Application of modified Logistic and Monod models in a single equations system framework to improve cell culture growth modeling and estimation

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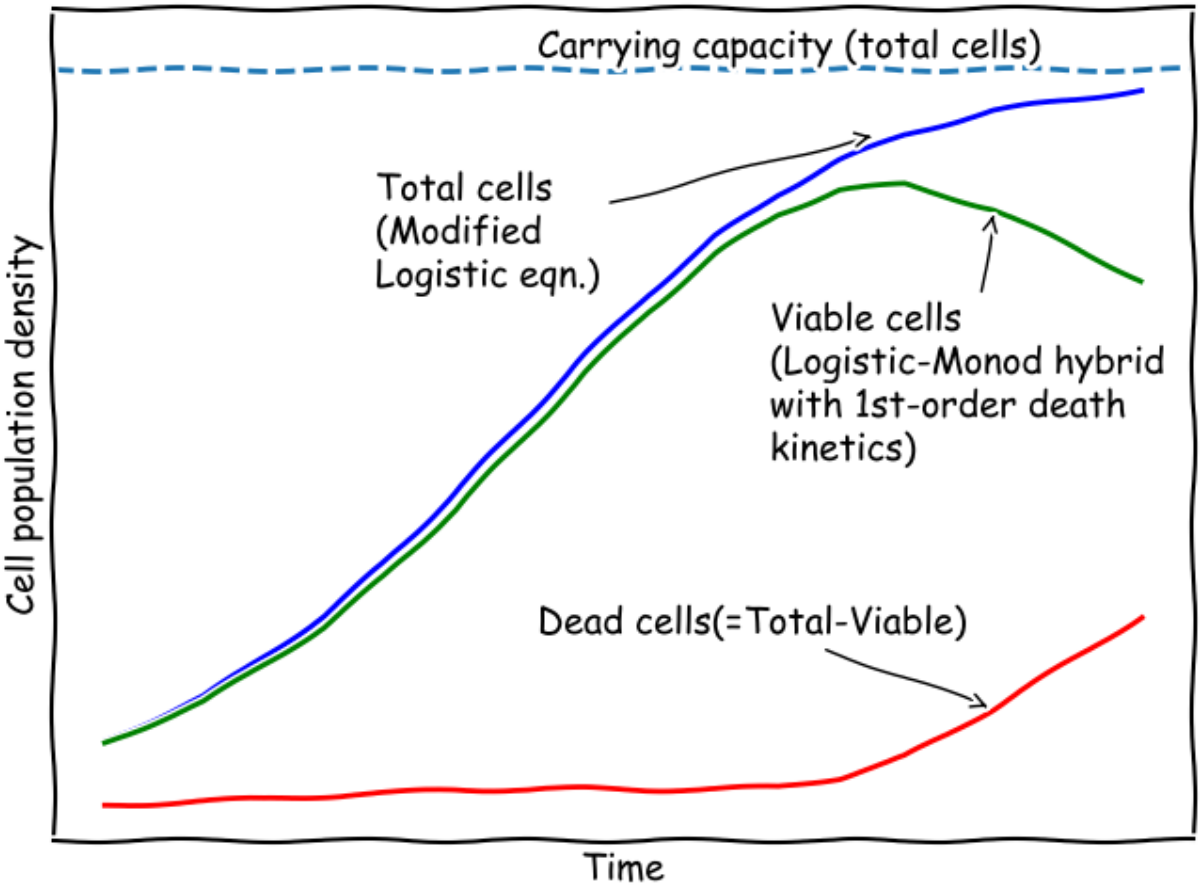
Abstract

In modeling cell culture growth using unstructured model, two types of equations are normally used: logistic and Monod. However, these two equations are known for their limitations to model death phase of cell culture growth and to account for dead cells accumulation data accurately. In this paper, we present a modeling framework whereby both Logistic and Monod equations can be used in a single set of equations system to overcome these limitations. First, it can be shown that the increase of total cell population that consists of viable and dead cells follows a logistic growth pattern with its own intrinsic growth rate and total carrying capacity. Furthermore, a hybrid Logistic-Monod equation with first-order decay kinetics can be used to model viable cell growth data with decline phase effectively. With this paradigm, a pseudo-rate equation can be written to account for dead cells accumulation data using population balancing with a simple understanding that dead cell population is simply the difference between total and viable cells. These equations can be adjoined with substrate consumption and product generation rate equations to depict complete batch growth data that covers exponential growth and death phases. This modeling framework has been fitted successfully to fit batch growth data of two cell lines from published literature with complete depictions of dead cell accumulation and cell viability profiles. The implication of this modeling framework for chemostat culture performance analysis is further investigated.

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

Graphical abstract:



Introduction

In modeling cell culture growth using unstructured model, two types of equations are normally used: logistic and Monod (Shirsat et al., 2015). In the case of logistic growth modeling, the model is simply fitted to cell growth data to determine two parameters that are of biological interest: specific intrinsic growth rate, k (1/h) and carrying capacity of the system, X_{max}. In rate form, the logistic 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 growth curve for cell population 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:

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

Here, μ_{max} is the maximum specific growth rate achievable when $S \gg K_s$. The parameter K_s which is also referred to as saturation constant 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 (Kovárová-Kovar & Egli, 1998; Kyriakopoulos et al., 2018). Based on Monod model, the cell growth rate equation can be written as follow:

$$\frac{dX}{dt} = \mu X = \frac{\mu_{max} S}{K_S + S} X \tag{3}$$

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

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

Coupled with substrate consumption rate equation, analytical solution for this set of equations system can be derived with the aid of advanced numerical software, e.g. Matlab as was demonstrated by Xu (2020). With this hybrid equation, cell growth and substrate consumption will progress initially through substrate-limiting phase that is modeled by Monod equation and gradually shifts towards self-inhibiting phase that is modeled by Logistic equation.

However, even with this hybrid form, the apparent drawback of this modeling framework is its inability to model decline phase of cell culture growth that is normally observed in cell culture experiments. Moreover, the mathematical depiction of dead cells accumulation data is rarely considered in practice due to its perceived unimportance as well as incomplete understanding of cell death kinetics at cellular and macroscopic levels. This can cause problem if the model is to be used to produce cell viability profile which is important in assessing the overall health of cell culture growth. It is quite often that the modeler will use different models to account for different phases of cell culture growth, i.e., exponential growth versus death phases with switching time point determined from visual inspection of experimental data that is always subjected to error. However, as simple as this crude approach is for model formulation, it can usually lead to poor model fit, notwithstanding the fact that it can inadvertently cause non-smoothing of state variable profiles at the switching point if special precaution is not taken to ensure that the adjoined models are smooth at that particular point. Furthermore, this modeling approach can cause further inconvenience if sensitivity analysis is to be conducted on the adjoined models using derivative-based approach.

In this contribution, we show how these two important equations in unstructured modeling arsenal can be used together in a single equations system to overcome these limitations. First, it can be shown that the growth of total cell population that consists of viable and dead cells follows a modified form of logistic equation with its own intrinsic growth rate, k_T and total carrying capacity $X_{T,max}$:

$$\frac{dX_T}{dt} = k_T X_V\left(1 - \frac{X_T}{X_{T,max}}\right) \quad X_T(0) = X_{T0} \quad (5)$$

where X_T is the total cell density and is simply the summation of viable X_V and dead cell X_D densities within the system. On the other hand, to account for the growth and eventual decline of viable cell population that depends on a growth-limiting substrate, a hybrid Logistic-Monod equations as proposed by Xu (2020) with the inclusion of first-order decay kinetics for cell viability degradation can be used as shown below:

$$\frac{dX_V}{dt} = (\mu_{hyb} - K_d)X_V \qquad X_V(0) = X_{V0} \tag{6}$$

where, for the sake of convenience we define

$$\mu_{hyb} = \frac{\mu_{max}S}{K_S + S}\left(1 - \frac{X_V}{X_{V,max}}\right) \tag{7}$$

In this formulation, 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 effects of inhibitory/toxic by-products and/or growth limiting substrates to cell viability degradation (Kyriakopoulos et al., 2018) which again are strictly empirical and so far, had received little experimental supports unlike Monod model.

With this paradigm, a pseudo-rate equation can be written based on population balancing to account for the accumulation rate of dead cells in the system with a simple understanding that dead cell population is simply the difference between total and viable cells:

$$\frac{dX_D}{dt} = k_T X_V\left(1 - \frac{X_V + X_D}{X_{T,max}}\right) - (\mu_{hyb} - K_d)X_V \qquad X_D(0) = X_{d0} \tag{8}$$

For this equation to make absolute sense, $dX_T/dt \geq dX_V/dt$ as the rate of growth of viable cells cannot outstripped the rate of growth of total cells. This equation can be expressed in the following pseudo first-order kinetic form based on viable cell density:

$$\frac{dX_D}{dt} = k_{X_D}X_V \tag{9}$$

where the pseudo-rate constant k_{XD} (1/h) can in turn be expressed as follow:

$$k_{X_D} = k_T\left(1 - \frac{X_V + X_D}{X_{T,max}}\right) - \mu_{hyb} + K_d \tag{10}$$

The advantage of this formulation is that it clearly expresses k_{XD} as a function of batch culture condition as exemplified by X_V, X_D, and S.

In most cases, a first-order decay term with respect to dead cell concentration is added to Equation (9) to account for lysis of dead cells that can manifest itself as a noticeable decline of total cell population that is normally observed nearing the end of batch culture growth:

$$\frac{dX_D}{dt} = k_{X_D}X_V - K_{lys}X_D \tag{11}$$

where K_{lys} (1/h) can be interpreted as the specific lysis rate of dead cells.

Equations (6) and (9) (or (11)) can be adjoined with suitable substrate consumption and product generation rate equations to give a complete depiction of cell culture growth profiles that includes decline phase of viable cells, dead cells accumulation, and cell viability profiles. The definition of cell viability is simply given as follow:

Cell viability: $$\mathrm{CV} = \frac{X_V}{X_V + X_D} \times 100 \tag{12}$$

Case studies

To demonstrate the capability of this modeling framework to fit and depict complete time course profiles of batch culture growth, experimental datasets of batch culture growth of two cell lines that produce important proteins will be used as sample cases in this study: 1) IgG-secreting murine hybridoma cell (Gao, Gorenflo, Scharer, & Budman, 2007) and 2) AGE1.HN.AAT cell for the production of Alpha-1 antitrypsin (AAT) (Ramos, Rath, Genzel, Sandig, & Reichl, 2020). These cell lines have been subjected to frequent modeling studies using structured modeling approach as can be seen from the works of Baughman, Huang, Sharfstein, and Martin (2010) and Selişteanu, Șendrescu, Georgeanu, and Roman (2015) for murine hybridoma cell and Ramos et al. (2020) for AGE1.HN.AAT cell. To the best of our knowledge, this is the first instance whereby these cell lines will be subjected to unstructured modeling analysis using our proposed modeling framework. The objective here is to see whether this approach can be used to model full time course profile of batch culture growth, from exponential growth to death phase, especially for viable and dead cells using only a single set of equations system. This capability can go a long way in producing a simple and yet effective generic mathematical model that can be used in process control for production-scale bioreactor system.

For IgG-secreting murine hybridoma cell, the corresponding concentration datasets are selected to fit the model equations: viable cells (X_V), dead cells (X_D), glutamine (which is the growth-limiting substrate, S), biomass (which is the growth-associated product, P_G), and secreted antibody, a non-growth associated product produced by the cells (P_{NG}). On the other hand, for AGE1.HN.AAT cell, the corresponding concentration datasets are selected to fit the proposed model: viable cells (X_V), dead cells (X_D), glucose (S), and AAT (P_G). Apart from Equation (6) to depict viable cell growth for both cell lines, Table 1 lists the complete modeling equations for other state variables for both cell lines. Of particular interest is the use of Equations (9) and (11) to depict dead cells accumulation rate for murine hybridoma and AGE1.HN.AAT cell culture growths, respectively. This selection is primarily guided by the model estimation result (see Results and discussion below) that indicates the non-identifiability of K_{lys} parameter for murine hybridoma cell growth i.e., small value and large uncertainty following statistical inferencing. Additionally, a first-order decay term is included to account for chemical decomposition of glutamine to ammonia (Ozturk & Palsson, 1990) for murine hybridoma cell with decomposition rate constant k_{dS} (1/h).

Table 1 List of model equations for batch culture growth modeling and estimation

Component	Murine hybridoma	AGE1.HN.AAT	
Dead cells:	Equation (9)	Equation (11)	
Growth-limiting substrate:	$\frac{dS}{dt} = -\frac{\mu_{hyb}}{Y_{X_v/S}} X_V - k_{d_S} S$	$\frac{dS}{dt} = -\frac{\mu_{hyb}}{Y_{X_v/S}} X_V$	Equation (13a and b)
Growth-associated product:	$\frac{dP_G}{dt} = \mu_{hyb} Y_{P_G/X_V} X_V$		Equation (14)
Non-growth associated product:	$\frac{dP_{NG}}{dt} = K_{P_{NG}} X_V$	-	Equation (15)

Numerical methods

In this work, the parameter estimation problem is solved using least-squares estimation strategy for differential equation model (Englezos & Kalogerakis, 2001). To ensure stability in the numerical solution, the corresponding ordinary differential equations (ODE) system is completely parameterized using 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 robust solver for large-scale NLP to solve the corresponding optimization problem. Due to its high-dimensional and nonlinear nature, multiple local solutions might exist that can produce suboptimal parameter estimation results. 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 model estimation result. The complete implementation of this parameter estimation strategy can be referred from the corresponding source code of Python programs that were posted in GitHub[1]. In that programs, 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 ODE model using third-party ODE solution package SUNDIALS (Hindmarsh et al., 2005) is further exploited for post-statistical inferencing of the fitted model parameters.

Results and discussion

The results of the parameter estimation using the proposed modeling framework for both cell culture growths data are shown in Table 2 together with their 95% confidence intervals as determined using student-t distribution statistics. For murine hybridoma cell, due to the non-identifiability of K_{lys} parameter in the model estimation, the phenomenon of dead cells lysis was considered not significant and the use of Equation (9) is sufficient to account for dead cells accumulation data. It is worth to mention that through experimental investigation, Goergen, Marc, and Engasser (1993) also found out that dead cell lysis was apparently not significant for murine hybridoma cell in a normal culture environment. The validity of this parameter estimation results is further proven with model simulations that indicates the ability of the fitted models to reproduce satisfactorily the corresponding experimental profiles as shown in Figures 1 and 2 for murine hybridoma cell and Figure 3 for AGE1.HN.AAT cell. The ability of this modeling framework to predict the decline phase of viable

[1] https://github.com/nazrifuad2020/modified-logistic-monod-fit

cell concentration as well as dead cells concentration and cells viability profiles is clearly apparent for both cell culture growths. These results clearly give credibility to this modeling framework to simulate all phases of batch culture growth using only a single set of equations system. Figure 4 shows the dynamic profiling of k_{XD} parameter for both cell culture growths as calculated using Equation (10) that shows distinct behavior of the corresponding parameter evolution across both cell lines.

Using the fitted models, we can carry out further model-based studies such as sensitivity analysis to ascertain the sensitivity of state profile of interest to model parameters. For instance, using a derivative-based approach i.e., forward sensitivity analysis, we can determine the impact of model parameters to dead cell accumulation profile as shown in Figures 5 for both cell culture growths. From these results, we can see that even though the sensitivities of the corresponding state profile to model parameters were quite different across both cell lines, however, one thing is clear from this result is that the effect of Monod saturation constant, K_S is quite significant to both dead cell accumulation profiles according to the fitted models.

Table 2 Estimated model parameters with their 95% confidence intervals

Parameter grouping	Parameter (unit)	Murine hybridoma			AGE1.HN.AAT		
		Value	95% confidence interval		Value	95% confidence interval	
Total cell growth (Modified Logistic)	k_T (1/h)	0.103	(0.0731	0.144)	0.0327	(0.0298	0.0358)
	$X_{T,max}$ ($\times 10^6$ cells/mL)	1.00	(0.941	1.07)	4.54	(4.19	4.91)
Viable cell growth (Logistic-Monod)	μ_{max} (1/h)	0.139	(0.115	0.167)	0.0383	(0.0337	0.0436)
	K_S (M)	1.42×10^{-4}	(1.88×10^{-5}	1.07×10^{-3})	3.11×10^{-3}	(8.63×10^{-4}	0.0112)
	$X_{V,max}$ ($\times 10^6$ cells/mL)	1.14	(0.870	1.49)	7.01	(5.03	9.78)
Cell death and lysis	K_d (1/h)	0.0290	(0.0214	0.0391)	7.23×10^{-3}	(5.26×10^{-3}	9.93×10^{-3})
	K_{lys} (1/h)	-	-	-	0.0221	(0.0108	0.0452)
Yield parameters (substrate and products)	$Y_{Xv/S}$ (10^6 cells/mmol)	1.62×10^{3}	(676	3.86×10^{3})	180	(156	209)
	$Y_{PG/Xv}$[a,b]	7.58×10^{-3}	(5.87×10^{-3}	9.79×10^{-3})	21.2	(18.4	24.4)
	K_{PNG} (mmol/10^6 cells/h)	3.96×10^{-9}	(3.58×10^{-9}	4.38×10^{-9})	-	-	-
Chemical decomposition	k_{DS} (1/h)	0.0192	(9.65×10^{-3}	0.0382)	-	-	-

[a]For murine hybridoma cell, the yield unit for biomass product is mmol/10^6 cells

[b]For AGE.HN.AAT, the yield unit for AAT product is μg/10^6 cells

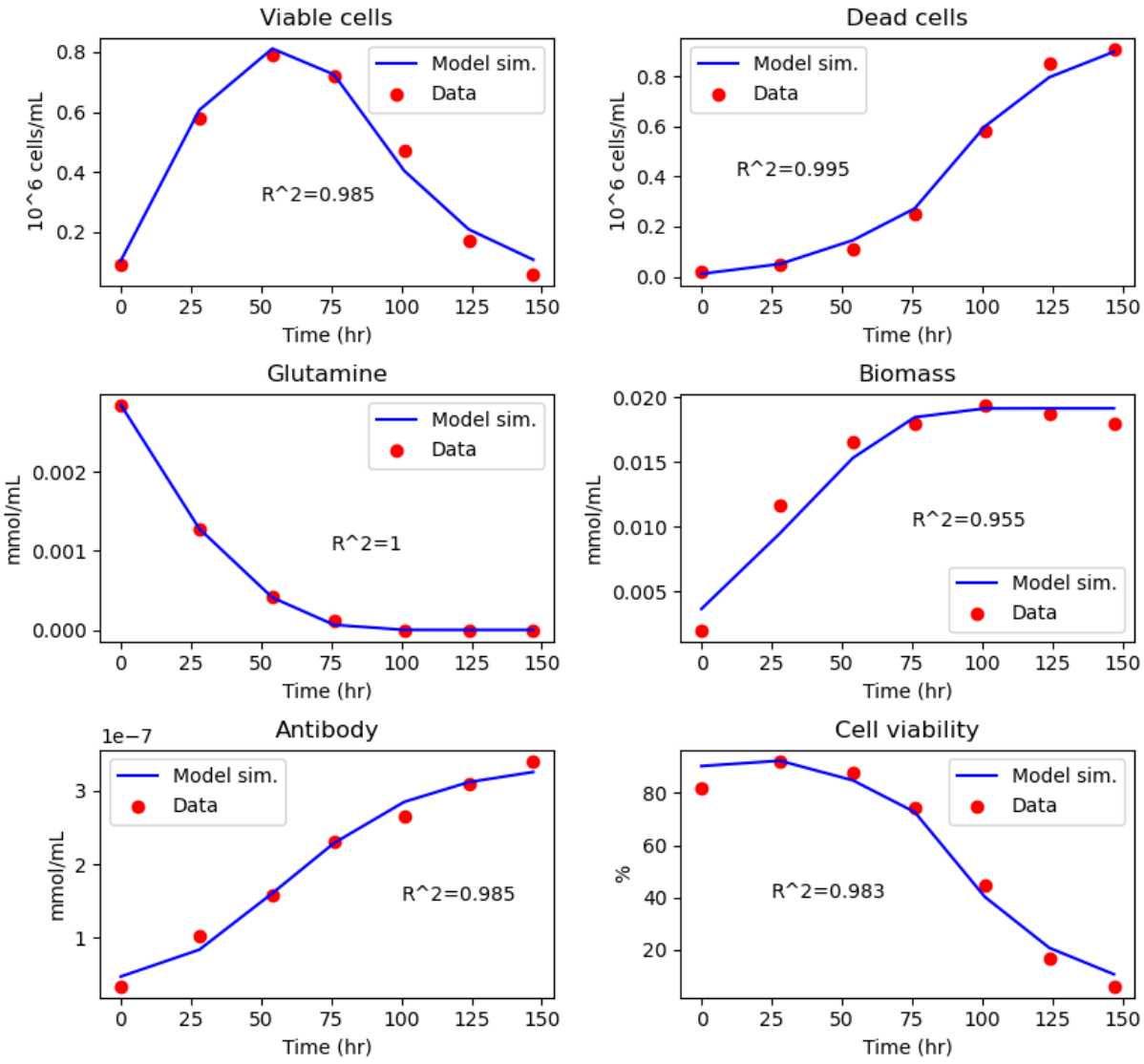


Figure 1 Comparison of model simulation with experimental data for viable cells, dead cells, glutamine, biomass, secreted antibody, and cells viability: murine hybridoma

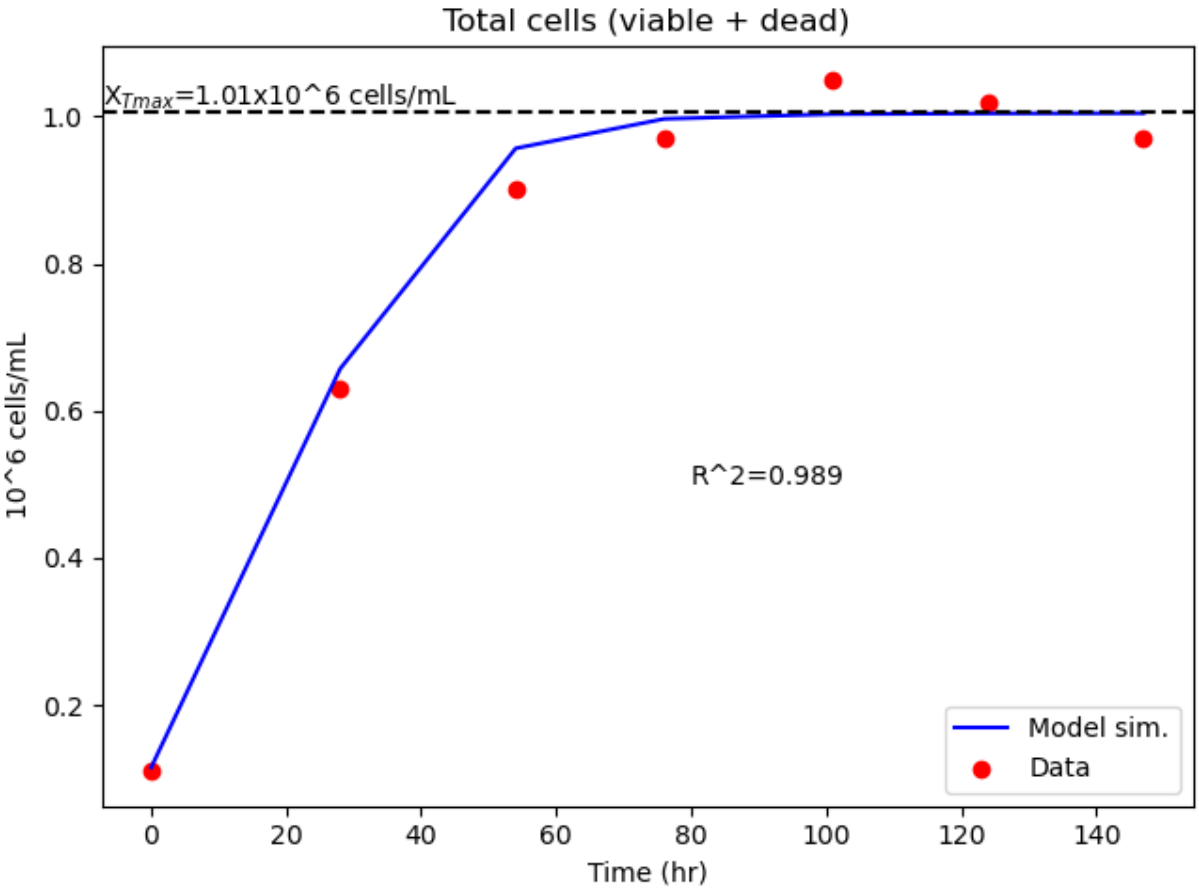


Figure 2 Comparison of simulation result and experimental data for total cells concentration: murine hybridoma

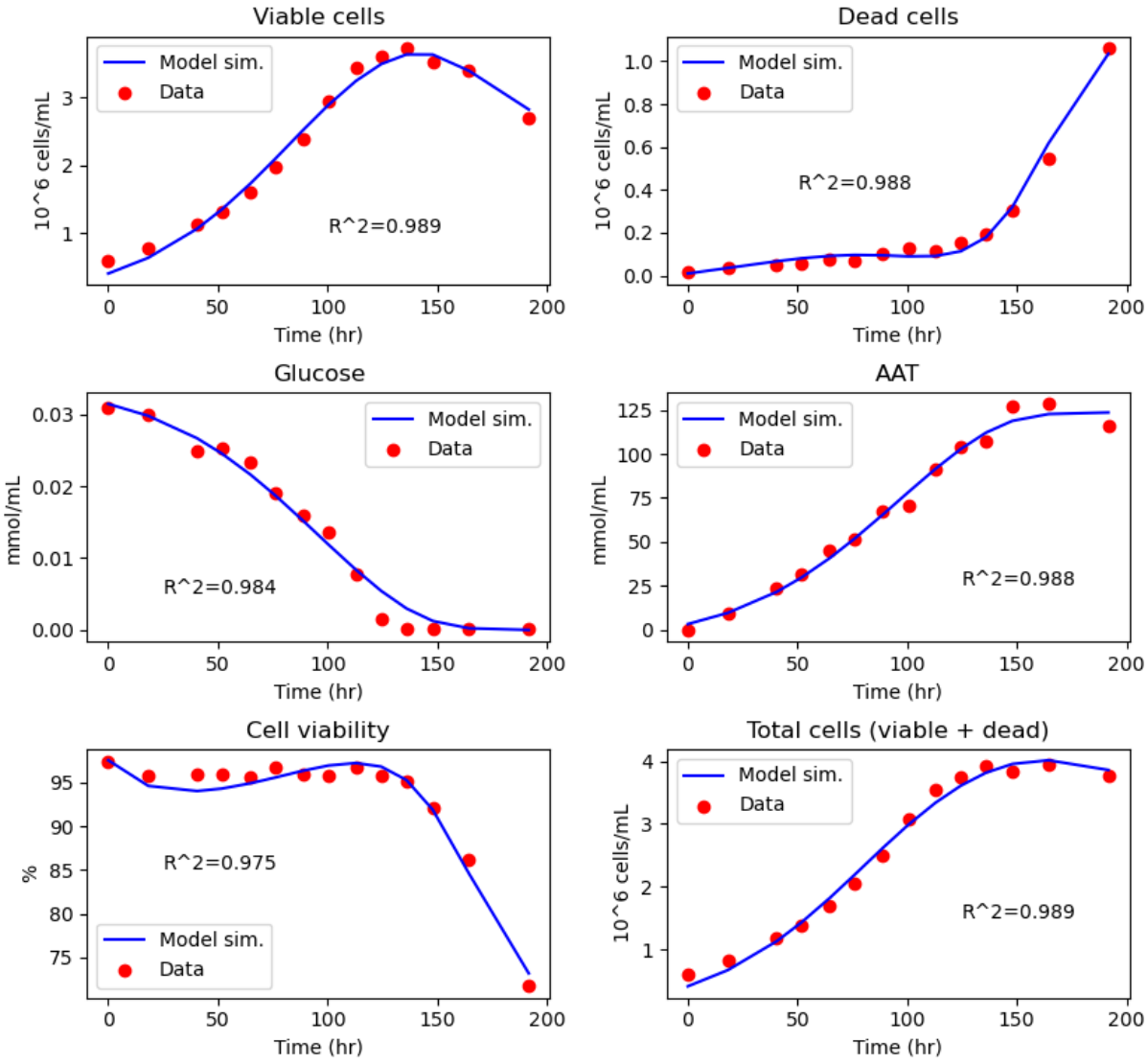
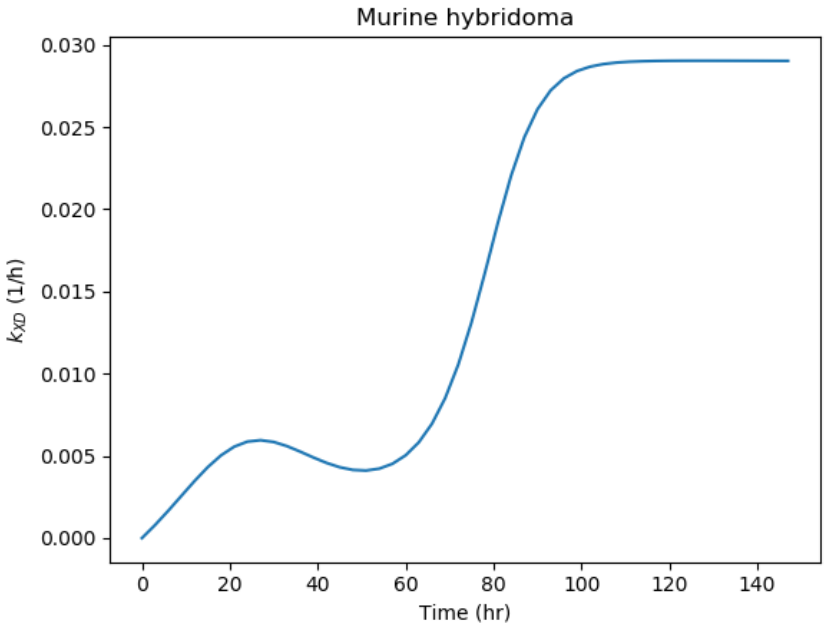


Figure 3 Comparison of model simulation with experimental data for viable cells, dead cells, glucose, AAT, cells viability, and total cell concentration: AGE1.HN.AAT

(a)



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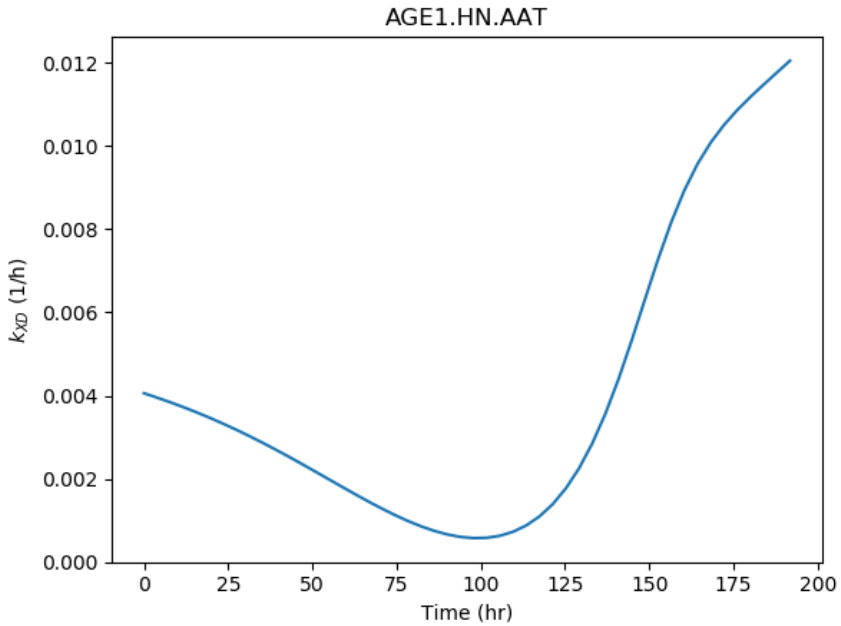
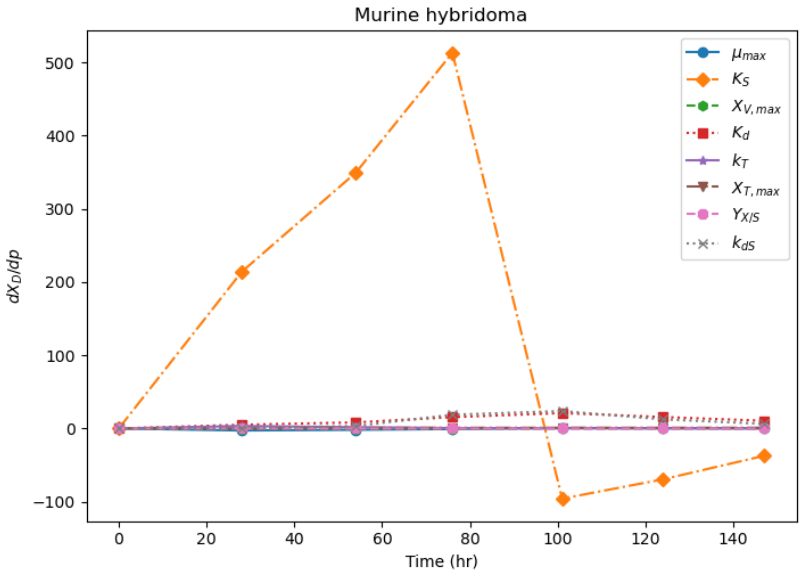


Figure 4 Dynamic profiling of pseudo-rate constant for dead cells generation, k_{XD}

(a)



(b)

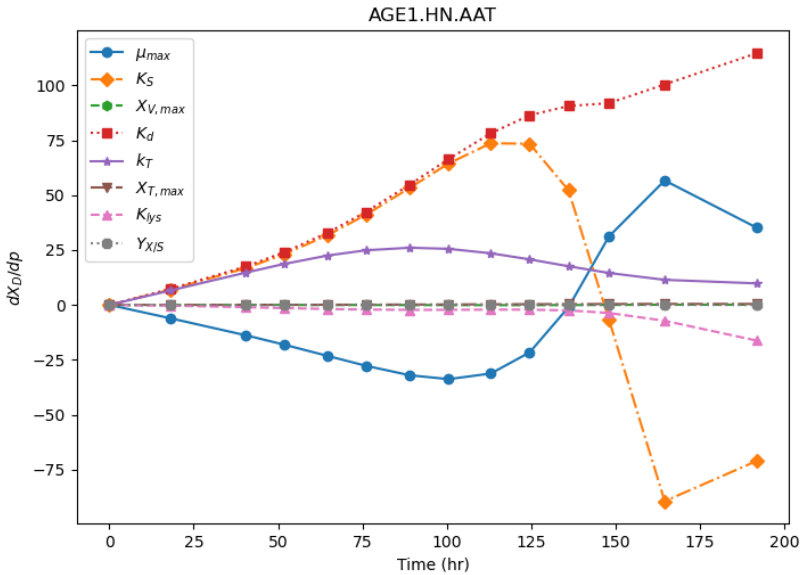


Figure 5 Sensitivities of dead cell accumulation profile to model parameters

Chemostat culture performance

The implication of the proposed modeling framework for continuous culture (chemostat) performance analysis is further investigated. Table 3 lists the modeling equations for chemostat culture based on the proposed modeling framework for viable cell, dead cell, growth-limiting substrate, and product concentrations, i.e., X_V, X_D, S, and P respectively. Parameter D is the dilution rate (1/h) and is defined as the ratio of inlet volumetric flowrate to volume of cell culture medium. These equations can be solved analytically as shown by Xu (2020) or numerically to yield steady-state relationships of chemostat culture variables to dilution rate, D until washout point. These relationships are shown for both cell lines in Figure 6 for cell concentrations (viable, dead, and total), cell viability, and pseudo-rate constant for dead cells generation, k_{XD}. Additionally, the dependence of substrate and product concentrations (as well as its production rate) to dilution rate are shown in Figure 7 for both cell lines. Generally, there exists a specific value of dilution rate that gives highest viable cell concentration in both chemostat cultures although this value does not necessarily correspond to peak cell viability (see Figure 6). However, for murine hybridoma cell (see Figure 6 (a)), this peak value occurs at low dilution rate, at the region that is also characterized by high sensitivity to variations in D. Moreover, due to the absence of dead cells lysis phenomenon, this low dilution rate region is also characterized by high dead cell concentrations. As IgG production is non-growth associated, its peak production rate also occurs at this peak viable cell concentration as shown in Figure 7 (a). This situation brings forth the issues of sensitivity, controllability, and reliability into consideration whenever the objective of maximum production rate is to be pursued for this continuous culture system. On the other hand, for AGE1.HN.AAT cell, the region of low dilution rate is characterized by low total cells concentration due to the postulated dead cells lysis phenomenon (see Figure 6 (b)). As shown in Figure 7 (b), the peak production rate of AAT, which is growth-associated seems to occur near the peak cell viability that occurs at higher dilution rate than peak viable cell concentration (see Figure 6 (a)). Finally, as shown in Figure 6, the dependence of pseudo-rate constant, k_{XD} to dilution rate for murine hybridoma cell is more complex than AGE1.HN.AAT cell which exhibits minimum value near the peak cell viability.

Table 3 List of model equations for chemostat culture

Component	Murine hybridoma	AGE1.HN.AAT	
Viable cells:	$\mu_{hyb} - K_d - D = 0$		Equation (16)
Dead cells:	$k_{X_D} X_V - D X_D = 0$	$k_{X_D} X_V - K_{lys} X_D - D X_D = 0$	Equation (17a and b)
Growth-limiting substrate:	$D(S_f - S) - \frac{\mu_{hyb}}{Y_{X_v/S}} X_V - k_{d_S} S = 0$	$D(S_f - S) - \frac{\mu_{hyb}}{Y_{X_v/S}} X_V = 0$	Equation (18a and b)
Growth-associated product:	$D(P_{G,f} - P_G) + \mu_{hyb} Y_{P_G/X_V} X_V = 0$		Equation (19)
Non-growth associated product:	$D(P_{NG,f} - P_{NG}) + K_{P_{NG}} X_V = 0$	-	Equation (20)

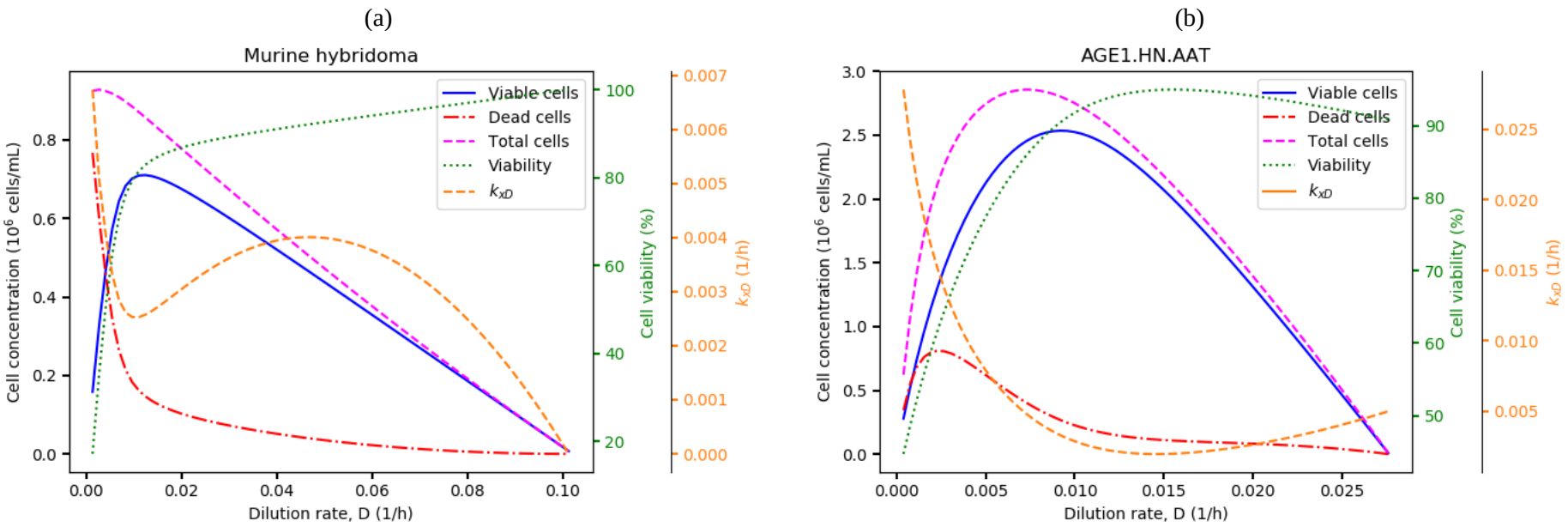


Figure 6 Steady-state relationships of chemostat culture variables to dilution rate until washout point: Cell concentrations (viable, dead, and total), cell viability, and pseudo-rate constant, k_{XD}

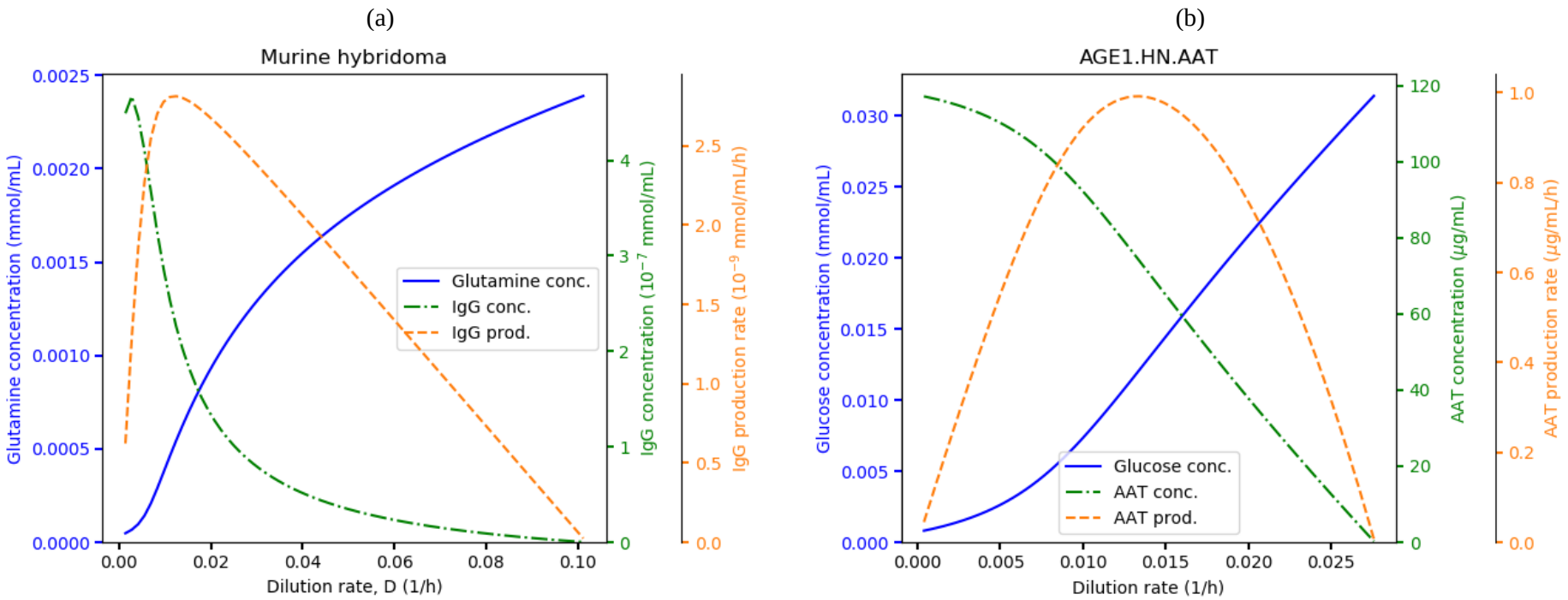


Figure 7 Steady-state relationships of chemostat culture variables to dilution rate until washout point: Substrate concentration, product concentration, and production rate

Conclusions

A modeling framework that incorporates both modified Logistic and Monod equations in a single equations system has been proposed to overcome the limitations of both modeling equations when applied separately to simulate cell culture growth in the presence of dead cells accumulation data. Using batch culture growth data of two cell lines as case studies, the effectiveness of this modeling framework to depict time-course profiles of important culture state variables that include dead cells accumulation and cell viability was successfully demonstrated. Using this modeling framework, the impact of model parameter perturbations to dead cell accumulation profile can be further investigated using derivative-based sensitivity analysis. Our analysis indicates that for the two cell lines under consideration, the dead cell accumulation profile is quite sensitive to Monod saturation constant of the growth-limiting substrate. Furthermore, using this modeling framework, more informative assessment of chemostat culture performance can be carried out. Here, we may discover the influence of dead cell accumulation and its lysis (or lack of it) on the performance of continuous culture system that can aid us in choosing more suitable region to operate our bioreactor in continuous production mode.

Acknowledgement

The authors would like to acknowledge the support that was provided by Universiti Kebangsaan Malaysia to carry out this research under Research University Grant with grant number: GUP 2018-009 and GUP-2020-011.

Appendix A

The modified Logistic equation for total cells population, i.e., Equation (5) can be derived from the following set of coupled ordinary differential equations:

$$\frac{dX_T}{dt} = \alpha S X_V \quad \text{(A1)}$$

$$\frac{dS}{dt} = -\beta\alpha S X_V \quad \text{(A2)}$$

where X_T is total cell density that is summation of viable X_V and dead cell X_D cell densities. S is the concentration of growth-limiting substrate. Parameters a and b refer, respectively to the rate of growth of viable cells per substrate concentration and amount of substrate needed to produce new cells. As Equations (A1) and (A2) only differ by a constant, the relationship between the derivatives can be expressed as follow:

$$\beta \frac{dX_T}{dt} = -\frac{dS}{dt} \quad \text{(A3)}$$

Integrating Equation (A3) yield:

$$S = S_0 - \beta(X_T - X_{T0}) \quad \text{(A4)}$$

Substituting Equation (A4) into Equation (A1) further yields:

$$\frac{dX_T}{dt} = \alpha X_V(S_0 - \beta X_T + \beta X_{T0}) \quad \text{(A5)}$$

Factoring the above equation then yields:

$$\frac{dX_T}{dt} = \alpha\beta\left(\frac{S_0}{\beta} + X_{T0}\right)X_V\left(1 - \frac{X_T}{\frac{S_0}{\beta} + X_{T0}}\right) \quad \text{(A6)}$$

By identifying

$$k_T = \alpha(S_0 + \beta X_{T0}) \quad \text{(A7)}$$

$$X_{T,max} = \frac{S_0}{\beta} + X_{T0} \quad \text{(A8)}$$

we recover the modified form of Logistic equation for total cells population growth as shown by Equation (5) in the manuscript.

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