Dynamic optimization of metabolic networks coupled with gene expression

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Mathematics for key technologies

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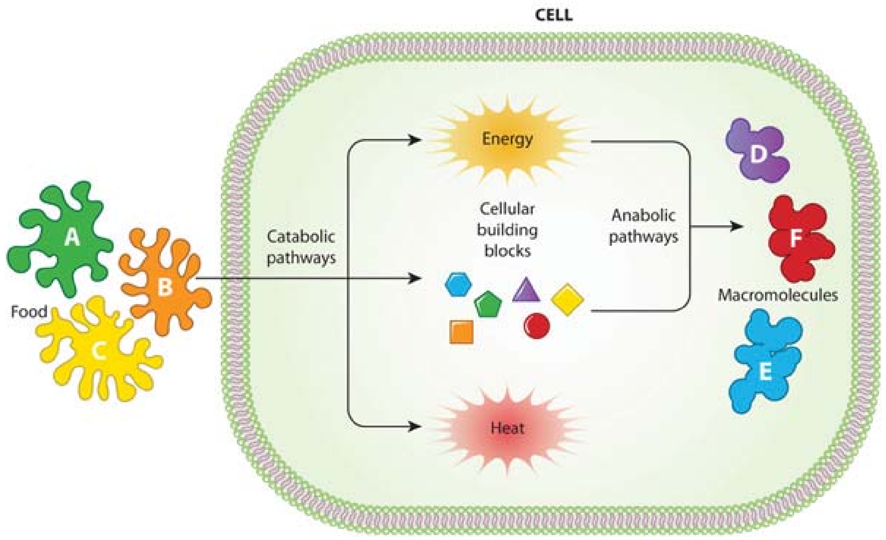
Cellular metabolism



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Metabolism and enzyme production

$$Y \underset{\uparrow P}{\xleftrightarrow{V_y}} X, \quad X \underset{\uparrow P}{\xrightarrow{V_x}} \tilde{X}, \quad X \underset{\uparrow P}{\xrightarrow{V_p}} P$$

- Molecular species
  - Extracellular nutrients and/or waste Y
  - Intracellular metabolites X
  - Macromolecules/enzymes P
- Reaction fluxes
  - Exchange reactions V_y
  - Internal metabolic reactions V_x
  - Biomass reactions V_p
- Stoichiometric matrices S_j^i (species i, reactions j)

Dynamic model

- Mass balance

$$\begin{aligned} \dot{Y} &= -S_y^y V_y \\ \dot{P} &= S_p^p V_p \\ \dot{X} &= S_y^x V_y + S_x^x V_x - S_p^x V_p \end{aligned}$$

- Macromolecule production is slow: small ε
- Macromolecules are made from many components: large α

$$\begin{aligned} \dot{Y} &= -S_y^y V_y \\ \dot{P} &= \varepsilon\, S_p^p V_p \\ \dot{X} &= S_y^x V_y + S_x^x V_x - \varepsilon\alpha\, S_p^x V_p \end{aligned}$$

Quasi steady state approximation

- ▷ Time-scale separation (using Tikhonov's theorem)

$$\begin{aligned}\dot{Y} &= -S_y^y V_y,\\ \dot{P} &= \varepsilon\, S_p^p V_p,\\ 0 &= S_y^x V_y + S_x^x V_x - \alpha\,\varepsilon\, S_p^x V_p.\end{aligned}$$

- ▷ Exchange reactions and biomass production coupled via quasi steady-state constraint for intracellular metabolism.
- ▷ Model reduction

Tikhonov's theorem

$$\begin{aligned}\dot{z} &= f(x,z,\varepsilon), \qquad (*)\\ \varepsilon\dot{x} &= g(x,z,\varepsilon),\\ (z(0),x(0)) &= (z_0,x_0),\end{aligned}$$

with f, g sufficiently smooth, under the following hypotheses:

(H1) there exists a unique solution $x = \bar{g}(z)$, sufficiently smooth, of $g(x,z,0) = 0$; the matrix $\partial g/\partial x(\bar{g}(z),z,0)$ has all eigenvalues with strictly negative real part;

(H2) the reduced system

$$\begin{aligned}\dot{z} &= f(\bar{g}(z),z,0), \qquad (**)\\ z(0) &= z_0,\end{aligned}$$

has a solution $z^0(t)$ on an interval $[0,T]$, for some $T > 0$;

(H3) x_0 is in the basin of attraction of the steady state $\bar{g}(z_0)$ of the fast system $\dot{\xi} = g(\xi, z_0, 0)$.

If these hypotheses are satisfied, system (*) admits a solution $(x^\varepsilon(t), z^\varepsilon(t))$ on $[0,T]$. In addition,

$$\lim_{\varepsilon\to 0+} z^\varepsilon(t) = z^0(t)$$

and

$$\lim_{\varepsilon\to 0+} x^\varepsilon(t) = x^0(t) = \bar{g}(z_0)(t),$$

uniformly on any closed interval contained in $(0,T]$.

Steady-state vs. dynamic optimisation

	Metabolism	+ Enzyme production
Steady state	Flux Balance Analysis (FBA) Varma/Palsson 94	Resource Balance Analysis (RBA) Goelzer et al. 11
Dynamic	Dynamic FBA (dFBA) Mahadevan et al. 02	Dynamic Enzyme Cost Analysis (deFBA) – TODAY –

Flux balance analysis (FBA)

- Goal: Determine metabolic fluxes maximizing a cellular objective.
- Network model: Metabolism & steady state

$$0 = S_y v_y + S_x v_x - S_{bm} v_{bm} \tag{1}$$

- Constraints: Bounds on fluxes

$$v_{i,min} \leq v_i \leq v_{i,max} \tag{2}$$

- Optimisation: Linear programming (LP)

$$\max_{v} v_{bm} \text{ such that } (1) \text{ and } (2)$$

Dynamic flux balance analysis (dFBA)

- Goal: Determine metabolic fluxes maximizing a cellular objective over time (based on biomass concentration $P(t)$).
- Network model: Metabolism & dynamic

$$\begin{aligned} \dot{y}(t) &= -S_y^y v_y(t)\, P(t) \\ \dot{x}(t) &= S_y^x v_y(t)\, P(t) + S_x^x v_x(t)\, P(t) - S_{bm}^x v_{bm}(t)\, P(t) \\ \dot{P}(t) &= v_{bm}(t)\, P(t) \end{aligned} \tag{3}$$

- Constraints: Bounds on fluxes & flux changes

$$v_{i,min}(y,P) \leq v_i(t) \leq v_{i,max}(y,P), \qquad |\dot{v}_i(t)| \leq \dot{v}_{i,max} \tag{4}$$

- Optimisation: Non-linear dynamic

$$\max_{v(t)} P(t_{end}) \text{ such that } (3) \text{ and } (4)$$

Resource balance analysis (RBA)

- Goal: Determine cell composition (protein concentrations p) and metabolic fluxes v maximizing the growth rate μ.
- Network model: Metabolism+enzyme production & steady st.

$$\begin{aligned} 0 &= S_y^x v_y + S_x^x v_x - \alpha\varepsilon S_p^x v_p \\ 0 &= \varepsilon S_p^p v_p - \mu p \end{aligned} \tag{5}$$

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j \in \mathscr{V}_i} |v_j(t)/k_j| \leq p_i, \qquad \sum_i c_i p_i \leq 1 \tag{6}$$

- Optimization: Iteratively solving LPs

$$\max_{v,p} \mu \text{ such that } (5) \text{ and } (6)$$

Dynamic enzyme-cost FBA (deFBA)

- Goal: Determine the dynamic cell composition and metabolic fluxes to maximize a cellular objective over a time interval
- Network model: Metabolism+enzyme production & dynamic

$$\begin{aligned} \dot{Y} = -S_y^y V_y, \qquad \dot{P} &= \varepsilon\, S_p^p V_p, \\ 0 = S_y^x V_y + S_x^x V_x &- \alpha\, \varepsilon\, S_p^x V_p \end{aligned} \tag{7}$$

- Constraints: Enzyme capacity & cellular composition

$$\sum_{j \in \mathscr{V}_i} |V_j(t)/k_j| \leq P_i(t), \qquad \sum_i c_i P_i(t) \leq 1 \tag{8}$$

- Optimization: Linear dynamic

$$\max_{V,Y,P} \int_0^{t_{end}} c^{\mathrm{T}} P(t)\, dt \text{ such that } (7) \text{ and } (8)$$

Constraints

- Enzyme capacity constraints

$$\left|\frac{v_1}{c_1}\right| + \cdots + \left|\frac{v_m}{c_m}\right| \le P_E$$

- Biomass-independent flux bounds

$$v_{min} \le v \le v_{max}$$

- Non-negativity of molecular species

$$Y \ge 0, \quad P \ge 0$$

- Biomass composition constraints

$$H_B P \le h_B$$

Dynamic optimization problem

Let $z = (Y, P)$.

$$\max_{\mathscr{V}(\mathscr{Z}, z_0)} \int_0^{t_{end}} \Phi(z(t), v(t))dt \; + \; \Psi(z(t_{end}))$$

$$\begin{aligned} \text{s.t. } & \dot{Y} = -S_y^y V_y, \quad \dot{P} = \varepsilon\, S_p^p V_p, \\ & S_y^x V_y + S_x^x V_x - \alpha\, \varepsilon\, S_p^x V_p = 0, \\ & z(0) = z_0, \\ & z(t) \ge 0, \\ & v_{min} \le v(t) \le v_{max}, \\ & H_C v(t) \le H_E P(t), \quad H_B P(t) \le h_B. \end{aligned}$$

Minimal example

- Components: Nutrient Y, metabolite X, generic enzyme P
- Reactions

$$V_y : Y \to X \;\text{(uptake)}, \quad V_p : \alpha X \to P \;\text{(biomass)}$$

- Enzymatic constraint

$$\frac{V_y}{k_y} + \frac{\varepsilon\, V_p}{k_p} \le P$$

- Approximate model (on long time-scales)

$$\dot{Y} = -V_y, \quad \dot{P} = \varepsilon\, V_p, \qquad V_y = \alpha\, \varepsilon\, V_p$$

Objective functionals

- Maximization of terminal biomass

$$J_1 = P(t_{end})$$

- Maximization of discounted biomass integral

$$J_2 = \int_0^{t_{end}} P(\tau) e^{-\mu\tau} d\tau$$

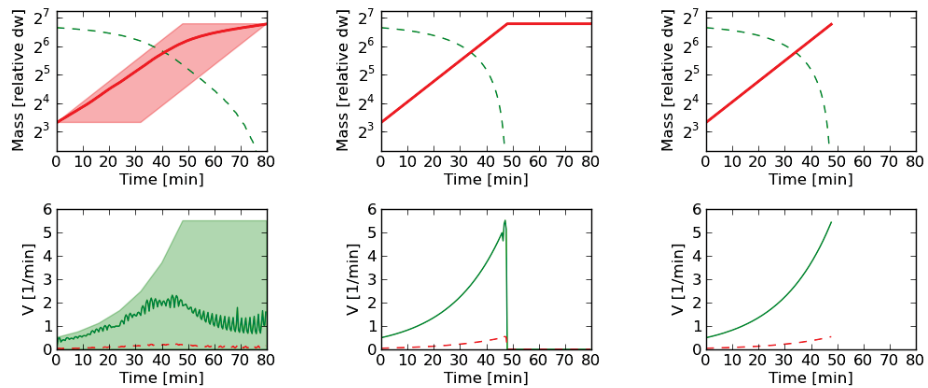
- Minimization of time to consume nutrients

$$J_3 = -\int_0^{t_{end}} d\tau = -t_{end}$$

with $Y(t_{end}) = 0$.

Optimization results for minimal network

Terminal biomass | Discounted biomass | Minimal Time



Analytical proof of existence and uniqueness for J_2 and J_3
⇝ mathematical optimum biologically meaningful?

Minimal network yields Monod model

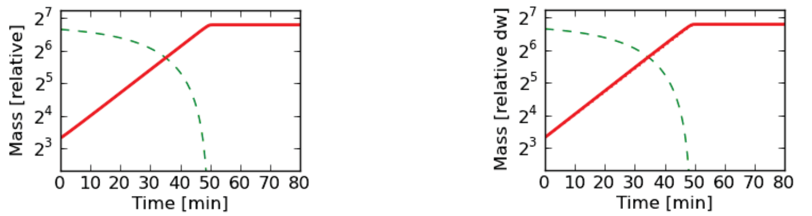
- Assume Michaelis-Menten kinetics

$$V_y = \frac{V_{m,y} P Y}{K_y \vartheta_e + Y}, \qquad \varepsilon V_p = \frac{V_{m,p} P X}{K_p + X}.$$

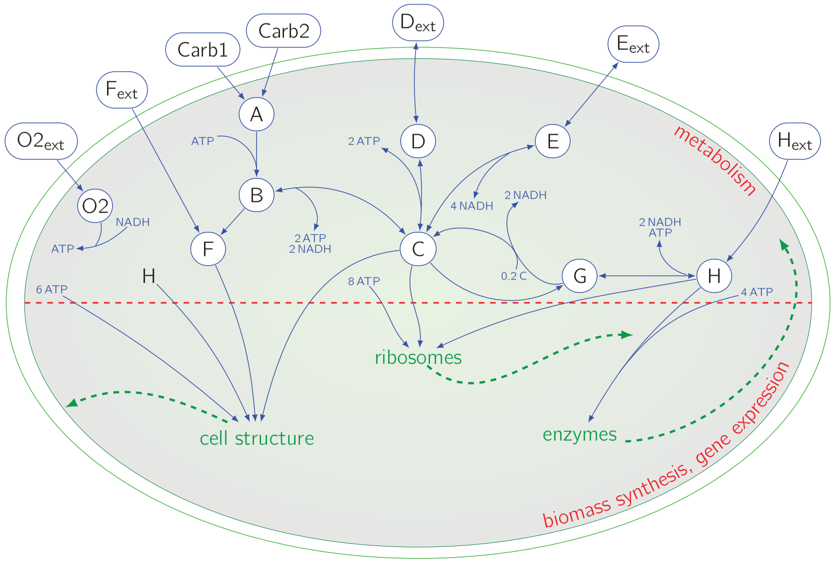
- Choose consistent parameters

$$V_{m,y} = \Big(\frac{1}{k_y} + \frac{1}{\alpha k_p}\Big)^{-1}, \qquad V_{m,p} = \Big(\frac{\alpha}{k_y} + \frac{1}{k_p}\Big)^{-1}.$$

Simulation (assuming kinetics) | Dynamic optimization (no kinetics assumed)



Core cellular network



Covert et al. 2001 (for the metabolic part)

Network specification

Reaction	Enz	k_{cat}
Exchange reactions		
Carb1 → A	T_{C1}	$3000\ \frac{1}{min}$
Carb2 → A	T_{C2}	$2000\ \frac{1}{min}$
F_{ext} → F	T_F	$3000\ \frac{1}{min}$
$O2_{ext}$ → O2	S	$1000\ \frac{1}{min}$
D ↔ D_{ext}	S	$1000\ \frac{1}{min}$
E ↔ E_{ext}	S	$1000\ \frac{1}{min}$
H_{ext} → A	T_H	$3000\ \frac{1}{min}$
Metabolic reactions		
A + ATP → B	E_B	$1800\ \frac{1}{min}$
B → C + 2 ATP + 2 NADH	E_C	$1800\ \frac{1}{min}$
B → F	E_F	$1800\ \frac{1}{min}$
C → G	E_G	$1800\ \frac{1}{min}$
G → 0.8 C + 2 NADH	E_N	$1800\ \frac{1}{min}$
C ↔ 2 ATP + 3 D	E_D	$1800\ \frac{1}{min}$
C + 4 NADH ↔ 3 E	E_E	$1800\ \frac{1}{min}$
G + ATP + 2 NADH ↔ H	E_H	$1800\ \frac{1}{min}$
NADH + O → ATP	E_T	$1800\ \frac{1}{min}$

Reaction	Enz	k_{cat}
Biomass reactions		
400 H + 1600 ATP → T_{C1}	R	$2.5\ \frac{1}{min}$
1500 H + 6000 ATP → T_{C2}	R	$0.67\ \frac{1}{min}$
400 H + 1600 ATP → T_F	R	$2.5\ \frac{1}{min}$
400 H + 1600 ATP → T_O	R	$2.5\ \frac{1}{min}$
400 H + 1600 ATP → T_D	R	$2.5\ \frac{1}{min}$
400 H + 1600 ATP → T_E	R	$2.5\ \frac{1}{min}$
400 H + 1600 ATP → T_H	R	$2.5\ \frac{1}{min}$
500 H + 2000 ATP → E_B	R	$2\ \frac{1}{min}$
500 H + 2000 ATP → E_C	R	$2\ \frac{1}{min}$
1000 H + 4000 ATP → E_D	R	$1\ \frac{1}{min}$
1000 H + 4000 ATP → E_E	R	$1\ \frac{1}{min}$
1500 H + 6000 ATP → E_F	R	$0.67\ \frac{1}{min}$
500 H + 2000 ATP → E_G	R	$2\ \frac{1}{min}$
2500 H + 10000 ATP → E_H	R	$0.4\ \frac{1}{min}$
500 H + 2000 ATP → E_N	R	$2\ \frac{1}{min}$
500 H + 2000 ATP → E_T	R	$2\ \frac{1}{min}$
2000 H + 4000 C + 16000 ATP → R	R	$0.2\ \frac{1}{min}$
250 H + 250 C + 250 F + 1500 ATP → S	R	$3\ \frac{1}{min}$

⇝ preferred carbon source Carb1

Case study: Carbon switch

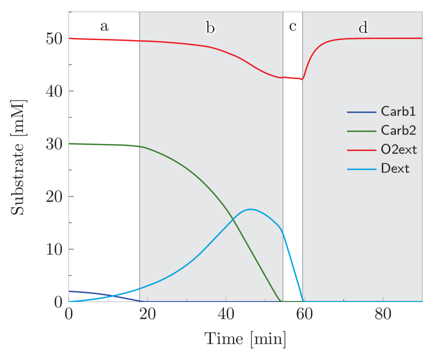
Growth scenario:
- Low amount of preferred carbon source C_1
- High amount of non-preferred carbon source C_2
- Ample oxygen supply

Objective: Discounted biomass

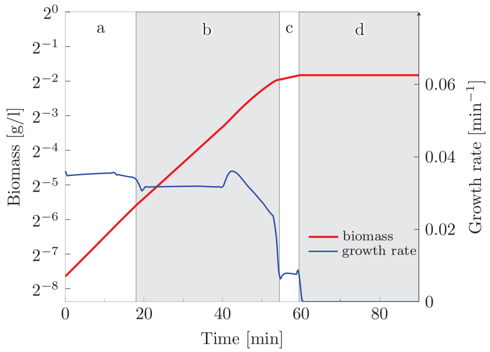
$$J = \int_0^{t_{end}} c_{bm}^{\mathrm{T}} P(t)\, e^{-\mu t} dt$$

Dynamic optimization results I

Substrates



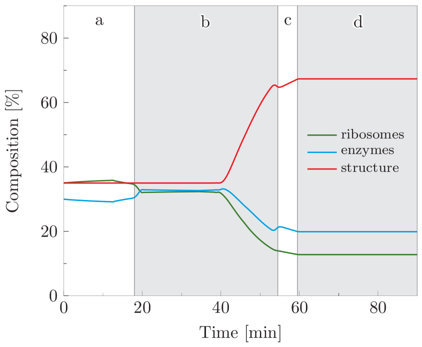
Biomass & Growth



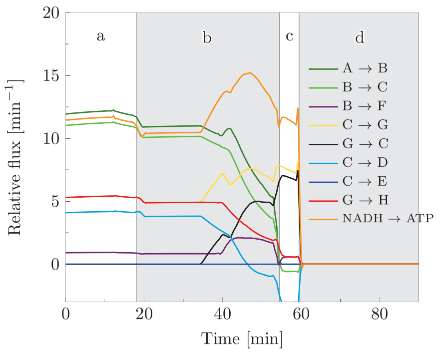
Four cellular growth phases

Dynamic optimization results II

Cell composition



Metabolic fluxes



Cellular reorganisation at the end of phase (b)

Discussion

Modeling metabolism including enzyme costs
- Mass balance ODE model for cellular metabolism and biomass production.
- Time-scale separation yields dynamic biomass model with quasi steady-state metabolic constraints.
- Dynamic optimisation framework: deFBA

Case studies
- Minimal network yields Monod growth model.
- Core network shows different exponential growth phases and pre-adaptation to impending nutrient depletion.
- Choice of the objective functional crucial for the results.