## Quantitative Microbiology Problem Set #4 due: Tuesday, March 14, 2023

[Problem #1 and #3 should be quite straightforward and will be weighted less. Main effort in this Problem Set should be devoted to Problem #2.]

1. Growth in continuous culture. The idea of a continuous culture setup is to allow a permanent and steady growth of bacteria by continuously (i) supply a growth chamber with fresh medium, and (ii) take away excess liquid, bacteria, and waste products. The simplest setup is depicted in the following.



Here a constant input flow (flow rate f in Volume per time) of fresh medium with input nutrient concentration  $[S]_{in}$  equals a constant output flow of medium. The volume V within the growth chamber is fixed and the medium chamber is well mixed and well-aerated. Such a setup ha been proposed most famously by Monod (his bactogène) as well as by Novick and Szilard (their chemostat) already in 1950. Nowadays, the term chemostat is mostly used.

To understand the robustness of this setup, possible applications and problems, we study the coupled dynamics of bacterial growth and substrate kinetics within this chemostat in the following.

(a) Suppose you start the chemostat with a certain density of bacteria  $\rho_0$ . Write down an ODE describing the dynamics of the bacterial density  $\rho(t)$  by considering the growth and outflow of bacteria over time. Show that the specific growth rate  $\lambda = D$ , with D = f/V defined as the dilution rate, is a possible steady state of the chemostat.

This behavior is remarkable as it allows to precisely control the growth rate simply by the dilution rate, without for example having to control the substrate concentration. However, we have to consider if this possible steady state is really taken by the system, and if it is in agreement with the Monod kinetics (below).

## The Monod growth law.

Monod observed empirically that the bacterial growth rate depends hyperbolically on the substrate concentration [S] as:

$$\lambda([S]) = \lambda^{\infty} \frac{[S]}{[S] + K_M}.$$
(1)

It is also known that the depletion of nutrients is linked to the growth of cells by,

$$\frac{d[S]}{dt} = -\lambda\rho/Y,\tag{2}$$

where  $\rho$  denotes the density of bacteria, and Y is the yield factor.

For *E. coli* cells growing in well aerated conditions in a minimal media with glucose as the growth limiting nutrient, we can use Y = 0.5 g cell biomass/g glucose,  $K_M = 10\mu M$ , and a maximal growth rate  $(\lambda^{\infty})$  of 1/hr.

- (b) Consider the substrate dynamics in the chemostat and determine the concentration  $[S]^*$  and the bacterial density  $\rho^*$  at the steady state where  $d\rho/dt = 0$  and d[S]/dt = 0. Assume the coupling between substrate and growth dynamics is given by Monod kinetics, Eqs. (1) and (2). Plot the result for varying dilution rate and the glucose input concentrations  $[S]_{in} = \{0.05\%, 0.1\%, 0.2\%\}$ . What is the maximum dilution rate  $D_{max}$  below which the chemostat is not washed out but a steady and finite bacterial concentration is reached?
- (c) Argue that the steady state solution derived before is the only stable steady state of the system and that the system reaches this state when starting with an arbitrary initial state. How does the system evolves if you for example start with a very low bacterial concentration  $\rho$ ?
- 2. Proteome allocation and Monod's growth law: In this problem, we will work out key elements of the proteome allocation analysis for bacterial growth step-by-step using a concrete example, growth of *E. coli* cells on lactose. With a few assumptions, we will *derive* Monod's growth law introduced in problem # 1.

In the following, all quantities correspond to amount derived from a 1-mL of culture at optical density (OD) = 1, referred to as "OD·mL" in short. 1 OD·mL of culture corresponds to  $10^8 \sim 10^9$  bacterial cells in typical culturing conditions. We will refrain from using per cell quantity because the amount per cell can vary 10x due to change of cell size in different conditions. Instead, amount per OD·mL is more invariant. In particular, total dry mass contained in 1 OD · mL of culture is approx 0.5 mg and total cytoplasmic water contained in 1 OD · mL of culture is approximately 1 mg (or  $1\mu L$  in volume) for almost all growth 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:  $N_i$  and  $M_i$  are, respectively, the number and mass of protein *i* per OD·mL of culture.  $M \approx 0.35 \ mg$  is the total mass of cellular proteins per OD·mL of culture.  $m_i$  is the molecular weight of protein *i*.  $\phi_i \equiv M_i/M$  is the mass fraction of protein *i* among all cellular proteins; it is also referred to as the "proteome fraction".

(a) Conversion between proteome fraction and concentration: The average intracellular concentration of a protein X, [X], can be taken as the number of proteins in OD  $\cdot$  mL of culture,  $N_X$ , divided by the total cytoplasmic water volume in OD  $\cdot$  mL of culture, V. For a protein with molecular weight  $m_X$ , derive a relation between its concentration [X] and its proteome fraction  $\phi_X$ . If the protein is 300 as in length (typical of many proteins), find its concentration in  $\mu M$  if its proteome fraction is 1%

(part per thousand). [Hint: It will be useful to convert the total protein abundance  $M = 0.35 \ mg/(\text{OD} \cdot \text{mL})$  into "no. of aa per  $\text{OD} \cdot \text{mL}$ ", using the average mass of an amino acid, 110 Daltons.]

Note: In the problems below, we will only refer to protein concentration in term of proteome fraction. It turns out that proteome fraction is more readily obtained experimentally (e.g., by proteomics and ribosome-profiling); it is also a more natural quantity to work with in models. The purpose of part (a) of this problem is to let you know that you can always convert proteome fraction to a more familiar concentration unit, e.g.,  $\mu M$ .

- (b) Protein synthesis flux: Let  $J_R$  denote the flux of protein synthesis, in unit of no. of aa polymerized per OD·mL of culture per time. For a culture growing exponentially at rate  $\lambda$ , this is just  $\lambda \cdot M$  (with the total protein mass M in  $\#aa/(OD \cdot mL)$ ). Molecularly, protein synthesis flux can be written as the product of the ribosome elongation rate (denoted as  $\varepsilon$ ) and  $N_R$ , the total number of ribosomes per OD·mL of culture. [Here we will assume that all ribosomes are engaged in translation and work at the same speed. This turns out to be a reasonably good approximation as long as the growth rate is not too slow.]
  - Show that the above leads to the growth law

$$\lambda = \gamma \cdot \phi_R,\tag{3}$$

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  $\gamma$  in terms of  $\varepsilon$  and  $m_R$ , the molecular weight of all r-proteins in a ribosome.

- Adding up the length of all r-proteins in a ribosomes gives 7336aa. However, for a 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  $m_R = 1.6 \times 7336aa$ . Further using  $\varepsilon = 16aa/s$ , find the value of  $\gamma$  in unit of  $h^{-1}$ .
- Explain why  $\gamma$  is an upper bound on the growth rate attainable. What is the corresponding doubling time? The fastest doubling time for *E. coli* is 20 min, when the culture is supplemented with many nutrient ingredients including all amino acids, nucleotides, and vitamins. What is the corresponding ribosomal fraction  $\phi_R$  at this fastest growth rate? The remaining fraction of the proteome, called  $\phi_Q$ , are comprised of obligatory proteins needed for house-keeping functions, e.g., synthesis of the lipid membrane and cell wall.
- (c) Carbon uptake flux: Consider growth of *E. coli* in minimal medium with a single substrate as a carbon source, without supplement of amino acid and other substances. Let  $J_C$  denote the flux of carbon uptake, in unit of no. of substrate molecules taken up per time per OD·mL. Molecularly, this can be written as a product of  $k_E$ , the turnover rate of the uptake enzyme, and  $N_E$ , the number of 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 enzyme E,  $m_E$ .

- The condition of *flux balance* can be stated as  $J_R = c \cdot J_C$ , where the coefficient c 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  $\lambda = \nu_E \cdot \phi_E$  and find an expression for  $\nu_E$  in terms of the parameters for the enzyme  $(k_E, m_E)$  and the coefficient c.
- Consider the case where lactose is the sole carbon substrate. 1 g of lactose is known to yield 0.5 g of dry mass. Based on the protein: dry mass ratio given above, work out the conversion factor c for lactose.
- Given that the turnover rate for the lactose transporter (LacY, the lac permease), is  $k_Y = 3/s$ , and the molecular weight of LacY is  $m_Y = 417 \ aa$ , work out the value of  $\nu_E$  for lactose uptake (called  $\nu_Y$ ) 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, and other enzymes not specific to lactose degradation. Let the proteome fraction of all these carbon catabolic proteins be  $\phi_C$ . Since the expression of LacY is co-regulated with these proteins (by cAMP-CRP),  $\phi_E$  is a fixed fraction of  $\phi_C$ . Suppose  $\phi_E = \alpha_Y \phi_C$ , with  $\alpha_Y < 1$  being a fixed portion, we can write

$$\lambda = \nu_C \cdot \phi_C,\tag{4}$$

where  $\nu_C = \alpha_Y \nu_Y$  if LacY is the bottleneck of lactose uptake.

For cells grown in minimal medium without the supplement of amino acids, etc, another significant fraction of the proteome is comprised of anabolic proteins (i.e., enzymes for biosynthesis of amino acids, etc.) Let the proteome fraction of all these enzymes be  $\phi_A$ . There is a linear relation between the growth rate  $\lambda$  and  $\phi_A$  similar to Eqs. (3) and (4):

$$\lambda = \nu_A \cdot \phi_A. \tag{5}$$

It turns out that  $\nu_A \approx \gamma$ .

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

$$\phi_R + \phi_C + \phi_A = \phi_{\max},\tag{6}$$

where  $\phi_{\text{max}} = 1 - \phi_Q$ ,  $\phi_Q$  being is the same fraction of obligatory proteins encountered in part (b).

• Combine Eqs. (3)-(6) to show that the growth rate depends on the parameter  $\nu_C$  (a measure of "nutrient quality") as

$$\lambda = \lambda_C \frac{\nu_C}{\nu_C + K_C}.\tag{7}$$

Express  $\lambda_C$  and  $K_C$  in terms of  $\gamma$  and  $\phi_{\text{max}}$ .

- For an "infinitely good" carbon source for which  $\nu_C \to \infty$ , what is the growth rate?
- For *E. coli* growing on lactose, the growth rate is found to be approximately 1/h. Find the corresponding value of  $\nu_C$ . Find the proteome fraction  $\phi_R$ ,  $\phi_C$ , and  $\phi_A$  devoted to ribosomal, catabolic, and anabolic enzymes during growth on lactose.

- Based on the value of  $\nu_Y$  you calculated in part (c), what share of catabolic proteins is LacY? What fraction of the entire proteome is LacY?
- (e) Next, let us consider the equilibration of lactose within the cytoplasm. As stated above, the influx of lactose is given by  $k_Y \cdot N_Y$ . The lactose brought into the cell is degraded by LacZ. The lactose degradation flux is  $k_Z \cdot N_Z[L]_{in}/([L]_{in} + K_Z)$ , where  $[L]_{in}$  is the intracellular lactose concentration,  $k_Z$  and  $K_Z$  are the turnover rate and binding constant of beta-galactosidase for lactose, and  $N_Z$  is the number of enzymes. To keep the intracellular lactose concentration at the level of  $K_Z$ , how much LacZ must be expressed? i.e., what is the proteome fraction  $\phi_Z$  for LacZ? What is the share of catabolic proteins do LacY and LacZ together comprise of?

[Properties of beta-galactosidase you need to know:  $k_Z = 60/s$ , molecular weight  $m_Z = 4100 \ aa$  (since LacZ has the length of 1024 aa, and functional enzyme is comprised of a LacZ tetramer).]

(f) Finally, we derive the Monod law relating the growth rate at different substrate concentrations in the medium. Let the lactose concentration in the medium be [L]. Then, the lactose turnover rate per LacY becomes

$$k_Y([L]) = k_Y^{\infty} \frac{[L]}{[L] + K_Y}$$
(8)

where  $k_Y^{\infty} = 3/s$  is the saturated turnover rate used above, and  $K_Y$  is the binding constant of lactose to LacY. Express  $\nu_C$  in terms of  $k_Y([L])$  and use it in Eq. (8) in Eq. (7) to derive Monod's growth law:

$$\lambda([L]) = \lambda^{\infty} \frac{[L]}{[L] + K_M}.$$
(9)

Express the Monod constant  $K_M$  in terms of  $K_Y$  and the basic parameters of the growth laws in Eqs. (3)-(5). Find the value of the Monod constant if  $K_Y = 0.3 \ mM$ . Can you explain why the Monod constant, which describes the lactose concentration at which the growth rate is half of the maximum value, is much smaller than the binding constant  $K_Y$ ?

3. Effect of antibiotics on cell growth: Bacteriostatic antibiotics slows down cell growth by interfering with a spectrum of bacteria-specific functions without killing cells. The drug efficacy can be quantified by the IC50 value, which is the concentration that slows down growth by 50%. In this problem, we will compute IC50 for antibiotics which targets protein synthesis using the bacterial growth laws you learned in class.

Let the fraction of proteome devoted to translational, catabolic, and anabolic processes be  $\phi_R$ ,  $\phi_C$ , and  $\phi_A$ , respectively. As we learned in Problem #2, flux balance conditions lead to simple linear relations between the growth rate  $\lambda$  and each of these 3 proteome fractions; see Eqs. (3) – (5), with the macroscopic parameters  $\gamma$ ,  $\nu_C$  and  $\nu_A$  related to molecular parameters. The 3 proteome fractions are also constrained by (6).

(a) By eliminating  $\phi_R$ ,  $\phi_C$ , and  $\phi_A$  in each the of the relations (3) – (5), find the growth rate  $\lambda$  as a function of  $\gamma$ ,  $\nu_C$ ,  $\nu_A$ , and  $\phi_{\text{max}}$ .

A large class of antibiotics target the ribosome, and the effect of a good number of them can be described as slowing down the translational process. We can model the effect of a sublethal dose of such translation-retarding drugs by a reduction in the ribosome's elongation rate, which would result in a reduction in the parameter  $\gamma$ ; see Problem #2b. Let the value of  $\gamma$  in the absence of drugs be  $\gamma_0$  (whose value was obtained in Problem #2b). [Note that the meaning of  $\gamma$  and  $\gamma_0$  here are different from those in the lecture notes.] Let the growth rate in the absence of drug be  $\lambda_0$ . For different carbon sources (different values of  $\nu_C$ ), the growth rate varies as  $\lambda_0(\nu_C)$ .

(b) Suppose the antibiotics binds to the ribosome with a dissociation constant  $K_D$  and reduces  $\gamma$  as described below:

$$\gamma = \frac{\gamma_0}{1 + [D]/K_D}$$

where [D] is the drug concentration. Show that the growth rate depends on drug concentration as

$$\lambda = \frac{\lambda_0}{1 + [D]/K_I}$$

Find the IC50 value,  $K_I$ , in terms of the dissociation constant  $K_D$  and the ratio of the drug-free growth rate  $\lambda_0(\nu_C)$  and the maximum drug-free growth rate  $\lambda_0(\nu_C \to \infty) \equiv \lambda_C$ .

(c) Rewrite your result in part (b) in terms of the doubling time T in the presence of drug, and the doubling time  $T_0$  in the absence of drugs. For a drug with a dissociation constant  $K_D = 5 \ \mu M$ , plot the doubling time T vs drug concentration [D] in 3 different growth medium, with carbon sources that support doubling time of  $T_0 = 60$ min, 90min, 120min, respectively in the absence of the drug. Indicate the value of  $K_I$  on the plot for each case. Explain qualitatively why the IC50 value should depend on the quality of the nutrient  $(\nu_C)$ , as manifested by its dependence on the drug-free growth rate  $\lambda_0(\nu_C)$ .