Strategies for Bioreactor Optimization I: Problem definition

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Introduction

We developed material balances for nutrients, products and cells for batch, fed-batch and continuous stirred tank bioreactors. However, we have not considered the *best* way to operate these bioreactors, for example, to maximize the amount of the product or the rate of product production in a given time period. To explore the *optimum* way to operate a bioreactor, we need to specify two things; first we need a model for the specific growth rate of cells, and second we need a performance objective for the process. Let's consider each of these in turn.

Specific growth rate models. One of the most widely used models for the specific growth of simple cells on a limiting nutrient i was suggested by Monod (1):

$$\mu = \mu_g^{max}\left(\frac{C_i}{K_{G,i}+C_i}\right) \tag{1}$$

where μ_g^{max} is the maximum specific growth rate (hr^{-1}), and $K_{G,i}$ is a saturation constant for nutrient i (mmol/L). However, there have been many modifications to the Monod equation developed to describe different scenarios such as product or nutrient *inhibition*. Generally, these modifications take the form:

$$\mu = \mu_g^{max}\left(\frac{C_i}{K_{G,i}+C_i}\right)\phi\left(\mathbf{C},\mathbf{k}\right) \tag{2}$$

where $\mathbf{C}$ and $\mathbf{k}$ denote the concentration *vector*, and a parameter vector, respectively. For example, the inhibition of growth rate by product P could be captured by terms of the form:

$$\phi\left(\mathbf{C},\mathbf{k}\right) = \frac{K_{I,P}}{K_{I,P}+C_P} \tag{3}$$

or

$$\phi\left(\mathbf{C},\mathbf{k}\right) = 1 - \frac{\alpha C_P^n}{1+\alpha C_P^n} \tag{4}$$

where α and $K_{I,P}$ are inhibition constants, and n is called a cooperativity parameter. Other formulations have been proposed to capture substrate inhibition. One of the most common is given by:

$$\mu = \mu_g^{max}\left(\frac{C_i}{K_{G,i}+C_i+C_i^2/K_{I,i}}\right) \tag{5}$$

where $K_{I,i}$ is an inhibition constant. While we'll consider only modified Monod models, there are many other models in the literature that have been developed for particular cases. Irrespective of the model, all contain parameters that we must estimate from experimental data before we can optimize a biological process.

Performance analysis of biological processes. Biological processes can be characterized by three performance criteria, the yield, the *productivity* and the *titer*. The titer is the easiest to understand, it is the concentration of the target product in the bioreactor during the process, or at some endpoint time. On the other hand, the yield and productivity describe the amount of product produced per unit of starting material consumed, and the rate of product production, respectively.

Continuous cultures. Before we can discuss these application of these performance metrics to continuous cultures, we need to explore the relationship between the dilution rate and the abundance of cells in the bioreactor. The dilution rate in a bioreactor is related to the growth rate of the organisms in the reactor through the cellmass balance. To see this, let's split the transport terms in the cellmass balance into input and output terms:

$$\sum_{s,in}^{\mathcal{S}} D_s X_s - \sum_{s,out}^{\mathcal{S}} D_s X_s + (\mu - k_d) X = 0 \tag{6}$$

The input terms describe the abundance of cells being carried into the reactor by flow. In most applications we do *not* have cells in any of the input streams. This condition (known as the sterile feed assumption) reduced the cellmass balance to:

$$-\sum_{s,out}^{\mathcal{S}} D_s X_s + (\mu - k_d) X = 0 \tag{7}$$

Since we are assuming a well mixed bioreactor, $X_s \simeq X$ which reduces our cellmass balance to:

$$(\mu - k_d) X - X \sum_{s,out}^{\mathcal{S}} D_s = 0 \tag{8}$$

or:

$$(\mu - k_d) = \sum_{s,out}^{\mathcal{S}} D_s \tag{9}$$

Equation (9) is an important relationship; it says that we, not the microorganism, can set how fast we want the cells to grow in the reactor. Thus, we can *externally* control the rate that *intracellular* metabolites, energy (e.g., ATP) and reducing power (e.g., NADH) are used to produce new cells or products. Of course, we can't increase the dilution rate forever as their is a maximum rate at which cells can divide. If we increase the dilution rate beyond the maximum specific growth rate, all of the cells will be carried out of the reactor and washed into downstream processing units (this is called the washout point).

We have already seen yields when we discussed batch cultures. The yield measures the

amount of product produced per unit of starting material consumed in a bioreactor. In a continuous bioreactor, we can redefine the the apparent yield of product i produced by the consumption of substrate j as:

$$Y_{ij} = \left| \frac{\sum_s v_s D_s C_{i,s}}{\sum_s v_s D_s C_{j,s}} \right| \tag{10}$$

which is a direct measure of the ratio of actual concentrations of starting materials and products in the bioreactor.

Example 1 (Apparent cellmass yield in a continuous culture.)

Calculate the apparent yield of cellmass (X) on glucose (G) in a well-mixed continuous bioreactor with two sterile input streams (S1 and S2) and a single output stream (S3). Glucose is carried into the reactor in stream 1, but not stream 2. Unreacted glucose and cells are carried out the reactor in stream 3. For our reactor, the apparent yield equation:

$$Y_{ij} = \left| \frac{\sum_s v_s D_s C_{i,s}}{\sum_s v_s D_s C_{j,s}} \right| \tag{11}$$

becomes:

$$Y_{XG} = \left| \frac{-D_3 C_{X,3}}{D_1 C_{G,1} - D_3 C_{G,3}} \right| \tag{12}$$

which reduces to:

$$Y_{XG} = \left| \frac{-D_3 C_X}{D_1 C_{G,1} - D_3 C_G} \right| \tag{13}$$

after invoking the well mixed assumption.

Lastly, productivity is a measure of the rate of production or consumption of a species in a reactor. For steady-state well mixed continuous bioreactors, we define the productivity as:

$$\mathcal{P}_j = C_j \sum_{s,out}^{\mathcal{S}} D_s \tag{14}$$

We can also define productivity for fed-batch cultures by consider the total inflow rate. Productivity has units of concentration per time e.g., mmol/L-hr, thus it is a measure of the rate of product production in the reactor. Often the optimization problem for continuous cultures is to choose the dilution rate D such that product or cellmass productivity is maximized. However, as we shall see, this is challenging as the optimum dilution rate is very close to the washout point for the bioreactor.

Fed-batch cultures. The problem of optimizing fed-batch cultures is much more difficult than a continuous culture because the bioreactor is not at a steady state. In this case, for a given choice of design variables (things we can change), we must solve a dynamic system of differential equations (the species balances) and evaluate the solution $\mathbf{x}(t)$ using our performance criteria, such as titer

or productivity. For fed-batch cultures, our design variables are typically the shape of the feed ramp (the volumetric flow rate into the bioreactor as a function of time), the starting abundance of nutrients and cells, and the length of time we run the culture. Given these design variables $\mathbf{u}$, we will maximize the performance function $J\left(T\right)$:

$$J\left(T\right) = \vartheta\left(\mathbf{x}\left(T\right), T\right) + \int_{t_0}^{T} \mathcal{L}\left(\mathbf{x}, \mathbf{u}, t\right) dt \tag{15}$$

where $\vartheta\left(\mathbf{x}\left(T\right), T\right)$ and $\mathcal{L}\left(\mathbf{x}, \mathbf{u}, t\right)$ are specific performance functions.

Estimating growth model parameters from experimental data. Before we can consider the optimization of either continuous or fed-batch bioreactors, we must estimate the unknown model parameters that appear in our specific growth rate models. The generation terms in the species material balances contain many unknown parameters that must be estimated before we can solve the balance equations. For example, we must know values for the yield coefficients or the parameters that appear in the kinetics i.e., the saturation constants. Often, there is no first principles way to obtain these values, so we must estimate them from experimental data.

How do we estimate the parameters that appear in saturation kinetics? The parameters that appear in saturation kinetics, for example the maximum rate of growth or uptake and the saturation constants, can be estimated in simple cases (single limiting substrate) by linearizing the rate form. Suppose we want to estimate μ_g^{max} and $K_{G,S}$ for a single limiting nutrient. We can run a series of continuos bioreactors at different dilution rates where we measure the steady-state substrate level in the reactor for each dilution rate. If we ignore cell death $\mu \gg k_d$, we can invert Eqn. (1) and collect terms:

$$\frac{1}{D} = \frac{K_{G,S}}{\mu_g^{max}} \frac{1}{C_S^*} + \frac{1}{\mu_g^{max}} \tag{16}$$

Eqn. (16) is a *linear* equation; if $1/D$ is the dependent variable (y-axis), and $1/C_S^*$ is the independent variable (x-axis), then $1/\mu_g^{max}$ is the y-intercept and $K_{G,S}/\mu_g^{max}$ equals the slope (Fig 1).

A second independent way to estimate μ_g^{max} uses data from batch cultures. During exponential growth in a batch reactor $\mu \simeq \mu_g^{max}$. Cell growth in this phase can be modeled using the exponential:

$$X(t) \simeq X_o \exp\Big[\left(\mu_g^{max} - k_d\right)\Delta t\Big] \tag{17}$$

for $S \gg K_g$ where we have assumed the reactor is well-mixed along with our other normal assumptions. Equation (17) can be decomposed into a linear form by taking the natural log of both sides:

$$\ln X\left(t\right) \simeq \ln X_o + \left(\mu_g^{max} - k_d\right)\Delta t \tag{18}$$

If $\mu_g^{max} \gg k_d$, then μ_g^{max} is the slope of a plot of the log-transformed cell mass as a function of time.

What is the true cell mass yield on substrate Y_{XS}^*? We have explored many ways to estimate the apparent yield, however, it is less clear how to calculate the true cell mass yield. One method to

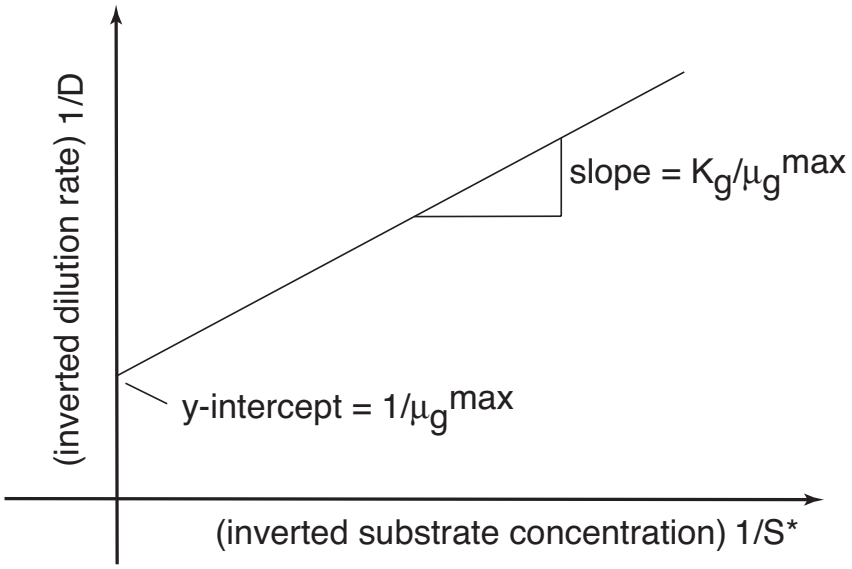

Fig. 1: Example rate inversion plot for growth on a single limiting nutrient in a well-mixed continuous bioreactor.

calculate yield coefficients is to compute the ratio of specific uptake or production rates. However, because of the limitations of treating cells as black boxes, there are many confounding factors that are hidden in this calculation. One of the most confusing concepts is known as *endogenous metabolism* or *maintenance*. Maintenance, first introduced by Pirt (2), is the utilization of carbon (and other nutrients) for functions *other* than growth or production formation. We can estimate the maintenance coefficient starting from the steady-state substrate balance:

$$D\left(C_{S,in} - C_S^*\right) - \frac{1}{Y_{XS}^*}\left(D + k_d\right)X^* - m_S X^* = 0 \tag{19}$$

Dividing both sides by the steady-state cell mass level gives:

$$D\left(\frac{C_{S,in} - C_S^*}{X^*}\right) - \frac{D + k_d}{Y_{XS}^*} - m_S = 0 \tag{20}$$

where the quantity:

$$\frac{1}{Y_{XS}} \equiv \frac{C_{S,in} - C_S^*}{X^*} \tag{21}$$

is the apparent yield. The apparent yield defines how much cell mass would be produced per unit substrate consumed if we simply measured substrate consumption and growth rates. Equation (20) can be rearranged to yield the relationship:

$$\frac{1}{Y_{XS}} = \frac{1}{Y_{XS}^*} + \frac{\hat{m}}{D} \tag{22}$$

where the quantity $\hat{m}$:

$$\hat{m} \equiv \frac{k_d}{Y^*_{XS}} + m_S \tag{23}$$

As the specific rate of cell death, and the maintenance *decreases* the apparent yield and the theoretical yield converge:

$$\lim_{k_d, m_S \to 0} \frac{1}{Y_{XS}} = \frac{1}{Y^*_{XS}} \tag{24}$$

References

1. Monod J (1949) The growth of bacterial cultures. Annu Rev Microbiol 3: 371 - 394.
2. Pirt SJ (1965) The maintenance energy of bacteria in growing cultures. Proc R Soc Lond B Biol Sci 163: 224-31.