Modelling Biochemical Reaction Networks

Lecture 6: Coupling uptake and growth

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Uptake model

- Recall: Last lecture, we derived equations for the rate of passive transport of glucose into yeast cells (v_t).
  We add a glucose utilization term (v_u) to the equation for the internal glucose:

$$\frac{d[\mathrm{G}_{(\mathrm{in})}]}{dt} = v_t / V_{\mathrm{cells}} - v_u$$

$$\text{and} \quad \frac{d[\mathrm{G}_{(\mathrm{out})}]}{dt} = -v_t / V_{\mathrm{medium}}$$

$$\text{with} \quad v_t = \frac{v_{t,\mathrm{max}}[\mathrm{G}_{(\mathrm{out})}]}{[\mathrm{G}_{(\mathrm{out})}] + K_t}$$

Growth modeling

- In cell cultures, we typically measure biomass or dry mass rather than the number of cells.
- The simplest way to model growth is to add a pseudo-reaction for the conversion of glucose to biomass (B):

$$\mathrm{G_{(in)}} \xrightarrow{v_u} Y_g\mathrm{B}$$

- Y_g is the biomass yield, the amount of biomass obtained per unit glucose metabolized.
- v_u is often of the Michaelis-Menten form to recognize the fact that there is a maximal rate of growth:

$$v_u = \frac{v_{u,\max}[\mathrm{G_{(in)}}]}{[\mathrm{G_{(in)}}] + K_u}$$

Growth modeling

- The rate equation for biomass growth is then

$$\frac{dB}{dt} = Y_g v_u$$

- Note: B is normally measured in g/L.

Connections between growth and uptake

- Several quantities that appear as parameters in fact depend on some of the variables:
  - $V_{\text{cells}} \propto B$
  - $v_{t,\max} = k_{\text{cat}} T_0$ and $T_0 \propto B$
    $[k_{\text{cat}} = k_3/(1 + K_2)]$
  - $v_{u,\max} \propto B$

Parameter estimates

Glucose transport by Hxt7

- $k_{\text{cat}} \approx 200\,\text{s}^{-1}$
  Source: Ye et al., Yeast **18**, 1257 (2001).
- $K_t \approx 2\,\text{mM}$
  Source: Ye et al., Yeast **18**, 1257 (2001).
- $T_0 \approx \frac{(2\times 10^4\ \text{molecules/cell})\,V_{\text{medium}}}{N_A(\text{biomass/cell})} B$
  Source for density of transporters: Ye et al., Yeast **18**, 1257 (2001).
  A typical yeast cell weighs about 60 pg (an oft repeated estimate, e.g. `http://www.weizmann.ac.il/plants/Milo/images/YeastSize-Feb2010.pdf`) of which 60% is water (Illmer et al., FEMS Microbiol. Lett. **178**, 135, 1999). The biomass per cell is therefore about 24 pg/cell.

Parameter estimates

Glucose transport by Hxt7

- Because the cells occupy only a small fraction of the total volume, $V_{\text{medium}} \approx V_{\text{culture}}$. This varies from experiment to experiment, but might typically be 200 mL for an experiment in a shaker flask.
  This gives $T_0 \approx (3 \times 10^{-10} \text{mol L g}^{-1})B$.
- Therefore $v_{t,\text{max}} \approx (6 \times 10^{-8}\,\text{mol L g}^{-1}\text{s}^{-1})B$
- The volume of cells in the solution can similarly be related to B assuming that yeast cells have a density (ρ_{cells}) similar to that of water (about 1000 g/L):

$$\begin{aligned} V_{\text{cells}} &= BV_{\text{culture}}/\rho_{\text{cells}} \\ &= \frac{0.2\,\text{L}}{1000\,\text{g/L}} B = (2 \times 10^{-4}\,\text{L}^2/\text{g})B \end{aligned}$$

Parameter estimates

Growth parameters

- $Y_g = 0.45\,\text{g biomass/g glucose}$
  Source: Ertugay and Hamamci, Folia Microbiol. **42**, 463 (1997).
- Write $v_{u,\max} = \mu_{\max} B$.
- μ is a first-order rate constant for glucose utilization, which is proportional to biomass production.
- $\mu_{\max} = 0.38\,\text{h}^{-1}$
  Source: Ye et al., Yeast **18**, 1257 (2001).
- Another estimate: μ is related to the doubling time t_2 by $\mu = \ln 2/t_2$.
- Minimum doubling time for yeast grown on glucose: 75 min
  Source: Tyson et al., J. Bacteriol. **138**, 92 (1979).
- $\mu = 0.55\,\text{h}^{-1}$

Parameter estimates
Growth parameters

- I have had no luck tracking down data that would allow me to calculate K_u.
- Leave it as a disposable parameter
- Investigate its effect
- Fit to data?

Initial conditions

- Starved cells: $[\mathsf{G}_{(\mathsf{in})}](0) = 0$.
- Ye and coworkers studied Hxt7 in 1% glucose solution, corresponding to about $[\mathsf{G}_{(\mathsf{out})}](0) = 55\,\mathrm{mM}$.
- They started their experiments with a culture diluted to an optical density (OD) of 0.2 at 600 nm.
- An OD of 1 corresponds to 3×10^7 cells/mL so we're starting with about 6×10^6 cells/mL.
  Source: Ausubel et al., *Short protocols in molecular biology*, 5th ed., Vol. 2.
- Using the typical cell mass of 60 pg and 60% water content, this gives $B(0) \approx 0.1\,\mathrm{g/L}$.