

PHYSICS 239 Spatiotemporal Biodynamics

Homework #3

Due Wednesday Feb 23, 2022

[Note: Those not from math/physics background need not attempt problem(s) indicated by *]

1. Proteome allocation and the Monod growth law. In class we went over key elements of the proteome allocation analysis for bacterial growth. In this problem, you will work them out step-by-step using a concrete example, for the growth of *E. coli* on lactose as the sole carbon source. From a few assumptions, we will obtain quantitatively the growth rate and the expression of the *lac* operon for different concentrations of lactose, i.e., Monod's growth law and the phenomenon of *catabolite repression*.

In the following, all quantities correspond to amount contained in 1-mL of exponentially growing culture at optical density (OD) = 1, referred to as "OD·mL" for short. 1 OD·mL of culture corresponds to $10^8 \sim 10^9$ bacterial cells depending on the specific culturing condition. We will refrain from using per cell quantity because the amount per cell can vary 10x due to change of cell size in different growth conditions (including for cells grown in different lactose concentrations to be studied here). Instead, amount per OD·mL is more invariant. In particular, 1 OD·mL of culture contains a total dry mass of ~ 0.5 mg and total cytoplasmic water content of ~ 1 mg (or 1 μ L in volume) for most conditions characterized. The total protein content in OD·mL varies moderately, from 0.3 mg at fast growth to 0.4 mg at slow growth. For simplicity, we will take total protein per OD·mL to be 0.35 mg.

Definition of symbols to be used below: N_X and M_X are, respectively, the number and mass of protein X per OD·mL culture. $M = 0.35$ mg is the total mass of cellular proteins per OD·mL of culture. m_X is the molecular weight of protein X. $\phi_X \equiv M_X/M$ is the mass fraction of protein X among all cellular proteins; it is referred to as the "proteome fraction", a measure of "protein abundance".

(a) Conversion between proteome fraction and concentration: The average intracellular concentration of a protein X, denoted as $[X]$, can be taken as the number of proteins in OD·mL, N_X , divided by the total cytoplasmic water volume in OD·mL, V . Derive a relation between the concentration $[X]$ and the proteome fraction ϕ_X in terms of the molecular weight m_X . For typical proteins 300 aa in length, find its concentration in μ M if the proteome fraction is 1‰ (part per thousand). You can take the average mass of an amino acid to be 110 Daltons.

[Note: Below we will only refer to protein concentrations as proteome fractions. It turns out that the latter is more readily obtained experimentally, e.g., by proteomics or by ribosome-profiling. It is also a natural quantity to work with in models. The above exercise is meant to let you know that you can always convert proteome fraction to a more familiar concentration unit, e.g., μ M.]

(b) Protein synthesis flux by ribosomes: Let J_R denote the flux of protein synthesis, in unit of # aa polymerized per OD·mL of culture. For a culture growing exponentially at the specific rate r , this is just $r \cdot M$ (with the total protein mass M expressed in # aa/(OD·mL)). Molecularly, protein synthesis flux can be written as the product of the ribosome elongation rate (denoted as ε) and

N_R , the total number of ribosomes per OD·mL of culture. [Here we have assumed that all ribosomes are engaged in translation at the same speed. This turns out to be a reasonably good approximation, breaking down only at very slow growth.]

- Show that the above leads to the growth law

$$\phi_R = r/k_R \quad (1)$$

where $\phi_R \equiv M_R/M$ is the proteome fraction of ribosomal proteins, M_R being the total mass of r-proteins per OD·mL of culture. Express k_R in terms of ε and m_R , the weight of all r-proteins in a ribosome.

- Adding up all r-proteins in the ribosome of *E. coli* gives 7336 aa. However, for the ribosomes to do its job, many helper proteins such as elongation factors are also needed. These proteins add up to another 60% in mass. Thus, we can take the “molecular weight” of an *effective* ribosome as $m_R = 1.6 \times 7336 \text{ aa}$. Further using the measured elongation rate of $\varepsilon = 16 \text{ aa/s}$, find the value of k_R in unit of h^{-1} .
- What is the theoretical maximum growth rate if a cell contains only ribosomes? What is the corresponding doubling time? [Note the factor of $\ln 2$ in the conversion.] The fastest doubling time observed for *E. coli* is $\sim 17 \text{ min}$, when the culture contains many nutrient ingredients including all amino acids and nucleotides. What is the corresponding ribosomal fraction ϕ_R at this fastest growth rate? The remaining fraction of the proteome (ϕ_Q) found at the fastest growth rate, are comprised of obligatory proteins needed for house-keeping functions. Empirically, ϕ_Q is found to be approximately growth-rate independent even though ϕ_R changes according to Eq. (1).

(c) Carbon uptake flux: Consider growth of *E. coli* in minimal medium with a single carbon source, without the supplement of amino acid and other substances. Let J_C denote the flux of carbon uptake, in unit of # substrate molecule taken up per time per OD·mL. Molecularly, this can be written as the product of ω_E , the specific rate of the uptake enzyme E, and N_E , the number of uptake enzymes per OD·mL of culture.

- Express J_C in term of the proteome fraction of the uptake enzyme, $\phi_E \equiv M_E/M$, and the molecular weight of the uptake enzyme, m_E .
- The condition of *flux balance* can be stated as $J_R = Y \cdot J_C$, where the yield Y represents the conversion factor from the substrate molecule to aa. Using flux balance and the expression you obtained above for J_R and J_C , derive the relation $\phi_E = r/k_E$ and find an expression for the parameter k_E in terms of the molecular parameters (ω_E, m_E) and the yield Y .
- Consider the case where lactose is the sole carbon substrate. 1 g of lactose is known to produce 0.5 g of dry mass. Based on the protein:dry mass ratio given above, work out the value of Y for lactose. Express it in unit of # aa/lactose molecule and in OD/mM lactose.
- Given that the specific uptake rate for the lactose transporter (LacY, the lac permease, 417aa in length) is $\omega_E = 3/s$ in saturating lactose concentration, write down the value of k_E for lactose uptake in unit of h^{-1} .

(d) The lactose transporter is one of a suite of “carbon catabolic proteins” expressed when *E. coli* is short of carbon supply. The other proteins include beta-galactosidase (LacZ) which degrades lactose into glucose and galactose (which then enter central metabolism), and other enzymes

not specific to lactose degradation. Let the proteome fraction of all these carbon catabolic proteins be ϕ_C . Since the expression of LacY is co-regulated with these other catabolic proteins and thus have the same growth-rate dependences, we can take the proteome fraction of LacY, ϕ_E , to be a fixed portion (η_E) of ϕ_C . This leads to

$$\phi_C = r/k_C \quad (2)$$

where $k_C = \eta_E k_E$.

For cells grown in minimal medium without the supplement of amino acids, etc., another significant fraction of the proteome is comprised of anabolic proteins, e.g., enzyme for biosynthesis of amino acids. Let the total proteome fraction of these enzymes be ϕ_A . Empirically, a linear relation between the growth rate r and ϕ_A similar to Eqs. (1) and (2) has been found,

$$\phi_A = r/k_A \quad (3)$$

with a coefficient k_A . It turns that numerically, $k_A \approx k_R$.

Finally, there is the constraint that sum of all proteome fractions add up to 1, i.e.,

$$\phi_R + \phi_C + \phi_A = \phi_{max} \quad (4)$$

where $\phi_{max} \equiv 1 - \phi_Q$, with ϕ_Q being the fraction of obligatory proteins encountered in **(b)**.

- Combine Eqs. (1)-(4) to show that the growth rate depends on the parameter k_C as

$$r = r_c \frac{k_C}{k_C + k_{RA}} \quad (5)$$

Express the lumped parameter r_c and k_{RA} in terms of k_R and ϕ_{max} and find their values. How would you interpret the meaning of r_c and how would you test this experimentally? Explain the sense by which the ratio $k_C:k_{RA}$ is regarded as a measure of “carbon quality”.

- For *E. coli* growing on saturating concentration of lactose, the growth rate is found to be $\sim 1/h$. Find the corresponding value of k_C . What is the ratio $k_C:k_{RA}$ for lactose? Find the proteome fraction ϕ_R, ϕ_A, ϕ_C during growth on lactose.
- Based on the value of k_E you calculated in **(c)**, what share of catabolic proteins is LacY? What fraction of the entire proteome is LacY?
- *E. coli* is found to grow on saturating glycerol at rate $\sim 0.7/h$ and on saturating galactose at rate $\sim 0.35/h$. What are the corresponding carbon quality index? Based on the development above, give two distinct molecular causes by which a substance may be of poor carbon quality.

(e) To derive the Monod growth law, we consider the lactose concentration in the medium to be maintained at a constant value $[L]$. Then the lactose uptake rate per LacY molecule is given by the Michaelis-Menten kinetics as

$$\omega_E([L]) = \omega_E \frac{[L]}{[L] + K_E} \quad (6)$$

where $\omega_E = 3/s$ is the uptake rate used above, and K_E is the equilibrium binding constant of lactose to LacY. Express k_C in terms of $\omega_E([L])$ and use it in Eq. (5) to derive the Monod growth law:

$$r([L]) = r_0 \frac{[L]}{[L] + K_M} \quad (7)$$

where r_0 is the growth rate under saturating concentration of lactose as given by Eq. (5). Express the Monod constant for lactose, K_M , in terms of K_E and the basic parameters of the growth laws in Eqs. (1)-(4). Find the value of the Monod constant if $K_E = 0.3 \text{ mM}$. Can you explain why the Monod constant is much smaller than the binding constant K_E ?

(f) Solve for $\phi_C([L])$, the fraction of catabolic proteins at different lactose concentration $[L]$. Show that

$$\phi_C([L]) = \phi_C^{max} \cdot (1 - r([L])/r_C) \quad (8)$$

and give the value of ϕ_C^{max} . Eq. (8) describes a linear decline in the abundance of catabolic proteins with increasing growth rate, referred to as the “C-line”. It is a quantitative statement of the phenomenon of “catabolite repression” ubiquitous in microbiology, wherein the expression of catabolic enzymes is inhibited in medium with improved carbon availability. Explain in your own words why should cells reduce the catabolic proteins when carbon is more available.

2. Competition for nutrient. Two species described by densities $\rho_1(t)$ and $\rho_2(t)$ grow on the same nutrient source, of concentration $n(t)$. Suppose the growth rate of species i is given by the Monod growth law, $r_i(n) = r_{i,0} \cdot n/(n + K_i)$, the death rate is given by μ_i , and the nutrient influx is j_0 . Find a criterion on the physiological parameters ($r_{i,0}, K_i, \mu_i$) in order for species i to survive in the steady state.

3*. MacArthur’s model of resource competition. MacArthur’s model applied to 2-species (of densities ρ_1, ρ_2) and 2 nutrients (of concentrations n_A, n_B) is

$$\dot{\rho}_1 = (v_{1A}n_A + v_{1B}n_B) \cdot \rho_1 - \mu_1\rho_1, \quad (1)$$

$$\dot{\rho}_2 = (v_{2A}n_A + v_{2B}n_B) \cdot \rho_2 - \mu_2\rho_2, \quad (2)$$

$$\dot{n}_A = \gamma_A n_A \cdot (1 - n_A/K_A) - (v_{1A}\rho_1 + v_{2A}\rho_2) \cdot n_A, \quad (3)$$

$$\dot{n}_B = \gamma_B n_B \cdot (1 - n_B/K_B) - (v_{1B}\rho_1 + v_{2B}\rho_2) \cdot n_B. \quad (4)$$

where $v_{i\alpha}$ is the consumption matrix indicating the uptake preference of species i for nutrient α , μ_i is the death rate of species i , and γ_α is the generation rate, K_α is the concentration scale of nutrient α in the habitat. (The yield factor has been omitted.)

(a) Assume the existence of a non-trivial steady state with $n_A^*, n_B^*, \rho_1^*, \rho_2^*$ all being non-zero. From $\dot{\rho}_i/\rho_i = 0$ in Eqs. (1) and (2), show that in the limit the death rate $\mu_i \rightarrow 0$, the steady state concentrations $n_\alpha^* \rightarrow 0$. Using this result in Eqs. (3) and (4), show that $\dot{n}_\alpha/n_\alpha = 0$ lead to the following equation for the steady state densities,

$$\begin{bmatrix} v_{1A} & v_{2A} \\ v_{1B} & v_{2B} \end{bmatrix} \cdot \begin{bmatrix} \rho_1^* \\ \rho_2^* \end{bmatrix} = \begin{bmatrix} \gamma_A \\ \gamma_B \end{bmatrix}$$

(b) Write down the solution of the above matrix equation for ρ_1^* and ρ_2^* . Show that the feasibility condition, i.e., $\rho_1^* > 0$ and $\rho_2^* > 0$, can be written as two conditions between the environmental parameters γ_A, γ_B , and $m_i \equiv v_{iB}/v_{iA}$, which describes the nutrient preference of species i . Plot the “ecological phase diagram” in the space (γ_A, γ_B) , marking clearly the region of coexistence, and the region of dominance/extinction.

(c) For a fixed environment parameterized by $\gamma \equiv \gamma_B/\gamma_A$ (which indicates the relative nutrient availability), plot the “physiological phase diagram” in the space (m_1, m_2) by indicating which regions of this space give coexistence, and which regions give dominance of species 1 or 2.

(d) What is the ‘optimal’ value of m_1 that species 1 should take on to maximize its existence (i.e., survival) if it expects species 2 to take on a random value of m_2 ? or if it expects species 2 to take on the ‘optimal’ value of m_2 ? If the m values of both species are close to this ‘optimal’ value, what would be the probability that one species becomes extinct if the environmental parameter γ can take on a value within a finite range δ about a mean value, $\bar{\gamma}$ with equal probability? [Assume the environment can vary rapidly while m_i , determined by genetics, is frozen over the scale of environmental variation.] What range of m_i should each species i take on to maximize its existence in a fluctuating environment if it can coordinate with the other species which is also interested in maximizing its existence? What danger is there if the other species ‘cheats’? [Note: Your response to (d) is not expected to be quantitative.]

4. Competition for essential nutrients. The dependence of the growth of bacterial species i on two essential nutrients A and B is given by

$$r_i(n_A, n_B) = \left[\frac{1}{v_{iA}n_A} + \frac{1}{v_{iB}n_B} \right]^{-1}$$

where $v_{i\alpha}$ is the single-nutrient consumption efficiency (when the other nutrient is in saturation) and n_α is the concentration of nutrient α as in Problem #2. Unlike substitutable nutrients, the uptake of nutrient α by species i is given by $r_i \cdot \rho_i/Y_{i,\alpha}$, where ρ_i is the density of species i , and $Y_{i,\alpha}$ is the yield of species i for nutrient α . This leads to the following set of consumer-resource equations

$$\begin{aligned} \dot{\rho}_1 &= r_1(n_A, n_B) \cdot \rho_1 - \mu\rho_1, \\ \dot{\rho}_2 &= r_2(n_A, n_B) \cdot \rho_2 - \mu\rho_2, \\ \dot{n}_A &= \mu \cdot (n_A^0 - n_A) - r_1(n_A, n_B) \cdot \rho_1/Y_{1,A} - r_2(n_A, n_B) \cdot \rho_2/Y_{2,A}, \\ \dot{n}_B &= \mu \cdot (n_B^0 - n_B) - r_1(n_A, n_B) \cdot \rho_1/Y_{1,B} - r_2(n_A, n_B) \cdot \rho_2/Y_{2,B}, \end{aligned}$$

for a chemostat-based system where μ is the dilution rate and n_α^0 is the inflow concentration of nutrient α . In this problem, you will derive the feasibility conditions for this system using Tilman’s graphical approach.

(a) Without solving the equations algebraically, sketch the conditions for $\dot{\rho}_i = 0$ in the (n_A, n_B) plane. Indicate the location of (n_A^*, n_B^*) where both ρ_1 and ρ_2 are finite. On the plot, also mark the point (n_A^0, n_B^0) which is proportional to the nutrient inflow. Next, find an algebraic expression for n_A^*, n_B^* in terms of the environmental and physiological parameters. [Hint: You can first use the matrix inversion formula for n_α^{-1} .]

(b) Show the balance of nutrient fluxes at (n_A^*, n_B^*) graphically using a vector relation among the nutrient influx \vec{J}_0 and the consumption fluxes \vec{J}_1, \vec{J}_2 , as done in class. Describe the condition for coexistence graphically, and write down the corresponding algebraic expression involving the constraint on n_A^0, n_B^0 .

(c) Show graphically what happens if (n_A^0, n_B^0) lies outside of the constraint, and write down the algebraic expression for the steady-state concentrations n_A^*, n_B^* and densities ρ_1^*, ρ_2^* corresponding to the two types of outcomes that would arise.

(d) Describe and explain the difference of the behavior obtained here compared to the ones obtained in class for two substitutable nutrients.