

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:

$$\frac{d[G_{(in)}]}{dt} = v_t/V_{\text{cells}} - v_u$$

and

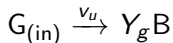
$$\frac{d[G_{(out)}]}{dt} = -v_t/V_{\text{medium}}$$

with

$$v_t = \frac{v_{t,\text{max}}[G_{(out)}]}{[G_{(out)}] + K_t}$$

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):



- ▶ 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:

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

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:
 - ▶ $V_{\text{cells}} \propto B$
 - ▶ $v_{t,\text{max}} = k_{\text{cat}} T_0$ and $T_0 \propto B$
[$k_{\text{cat}} = k_3/(1 + K_2)$]
 - ▶ $v_{u,\text{max}} \propto B$

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

- ▶ $Y_g = 0.45$ 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: $[G_{(in)}](0) = 0$.
- ▶ Ye and coworkers studied Hxt7 in 1% glucose solution, corresponding to about $[G_{(out)}](0) = 55 \text{ mM}$.
- ▶ 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 $B(0) \approx 0.1 \text{ g/L}$.