Engineering of central metabolisms

Solis-Escalante et al. (2015) A Minimal Set of Glycolytic Genes Reveals Strong Redundancies in *Saccharomyces cerevisiae* Central Metabolism. doi: 10.1128/EC.00064-15.

Kuipers et al. (2016) Pathway swapping: Toward modular engineering of essential cellular processes. doi: 10.1073/pnas.1606701113.

Sarah Bluhm, Klara Hamit, Juulia Mikkola, Joel Rouste

9/5/2023

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Introduction

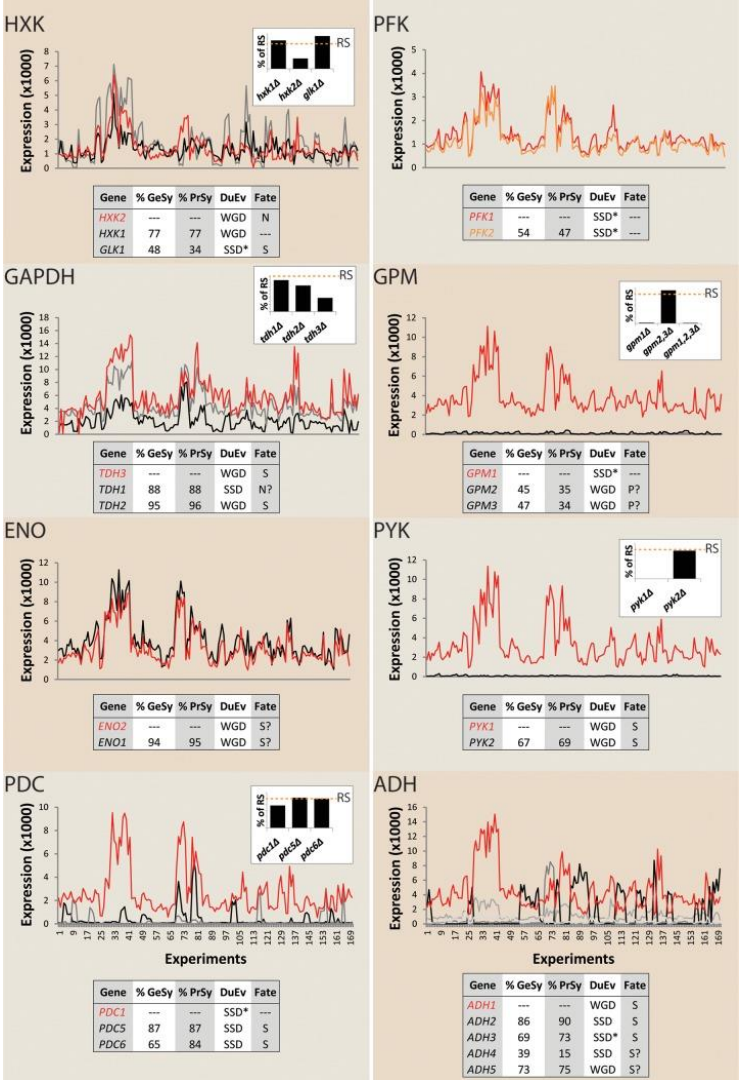
- **Microbes equipped with novel-to-nature capabilities required for replacement of petrochemistry by bio-based processes**
- **Engineering of microbes' central metabolisms difficult due to complexity developed through evolution**
  - **Deletion of functionally redundant paralogs to construct a "minimal glycolysis" strain (MG)**
  - **Pathway swapping**
    - **Transplantation of essential metabolic routes in *S. cerevisiae***

Aims

- **Make CENTRAL METABOLISM:**
- **Less complex**
- **Simulatable**
- **More modular, engineerable**
  - **Single-locus (not mosaic)**
  - **No redundancies (paralogs)**

Separation of major and minor glycolytic paralogs

- **Gene deletions of minor paralogs**
- Original fate of paralog: pseudogenization, subfunctionalization, or neofunctionalization
- Expression levels of paralogs
- Activities of knockouts



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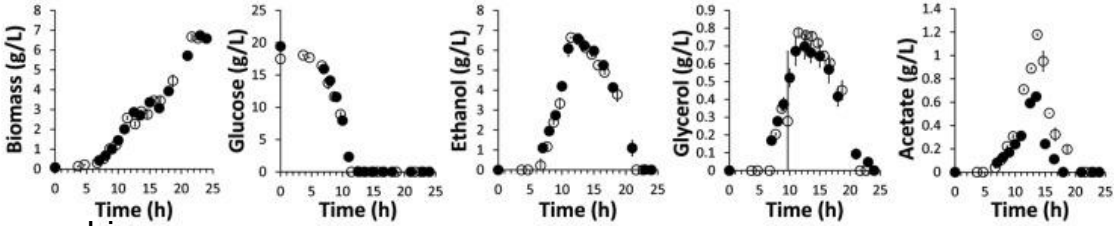
Biomass production and extracellular metabolites

- **Comparison: minimal glycolysis (MG) strain with reference**
- **13 minor glycolytic paralogs deleted**
- Specific growth rate, glucose consumption, ethanol production, glycerol production, and acetate production

Aerobic

A

	μ^*	q_s	q_{EtOH}	q_{Glyc}	q_{Ace}^*
MG	0.357±0.013	15.362±0.578	21.748±2.097	1.177±0.077	0.632±0.144
C	0.335±0.011	15.770±0.451	23.816±1.068	1.113±0.031	0.411±0.066



Anaerobic

B

	μ	q_s	q_{EtOH}	q_{Glyc}	q_{Ace}
MG	0.332±0.009	17.039±2.179	27.130±1.814	3.731±0.199	0.420±0.047
C	0.319±0.017	18.427±0.301	27.904±0.121	3.870±0.268	0.403±0.123

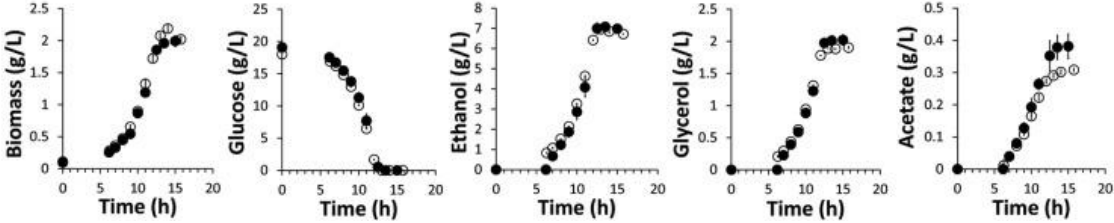


Figure 3 – Article 2

In vitro enzyme activities and intracellular metabolite concentrations: Minimal glycolysis (MG) vs congenic reference chain

(A) Average values of glycolytic enzyme activity of MG strain (white bars) and prototropic reference chain.

(B) Glycolytic metabolite profile of MG strain (open circle) and reference chain (close circle)

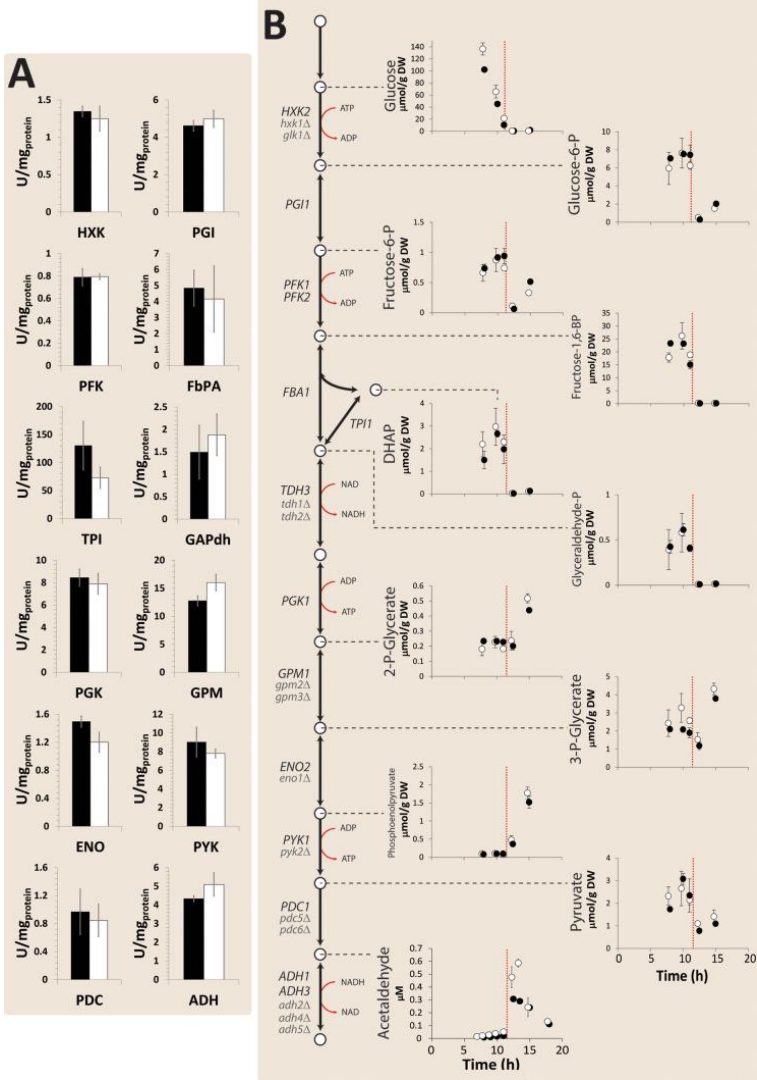


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Figure 4 – Article 2

Growth of MG strain and reference strain under different growth conditions

(A) Specific growth rates of MG strain (white bars) and reference strain (black bars)

(B) Growth (change in OD at 660 nm) of MG strain (open circles) and reference strain (closed circles) during carbon source switches

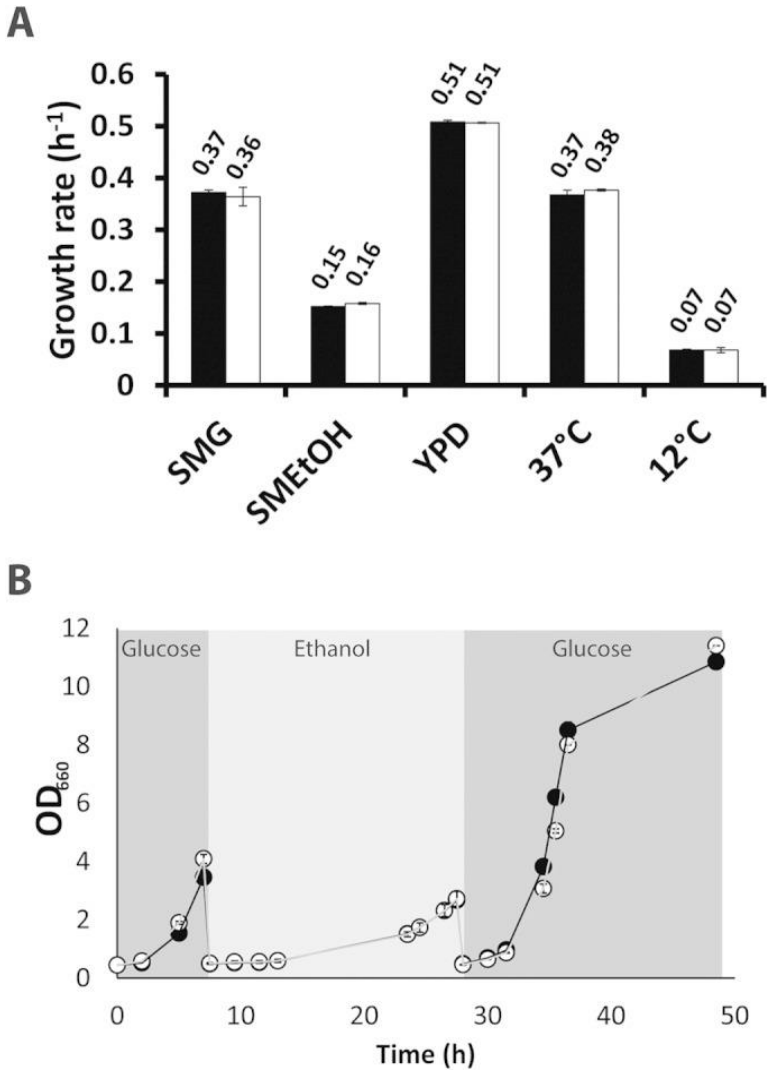

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Figure 5 – Article 2

Growth of MG strain and reference strain on different solid media

Serial dilutions of cell suspensions plated on agar media with 24 different compositions

(A-C) osmotic stress

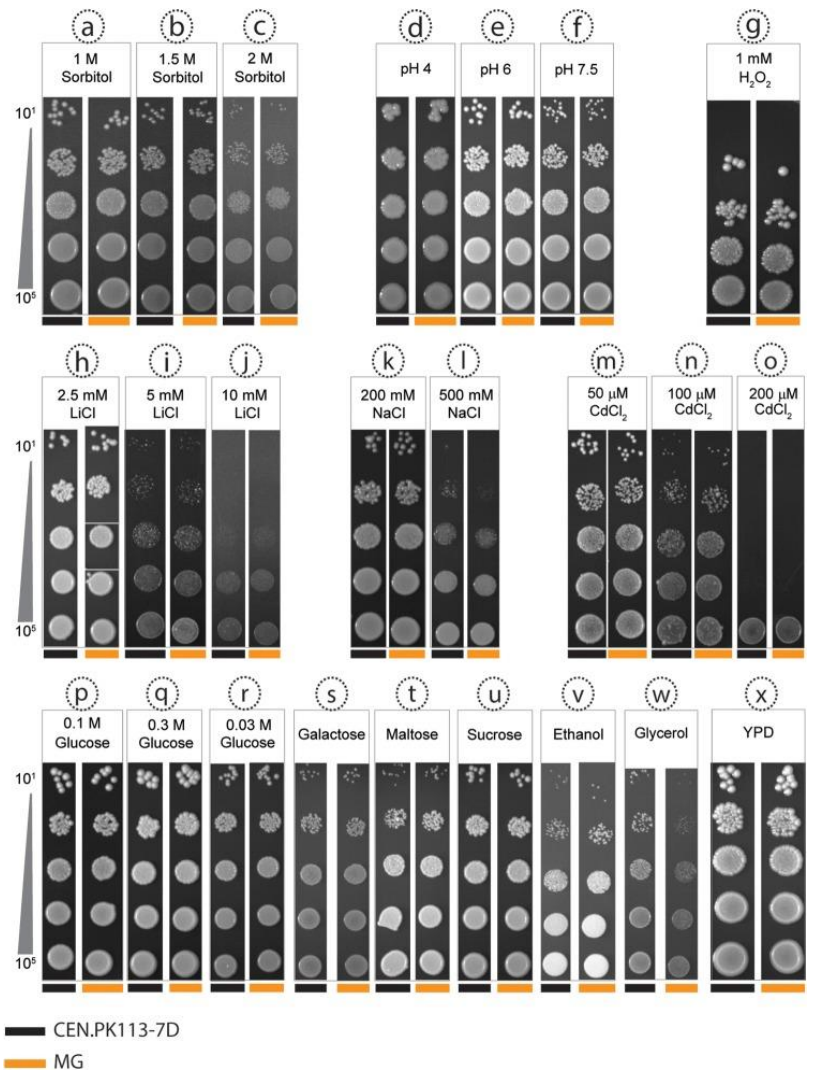
(D-F) pH differences

(G) oxidative stress

(H-O) three different salts

(P-W) six different carbon sources

(X) complex medium



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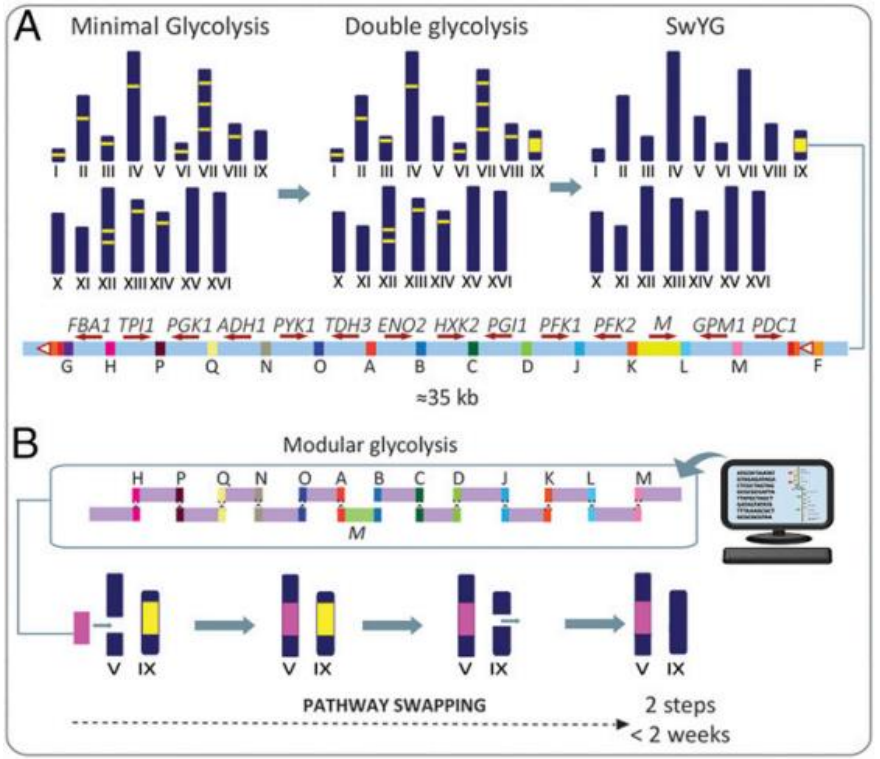
Schematic overview of the glycolysis swapping approach

- **A: Construction of a platform for glycolysis pathway swapping**
  - The genes involved in glycolysis are scattered over the chromosomes
  - In the SwYG glycolytic genes are expressed from a single locus and scattered native genes are removed
- **B: The in silico designed glycolytic gene cluster was assembled and integrated to chromosome V, followed by the removal of the endogenous glycolysis on chromosome IX**



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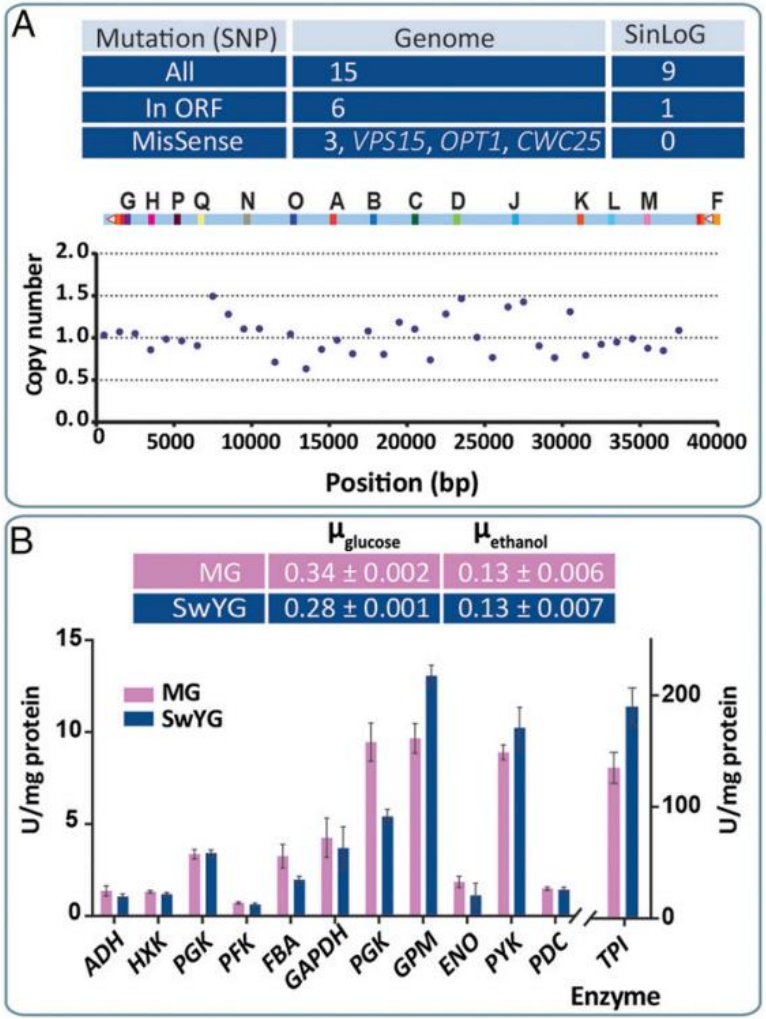
Characterization of SwYG

- **A: Next-gen sequencing and copy number analysis**
  - The in vivo assembled gene cluster followed the in silico blueprint
  - Copy number analysis revealed absense of duplicated glycolytic genes
- **B: Physiological characterization in shake-flask culture**
  - The specific growth rate per hour and enzyme activity data compared to the MG strain



Characterization of yeast strains with remodeled glycolysis

- **Decreased specific growth in strain IMX637, fixed upon overexpression of *SkTDH1* (IMX652)**
  - Effect on GAPDH activity
- **Activities of TPI, PGK, and ADH 50% lower in the mosaic strain in comparison to the SwYG**
  - No effect on growth rate
- **Overall prominent results in enzyme activities with all remodeled glycolysis strains**

A

SinLoG-V (IMX605)

ScADH1, ScHXK2, ScPGI1, ScPFK1, ScPFK2, ScFBA1, ScTDH3, ScPGK1,ScGPM1, ScENO2, ScPYK1, ScPDC1, ScTPI1

***Sk*-SinLoG-V (IMX637 & IMX652)**

SkADH1, SkHXK2, SkPGI1, SkPFK1,SkPFK2, SkFBA1, SkTDH1, SkPGK1, SkGPM1, SkENO2, SkPYK1, SkPDC1, SkTPI1

IMX652: + overexpression of SkTDH1

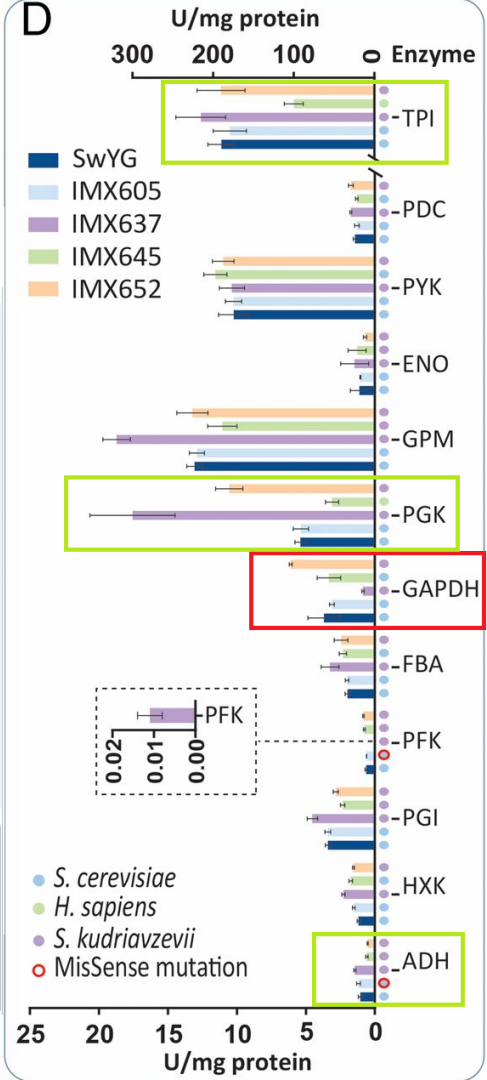
Mosaic-SinLoG-V (IMX645)

ScHXK2, ScFBA1, ScTDH3, ScPYK1,ScPDC1 SkADH1, SkPGI1, SkPFK1, SkPFK2, SkGPM1, SkENO2

HsTPI1, HsPGK1

C

	μ (h^{-1})
IMX605	0.28 ± 0.000
IMX637	0.13 ± 0.000
IMX652	0.25 ± 0.000
IMX645	0.28 ± 0.002



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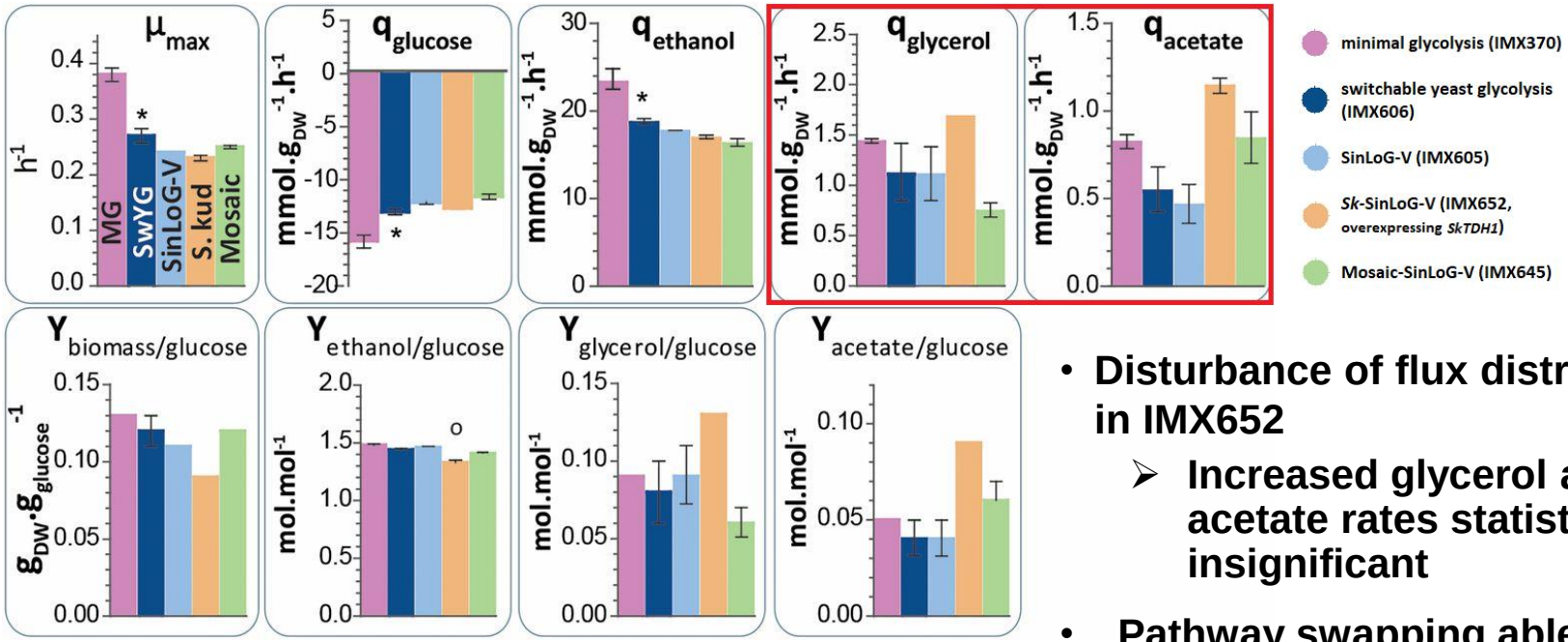
Physiological characterization during aerobic batches in bioreactors



- **Disturbance of flux distribution in IMX652**
  - ➢ **Increased glycerol and acetate rates statistically insignificant**
- **Pathway swapping able to identify interesting regulatory phenomena**

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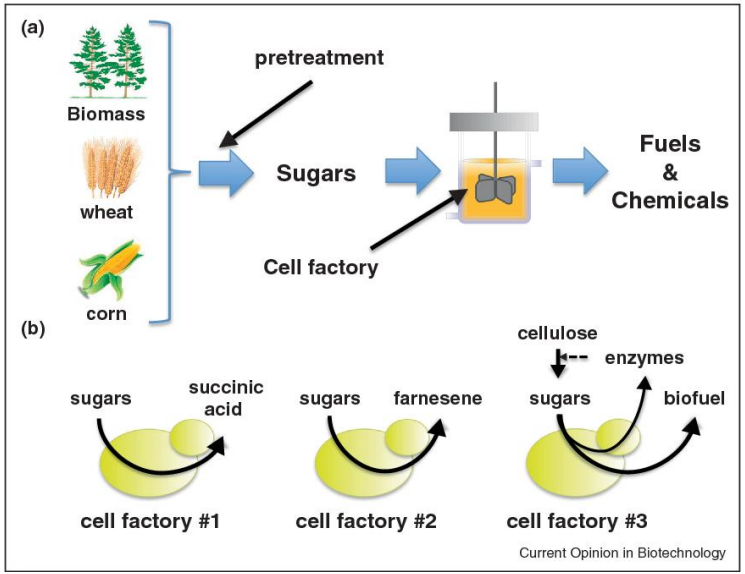
Conclusions & future prospects

- **A yeast strain with simplified glycolysis was constructed and taken advantage of to swap the metabolic pathway**
  - Modular design of synthetic genomes
  - Well-defined platform for future studies and applications
  - Could prove useful in streamlining design of microbes for bioprocesses such as petrochemical production



Nielsen, Jens et al. “Metabolic engineering of yeast for production of fuels and chemicals.” *Current opinion in biotechnology* 24 3 (2013): 398-404 .