Analysis of Well-Mixed Batch Cultures using Unstructured Models

Jeffrey D. Varner*

School of Chemical Engineering*
Purdue University, West Lafayette IN 47907

Introduction

Batch cultures are the simplest type of liquid culture that can be run. In a batch culture, there is not flow into or from the reaction vessel. Thus, there are no pumps or control systems to monitor and adjust flow rates, you simply fill a flask with nutrient rich broth, inoculate the flask with cells, and let the culture proceed. While batch cultures are experimentally simple to run, they are *more complex* than continuous cultures because they are dynamic. In this lecture, we'll develop a mathematical description of batch cultures using the general material balances, and then explore which model parameters we can be estimated with batch culture data.

General model equations for batch cultures. Let's start with the general material balances we derived previously:

$$\frac{dC_j}{dt} = \sum_{s=1}^{\mathcal{S}} v_s D_s C_{j,s} + \left(\sum_{r=1}^{\mathcal{R}} \sigma_{jr}\hat{r}_r\right) + \left(\sum_{k=1}^{\mathcal{T}} \tau_{j,k} q_k\right) X - \frac{C_j}{V}\frac{dV}{dt} \qquad j = 1, 2, \ldots, \mathcal{M} \tag{1}$$

$$\frac{dX}{dt} = \sum_{s=1}^{\mathcal{S}} v_s D_s X_s + (\mu - k_d)\, X - \frac{X}{V}\frac{dV}{dt} \tag{2}$$

$$\frac{dV}{dt} = \sum_{s=1}^{\mathcal{S}} v_s \frac{\rho_s}{\rho} F_s - \frac{V}{\rho}\frac{d\rho}{dt} \tag{3}$$

where the quantity D_s, called a *dilution rate* (hr^{-1}), is given as:

$$D_s \equiv \frac{F_s}{V} \qquad s = 1, 2, \ldots, \mathcal{S} \tag{4}$$

The quantity C_j denotes the concentration of the jth extracellular metabolite, V denotes the working volume of the culture and X denotes the cellmass. In a batch culture, there is not flow into or from the culture vessel, thus $D_s = 0\ \forall s$. Because there is no flow, there is no volume change ($dV/dt = 0$), and all the dilution terms in the material balances vanish leaving:

$$\frac{dC_j}{dt} = \left(\sum_{r=1}^{\mathcal{R}} \sigma_{jr}\hat{r}_r\right) + \left(\sum_{k=1}^{\mathcal{T}} \tau_{j,k} q_k\right) X \qquad j = 1, 2, \ldots, \mathcal{M} \tag{5}$$

$$\frac{dX}{dt} = (\mu - k_d)\, X \tag{6}$$

Analysis of a simple batch culture. To better understand the dynamics of a batch culture, let's simplify the general equations by assuming a Monod growth model (1), a single limiting nutrient S, and by neglecting product formation and maintenance utilization of substrate. With these assumptions the general batch balances reduce to:

$$\frac{dS}{dt} = -\frac{1}{Y^*_{X/S}}\mu\, X \tag{7}$$

$$\frac{dX}{dt} = (\mu - k_d)\, X \tag{8}$$

where μ is given by:

$$\mu = \mu_g^{max}\left(\frac{S}{K_g + S}\right) \tag{9}$$

The cellmass and substrate balances are coupled nonlinear differential equations which can be solved numerically using common packages such as MATLAB or JULIA (2) (Fig. 1). Cell mass growth occurs in three phases: lag, exponential and stationary/death phases. During the lag-phase, cells are manufacturing the proper systems internally to process the substrate. Once this adaptation phase is complete, cells begin to process substrate as quickly as possible which leads to a phase of maximum growth called the exponential phase. Once the substrate is exhausted, cells no longer divide, and growth stops leading to the stationary/death phase of the culture.

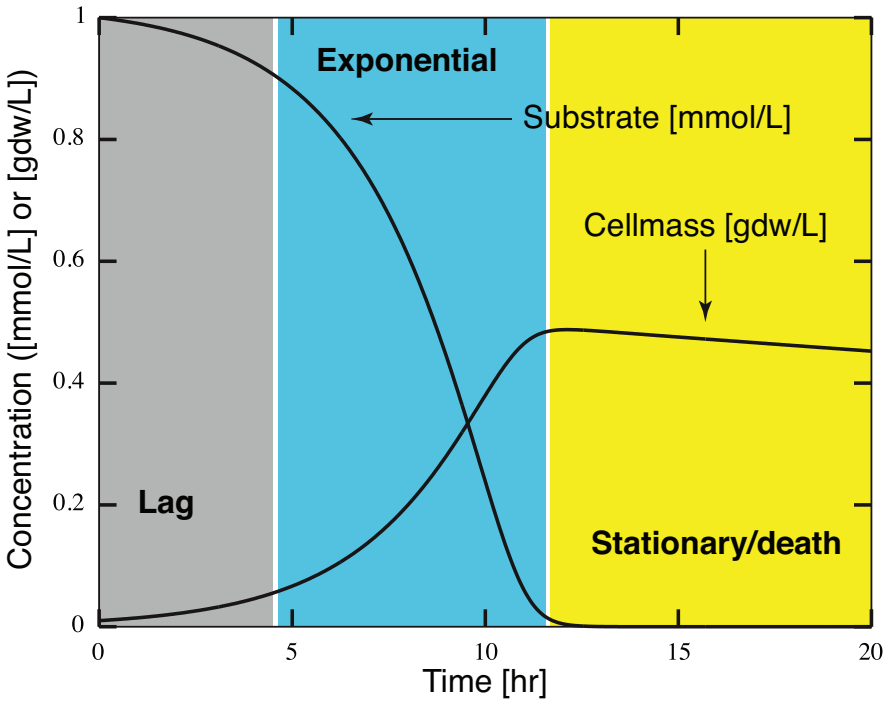

Fig. 1: Theoretical growth and substrate curve for cells in a well-mixed batch culture as a function of time. The x-axis denotes time [hr] while the y-axis denotes the cellmass (X) [gdw/L] or substrate (S) [mmol/L] concentration.

0.0.1 Estimation of model parameters. The simplified batch model equations with Monod growth kinetics have four unknown parameters; the maximum specific growth rate μ_g^{max}, the saturation coefficient K_g, the cell death constant k_d and the cellmass yield on substate $Y^*_{X/S}$. We can use measurements of the cellmass (X) and substrate (S) to estimate these parameters.

To estimate the yield, we take the ratio of the difference between the substate and cellmass measurements at the beginning of the culture and at the end of the exponential growth phase. Recall, the definition of the cellmass yield on substrate is given by:

$$Y_{X/S} = -\frac{\Delta X}{\Delta S} \tag{10}$$

Expanding the differences gives:

$$Y_{X/S} = -\frac{X(T) - X(0)}{S(T) - S(0)} \simeq \frac{X(T)}{S(0)} \tag{11}$$

where $t = T$ denotes the end of exponential phase. Note that we have not estimated $Y^*_{X/S}$, rather we have estimated the apparent yield. The difference between these quantities is associated with the maintenance utilization of substrate. In out case, the true yield ($Y^*_{X/S}$) and the apparent yield ($Y_{X/S}$) are equal (becuase we have neglected maintenance), however this is not generally true.

The cell death rate constant can be estimated from the cellmass measurements during the stationary/death phase of the batch culture. During this phase, $\mu = 0$, thus the cellmass balance reduces to:

$$\frac{dX}{dt} \simeq -k_d X \tag{12}$$

Rearranging and expanding the derivative gives:

$$-\frac{1}{X(t_0)} \frac{X(t_1) - X(t_{-1})}{t_1 - t_{-1}} \simeq k_d \tag{13}$$

where t_{-1}, t_0 and t_1 are three sequential time points during the stationary/death phase, and $X(t_*)$ denotes a cellmass measurement at time point t_*.

To estimate both K_g and μ_g^{max} we need to make use of the growth model. We know that at *anytime* during the culture the growth rate is given by Eqn (9). Thus, we can rearrange the growth model to give:

$$\frac{1}{\mu} = \left(\frac{K_g}{\mu_g^{max}}\right) \frac{1}{S} + \frac{1}{\mu_g^{max}} \tag{14}$$

Eqn (14) is called a *double reciprical*; we use this relationship in combination with estimates of the growth rate and substrate measurements to uniquely determine *both* K_g and μ_g^{max} from the slope and intercept of the double reciprocal plot (1/S versus 1/μ).

A quick and dirty approximation of μ_g^{max} can also be obtained by estimating the growth rate during the exponential phase; during the exponential phase the culture growth rate is approximately

$\mu \simeq \mu_g^{max}$ which reduces the cellmass balance to:

$$\frac{dX}{dt} \simeq \left(\mu_g^{max} - k_d\right) X \tag{15}$$

Assumming that we have already estimated k_d, the only unknown in Eqn (15) is μ_g^{max}:

$$\frac{1}{X}\frac{dX}{dt} + k_d \simeq \mu_g^{max} \tag{16}$$

or

$$\frac{1}{X(t_0)}\frac{X(t_1) - X(t_{-1})}{t_1 - t_{-1}} + k_d \simeq \mu_g^{max} \tag{17}$$

where t_{-1}, t_0 and t_1 are three sequential time points during the exponential phase, and $X(t_*)$ denotes a cellmass measurement at time point t_*.

References

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