BIOETHANOL PRODUCTION – FROM LAB MEDIUM TO LARGE SCALE LIGNOCELLULOSIC FERMENTATIONS

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Bioethanol production from lignocellulose

Chipping/ grinding

Pretreatment

SHF

Enzymatic hydrolysis

Fermentation

Enzyme production

SSF

Enzymatic Hydrolysis

Fermentation

CBP

Enzyme Production & Hydrolysis Fermentation

Acid hydrolysis

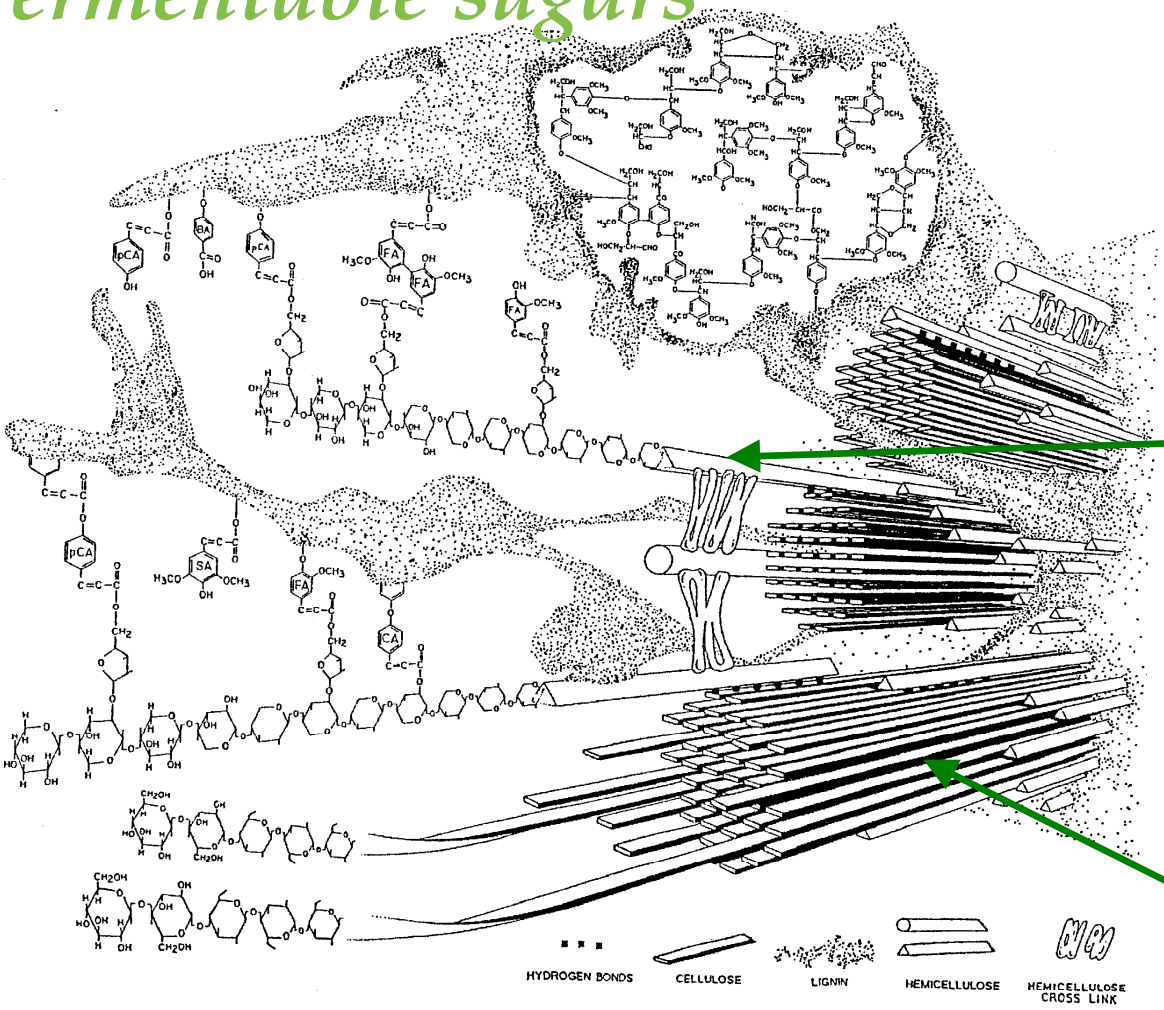
Acid recovery

Fermentation

Destillation

Ethanol

Lignocellulosic material can be degraded to fermentable sugars



Hemicellulose is degraded to mainly glucose, galactose, mannose, xylose and arabinose

Cellulose is hydrolysed to glucose units

Lignocellulosic material is recalcitrant and after degradation inhibitory compounds are formed

(H. Joergensen)

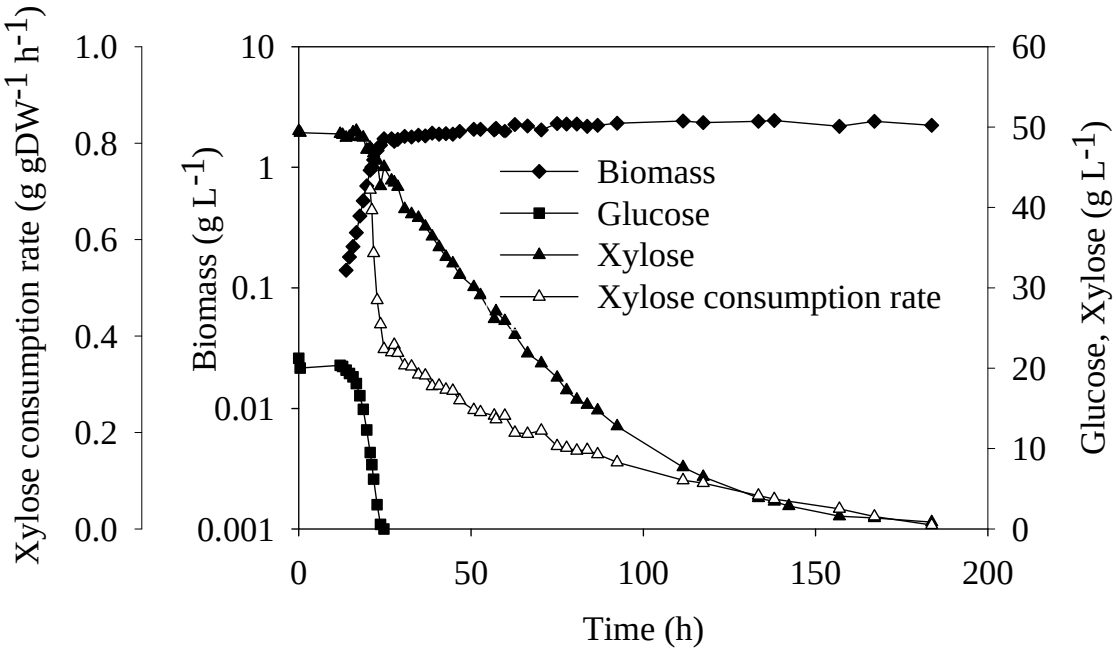
Important properties for a microbial cell factory to ferment lignocellusics

- High ethanol yield and productivity
- Minimal by-product formation
- Broad substrate range and simultaneous use of carbon sources
- High tolerance towards inhibitors

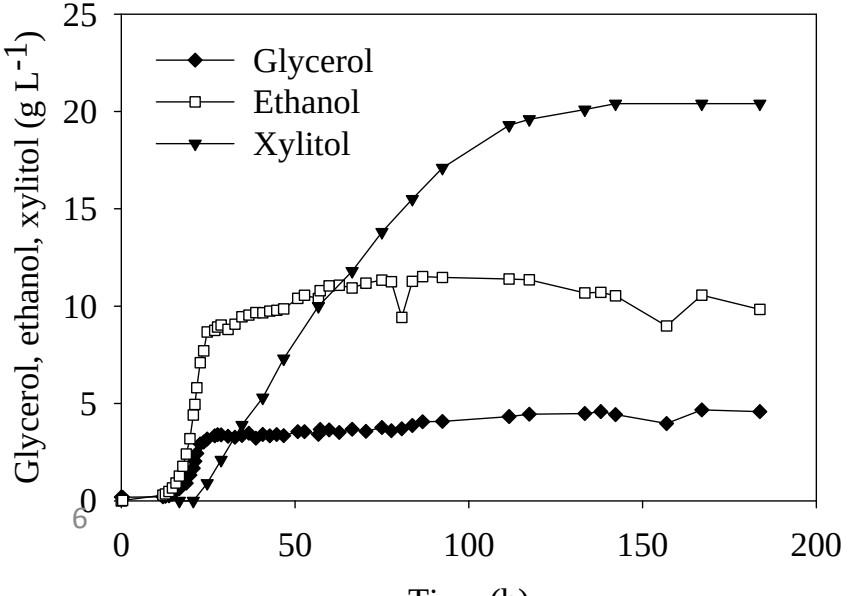
To meet these demands we have chosen to work with *Saccharomyces cerevisiae*

Zaldivar et al. (2001) AMB, **56,** 17

Targets for metabolic engineering



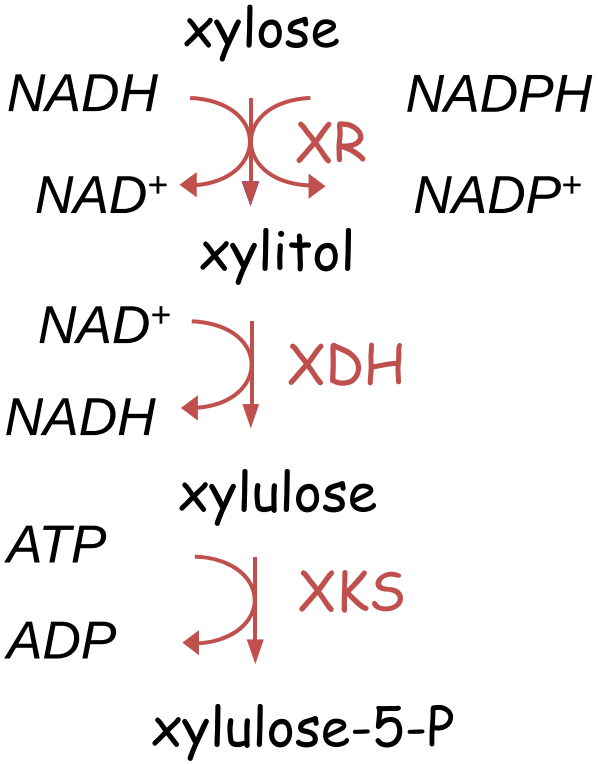
Conditions: Defined medium, 50 g/l xylose + 20 g/l glucose



S. cerevisiae TMB 3001 contains XR and XDH from *P. stipitis* and endogenous XKS (Eliasson *et al.* 2000)

Roca et al (2004) AMB, **63,** 578

By –product formation and redoxbalance



- Xylitol production results as a by-product due to differences in co-factor dependence during xylose utilisation

- Glycerol production results due to redox imbalances occuring during growth

Redox constraints limit ethanol production in *Saccharomyces cerevisiae*

Biochemical Pathways

325 biochemical reactions involve oxidations/reductions; redox cofactors mediate electron flow in the cell

NADPH: reductive reactions (biosynthetic)

NAD^+: oxidative reactions, sink for electrons

Regeneration of cofactors necessary

No transport of redox equivalents between the cellular compartments

Redox constraints limit ethanol production in *Saccharomyces cerevisiae*

Redirecting redox fluxes during ammonia assimilation in xylose utlising yeast led to:

Recombinant strain had:

- 12 % improved xylose uptake rate
- 16 % increased ethanol yield on xylose
- 45 % decreased xylitol yield

And

- 8 % increased ethanol yield on glucose
- 35 % decreased glycerol yield

The cell reacted to the genetic changes by modification of its redox fluxes in unrelated parts of the metabolism

Roca et al (2003) AEM, **69,** 4732, Nissen et al.

Use of undirected methods for strain improvement

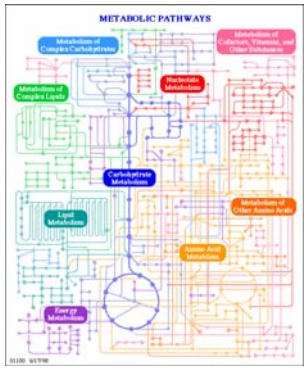
Why?
When the genetic background for the properties that needs to be improved not are know

How?

- Directed evolution
  - ✓Survival of the fittest under specifically designed growth conditions

- What?

✓Improved xylose utilisation rate
✓Improved inhibitor tolerance

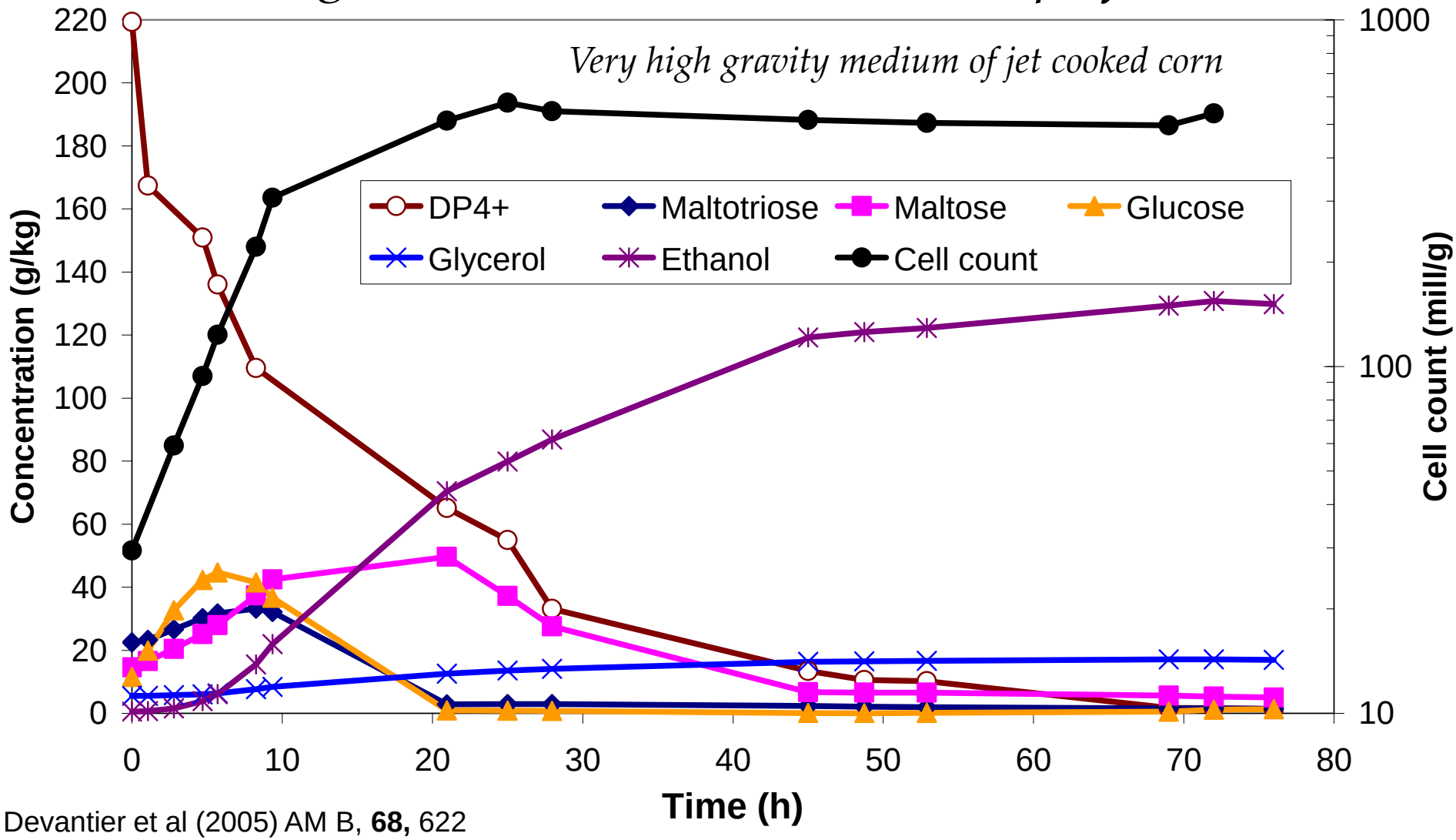


Important properties for a microbial cell factory to ferment lignocellusics

- Robust strain background
- Stress tolerance
- High product tolerance
- Process robustness
- Propagation procedure
- Nutrional requirements

Zaldivar et al. (2001) AMB, **56,** 17, Albers, unpublished results

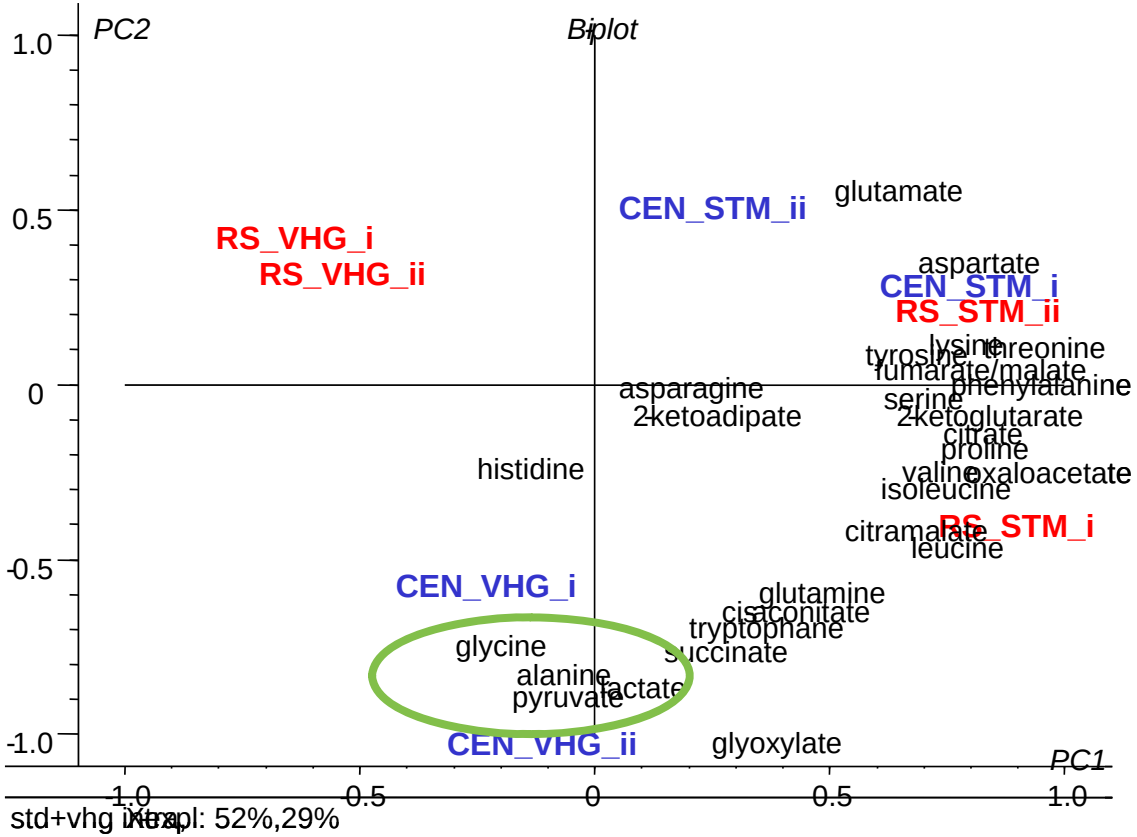
Industrial like conditions put a lot of stress onto the microorganism which leads to reduced performance



Devantier et al (2005) AM B, **68,** 622

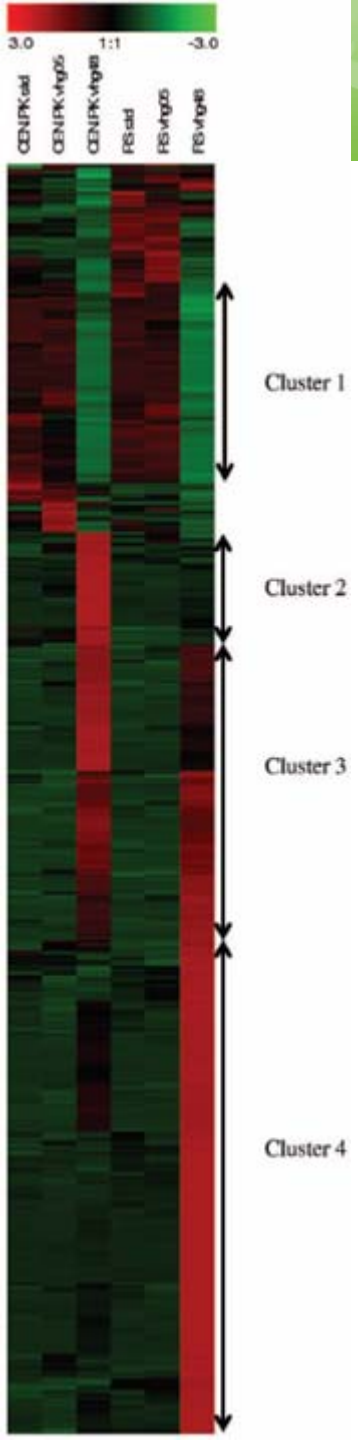
Metabolome analysis showed that:

- *The metabolite profile is characterised by the growth conditions*
- *Very high gravity fermentations is characterised by a high energy demand*



Devantier et al (2005) BB, **90,** 703

Strain background

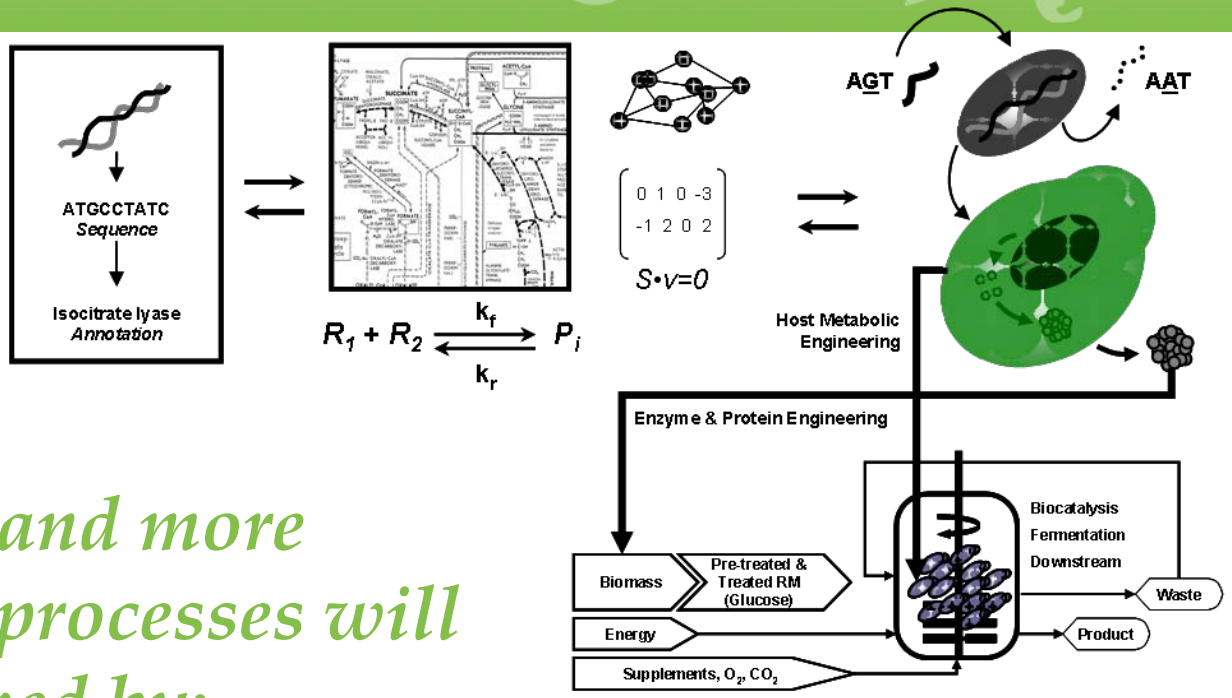


Physiological characterisation showed that the laboratory strain grew poorly under industrial conditions

The difference between stationary phase and growth phases was much larger than between media

The industrial Red Star strain up-regulated many more stress-related genes in stationary phase than the lab strain

Devantier et al (2005) Ind Biotechnol, **1,** 51



Cheaper and more efficient processes will be achieved by:

- Full use of the technology platform resulting in the post-genomic era
- Design of cell factories suitable for renewable raw materials
- Create knowledge about stress factors influence on cellular behaviour and identify cellular traits that lead to increased robustness
- Understanding of the whole processes and the interplay between different process steps

Otero et al (2007) AdvBiotechnol Biochem Eng 108, 1

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