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Validation of a Saccharomyces cerevisiae Dynamic Flux Balance Model

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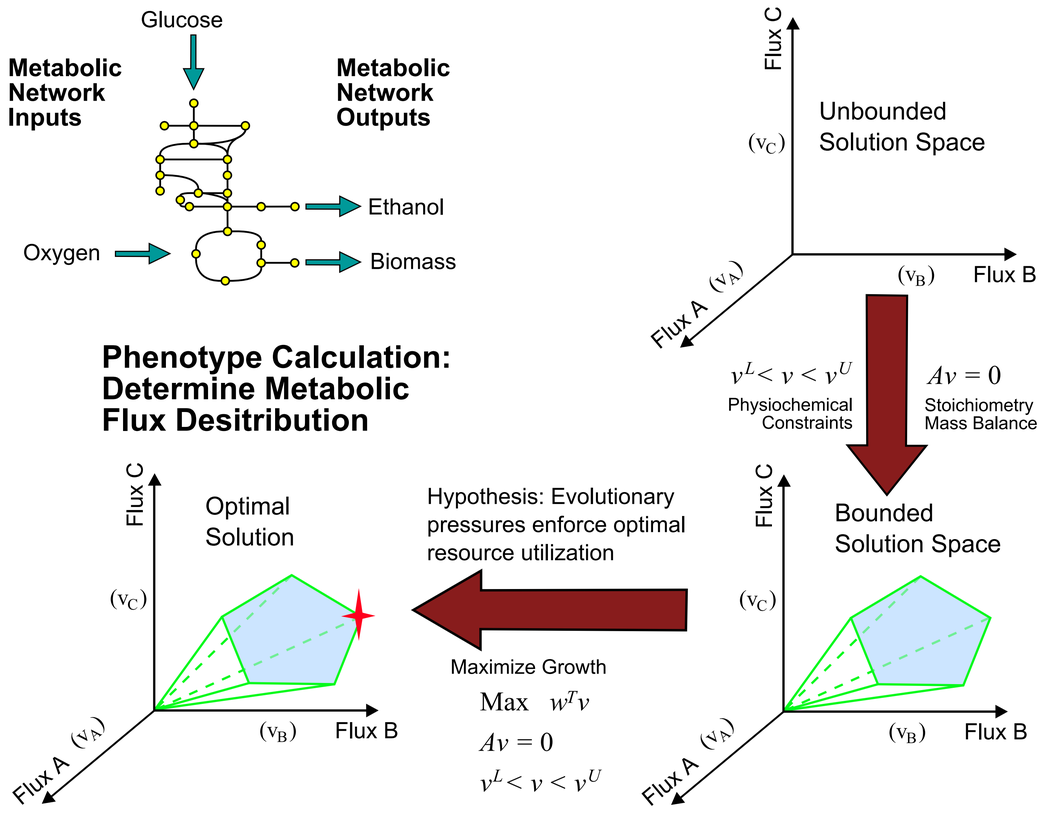
Classic flux balance analysis



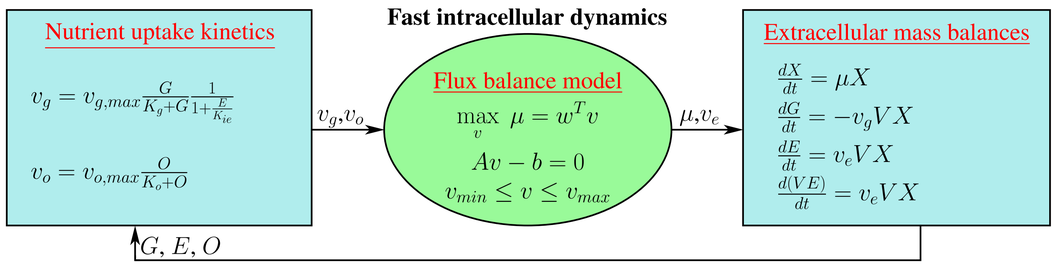
Limitations

- Strictly applicable only to balanced growth
- Cannot account for batch and fed-batch cultures
- Limited capability for including cellular regulation
- Inadequate for feeding policy optimization & cellular engineering in fed-batch culture

S. cerevisiae Genome-scale metabolic network

- *Saccharomyces cerevisiae i*ND750 (Duarte *et al.*, 2004)
- Genome-scale reconstruction of genes, transcripts, & reactions in *S. cerevisiae* metabolism
- 750 genes & 1149 reactions; compartmentalized and fully charge & elementally mass balanced
- 646 unique metabolites, 1059 balanced species & 1249 fluxes

Dynamic flux balance analysis



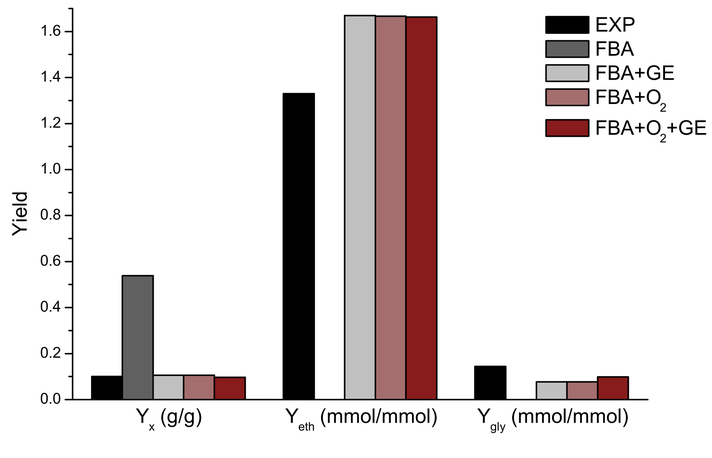
- Sequential solution proved inefficient & unreliable
- Simultaneous solution (Hjersted *et al.*, 2007)
  - Embed LP problem within integration subroutine
  - Use MOSEK (LP solver) interface to Matlab
- Computation time
  - Small-scale metabolic network: 7.3 sec
  - Genome-scale metabolic network: 13.5 sec
  - Inner flux balance model scales well

Objectives

- **Analysis of regulatory effects**
  - Consider gene expression data from a recent study by Åkesson *et al.* (2004)
  - Evaluate the effect of genetic regulation on dynamic flux balance model predictions by constraining reaction fluxes that are exclusively associated with experimentally absent genes
  - Analyze model predictions with respect to the oxygen uptake rate
- **Evaluation of metabolic engineering strategies**
  - Apply previously identified metabolic engineering strategies to the dynamic flux balance model
  - Assemble and directly screen a library of candidate gene insertion targets for fed-batch ethanol performance
- **Preliminary experimental validation**
  - Estimate model parameters from experiments and compare model predictions

Analysis of regulatory effects

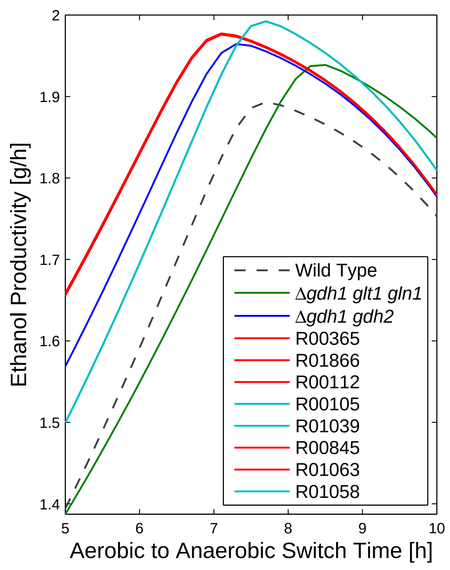
Quantitatively accurate predictions can still be obtained in the absence of detailed gene expression data by constraining oxygen uptake



Comparison of batch culture yields for biomass, ethanol, and glycerol from Åkesson *et al.* (2004) (EXP, FBA, FBA + GE) to two additional cases (FBA + O_2, FBA + O_2 + GE). The legend labels indicate experimental data (EXP), unconstrained prediction (FBA), gene expression constrained prediction (FBA+GE), oxygen uptake constrained prediction (FBA + O_2), and oxygen uptake and gene expression constrained prediction (FBA + O_2 + GE).

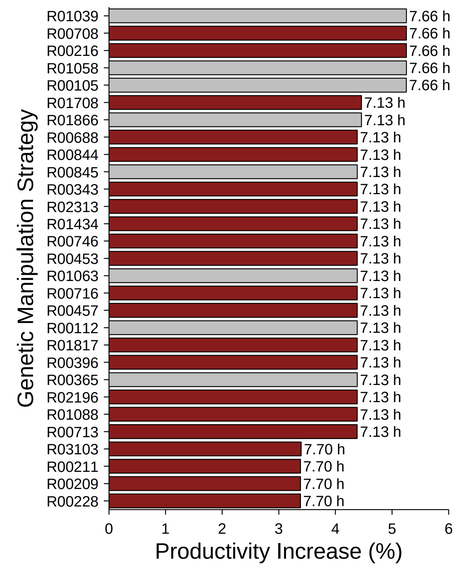
Evaluation of metabolic engineering strategies

Optimal performance depends on both the metabolic engineering strategy and the fed-batch operating policy



Sensitivity of the ethanol productivity to the aerobic-anaerobic switching time (t_s) for the wild-type and 10 genetic manipulation strategies. The dotted line indicates the optimal switching time for the wild type strain.

Gene insertions for ethanol overproduction in fed-batch culture can be directly identified



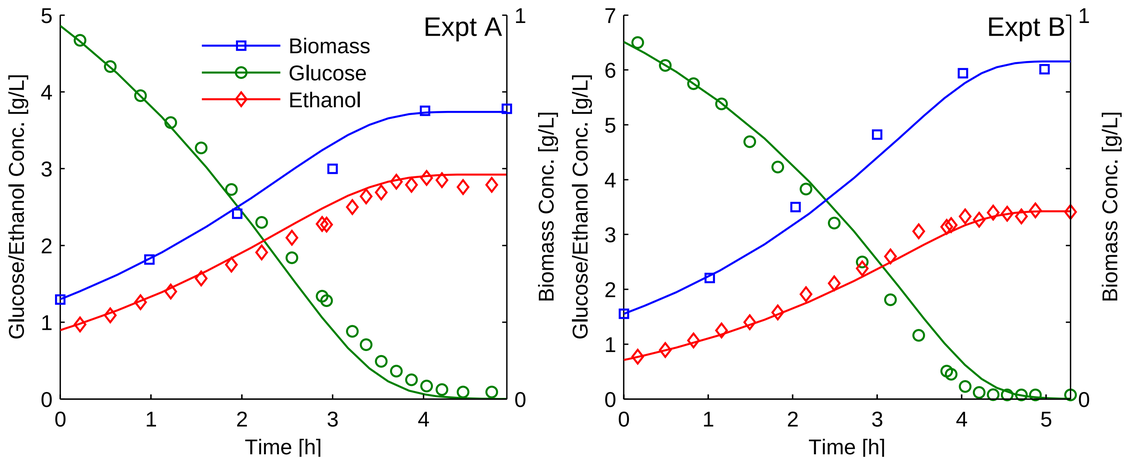
Dynamic screening of a gene insertion library derived from the KEGG database for optimal fed-batch ethanol productivity. Insertions proposed by Bro *et al.* (2006) are shown as black bars. The number indicated to the right of each bar indicates the optimal aerobic-anaerobic switching time.

Preliminary experimental validation

- Anaerobic batch experiments were conducted to compare with model predictions
- Wild-type *S. cerevisiae* (ATCC 32167) was grown with a defined medium in a nitrogen sparged environment
- Biomass was directly weighed following centrifugation and drying
- Ethanol and glucose were measured online with a YSI 2700 biochemistry analyzer
- A dynamic programming approach was used to estimate the glucose uptake parameters , v_{gm} and K_g

Parameter	Previous Value	Experiment A	Experiment B	Average Value	Units
v_{gm}	20.0	20.9	22.9	21.9	mmol/g/h
K_g	0.50	0.82	0.70	0.76	g/L

Dynamic flux balance model predictions show quantitative agreement with anaerobic batch experiments



Comparison of model predictions (lines) and experimental measurements (symbols) for batch A (left) and batch B (right) where the average of the estimated parameter values were used for both simulations.

Conclusions

- **Analysis of regulatory effects**
  - Showed that quantitatively accurate predictions of cellular growth and the exchange rates for primary metabolites such as ethanol and glycerol can be obtained in the absence of detailed regulatory data
- **Evaluation of metabolic engineering strategies**
  - Showed that metabolic engineering strategies can be dynamically screened for fed-batch performance
  - Uncovered several new experimentally testable genetic manipulation targets for enhanced ethanol production in fed-batch culture
- **Preliminary experimental validation**
  - Demonstrated quantitative agreement between anaerobic batch experiments and model predictions
- **Future work**
  - Incorporate dynamic regulatory effects
  - Develop more sophisticated dynamic optimization strategies
  - Experimentally evaluate computationally identified mutants for ethanol overproduction

References

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