Applying Logistic and Monod models in a single equations system framework for cell culture growth modeling and estimation

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Abstract

In modeling cell culture growth, two types of modeling equations are preferentially used: logistic and Monod. These two equations are known for their strengths and weaknesses in modeling cell culture growth. In this contribution, we show how these equations can be used in a single equations system framework to model cell culture growth that is supported by experimental observation. Specifically, we propose that logistic equation is used to model the dynamic of total cells growth that is simply the summation of viable and dead cells populations in the system. Subsequently, Monod equation is used to model the dynamic of viable cells growth that is subjected to growth-limiting substrate and cells death rate term. With this paradigm, a rate equation can be written for the accumulation of dead cells in the system with a simple understanding that dead cells population is simply the difference between total and viable cells. These equations can be adjoined with appropriate substrate consumption and product generation rate equations to depict a complete time course profiles of batch culture experiment. This modeling framework has been fitted successfully to depict a batch growth data of IgG-secreting murine hybridoma cell from published literature.

Keywords: Logistic model; Monod model; Cell growth model; Parameter estimation

Introduction

In modeling cell culture growth using unstructured kinetic model, two types of equations are preferentially used: logistic and Monod (Shirsat et al., 2015). In Logistic modeling, cells growth is dictated by two parameters that are of biological significance: specific intrinsic growth rate, k (1/h)

and carrying capacity of the system, X_{max} (cells/mL). The corresponding equation is normally written as follow:

$$\frac{dX}{dt} = kX\left(1 - \frac{X}{X_{max}}\right) \qquad X(0) = X_0 \tag{1}$$

This equation can be solved analytically to yield a logistic curve for cell culture growth that leads to a stationary population size of X_{max}.

Meanwhile, in Monod modeling, the specific growth rate, μ (1/h) is subjected to a growth limiting substrate with concentration S and its formulation was fashioned after the Michaelis-Menten kinetics, the expression of which for microbial and cell population growth is given as follow:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{2}$$

Here, μ_{max} is the maximum specific growth rate achievable when $S \gg K_s$. The parameter K_s which has the same unit as substrate concentration is also referred to as saturation constant and can be interpreted as the concentration of the substrate at which the specific growth rate is half of its maximum value μ_{max}. However, unlike Logistic model that is rooted in some mechanistic principle of biological population growth, Monod model is strictly empirical and borne out of necessity to fit experimental data of cell growth rate versus growth-limiting substrate concentration (Liu, 2007). Nevertheless, its utility to model microbial and cell population growth in various settings has been widely recognized and acknowledged by countless researchers. Based on the Monod model, the cell growth rate equation can be written as follow:

$$\frac{dX}{dt} = \mu X = \frac{\mu_{max} S}{K_S + S} X \qquad X(0) = X_0 \tag{3}$$

Recognizing the strengths and weaknesses of both modeling equations, there has been attempt to merge these two equations into a single expression that can describe the specific cell growth rate as a function of both growth-limiting substrate as well as self-inhibiting factor due to carrying capacity of the system (Xu, 2020):

$$\frac{dX}{dt} = \mu_{max}\left(\frac{S}{K_S + S}\right)\left(1 - \frac{X}{X_{max}}\right) X \qquad X(0) = X_0 \tag{4}$$

Coupled with a suitable substrate consumption rate equation, analytical solution for these equations can been derived with the aid of advanced numerical software, e.g. Matlab as was shown previously in the corresponding work (Xu, 2020). The only drawback with this modeling approach is its failure to predict the decline phase of viable cell concentration that is normally observed in cell culture experiments.

In this contribution, we propose a modeling framework whereby both Logistic and Monod equations can be used in a single equations system to model the complete time-course profile of batch cell culture growth. Specifically, we propose that logistic equation is used to model the dynamic of total cells growth with concentration, X_T that is simply the summation of viable and dead cells concentrations, X_v and X_d respectively, i.e. $X_T = X_v + X_d$. Subsequently, Monod-based equation is used to account for the viable cells growth with cells death rate term included to account for the decline of viable cell concentration that is normally observed in cell culture experiments:

$$\frac{dX_v}{dt} = (\mu - K_d)X_v = \left(\frac{\mu_{max} S}{K_S + S} - K_d\right) X_v \qquad X_v(0) = X_{v0} \tag{5}$$

Here, K_d (1/h) is commonly interpreted as the specific death rate of viable cells. In simplest term, this parameter can be treated as a constant although various ad-hoc expressions have been derived to account for the possible effect of inhibitory/toxic by-products and/or growth limiting substrates to cell viability which again are strictly empirical and so far have received little experimental verifications unlike Monod model (Kyriakopoulos et al., 2018).

To account for the fact that in most batch culture experiments, viable and dead cell concentrations are usually reported, we can write a rate equation for the accumulation of dead cells in the system with a simple understanding that dead cells concentration is simply the difference between total and viable cells concentrations, i.e. $X_d = X_T - X_v$ so that the rate equation for dead cells accumulation can be written as follow:

$$\frac{dX_d}{dt} = k\left(1 - \frac{X_v + X_d}{X_{T,max}}\right)(X_v + X_d) - \left(\frac{\mu_{max} S}{K_S + S} - K_d\right) X_v \qquad X_d(0) = X_{d0} \tag{6}$$

where $X_{T,max}$ is now interpreted as the total carrying capacity of the system that includes the totality of viable and dead cells populations. The advantage of this modeling approach is that while Logistic

equation is used appropriately to account for the totality of cells population growth, Monod equation is now used exclusively to account for the effect of growth-limiting substrate to viable cells growth, as should be the case since this is the only cell type in the system that consumes nutrient. Therefore, each equation is applied in their respective domain and the only place where they are used together is when they are applied in Equation (6) to account for the accumulation of dead cells in the system. These equations can be completed with suitable substrate consumption and product generation rate equations to give a complete depiction of cell culture growth evolution that includes the decline phase of viable cells concentration.

Computational methods

To demonstrate the capability of the combined modeling framework to depict the complete time course profiles of batch culture experiment, the corresponding model equations are fitted to the dataset of batch growth data of IgG-secreting murine hybridoma cell (Gao, Gorenflo, Scharer, & Budman, 2007) that is frequently used in cell culture modeling studies (Baughman, Huang, Sharfstein, & Martin, 2010; Selişteanu, Şendrescu, Georgeanu, & Roman, 2015). Although the complete dataset includes the time course profiles of 11 metabolite species, however we are only interested in the modeling of crucial state variables that consist of viable cells, dead cells, glutamine (which is the growth-limiting substrate), biomass, and secreted antibody. Therefore, the complete modeling equations for this system consist of Equations (5) and (6) for viable and dead cells populations, respectively, and three more equations for limiting substrate consumption and biomass and antibody productions rates, respectively as shown below:

Substrate: $$\frac{dS}{dt} = -\mu_{max}\left(\frac{S}{K_S + S}\right)\left(\frac{1}{Y_{X_v/S}}\right)X_v \qquad S(0) = S_0 \tag{7}$$

Biomass: $$\frac{dP_1}{dt} = \mu_{max}\left(\frac{S}{K_S + S}\right)Y_{P_1/X_v}X_v \qquad P_1(0) = P_{10} \tag{8}$$

Antibody: $$\frac{dP_2}{dt} = K_{P_2}X_v \qquad P_2(0) = P_{20} \tag{9}$$

In this work, the parameter estimation problem is solved using least-squares estimation strategy for differential equation model. To ensure stability in the numerical solution, the corresponding ordinary differential equations (ODE) system is completely parameterized with orthogonal collocation method on finite elements to transform the dynamic estimation problem into a nonlinear programming (NLP) problem that can be solved using large-scale NLP solver. In this work, we used IPOPT (Wächter & Biegler, 2006), a widely used large-scale NLP solver to solve the corresponding NLP problem. Due to its high-dimensional and nonlinear nature, multiple local solutions might exist. Therefore, to circumvent this problem, multi-start strategy is adopted whereby multiple initial guesses of the unknown parameters are generated using Latin hypercube sampling and the NLP problem is solved for each of these initial parameters guesses. The result of the best NLP run is then selected to represent the solution of the model estimation problem. The complete implementation of this parameter estimation strategy can be referred from the source code of the accompanied Python program. In that program, we used CasADi (Andersson, Gillis, Horn, Rawlings, & Diehl, 2019) which is an open-source tool for algorithmic differentiation as the intermediary between problem formulation codes in Python and IPOPT solver. The capability of CasADi to perform local sensitivity analysis for the ODE model using third-party ODE solution package, i.e. SUNDIALS (Hindmarsh et al., 2005) is further exploited for post-statistical inferencing.

Finally, we were able to determine the crucial parameters of this model together with their 95% confidence intervals as determined using student-t distribution statistics. These values are listed in Table 1. Aside from significant uncertainty associated with Monod kinetic parameters, the rest of the model parameters were estimated with good precision. The validity of this parameter estimation result is further proven with model simulation that indicates the ability of the model to fit satisfactorily the corresponding experimental dataset as shown in Figure 1. The ability of this model with the corresponding parameter values to predict the decline phase of viable cell concentration as well as time profiles of dead cells concentration and cell viability is clearly apparent. Another point of interest is the estimated value of the total carrying capacity, $X_{T,max}$ that agrees quite well with the observation from the total cells data as shown in Figure 2. These results clearly give credibility to this modeling

framework that exploits the strength of both Logistic and Monod equations to simulate full batch growth data within a combined equations system.

Table 1 Estimated model parameters with their 95% confidence intervals

Parameter (unit)	Value	95% confidence interval
μ_{max} (1/h)	0.582	(0.0199 , 16.999)
K_S (mmol/mL)	0.0136	(2.93×10^{-4} , 0.633)
K_d (1/h)	0.0227	(0.0172 , 0.0298)
k (1/h)	0.0836	(0.0613 , 0.114)
$X_{T,max}$ ($\times10^6$ cells/mL)	1.02	(0.933 , 1.11)
$Y_{Xv/S}$ (10^6 cells/mmol)	621	(480 , 803)
$Y_{P1/S}$ (mmol/10^6 cells)	9.60×10^{-3}	(7.59×10^{-3} , 0.0121)
K_{P2} (mmol/10^6 cells/h)	3.96×10^{-9}	(3.46×10^{-9} , 4.52×10^{-9})

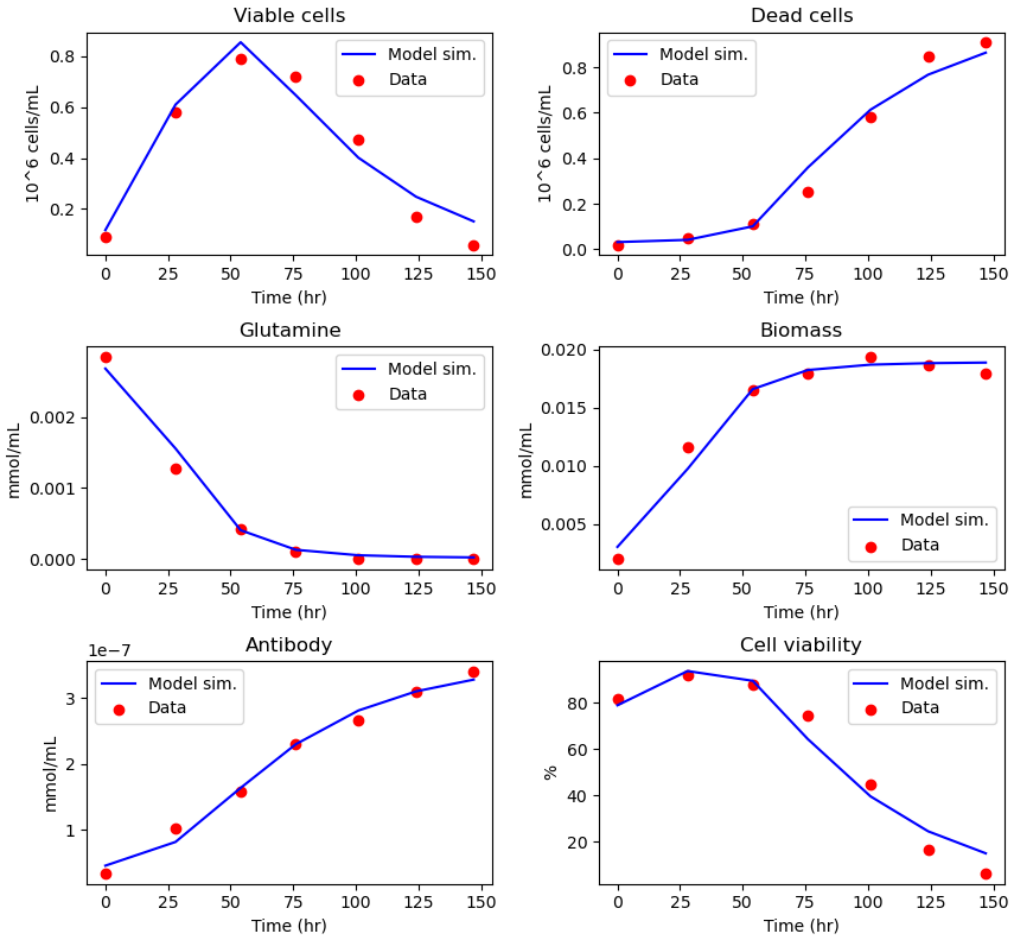

Figure 1 Comparison of model simulation with experimental data for viable cells, dead cells, glutamine, biomass, secreted antibody, and cell viability

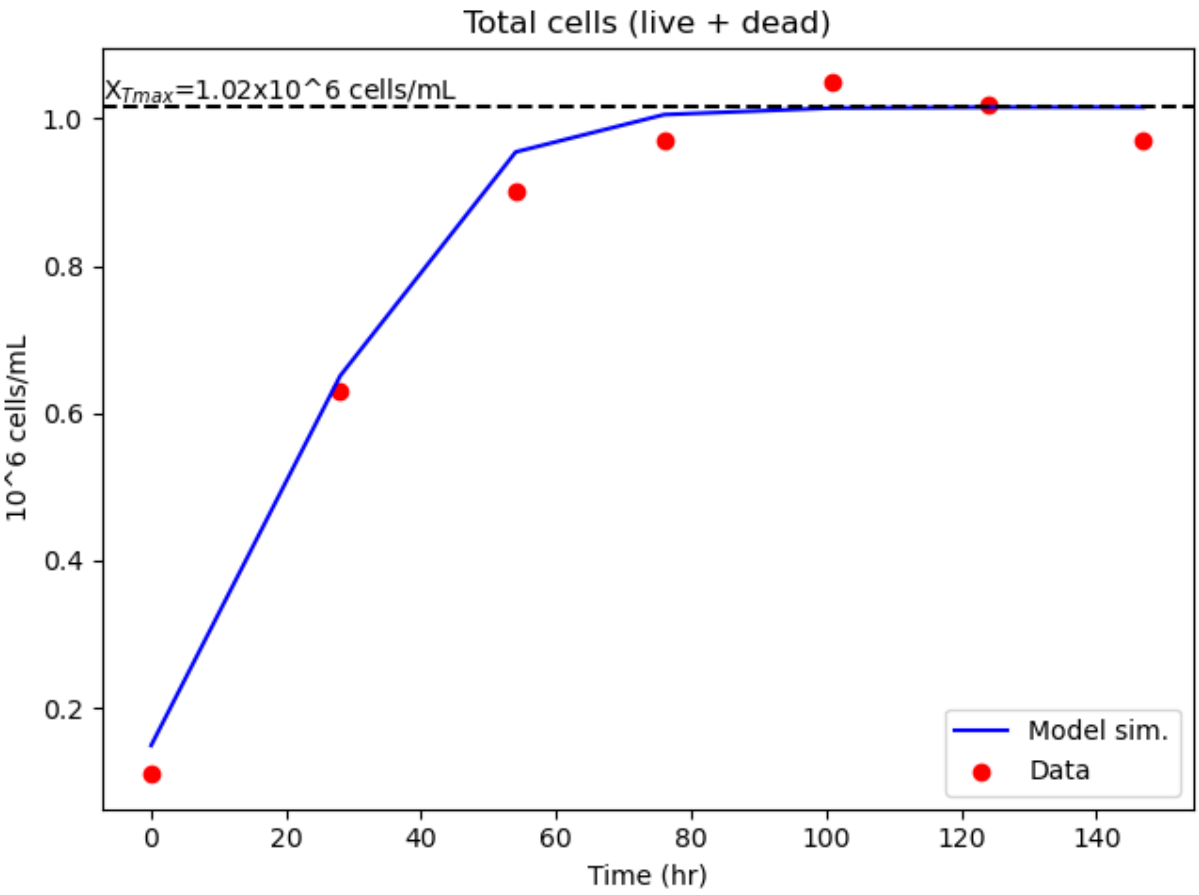

Figure 2 Comparison of simulation result and experimental data for total cells concentration

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