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Mannitol production in *Lactococcus lactis*: dynamic modeling, metabolic control and regulation

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1 Abstract

The dynamic modeling of metabolic networks constitutes a major challenge in systems biology. A top-down approach can be conducted using experimental time series of metabolite concentrations, obtained through Nuclear Magnetic Resonance (NMR), and defining the model structure as a system of non-linear coupled differential equations. An important class of equations under Biochemical Systems Theory is used, where rates are modeled with power-law functions.

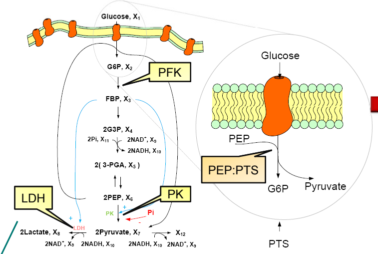
2 Introduction

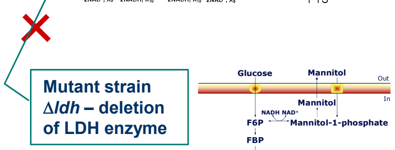
This work addresses the modeling of glycolysis in *Lactococcus lactis*, expanding a study recently conducted (Voit et al. 2006). The metabolic data used correspond to time series representing metabolite concentrations obtained by NMR (Neves et al. 2002).

The estimation of the parameters constitutes a major difficulty given the innumerous local minima and rough error surface. Although the obtained model might not correspond to global optima, it can nevertheless provide important insights into the design of the pathway and the function of specific feedforward and feedback activations and inhibitions.

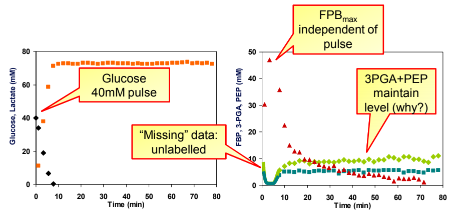
3 Methods

➢ Network topology

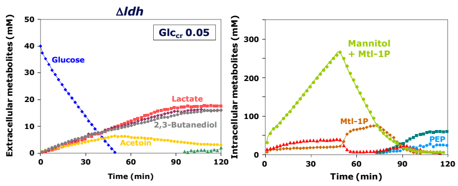




➢ Time series



Time course for [1-^{13}C]glucose (40mM) consumption/product formation and concentrations of intracellular metabolites under anaerobiosis in non-growing suspensions of *L. lactis* strain MG1363.



Metabolism of [1-^{13}C]glucose monitored by ^{13}C-NMR in non-growing suspensions of *L. lactis* strain MG1363Δ*ldh*. Kinetics of glucose consumption/end product formation and pools of intracellular metabolites.

➢ Differential equations

Biochemical systems theory

Models fluxes with differential equations

$$\dot{X}_i = \frac{dX_i}{dt} = f(X_1, X_2, \ldots)$$

taking Taylor series approximation in log space

- Scale properties (allometric, telescopic)
- Parameters have biochemical meaning
- Shown to be flexible to accommodate rich non-linear behavior

S-system

Kinetic orders

$$\dot{X}_i = \alpha_i \prod_{j=1}^{n} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n} X_j^{h_{ij}}$$

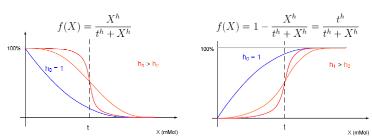
Rate constants

Generalized mass action

$$\dot{X}_i = \sum_{k=1}^{m} \pm \gamma_{ik} \prod_{j=1}^{n} X_j^{f_{ijk}}$$

Hill functions

New feature – introduction of control signals through switch-like function.
Can be modeled as a piecewise power-laws



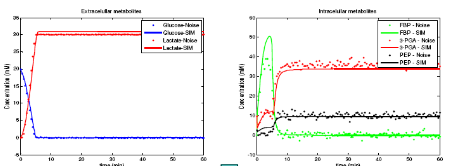
4 Results

$$\dot{X}_1 = -\beta_1 \frac{k_0 X_1}{t_0 + X_1} {X_6}^{\gamma_2}$$
$$\dot{X}_2 = \beta_1 \frac{k_0 X_1}{t_0 + X_1} {X_6}^{\gamma_2} - \beta_2 {X_2}^{\gamma_3}$$
$$\dot{X}_3 = \beta_2 {X_2}^{\gamma_3} - \beta_3 {X_3}^{\gamma_4}$$
$$\dot{X}_4 = 2\beta_3 {X_3}^{\gamma_4} - \beta_4 {X_4}^{\gamma_5} {X_9}^{\gamma_6} {X_{11}}^{\gamma_{106}}$$
$$\dot{X}_5 = \beta_4 {X_4}^{\gamma_5} {X_9}^{\gamma_6} {X_{11}}^{\gamma_{106}} + \beta_6 {X_6}^{\gamma_8} - \beta_5 {X_5}^{\gamma_7}$$
$$\dot{X}_6 = \beta_5 {X_5}^{\gamma_7} - \beta_1 \frac{k_0 X_1}{t_0 + X_1} {X_6}^{\gamma_2} - \beta_6 {X_6}^{\gamma_8} - \beta_7 X_6^{\gamma_9} \frac{t_1^{h_1}}{t_1^{h_1} + {X_{11}}^{h_1}} \frac{X_3^{h_3}}{t_3^{h_3} + {X_3}^{h_3}}$$
$$\dot{X}_7 = \beta_1 \frac{k_0 X_1}{t_0 + X_1} {X_6}^{\gamma_2} + \beta_7 X_6^{\gamma_9} \frac{t_1^{h_1}}{t_1^{h_1} + {X_{11}}^{h_1}} \frac{X_3^{h_3}}{t_3^{h_3} + {X_3}^{h_3}} - \beta_8 {X_7}^{\gamma_{10}} {X_{10}}^{\gamma_{11}} \frac{{X_3}^{h_2}}{{t_2}^{h_2} + {X_3}^{h_2}}$$
$$\dot{X}_8 = \beta_8 {X_7}^{\gamma_{10}} {X_{10}}^{\gamma_{11}} \frac{{X_3}^{h_2}}{{t_2}^{h_2} + {X_3}^{h_2}}$$
$$\dot{X}_9 = \beta_{11} {X_{10}}^{\gamma_{16}} + \beta_8 {X_7}^{\gamma_{10}} {X_{10}}^{\gamma_{11}} \frac{{X_3}^{h_2}}{{t_2}^{h_2} + {X_3}^{h_2}} + \beta_9 {X_7}^{\gamma_{12}} {X_{10}}^{\gamma_{13}} - \beta_4 {X_4}^{\gamma_5} {X_9}^{\gamma_6} {X_{11}}^{\gamma_{106}} - \beta_9 {X_7}^{\gamma_{12}} {X_{10}}^{\gamma_{13}}$$
$$\dot{X}_{10} = \beta_4 {X_4}^{\gamma_5} {X_9}^{\gamma_6} {X_{11}}^{\gamma_{106}} - \beta_9 {X_7}^{\gamma_{12}} {X_{10}}^{\gamma_{13}} - \beta_{11} {X_{10}}^{\gamma_{16}} - \beta_8 {X_7}^{\gamma_{10}} {X_{10}}^{\gamma_{11}} \frac{{X_3}^{h_2}}{{t_2}^{h_2} - {X_3}^{h_2}}$$
$$\dot{X}_{11} = \beta_{pi} (P_0 - X_{11})^{\gamma_{pi}} - \beta_4 {X_4}^{\gamma_5} {X_9}^{\gamma_6} {X_{11}}^{\gamma_{106}}$$
$$\dot{X}_{12} = \beta_9 {X_7}^{\gamma_{12}} {X_{10}}^{\gamma_{13}}$$

• MODEL
Infer parameters that better adjust experimental data

Hard problem!

• SIMULATE
In silico experiments - prediction capability: does model accommodate new experiments?



- The preliminary model adjusts well to different initial conditions
- glucose consumption rate similar to experimental data
- FBP saturates near 50mM independently of the glucose initial conditions
- Enzyme allosteric effect, like PFK and LDH, are well modelled as a switch. These enzymes are activated by FBP and responsible for the stationary behaviour of 3-PGA and PEP before glucose exhaustion.

5 Conclusions and Future work

- The new features of the model proposed: 1) introduction of control signals that correspond to genetic regulation through Hill functions on enzyme behavior; 2) dynamics of glucose uptake modeling by the PEP:PTS system with saturation phenomenon; 3) inclusion of new reactions in order to model mannitol production.
- Preliminary results show that this model is useful for optimization purposes
- An automatic procedure for parameter estimation using complete time series data is still under development, which constitute a major challenge in this area.

References

- Neves AR et al (2002) J. Biol. Chem. 277, 28088-28098.
- Voit EO et al (2006) IEE Proc Syst Biol 153, 286-298.

Acknowledgments

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