

MEMORE

Metabolic Engineering and Metabolic Modeling of *Escherichia coli* for the Production of Chemicals from Renewable Resources

Jo Maertens

Metabolic Engineering VII



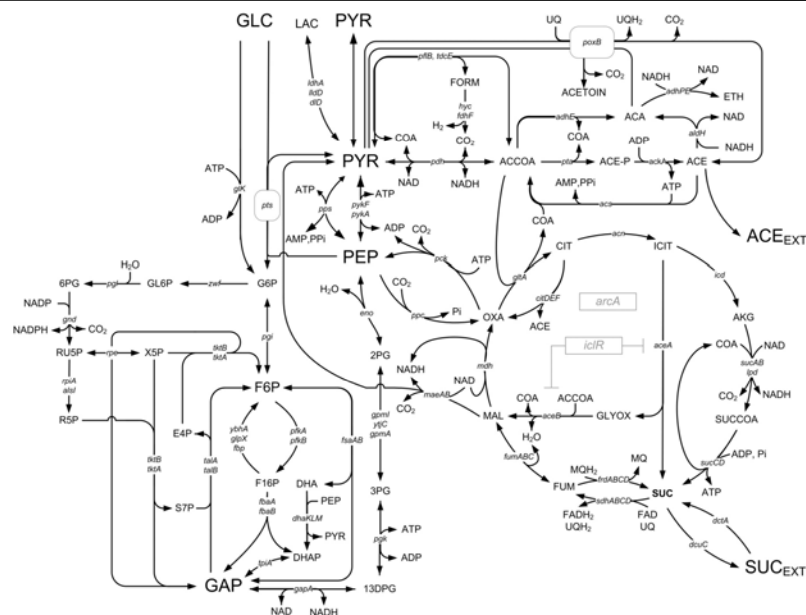
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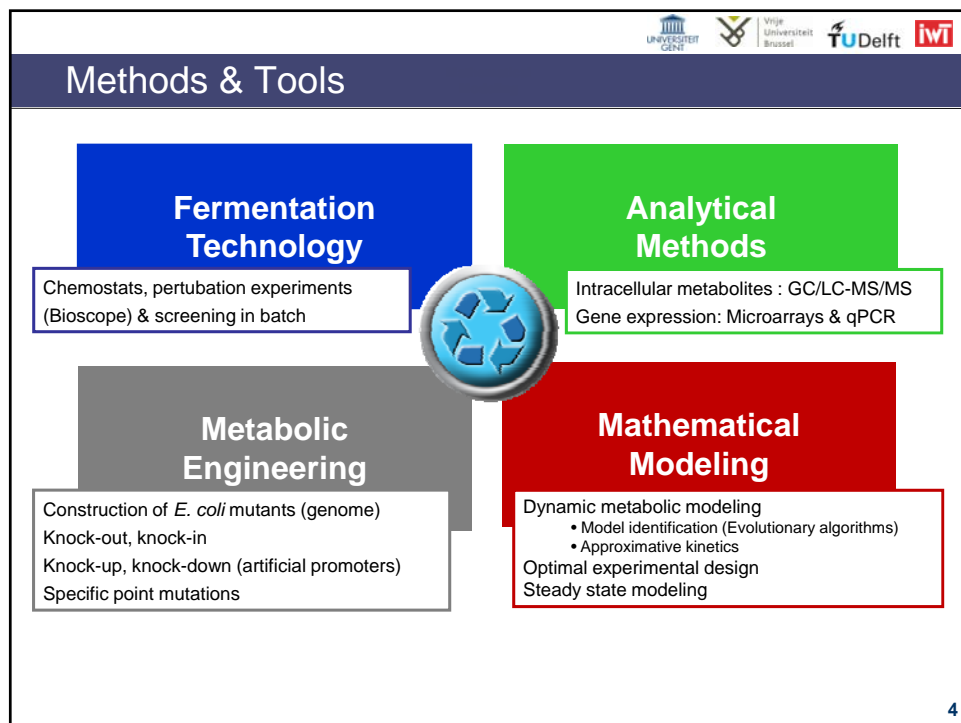
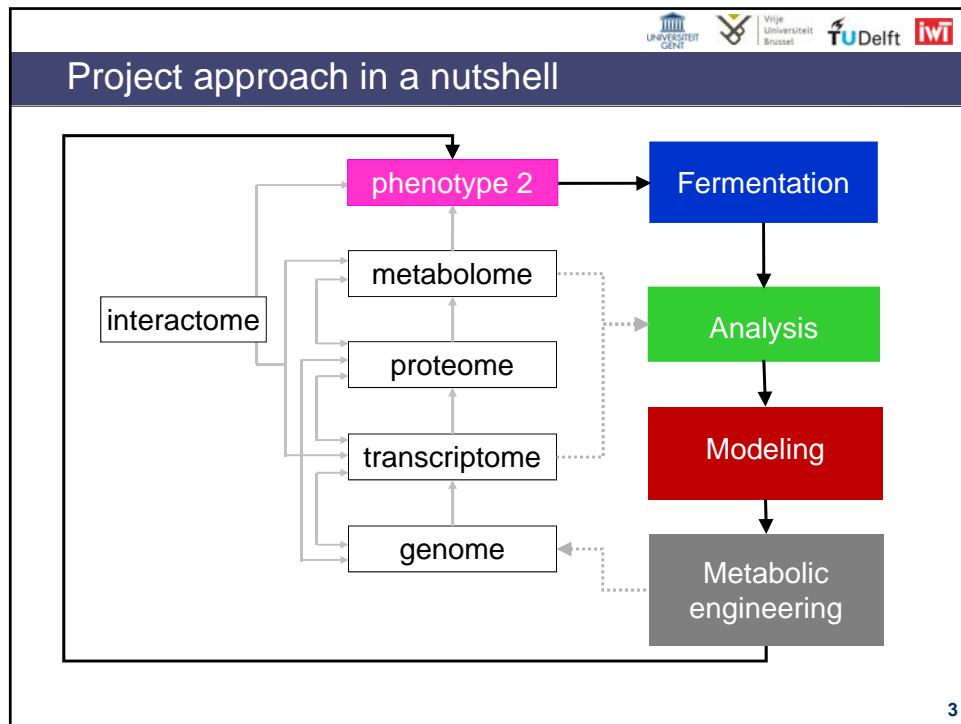
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E. coli metabolism



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The simple life: Garbage in = garbage out

- Variables not considered
- Negligibility assumptions
- Inaccurate data
- Accurate data

Exact and detailed mathematical analysis

Misleadingly wrong conclusions

The simple life: Garbage in = garbage out





- Variables not considered
- Negligibility assumptions
- Inaccurate data
- Accurate data

Need for reliable data,

Good Modeling Practice, ...

Exact and detailed mathematical analysis

Misleadingly wrong conclusions

Methods & Tools

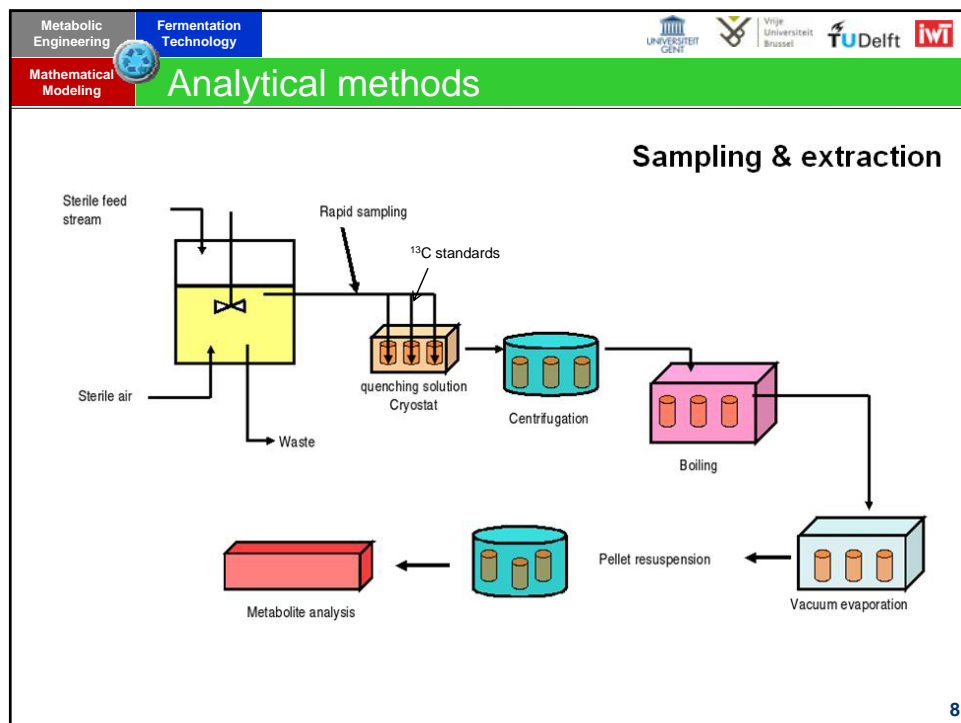
Metabolic Engineering

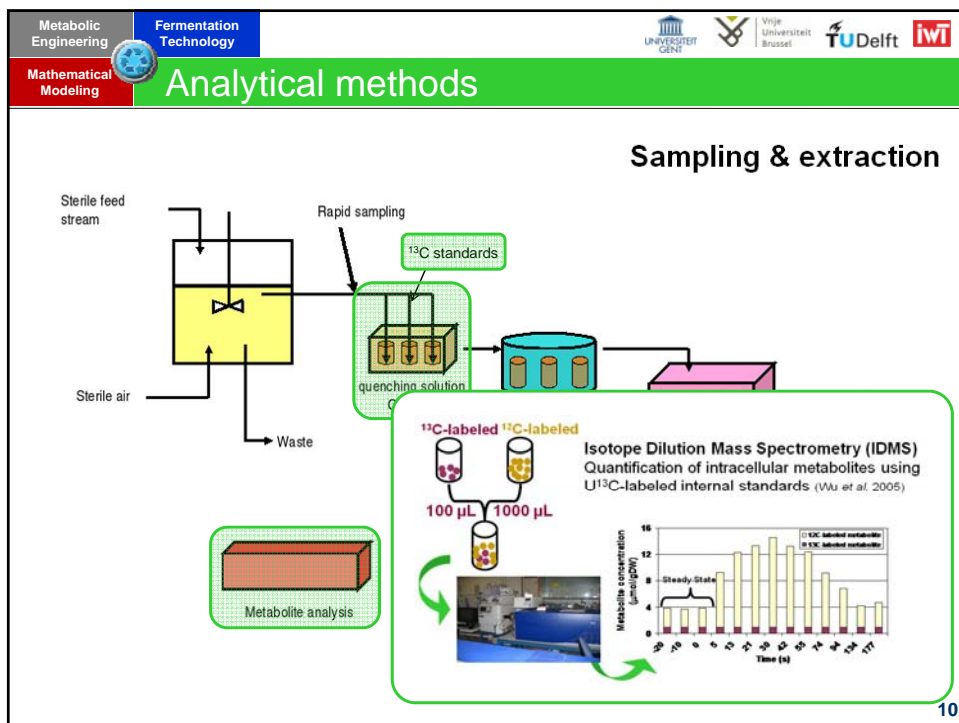
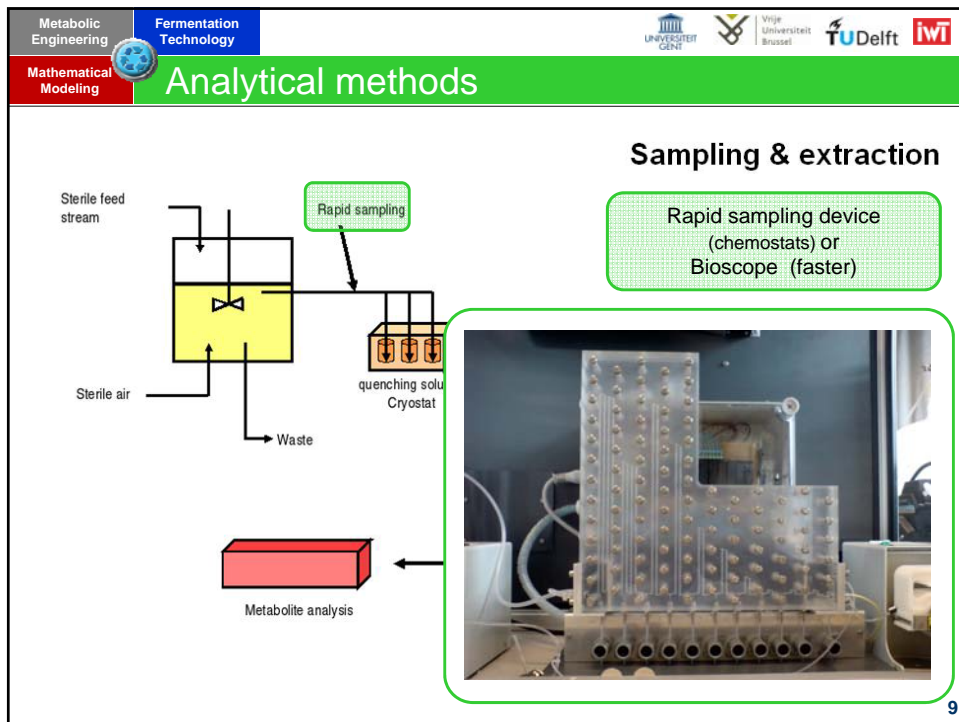
Fermentation Technology

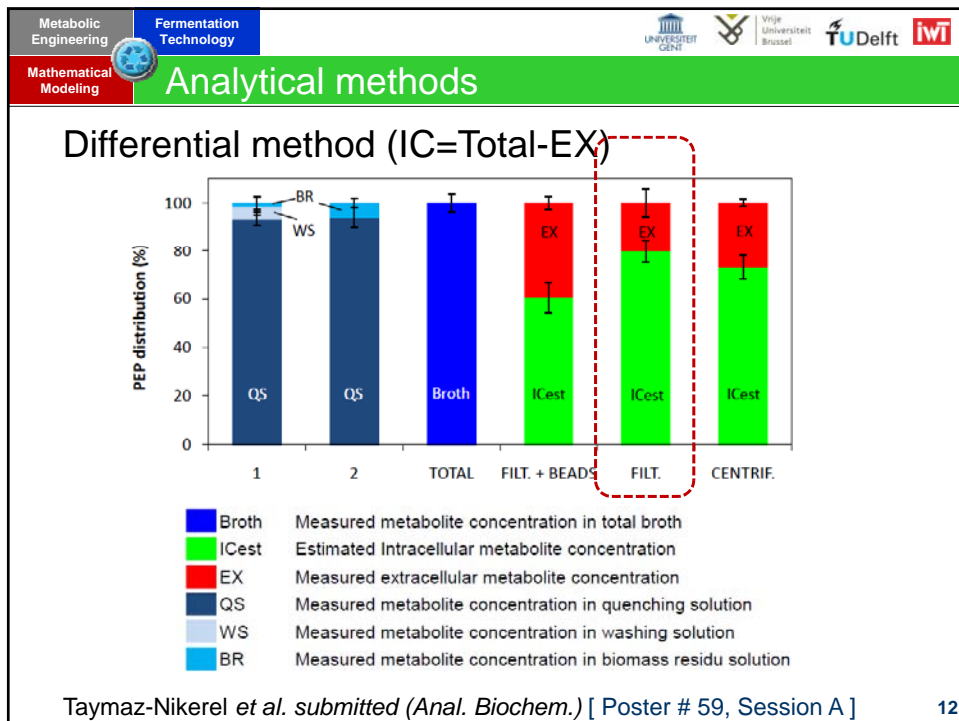
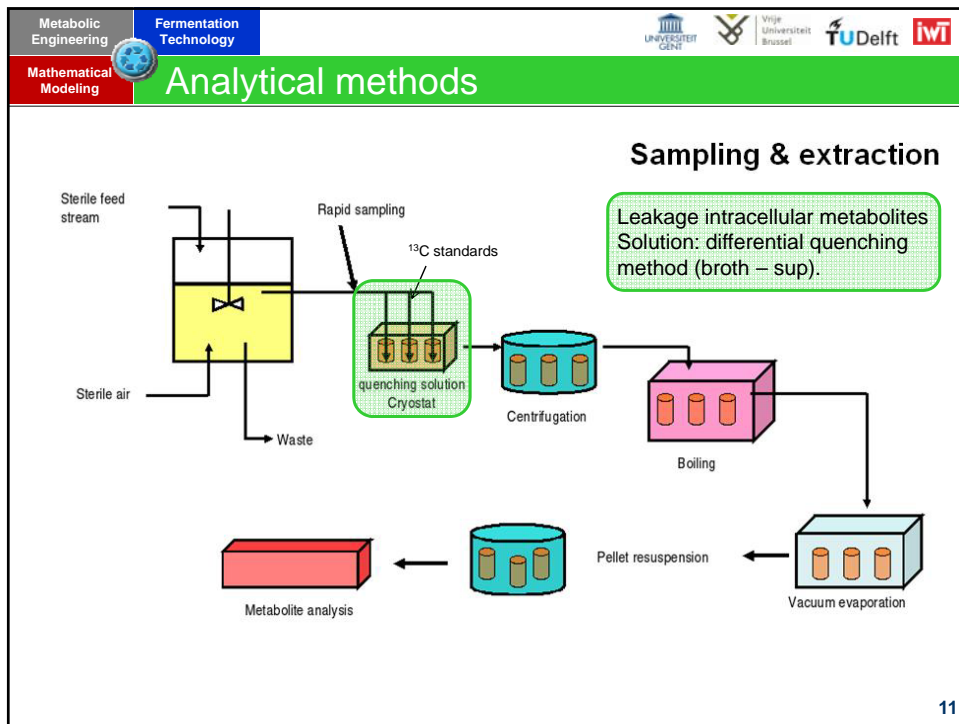
Mathematical Modeling

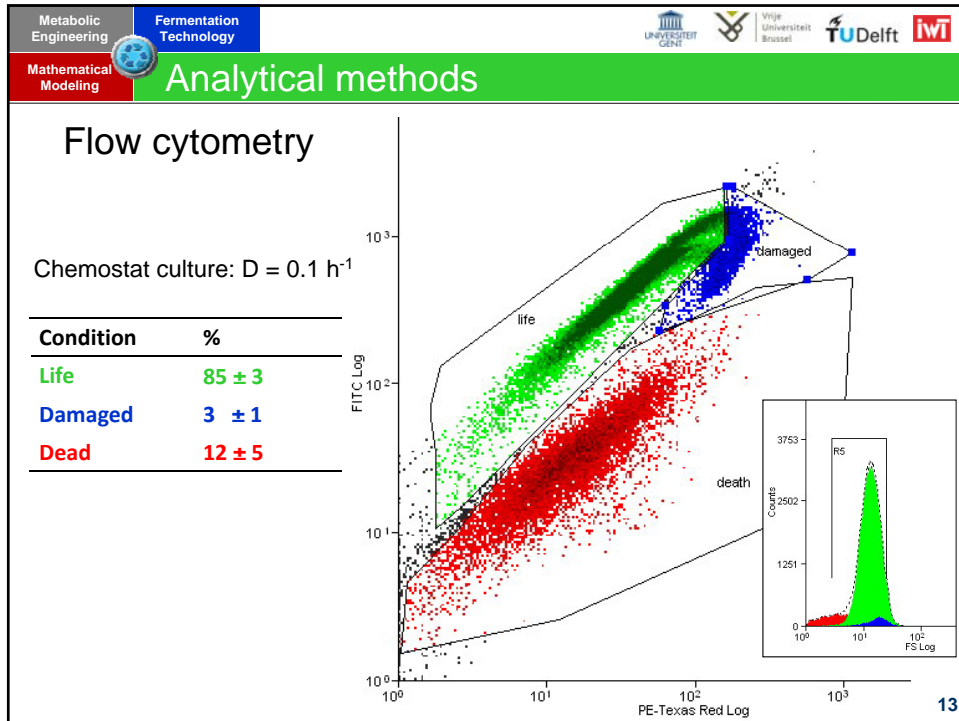
Analytical Methods

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Fermentation Technology

Mathematical Modeling

Analytical Methods

Methods & Tools

Mathematical Modeling

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Fermentation Technology

Metabolic Engineering

Analytical Methods

Mathematical Modeling

Metabolic modeling

- Dynamic metabolic modeling
 - Model structure identification (Evolutionary approaches)
Genetic programming (GP), Genetic algorithms (GA)
 - Optimal experimental design for model discrimination (OED-MD)
 - Optimal experimental design for parameter estimation (OED-PE)
 - Approximative kinetics [Poster # 57, Session A] & Cybernetic modeling
 - Hidden Markov models (HMM)
- Steady state metabolic modeling
 - Elementary flux modes, Metabolic flux analysis, Flux balance analysis
- Multivariate data analysis (MVDA)
 - Partial least squares (PLS)
 - Graph theory (GT)

Modeling based metabolic
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Dynamic metabolic modeling approach

```

graph TD
    A[Initial candidate models] --> B[population of models]
    B --> C[EA operations: crossover, mutation]
    C --> D[Selection of models]
    D --> B
    B --> E[Fitness calculation]
    E --> F[Best model(s)]
    F --> G[Optimal experimental design for model discrimination]
    G --> H[Best model]
    
```

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Genetic programming (GP)

Candidate model

$$a_1 - (S_1 / a_2) + a_3 * \ln(S_2)$$

Time (s)

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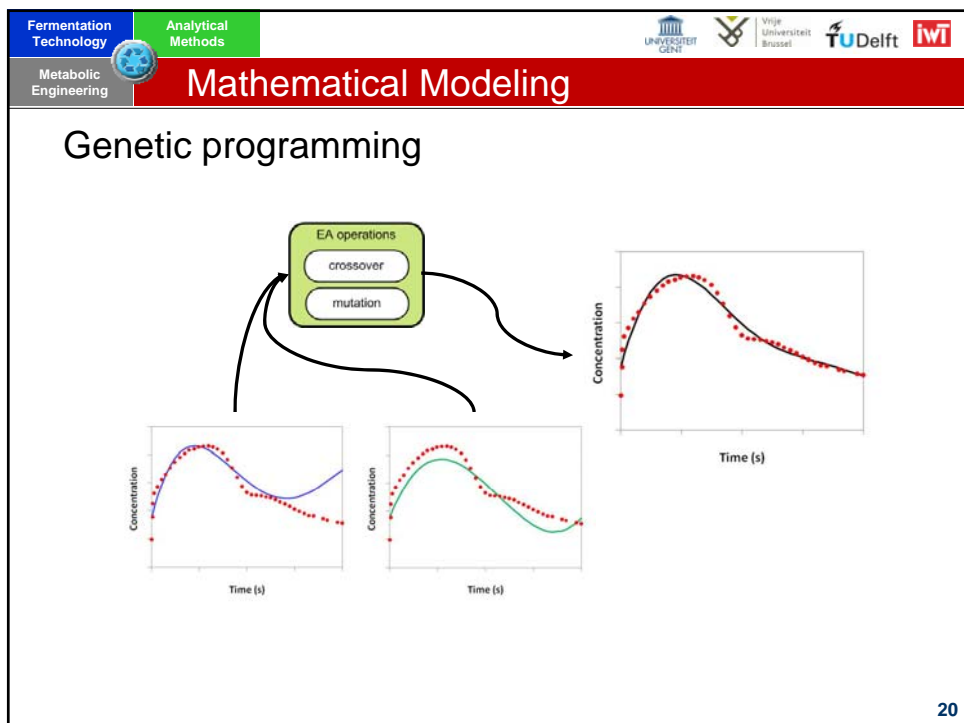
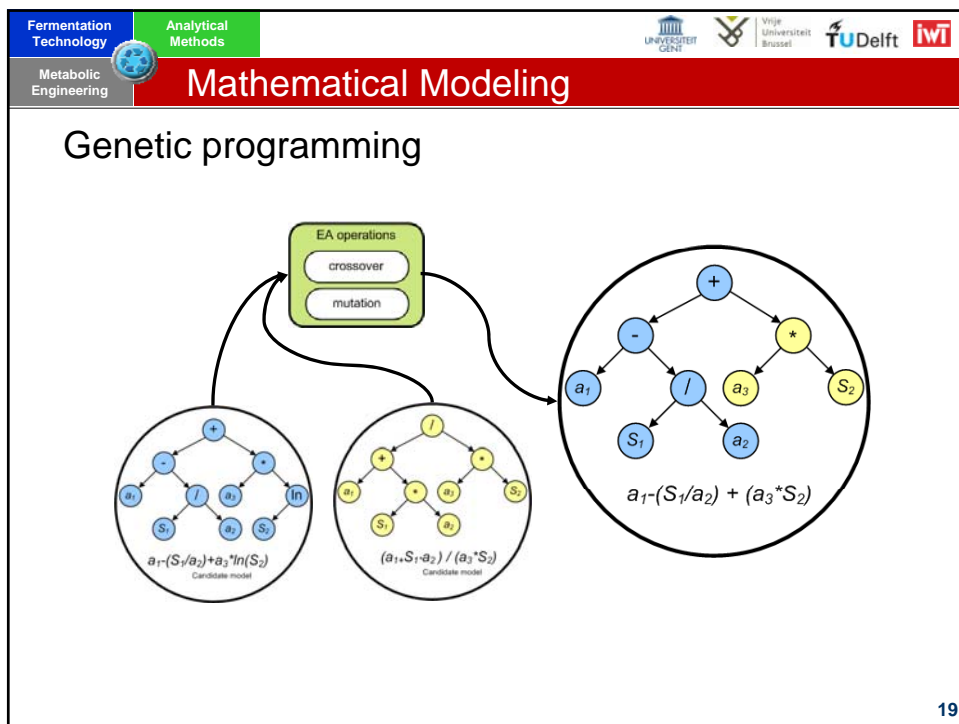
Genetic programming (GP)

Candidate model

$$(a_1 + S_1 \cdot a_2) / (a_3 \cdot S_2)$$

Time (s)

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Genetic algorithms (GA) based kinetic model structure identification: Glucose-6-phosphate dehydrogenase (v_{G6PDH})

```

graph LR
    GLC --> G6P
    G6P -.->|glycolysis| Glycolysis
    G6P -- NADP --> 6PG
    6PG -- NADPH --> G6P
    6PG -.->|pentose phosphate pathway| P5P
    6PG -.->|Entner-Doudoroff pathway| ED
    
```

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Genetic algorithms (GA) based kinetic model structure identification: Glucose-6-phosphate dehydrogenase (v_{G6PDH})

Time (s)	Concentration (mM)
0	3.5
5	4.8
10	5.1
12	5.2
15	5.1
20	4.5
25	4.2
30	4.0
35	3.8
40	3.7

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Genetic algorithms (GA) based kinetic model structure identification: Glucose-6-phosphate dehydrogenase (v_{G6PDH})

Kinetic model

$$v_{G6PDH} = \frac{1.2559 \cdot C_{NADPH}^2 + 1.2206 \cdot C_{NADP} \cdot C_{G6P} + 1.1527 \cdot C_{G6P} \cdot C_{NADPH} + 1.4743 \cdot C_{G6P} \cdot C_{NADP} \cdot C_{NADPH} + 1.2348 \cdot C_{G6P} \cdot C_{G6P} \cdot C_{NADPH} + 0.89416 \cdot C_{NADP} \cdot C_{G6P} \cdot C_{NADPH}}{C_{G6P} + 0.89562 \cdot C_{NADPH}^2 + 1.2638 \cdot C_{G6P} \cdot C_{NADP} + 0.89712 \cdot C_{G6P} \cdot C_{G6P} + -0.2745 \cdot C_{G6P}^2 \cdot C_{G6P} + 0.85057 \cdot C_{NADP}^2 \cdot C_{NADPH} + 1.209 \cdot 1}$$

Generation: 15, Fitness: 1.246379e-001, Complexity: 13

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Analytical Methods

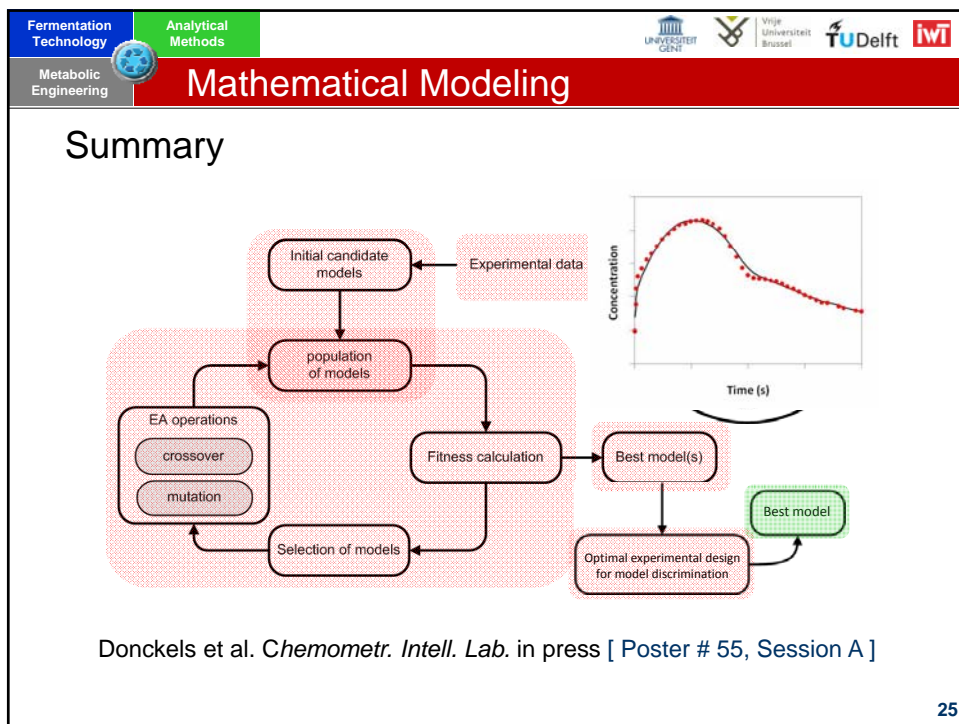
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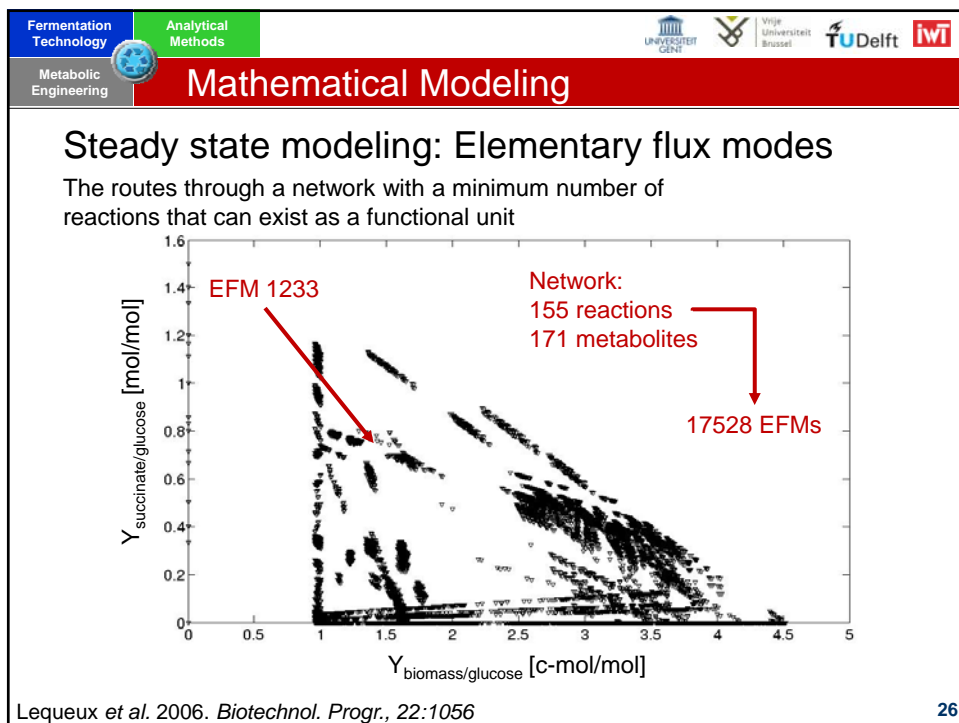
OED for model discrimination (v_{G6PDH})

Donckels et al. *Chemometr. Intell. Lab. in press* [Poster # 55, Session A]

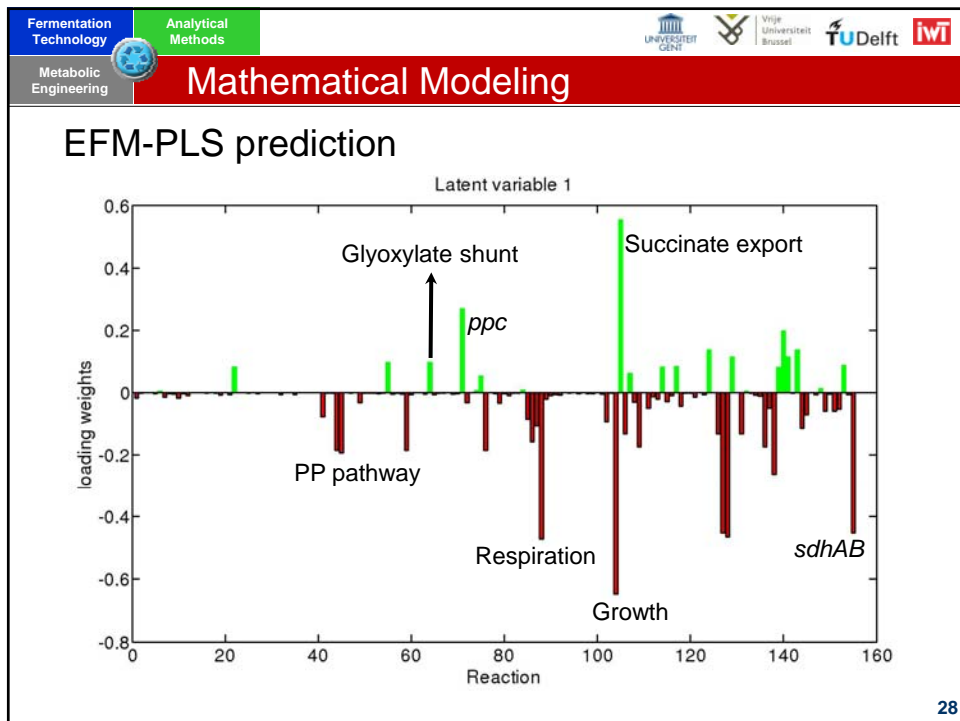
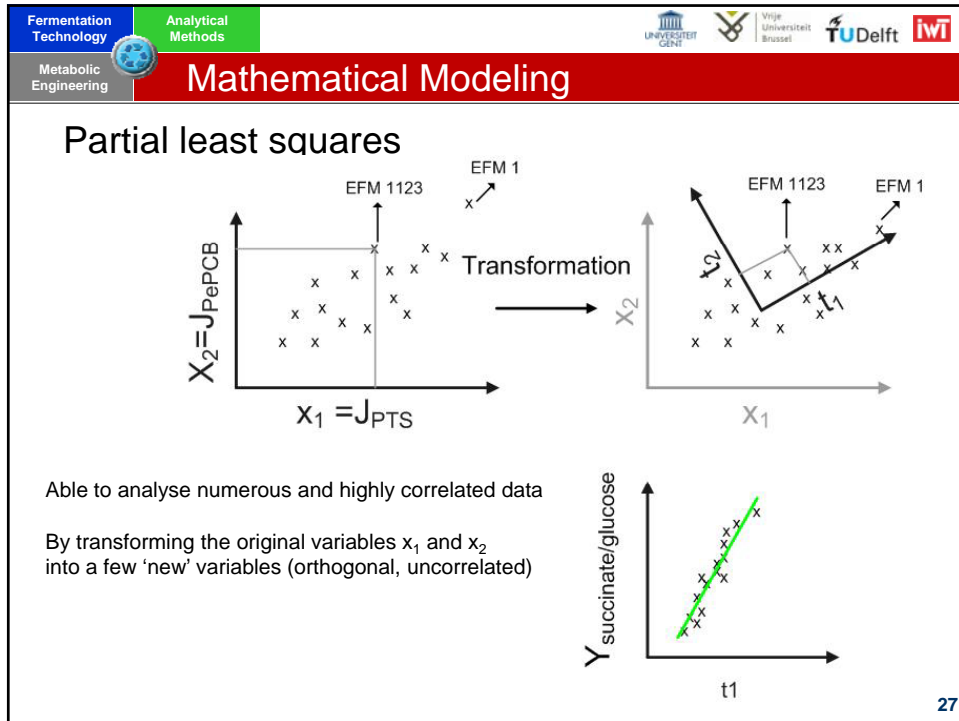
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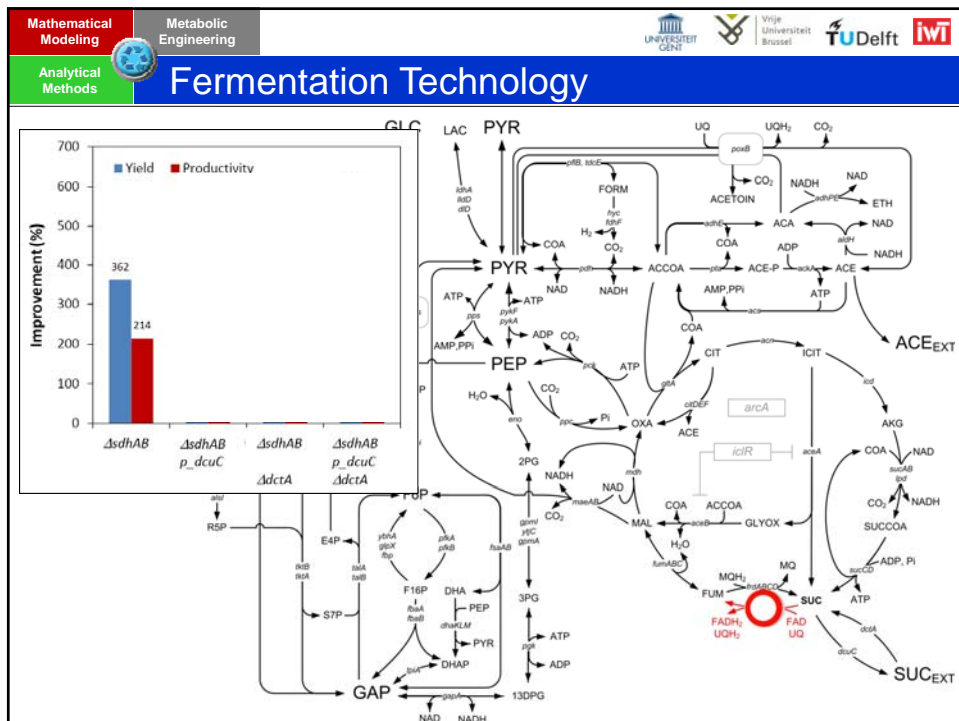
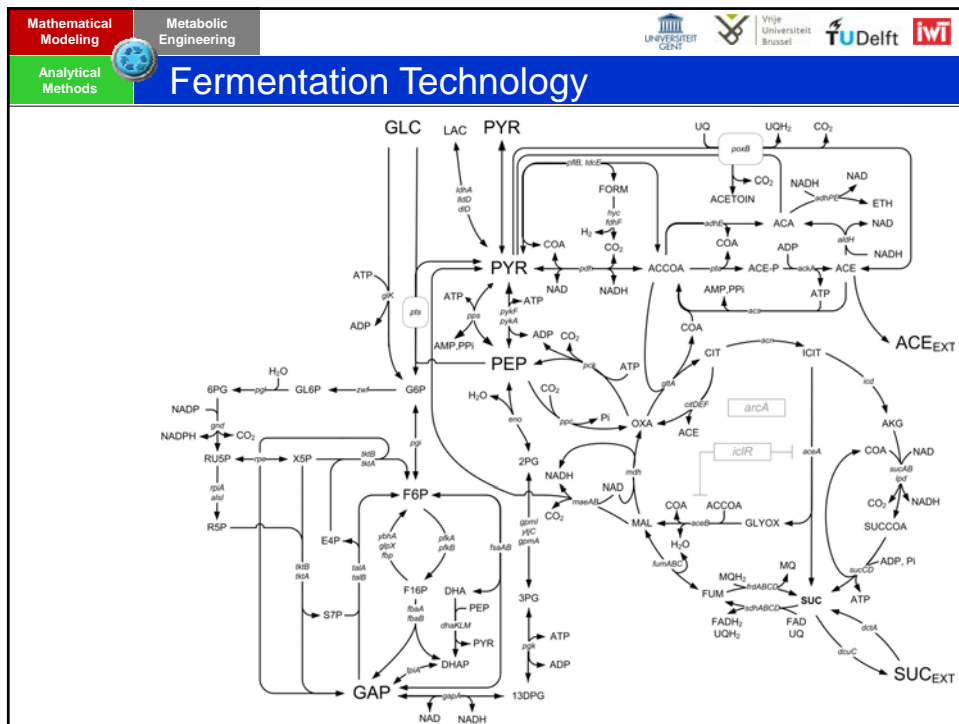


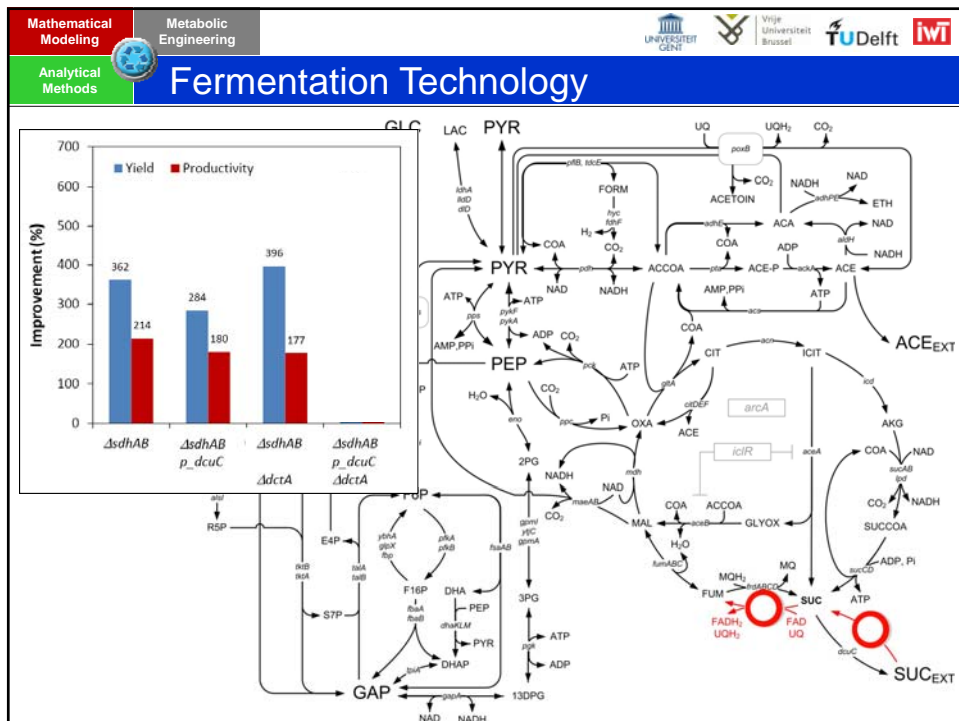
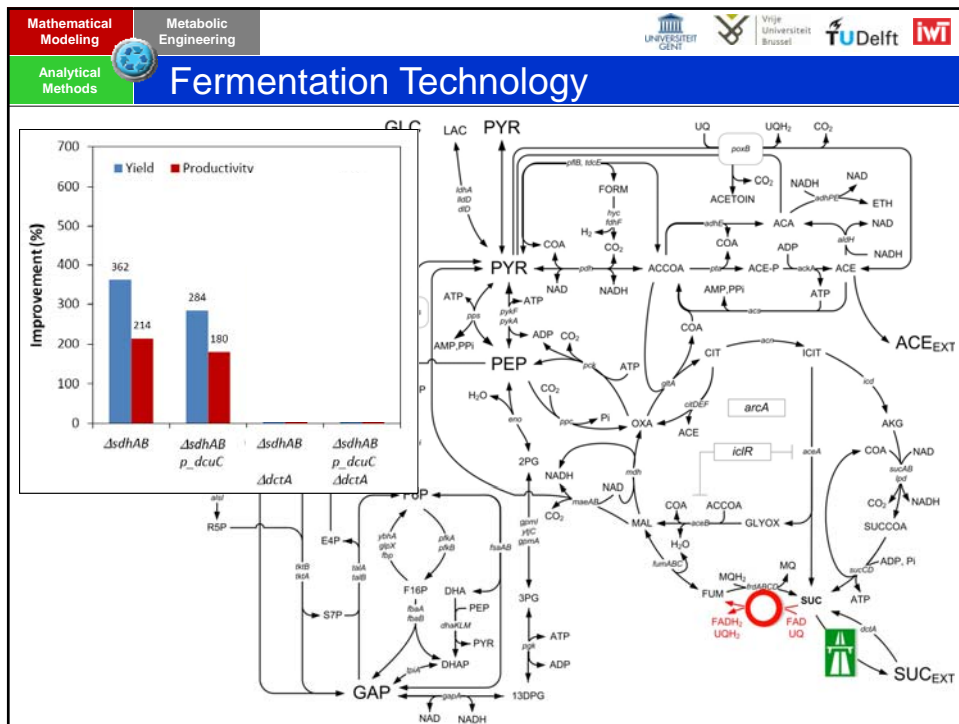
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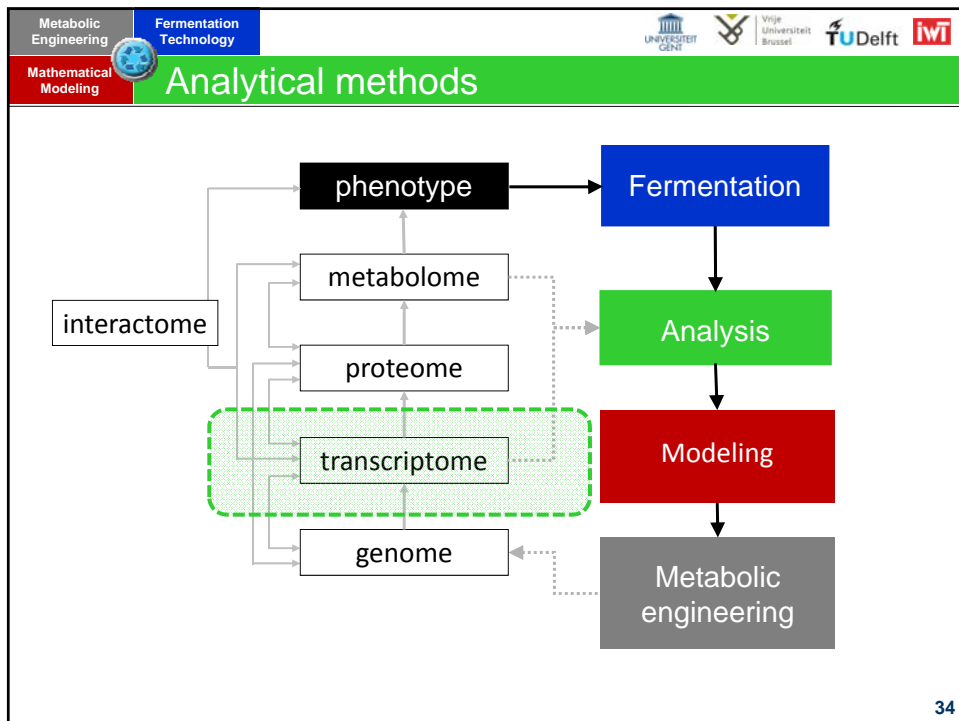
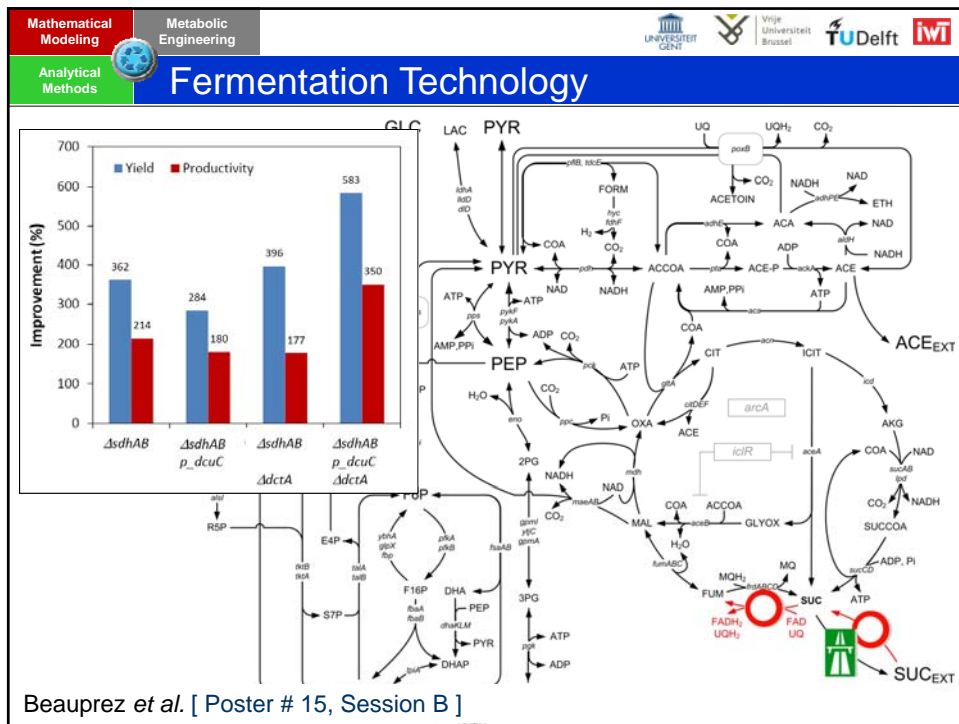


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Analytical methods



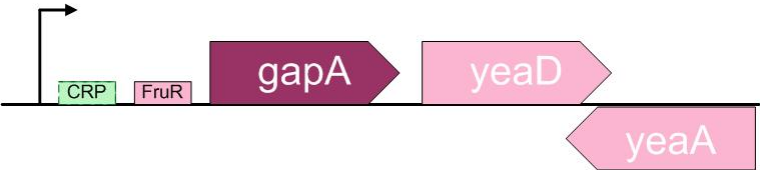
RT-qPCR: normalization

RT-qPCR normalization

- Eliminate non-biological variation
- Variation due to e.g.
 - Extraction efficiencies
 - cDNA synthesis

Reference genes

- Should be stably expressed
- Should not show variations under studied conditions
- At present: 16S rRNA, gapA




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RT-qPCR: reference genes

ATCTCGACGA AATGGCTGCA CCTAAATCGT GATGAAAATC ACATTTTAT [-250 -201]
CRP

- Transcription factor binding sites (TFBS)
 - Are well-conserved between diverse species
 - Show a slower rate of change than in other parts
- Predicting presence of TFBS
- Detection
 - Multiple (n-way) alignment
 - PhastCons (Phylogenetic Analysis with Space/Time models)

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Analytical methods

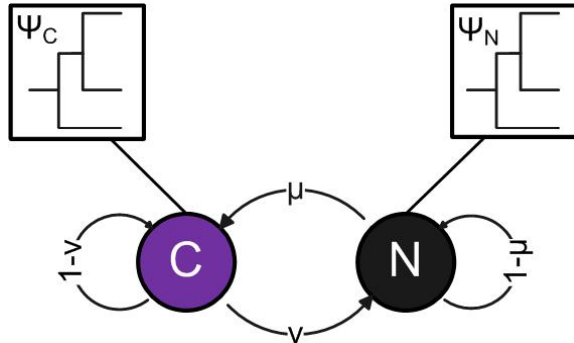
PhastCons

Phylo-HMM

States:
Conserved
Non-conserved

Observations:
A,C,G,T

Space:



Specie 1:GACGAAATGGCTGCACCTAAATCGTGATGAAATCACATT [-250 -201]

Specie 2:GACGAA?TGGCTGCACTAAATCGTGATGAA?ATCACATT [-250 -201]

Analytical methods

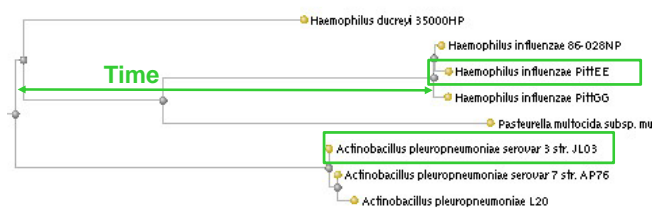
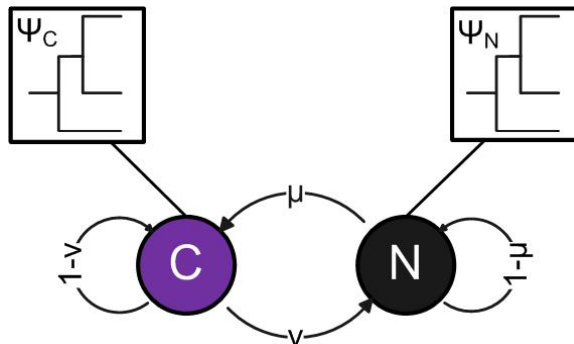
PhastCons

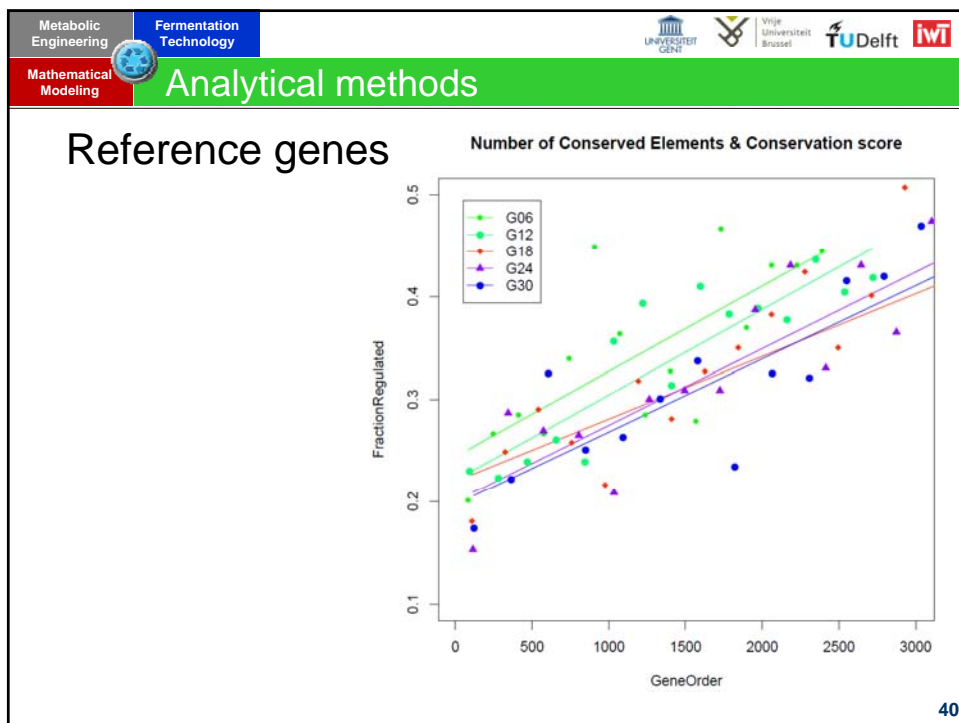
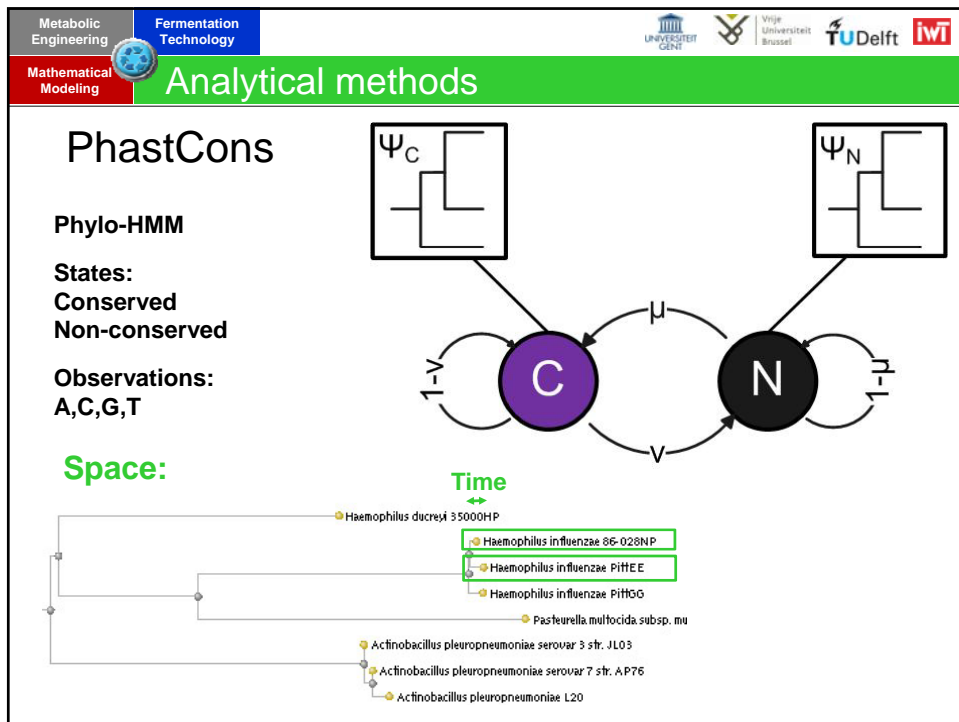
Phylo-HMM





States:
Conserved
Non-conserved

Observations:
A,C,G,T

Time:











Methods & Tools

Analytical
Methods





Fermentation
Technology

Mathematical
Modeling



Metabolic Engineering


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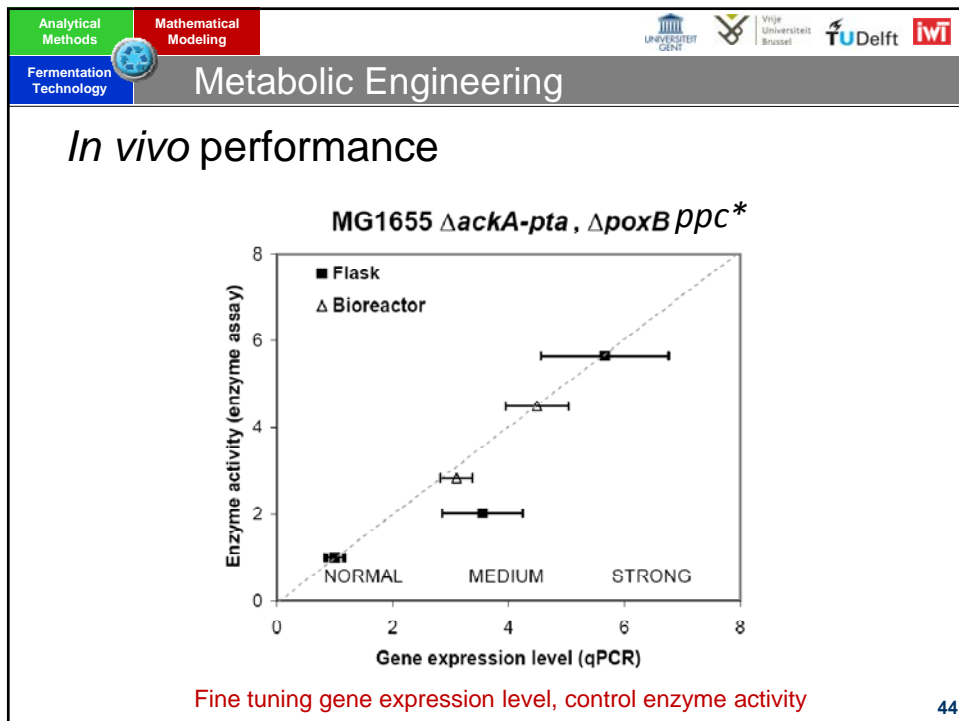
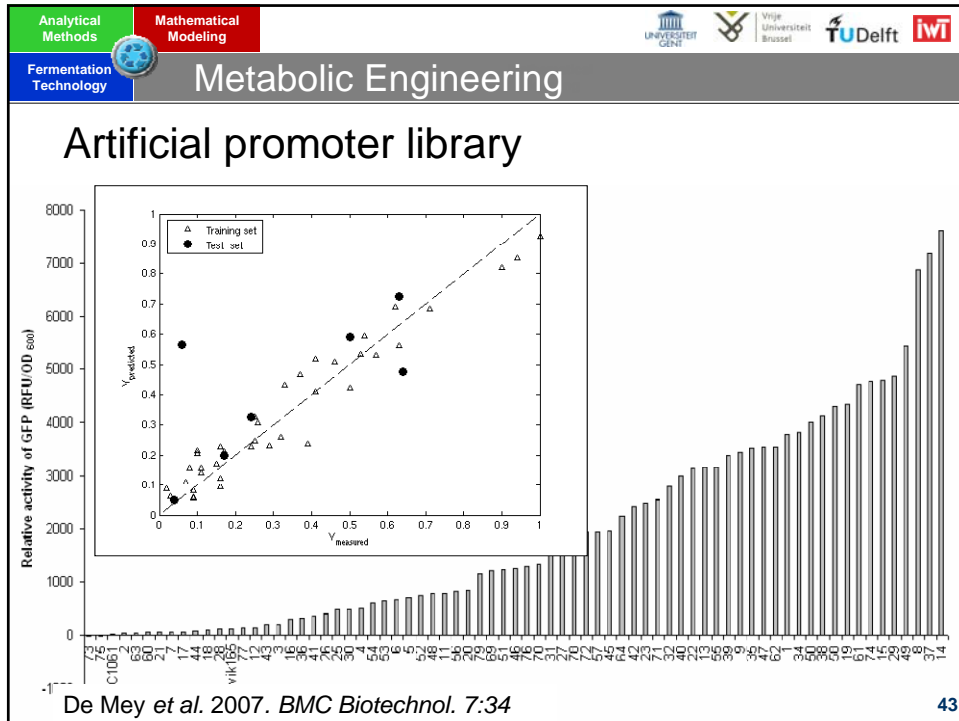
Metabolic Engineering

Genetic modification toolbox

- Protocol for (multiple) gene knock-outs
 - fast and reproducible
 - no antibiotic resistance left in mutant strain
- Protocol for (multiple) gene knock-ins
 - fine-tuning of gene expression for control of enzyme activity

→ Artificial promoter library
- Protocol for specific point mutations
 - Remove regulatory constraints
 - High efficiency strategy has been developed

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Summary

- metabolic engineering is more than just the math.
- metabolic engineering is more than just the analytics.
- metabolic engineering is more than just the fermentations.
- metabolic engineering is more than just the modifications.

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The MEMORE team



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<http://biomath.ugent.be/~memore>