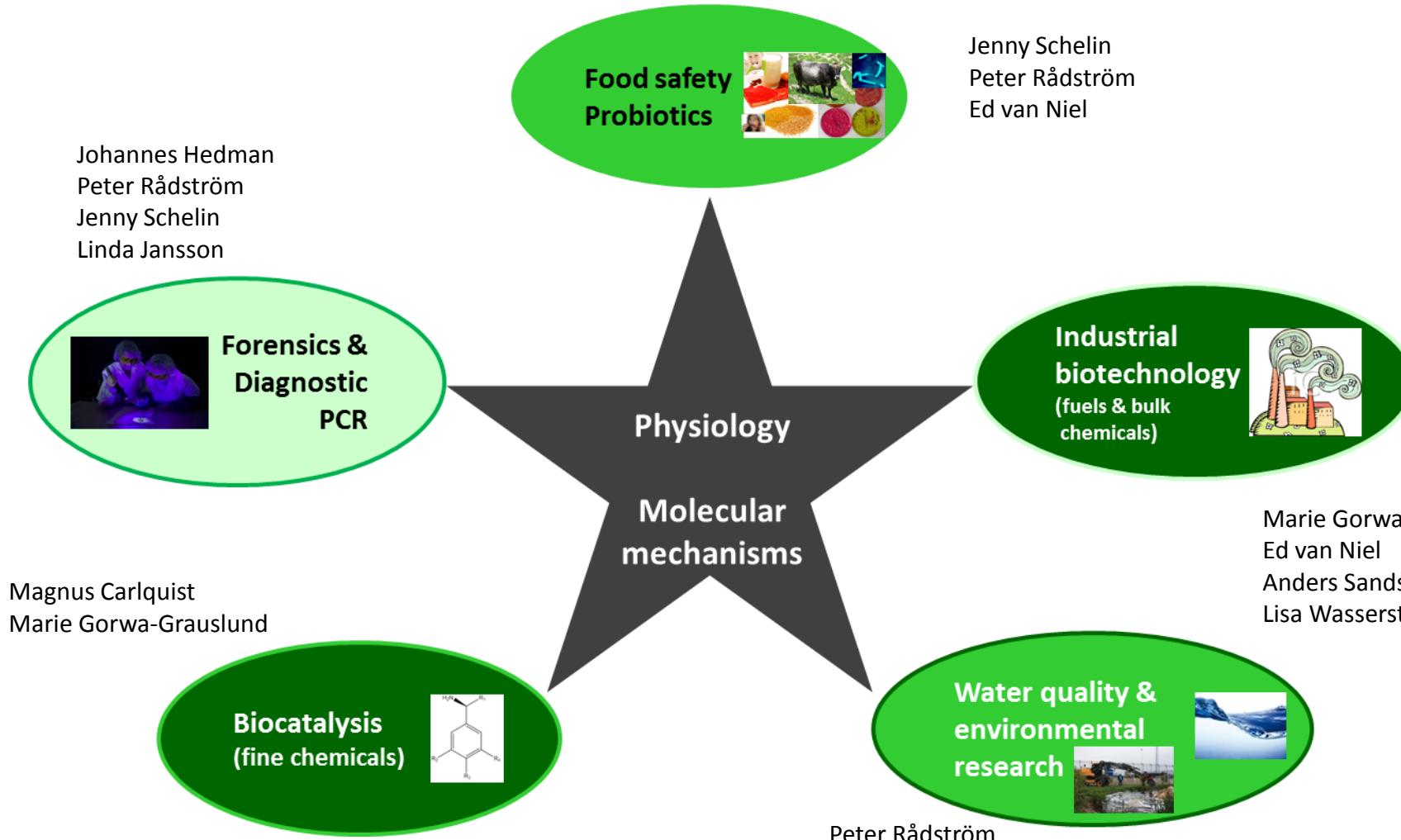


LUND  
UNIVERSITY

<http://www.tmb.lth.se>



# Research at Applied Microbiology



# Carboxylic acid production in 2<sup>nd</sup> generation biorefineries

**AIM:** Engineer *Saccharomyces cerevisiae* to produce carboxylic acids from biomass rich in C5 and C6 sugars



## Production of carboxylic acids

- from lignocellulosic biomass
- using *Saccharomyces cerevisiae*

# Carboxylic acids – *Why?*

## Polymers



*Polyethylene  
Terephthalate : PET*

## Food



*Acetic acid*

## Medicin



*Gluconic acid*

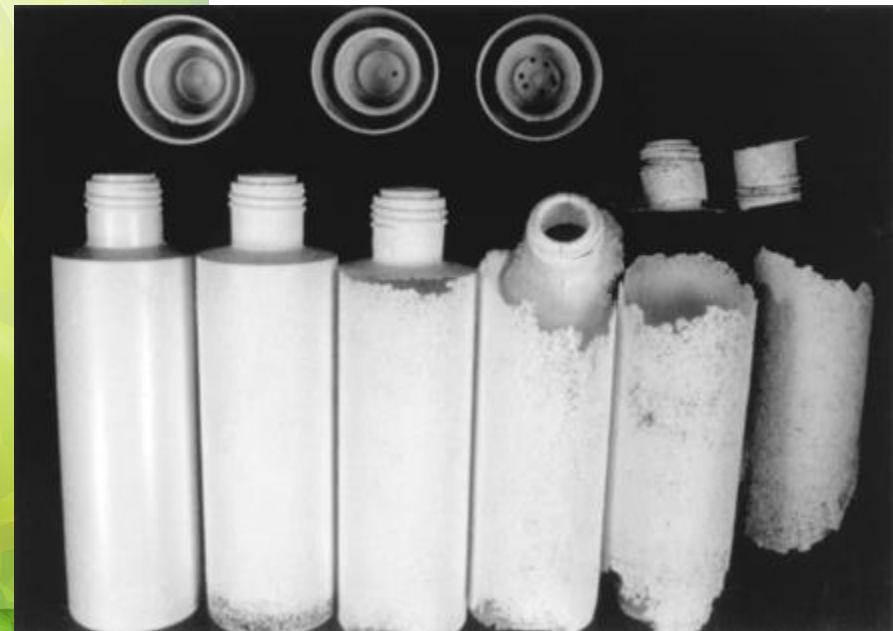
*Citric acid*

# Carboxylic acids and plastics

**BIOSUSTAINABLE  
PLASTICS**



**BIODEGRADABLE  
Polyhydroxybutyrate (PHB)**

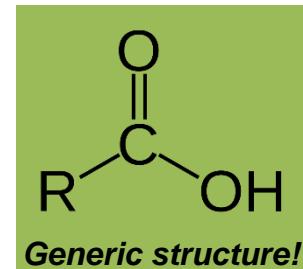
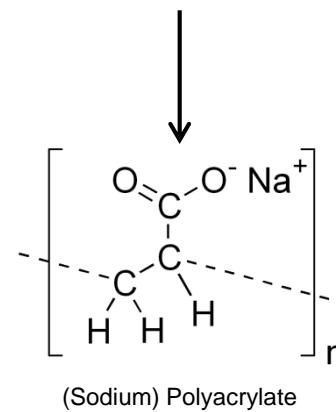
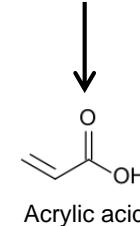
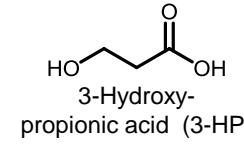
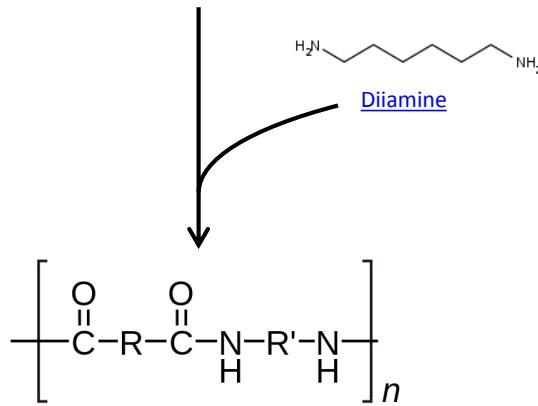
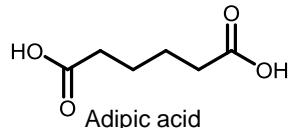
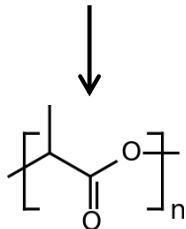
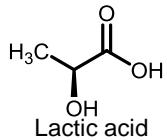


0 – 10 weeks in aerobic sewage sludge  
(average temperature, 20°C)

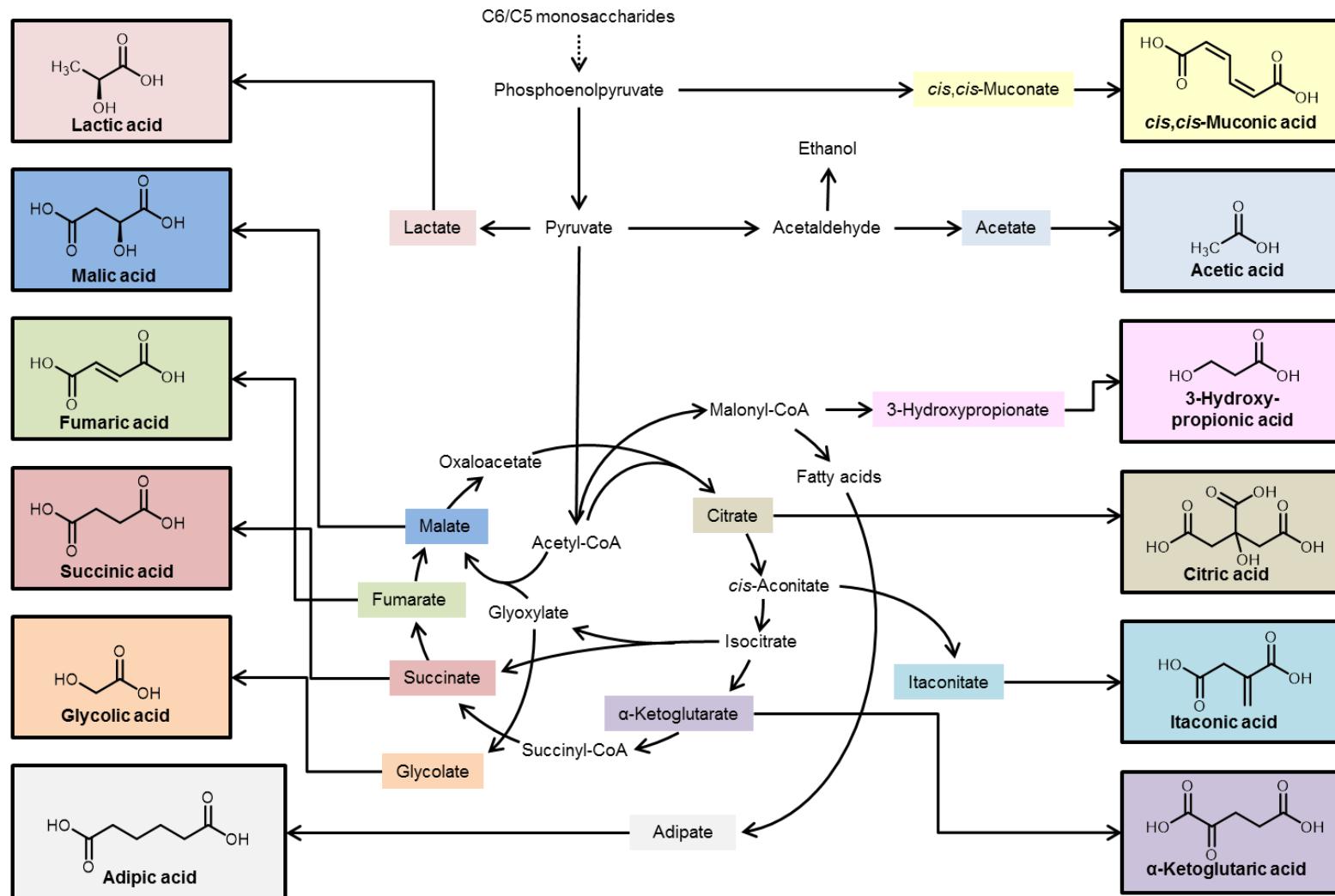
# Plastic garbage patches



# Carboxylic acids and bio-plastics



# Diversity of the metabolism

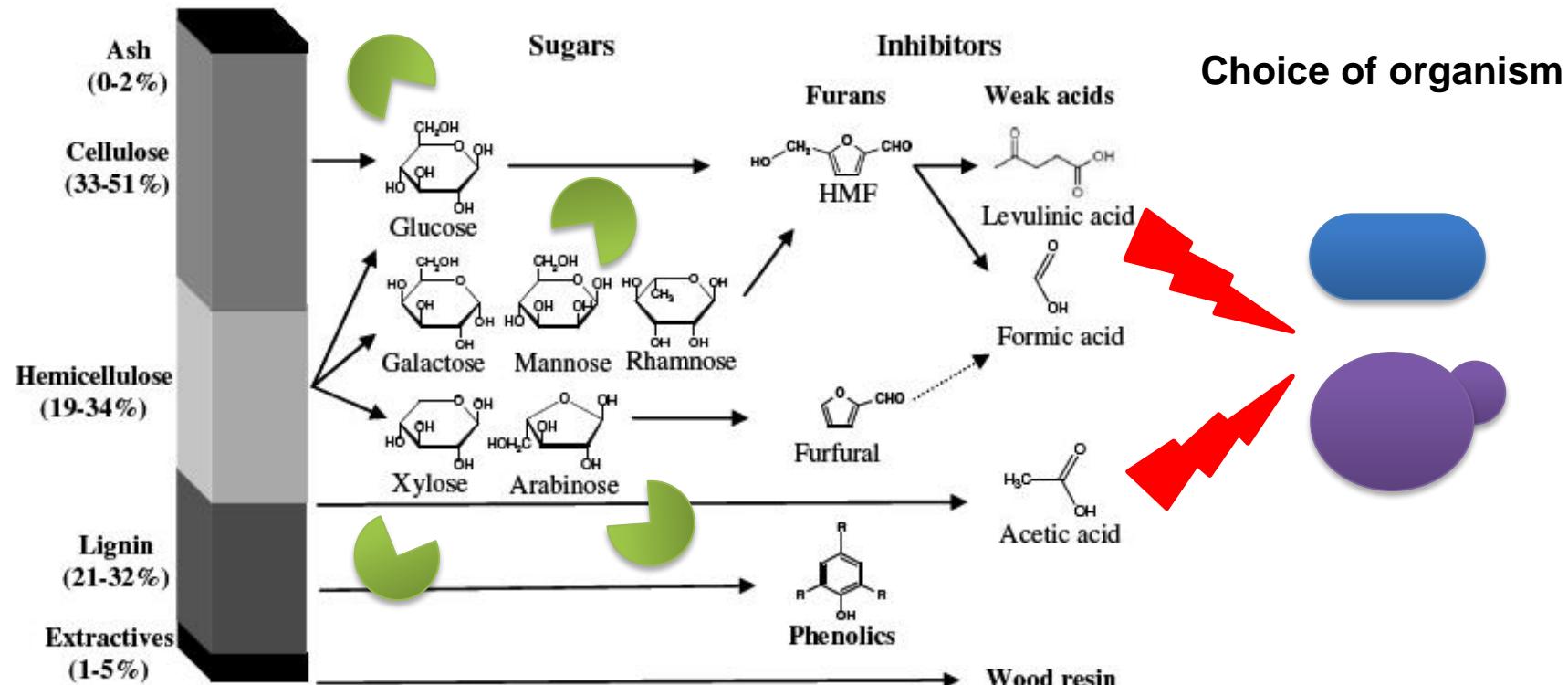


# Lignocellulosic biomass – Why?

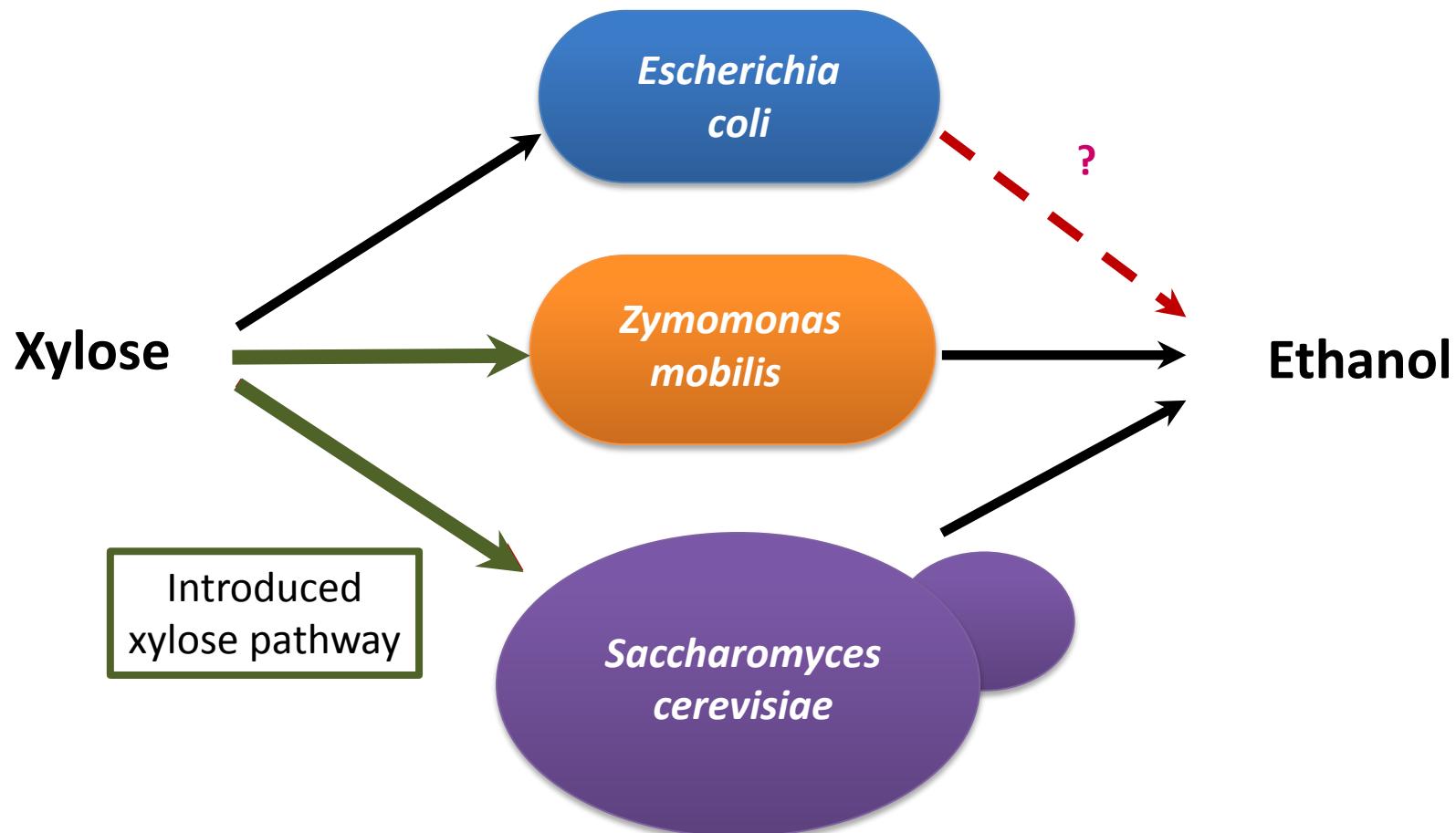


2<sup>nd</sup> generation Biorefineries

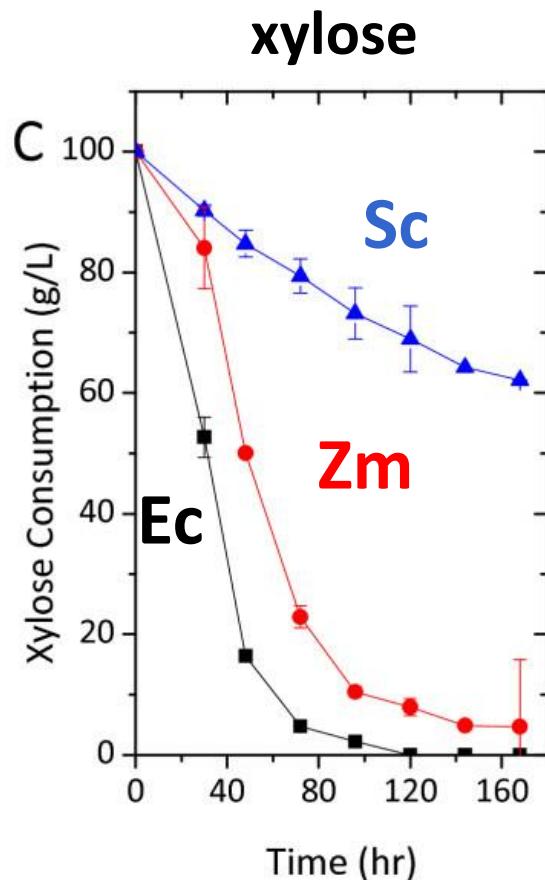
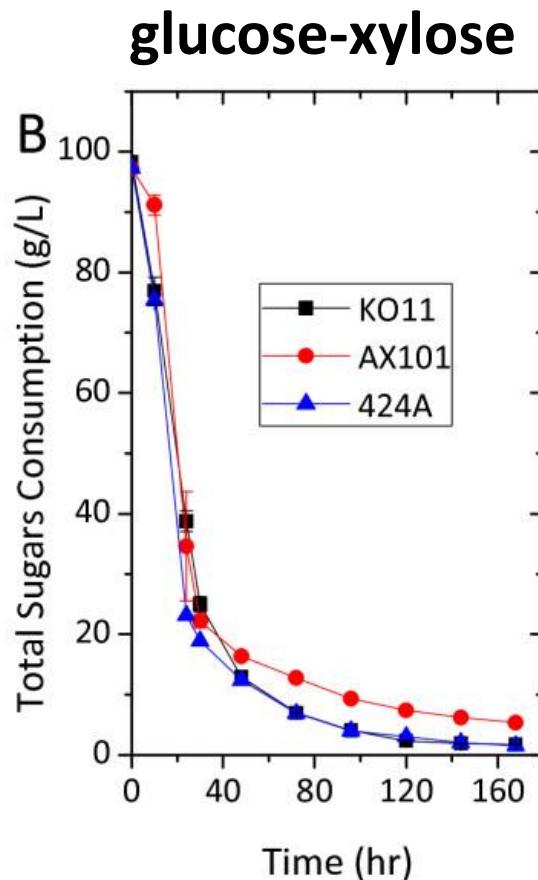
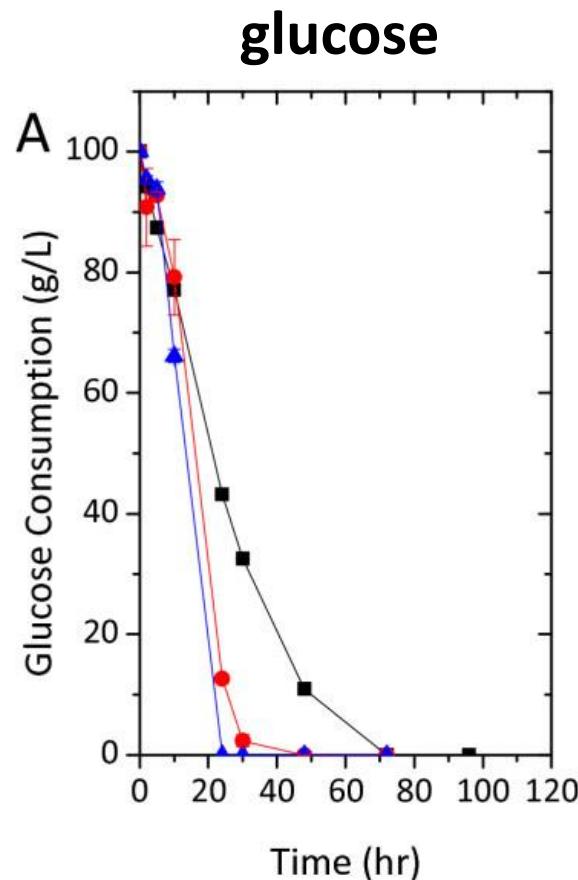
Lignocellulose



# Potential microorganisms for several bioprocesses



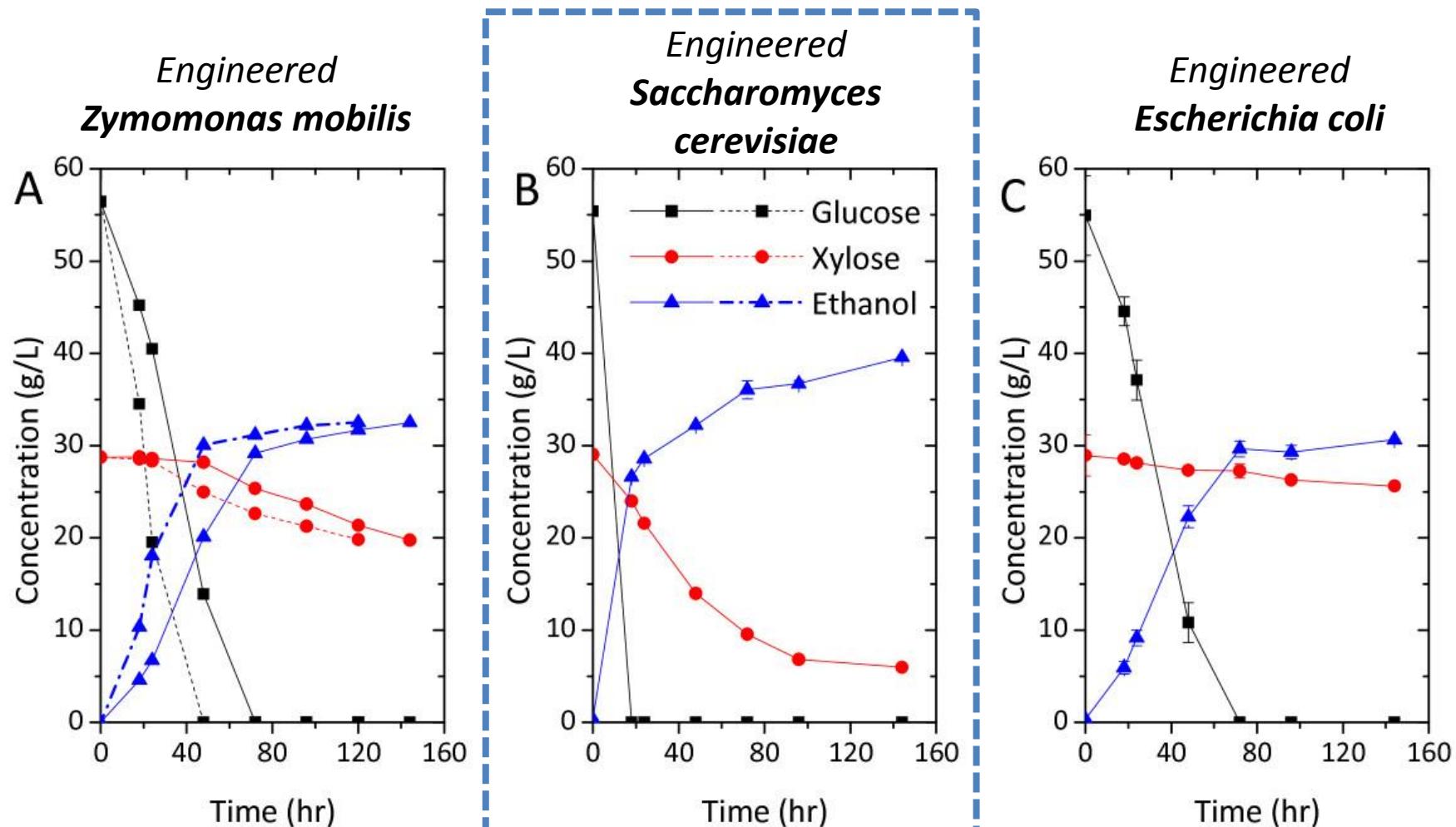
# Organism comparison



Lau et al 2010 Biotech Biofuels 3:11

**Performances in defined medium**

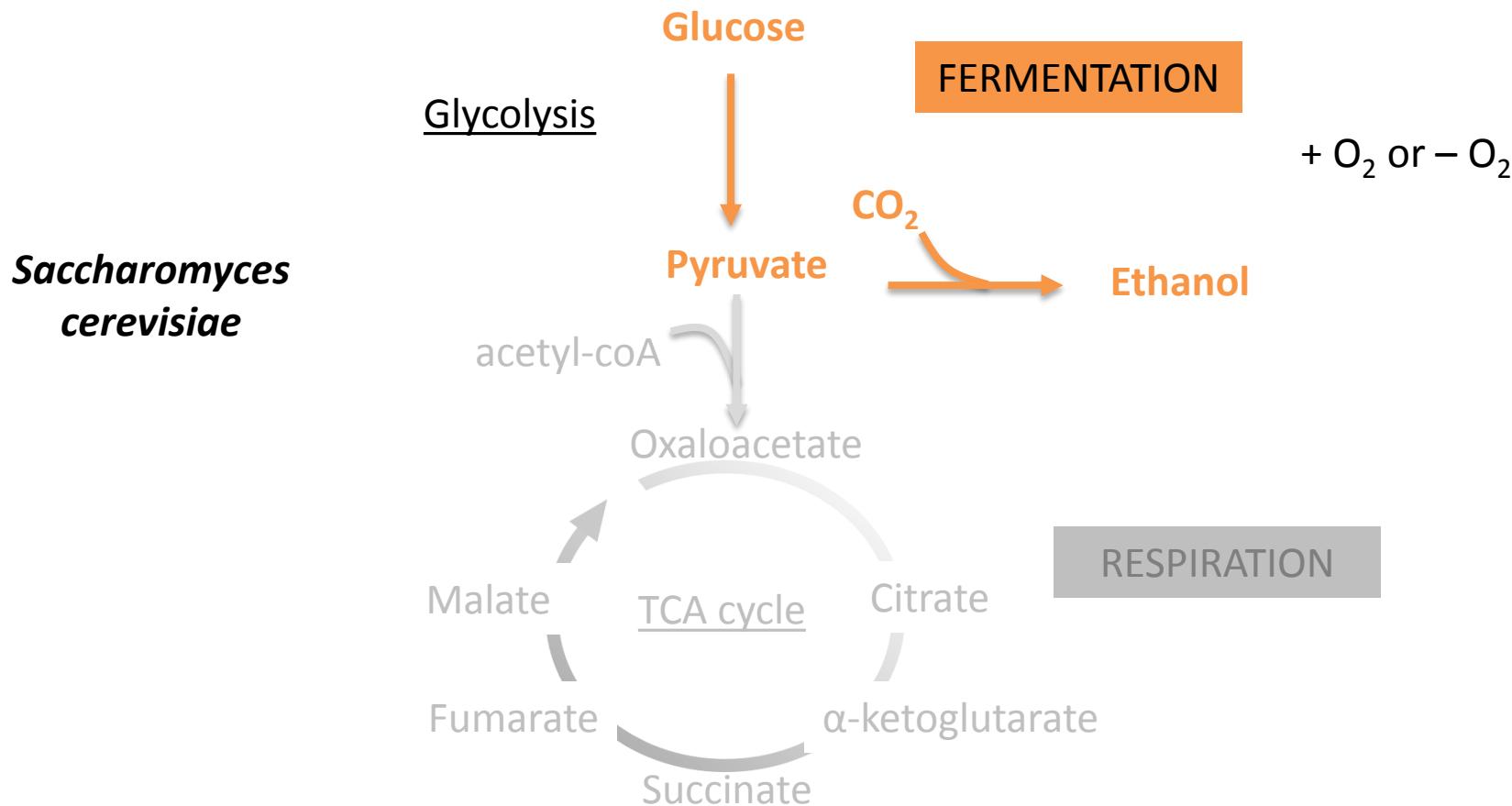
# S.c preforms best in hydrolysate



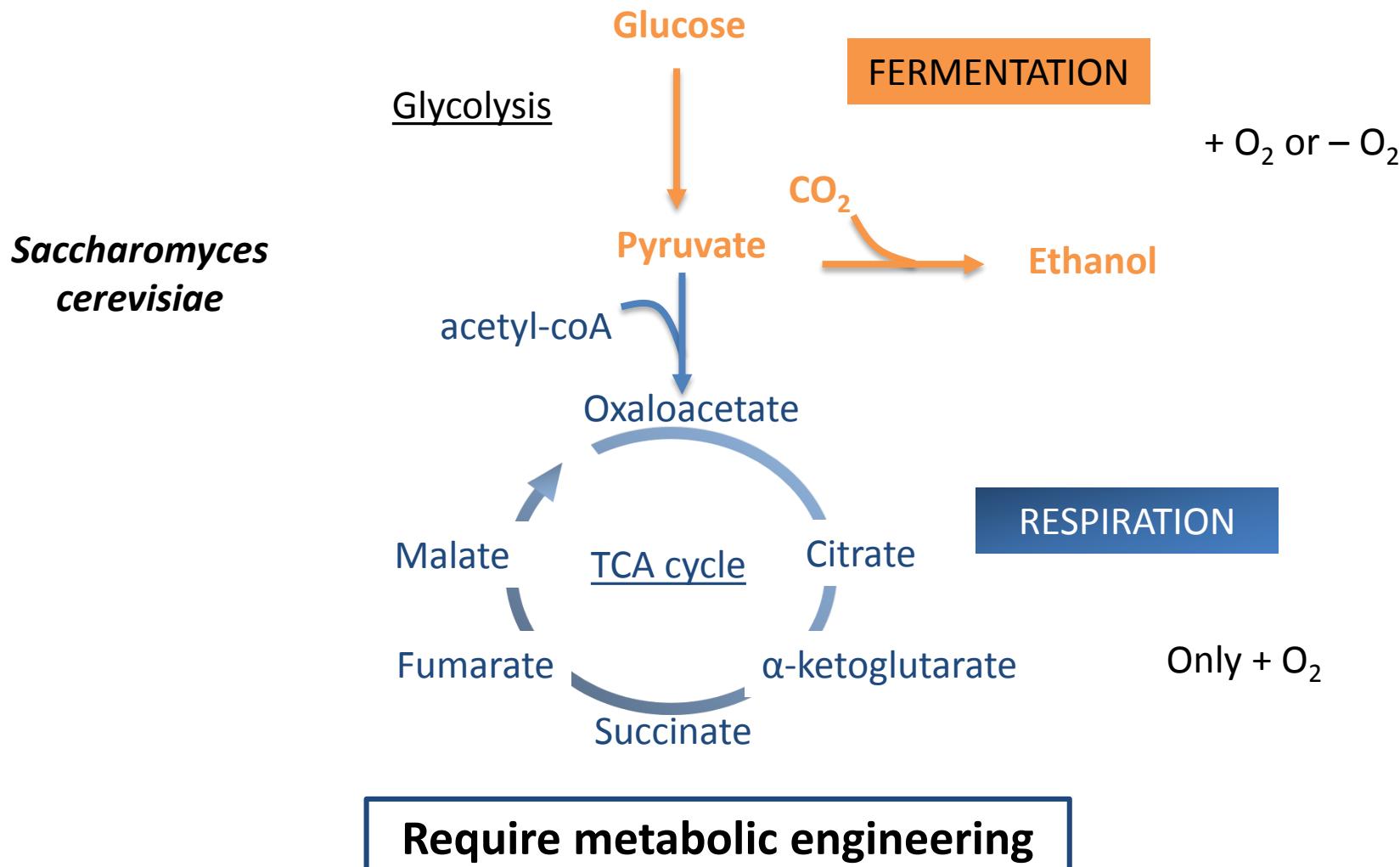
Corn stover hydrolysate

(From Lau et al 2010 Biotech Biofuels 3:11)

# Alcoholic fermentation even under aerobic conditions

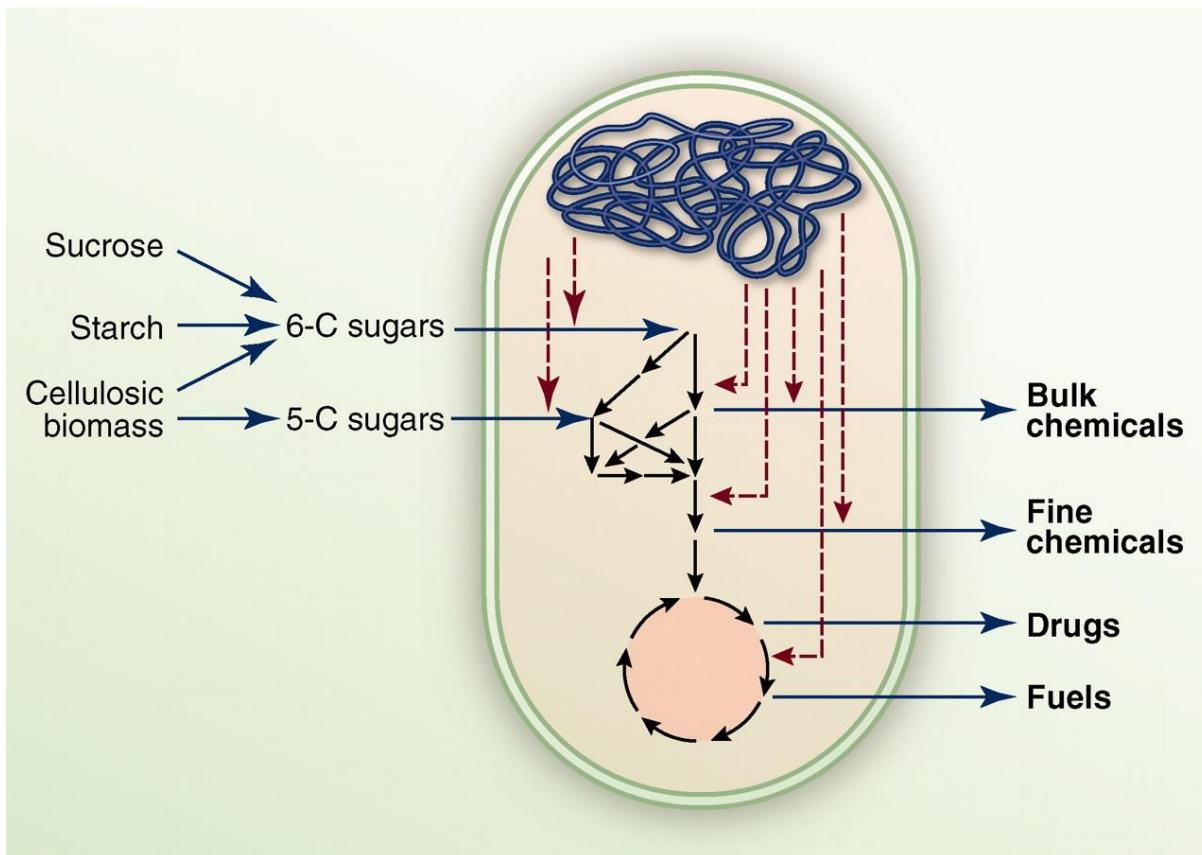


# Alcoholic fermentation even under aerobic conditions

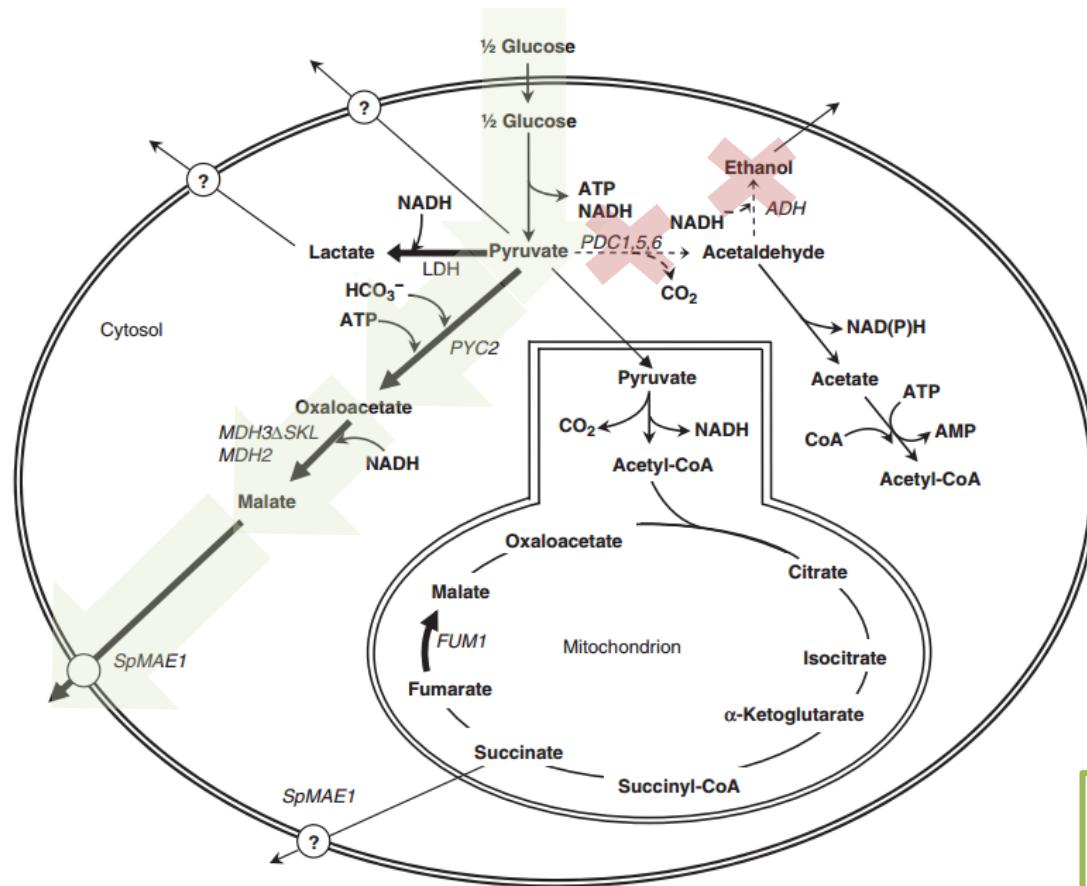


# What is metabolic engineering?

*"optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance"*



# Example of malic acid production in Yeast



## IMPORTANT FEATURES

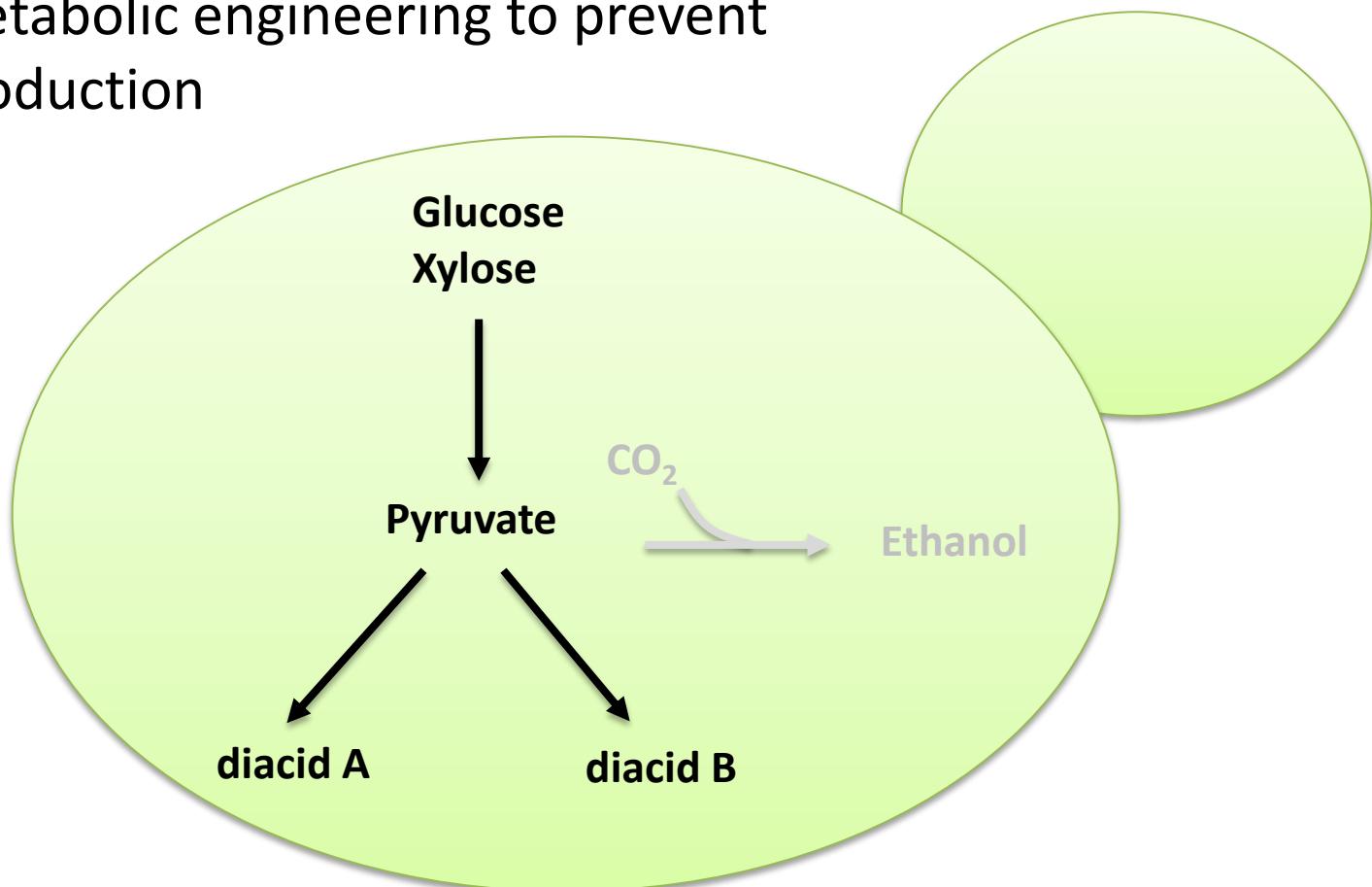
1. No ethanol production ( $\Delta pdc1,5,6$ )
2. Overexpress pyruvate carboxylate (*PYC2*)
3. Overexpress cytosolic malate dehydrogenase (*MDH3\Delta SKL*)
4. Malate transporter *SpMAE1*

## RESULT

0.42 mol malate/mol glucose  
59 g/L

# Outline of BioREFINE-2G

- *S. cerevisiae* best host since tolerant to low pH and inhibitors
- Require metabolic engineering to prevent ethanol production



• **Thank you for your attention !!**

## Acknowledgments

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Lisa Wasserstrom  
[Lisa.wasserstrom@tmb.lth.se](mailto:Lisa.wasserstrom@tmb.lth.se)  
+46733142242

