Workshop on Modelling of Animal Cells Processes

Elisabeth F. P. Augusto

Unifesp – Universidade Federal de São Paulo

elisabeth.augusto@unifesp.br

Why modelling?

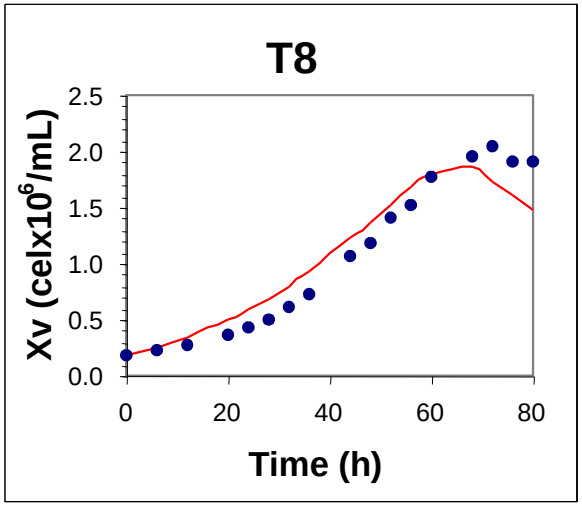
a) To **predict** system **behaviour** under different culture conditions,

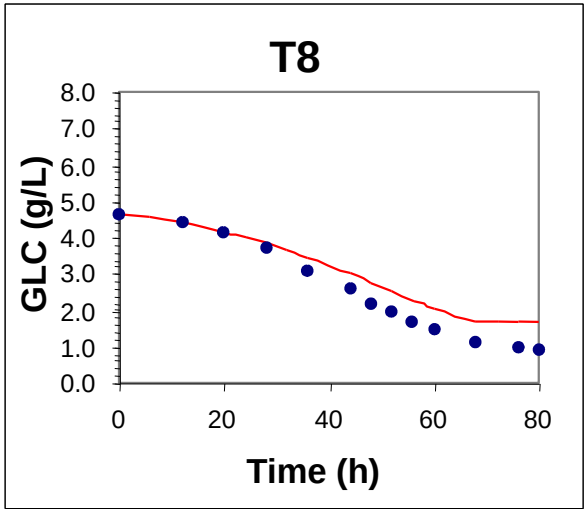
- ➢ Allows process simulation and control;
  - ✓ Improvement of product quality and reproducibility;
- ➢ Identifies limiting and inhibiting factors;
  - ✓ Helps process optimisation

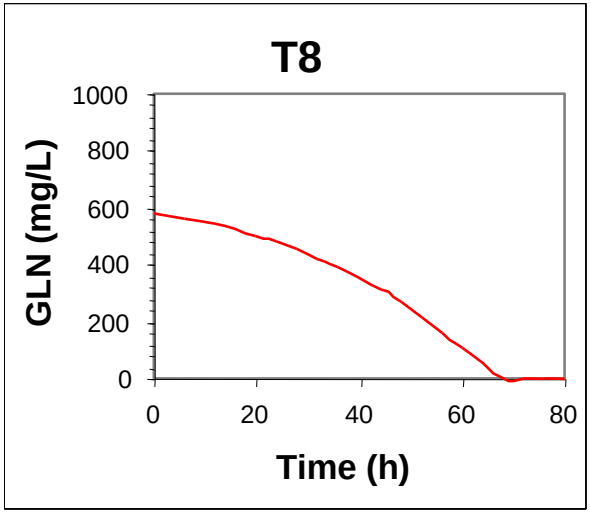
b) To **reduce** the number of **experiments** necessary to optimisation;

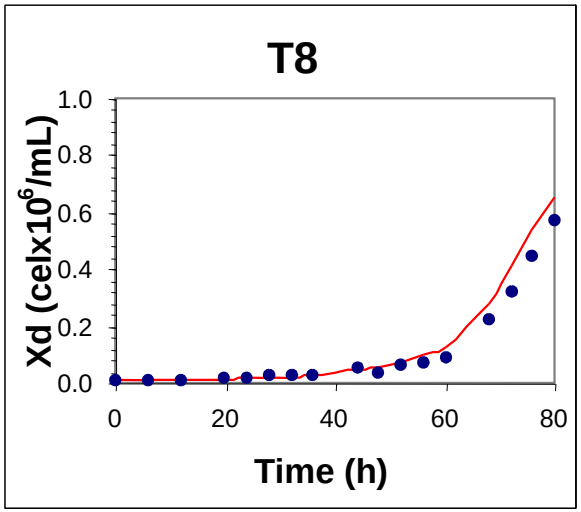
c) To help **organizing ideas** and concepts;

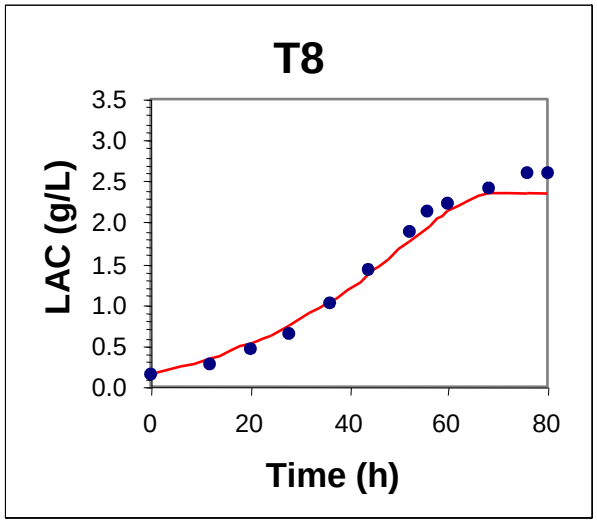
Model Simulation - example

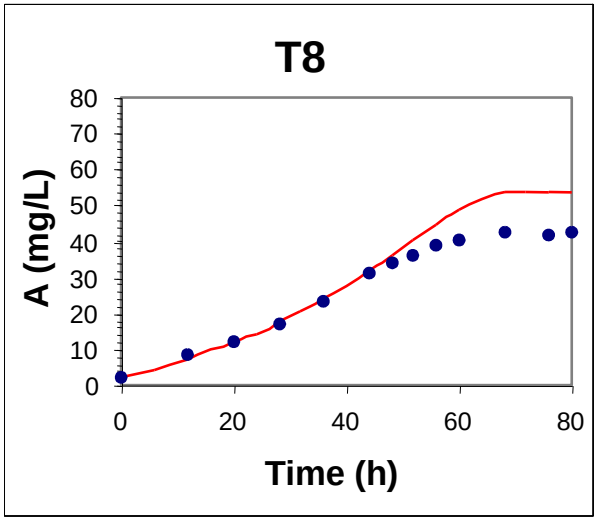












Hybridoma cells growth on serum added medium (GLN_0 = 584 $mg.L^{-1}$; GLC_0 = 4.5 $g.L^{-1}$. **Symbols** – experimental data; **Lines** – model simulation

Equations for the previous data (a batch operated process

$$\frac{dX_v}{dt} = \mu X_v - \mu_d . X_v \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$\frac{dX_d}{dt} = \mu_d X_v \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$\frac{dGLC}{dt} = -\frac{1}{y_{(X_v+X_d)/GLC_X}} \mu X_v - \frac{1}{y_{LAC/GLC_{LAC}}} \boxed{\alpha_1} \mu X_v \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$\frac{dGLN}{dt} = -\boxed{k} GLN - \frac{1}{y_{(X_v+X_d)/GLN_X}} \mu X_v - \frac{1}{y_{A/GLN_A}} \frac{\beta_m GLN}{K_{\beta,GLN} + GLN} X_v \quad (4)$$

$$\frac{dLAC}{dt} = \alpha_1 \mu X_v \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

$$\frac{dNH4}{dt} = \boxed{\gamma} kGLN + \boxed{\alpha_2} \mu X_v + \frac{\boxed{\beta_m} GLN}{\boxed{K_{\beta,GLN}} + GLN} X_v \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Kinetic equations

$$\mu = \boxed{\mu_{max}} \left(\frac{GLC}{\boxed{K_{GLC}} + GLC} \right) \left(\frac{GLN}{\boxed{K_{GLN}} + GLN} \right) \left(\frac{K_{NH4}}{\boxed{K_{NH4}} + NH_4} \right) \left(\frac{K_{LAC}}{\boxed{K_{LAC}} + LAC} \right) \quad \ldots\ldots \quad (7)$$

$$\mu_d = \boxed{K_d} \left(\frac{NH_4}{\boxed{K_{d,NH4}} + NH_4} \right) \left(\frac{LAC}{\boxed{K_{d,LAC}} + LAC} \right) \left(\frac{K_{d,GLN}}{\boxed{K_{d,GLN}} + GLN} \right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

6 state variables: X_V; X_d; GLC; GLN; LAC; A;

16 adjustable parameters: marked in this and previous page;

4 fixed parameters: $Y_{LAC/GLC}$, K, γ, $K_{\beta,GLN}$

Parameter	Preliminary estimation	Final estimation		Unity
	Model_1	**Model_1**	**Model_2**	
α_1	1,205	1,267	1,276	10^{-3} g LAC/10^6cel
α_2	7,74	19,44	13,713	10^{-3} mg A/10^6cel
β_m	0,0013	0,00202		10^{-3} mg A/10^6cel.h
Y_{Xt/GLC_X}	2,794	3,0755	3,22	10^6 cel/10^{-3} g GLC
Y_{Xt/GLN_X}	0,0057	0,0298	0,0379	10^6 cel/10^{-3} mg GLN
Y_{A/GLN_A}	0,00068	0,000165	0,0424	mg A/mg GLN
μ_{max}	0,0421	0,0514	0,0548	Cel/cel.h
K_{GLC}	0,083	0,149	0,157	g GLC/L
K_{GLN}	13,3	6,455	19,425	mg GLN/L
K_A	115,7	249,8	211,9	mg A/L
K_{LAC}	12,37	12,80	11,09	g LAC/L
K_d	0,1	0,0293	0,0253	Cel_d/ cel_V.h
$K_{d,A}$	30,93	0,805	0,509	mg A/L
$K_{d,LAC}$	1,36	0,741	0,102	g LAC/L
$K_{d,GLN}$	64,4	199,3	141,74	mg GLN/L
$K_{d,GLC}$	0,3	1,202	0,842	g GLC/L
Nºinterations	**-**	**29**	**38**	
Global residue	**-**	**83,15**	**79,43**	

Steps for mathematical modelling

1. Model formulation: establishes state variables, operational variables and parameters relevant to the process, related in a set of kinetic equations;

2. Mass balances: for each component, considering the state and operational variable selected

3. Parameters fitting: Definition of optimal values for parameters that minimise the discrepancies between experimental and calculated values;

4. Model validation: a comparative statistical analysis between real values (experimental) and calculated values (model simulation).

1. Model formulation … depends on several tasks,

a) Definition of representative variables: state variables and operational variables;

b) Definition of operation mode: batch, fedbatch, continous etc.

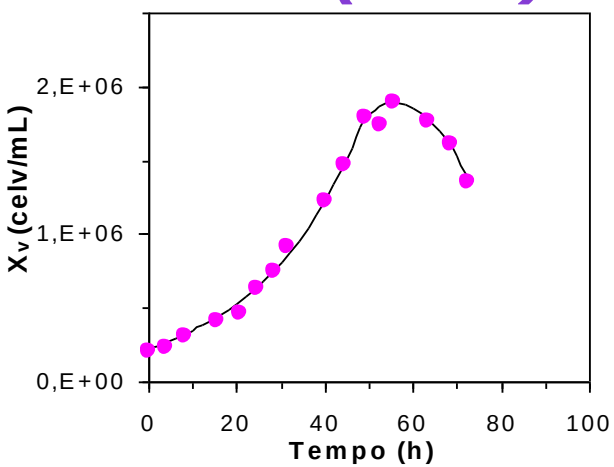
c) Kinetics analysis of experimental data,

- Definition of relevant kinetic variables,
  - specific rates
  - yield coefficients;
- Identification of dominant phenomena, i.e., limitation or inhibition factors;

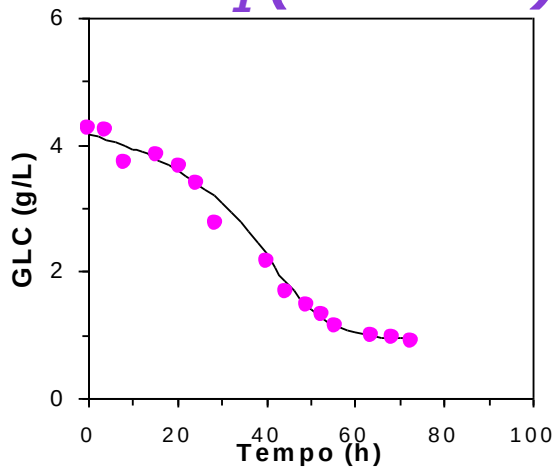
d) Selection of mathematical equations for the specific rates;

Definition of state variables

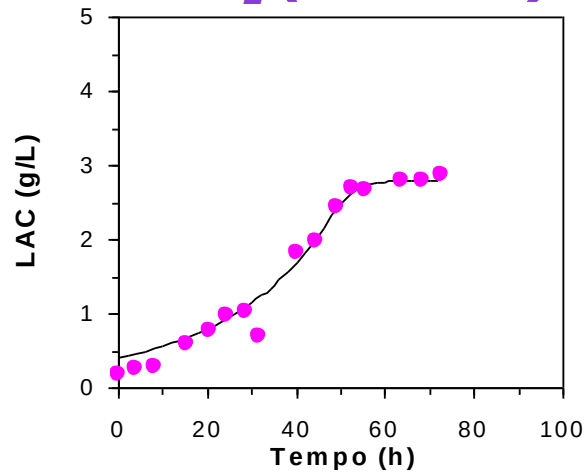
X (Cells)



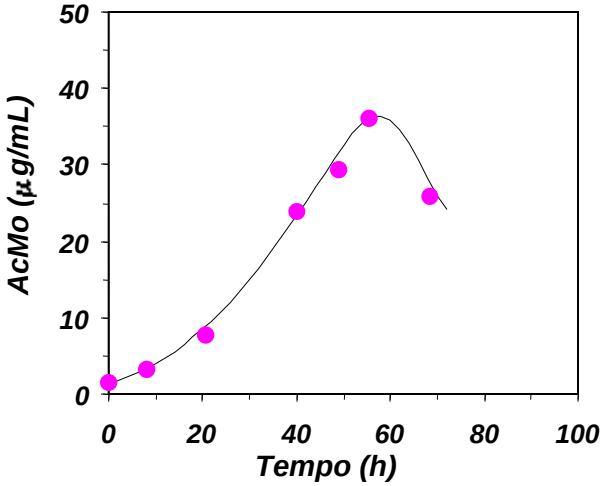
S_1 (Glucose)



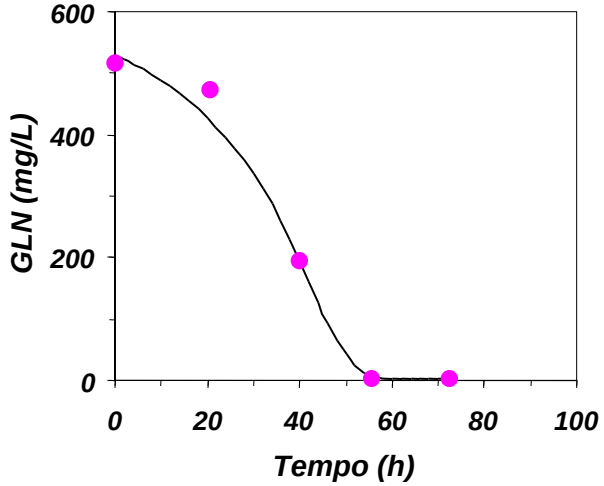
sP_1 (Lactate)



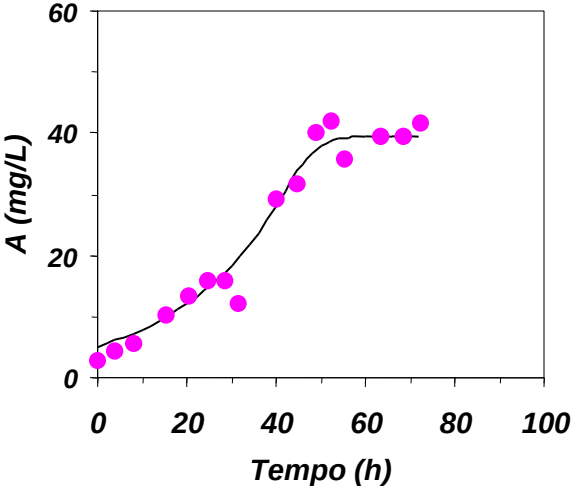
P (MAb)



S_2 (Glutamine)



sP_2 (Ammonium)



Growth of an hybridoma in a 2L bubble free bench bioreactor; Batch mode operation (MAb = monoclonal antibody)

Defining representative variables

State variables

X – Cells concentration (X_{Viable}, X_{Dead}, X_{Total}); ⇨ **Catalyst**

S – Substrates concentration (C, N, P, etc.);

P – Products concentration: proteins;

bP – By-products (inhibitors or not) concentration:

LAC, A (Ammonium), etc.;

pO_2 – O_2 partial pressure (Dissolved O_2)

} **f (Cell, Metabolism)**

Operational variables

X_0 – Initial cells concentration.;

S_0 – Initial substrates concentration;

F_S – Feed rate;

D – Specific feed rate;

T – Temperature;

pO_2 – Oxygen partial pressure (Dissolved Oxygen)

pH

1. Steps for Model formulation … depends on several tasks,

a) ~~Definition of representative variables: state variables and operational variables;~~

b) Definition of **operation mode**: batch, fedbatch, continous etc.

c) Kinetics analysis of experimental data,

- Definition of relevant **kinetic variables**,
  - specific rates
  - yield coefficients;
- Identification of dominant phenomena, i.e., limitation or inhibition factors;

d) Selection of mathematical equations for the specific rates;

1. Steps for Model formulation … depends on several tasks,

~~a) Definition of representative variables: state variables and operational variables;~~

~~b) Definition of **operation mode**: batch, fedbatch, continous etc.~~

c) Kinetics analysis of experimental data,

- Definition of relevant **kinetic variables**
  - specific rates
  - yield coefficients;
- Identification of dominant phenomena, i.e., limitation or inhibition factors;

d) Selection of mathematical equations for the specific rates;

Specific rates defined for animal cells bioprocesses

a) Cell growth ..... $\mu_X = \frac{1}{X_V} \cdot \frac{dX_V}{dt} = \frac{1}{X_V} \cdot (r_X)$

b) Cell death ..... $k_d = \frac{1}{X_v} \cdot \frac{dX_d}{dt}$

c) Substrate consumption ..... $\mu_{GLC} = \frac{1}{X_V} \cdot \left(-\frac{dGLC}{dt}\right) = \frac{1}{X_V} \cdot (-r_{GLC})$

$\mu_{GLN} = \frac{1}{X_V} \cdot \left(-\frac{dGLN}{dt}\right) = \frac{1}{X_V} \cdot (-r_{GLN})$

d) Product synthesis ..... $\mu_{MAb} = \frac{1}{X_V} \cdot \frac{dMAb}{dt} = \frac{1}{X_V} \cdot (r_{MAb})$

$\mu_{NH4} = \frac{1}{X_V} \cdot \frac{dNH4}{dt} = \frac{1}{X_V} \cdot (r_{NH4})$

$\mu_{LAC} = \frac{1}{X_V} \cdot \frac{dLAC}{dt} = \frac{1}{X_V} \cdot (r_{LAC})$

1. Steps for Model formulation … depends on several tasks,

~~a) Definition of representative variables: state variables and operational variables;~~

~~b) Definition of **operation mode**: batch, fedbatch, continous etc.~~

c) Kinetics analysis of experimental data,

- Definition of relevant **kinetic variables,**
  - ~~specific rates;~~
  - yield coefficients;
- Identification of dominant phenomena, i.e., limitation or inhibition factors;

d) Selection of mathematical equations for the specific rates;

Yield coefficients defined in animal cell systems:

$$Y_{X/GLC} = \frac{\Delta X}{-\Delta GLC} = \frac{r_X}{r_S} = \frac{\mu_X}{\mu_{GLC}}$$

$$Y_{X/GLN} = \frac{\Delta X}{-\Delta GLN} = \frac{r_X}{r_{GLN}} = \frac{\mu_X}{\mu_{GLN}}$$

$$Y_{LAC/GLC} = -\frac{r_{LAC}}{r_{GLC}} = -\frac{\mu_{LAC}}{\mu_{GLC}}$$

$$Y_{NH4/GLN} = -\frac{r_{NH4}}{r_{GLN}} = -\frac{\mu_{NH4}}{\mu_{GLN}}$$

Other relations:

$$Y_{NH4/X} = -\frac{r_{NH4}}{r_X} = -\frac{\mu_{NH4}}{\mu_X}$$

$$Y_{O_2/GLC} = \frac{\mu_{O_2}}{\mu_{GLC}}$$

Etc…

1. Steps for Model formulation … depends on several tasks,

a) ~~Definition of representative variables: state variables and operational variables;~~

b) ~~Definition of **operation mode**: batch, fedbatch, continous etc.~~

c) Kinetics analysis of experimental data,

- ~~Definition of relevant **kinetic variables,**~~
  - ~~specific rates;~~
  - ~~yield coefficients;~~
- Identification of dominant **phenomena**, *i.e.*, limitation or inhibition factors;

d) Selection of mathematical equations for the specific rates;

1. Steps for Model formulation … depends on several tasks,

a) ~~Definition of representative variables: state variables and operational variables;~~

b) ~~Definition of **operation mode**: batch, fedbatch, continous etc.~~

c) ~~Kinetics analysis of experimental data,~~

- ~~Definition of relevant **kinetic variables,**~~
  - ~~specific rates;~~
  - ~~yield coefficients;~~
- ~~Identification of dominant **phenomena**, *i.e.*, limitation or inhibition factors;~~

d) Selection of mathematical **equations** for the specific rates;

Model formulation

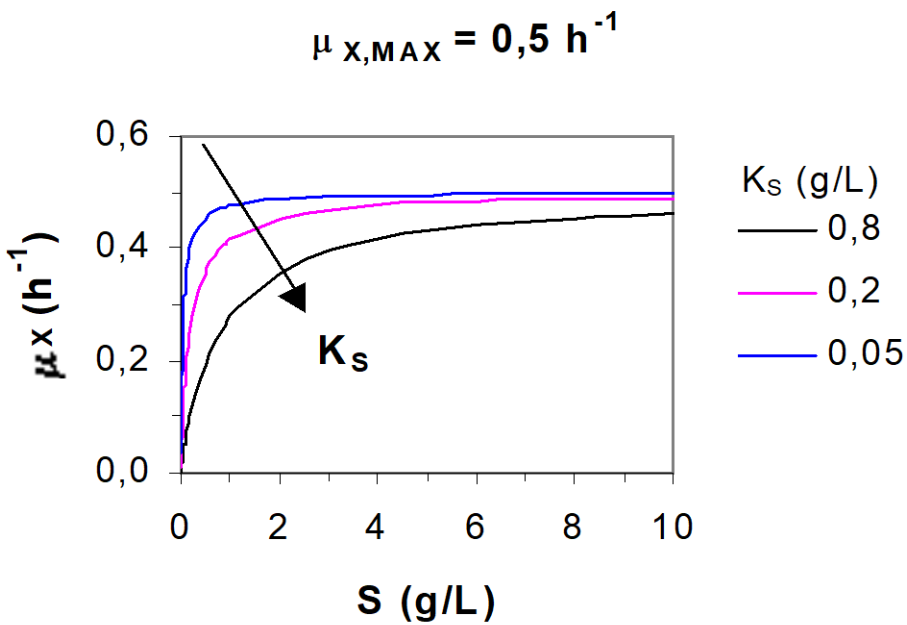
Models for cell growth

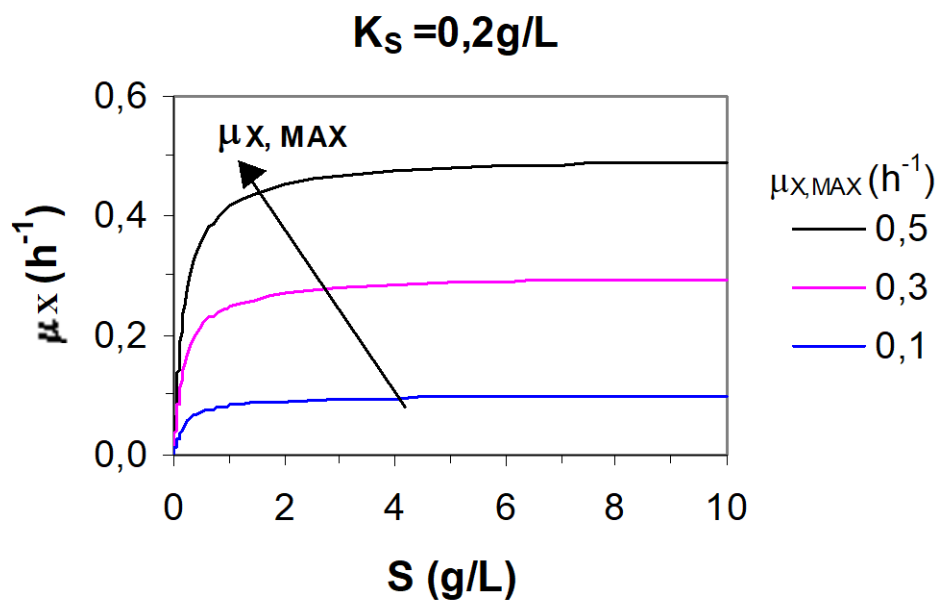
$$\mu_X = \mu_{X,max}\left(\frac{GLN}{K_{GLN} + GLN}\right)$$

one limiting substrate formulation

Parameters

(Monod model)





Models for cell growth…

$$\mu_X = \mu_{X,max} \cdot \boxed{\frac{GLN}{k_{GLN} + GLN}}$$

one limiting substrate

$$\mu_X = \mu_{X,max} \boxed{\frac{GLC}{k_{GLC} + GLC}} \cdot \boxed{\frac{GLN}{k_{GLN} + GLN}}$$

multiple limiting substrate

one limiting substrate

and

one inhibiting by-product

$$\mu_X = \mu_{X,max} \boxed{\frac{GLC}{k_{GLC} + Glc}} \cdot \boxed{\frac{k_{i,LAC}}{k_{i,LAC} + LAC}}$$

Parameters

$$\mu_X = \mu_{X,max} \frac{GLN}{k_{GLN} + GLN} \cdot \frac{k_{i,NH_3}}{k_{i,NH_3} + NH_3} \cdot \frac{k_{i,LAC}}{k_{i,LAC} + LAC}$$

$$\mu_X = \mu_{X,max} \cdot \frac{GLC}{k_{GLC} + GLC} \cdot \frac{GLN}{k_{GLN} + GLN} \cdot \frac{k_{i,NH_3}}{k_{i,NH_3} + NH_3} \cdot \frac{k_{i,LAC}}{k_{i,LAC} + LAC}$$

Models for cell growth…

$$\mu_x = \boxed{D} + d_0 \exp\left(\frac{d_1}{\mu}\right)$$

D = Specific feed rate

$$\mu = 0.043\left(\frac{\boxed{B} - 0.07}{\boxed{B}}\right)$$

B = Base medium concentration

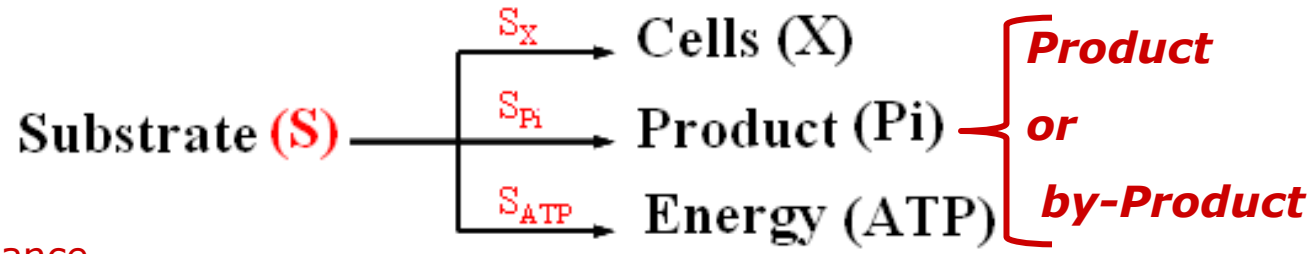
$$\mu_X = \mu_{X,max} \cdot \boxed{(Serum)} \cdot \frac{GLC}{k_{GLC} + GLC}$$

$$\mu_X = \mu_{X,max} \cdot \left(\frac{\boxed{Serum}}{\boxed{(Serum} + (k_{Serum})_0 \cdot X^{-\beta *}}\right) \cdot \frac{GLN}{k_{GLN} + GLN} \cdot \frac{k'_{i,NH_3}}{k'_{i,NH_3} + (NH_3)^2}$$

$$\begin{cases} \mu_X = \mu_{X,min} + (\mu_{X,max} - \mu_{X,min}) \cdot \dfrac{(GLC - \boxed{GLC_{thres}})}{k_{GLC} + (GLC - \boxed{GLC_{thres}})} & \text{..... } GLC \geq GLC_{thres} \\ \mu_X = 0 & \text{..... } GLC < GLC_{thres} \end{cases}$$

Model for substrate consumption

$$\mu_S = \frac{1}{(Y_{X/S}^{max})} \cdot \mu_X$$



$$\mu_S = \frac{1}{(Y_{X/S}^{max})} \cdot \mu_X + \overbrace{m_S}^{\text{maintenance}}$$

$$\mu_S = \frac{1}{(Y_{X/S}^{max})} \cdot \mu_X + m_S \frac{S}{k_S^S + S}$$

$$\mu_S = \frac{1}{(Y_{X/S})} \cdot \mu_X + \frac{\gamma}{(Y_{P/S})} \cdot \mu_X$$

$$\mu_S = \frac{h_1 \cdot S}{h_2 + S}$$

$$\mu_S = \mu_S^* + \mu_S^E \begin{cases} \mu_S^* = \dfrac{\mu_X}{Y_S^{max}} + m_S \\ \mu_S^E = \Delta\mu_S^{max} \dfrac{S - S^*}{S - S^* + k_S^S} \end{cases}$$

specific uptake rate under substrate limitation conditions

increment or decline of specific uptake rate due to substrate excess **→OVERFLOW**

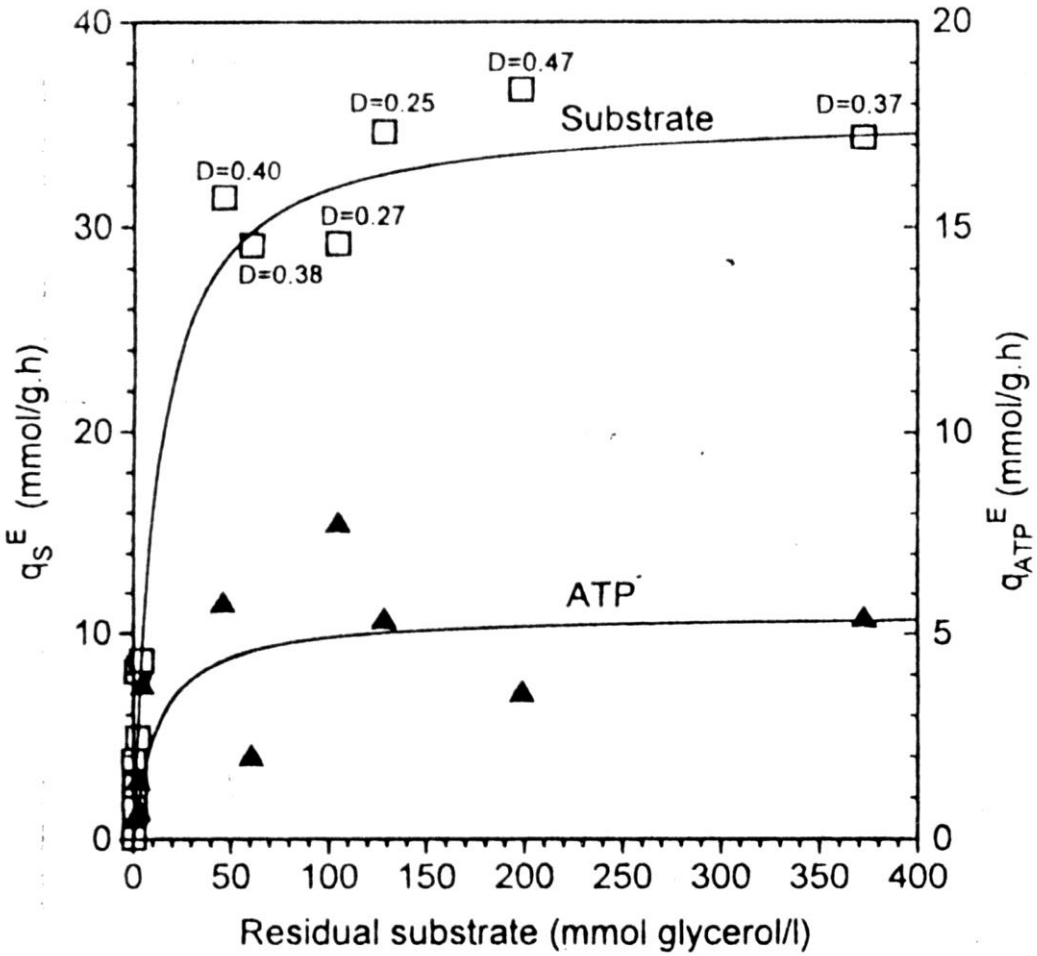


Figure 6. Effect of residual glycerol concentration on the excess of glycerol and ATP consumption rates of *K. pneumoniae* at different dilution rates (D, h^{-1}) in continuous culture.

(Zeng & Deckwer, 1995)

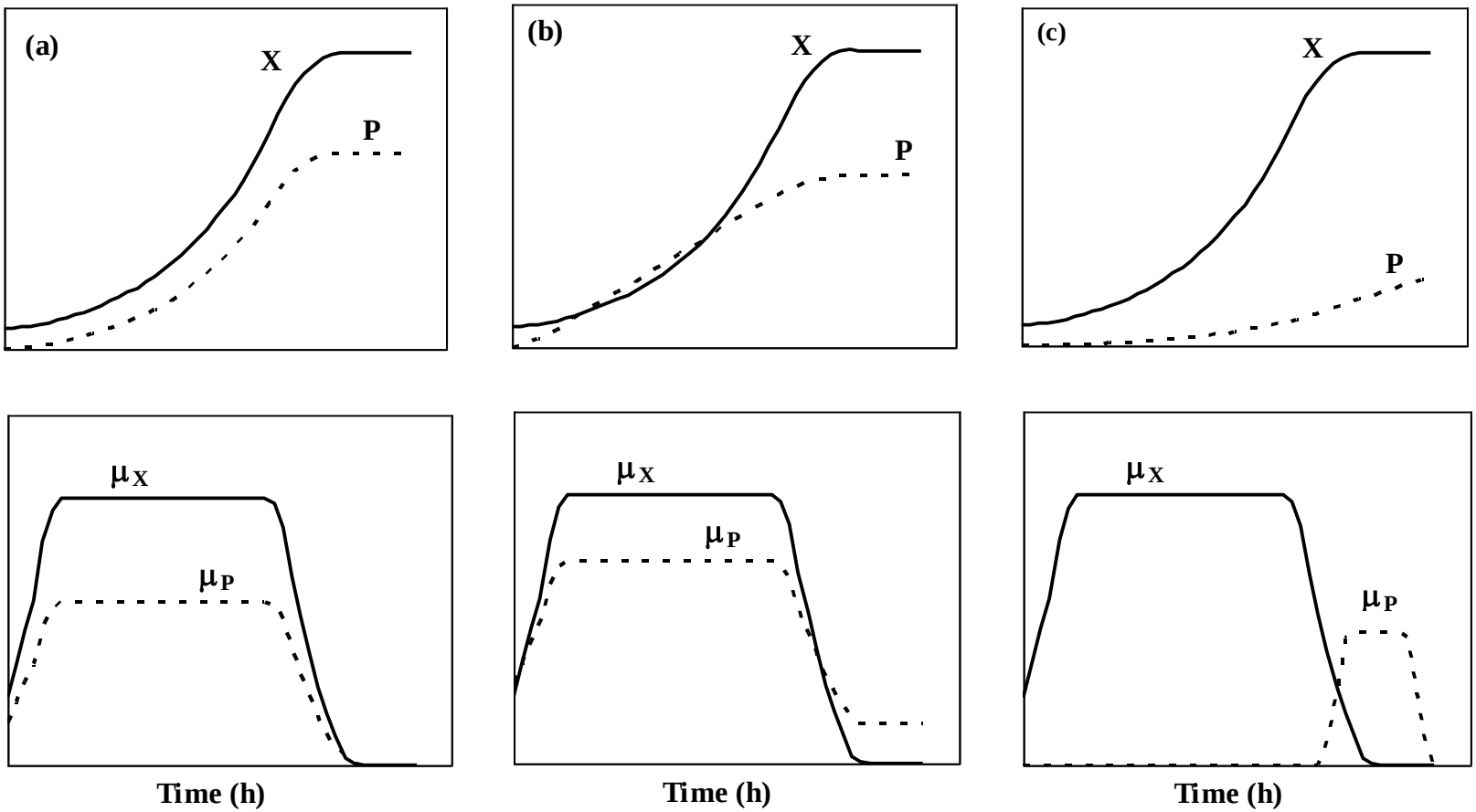
$$\mu_S = \mu_S^* + \mu_S^E$$

$$\mu_S^* = \frac{\mu_X}{Y_S^{max}} + m_S$$

$$\mu_S^E = \Delta\mu_S^{max} \frac{S - S^*}{S - S^* + k_S^S}$$

Models for production (Luedeking & Piret)

$$\mu_P = \alpha\mu_X \qquad \mu_P = \alpha\mu_X + \beta \qquad \mu_P = \beta$$



(a) Independent of cell growth; (b) Associated to cell growth; (c) Partially associated to cell growth. X- Cells concentration; P- Product conc. (ammonium);μ- Specific growth rate; μP- Specific production rate.

Model for production: Protein

$$\mu_{MAb} = \beta$$

$$\mu_{MAb} = \alpha \cdot \boxed{\mu_X} + \beta$$

$$\mu_{MAb} = \alpha_1 \cdot \boxed{k_d} + \beta_1$$

$$\mu_{MAb} = \frac{\alpha_0}{k_\mu + \mu_X} \cdot \mu_X + \beta$$

$$\mu_{MAb} = (\alpha_1 \cdot k_d + \beta_1) \frac{GLN}{k_{GLN}^{MAb} + GLN} \cdot \frac{k_{i,GLC}^{Mab}}{GLC - GLC^* + k_{i,GLC}^{MAb}} \overbrace{\left(F_1 + e^{-F_2 \cdot \Delta t}\right)}^{\text{Loss of production capacity within the population}}$$

$$\mu_{MAb} = \beta_2 \cdot (Serum) \frac{GLN}{k_{GLN}^{MAb} + GLN}$$

$$\mu_{MAb} = (\alpha_1 \cdot k_d + \beta_1) \cdot \frac{GLN}{k_{GLN}^{MAb\#} \cdot X_v + GLN} \cdot \frac{k_{i,GLC}^{MAb\#} \cdot X_v}{GLC + k_{i,GLC}^{MAb\#} \cdot X_v}$$

Model for production: ***By-products*** ***(****Inhibitor****)***

$$\mu_{LAC} = \mu_{LAC,max} \cdot \frac{GLC}{k_{GLC}^{LAC} + GLC}$$

$$\mu_{NH_3} = \frac{E_1 + E_2 \cdot GLC}{E_3 + GLC}$$

$$\mu_{NH_3} = \frac{G_1 + G_2 \cdot LAC}{G_3 + LAC}$$

$$\mu_I = \mu_I^* + \mu_I^E = \underbrace{m_I + Y_{I/X}}_{\text{Limitation of substrate}} + \underbrace{\Delta\mu_I^{max}\left(\frac{S - S^I}{S - S^I + K_S^I}\right)}_{\text{Excess of substrate}}$$

(Zeng, 1995; Zeng and Deckwer, 1995a)

Steps for model definition:

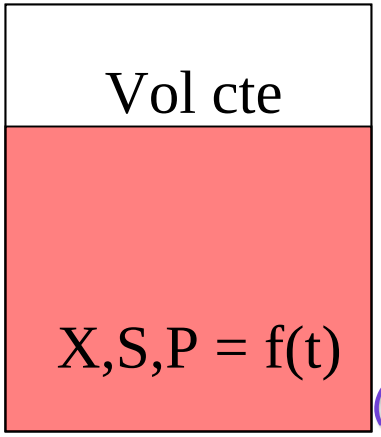
1. ~~**Model formulation:** establishes state variables, operational variables and parameters relevant to the process, related in a set of kinetic equations;~~

2. Mass balances: for each component, considering the state and operational variable selected

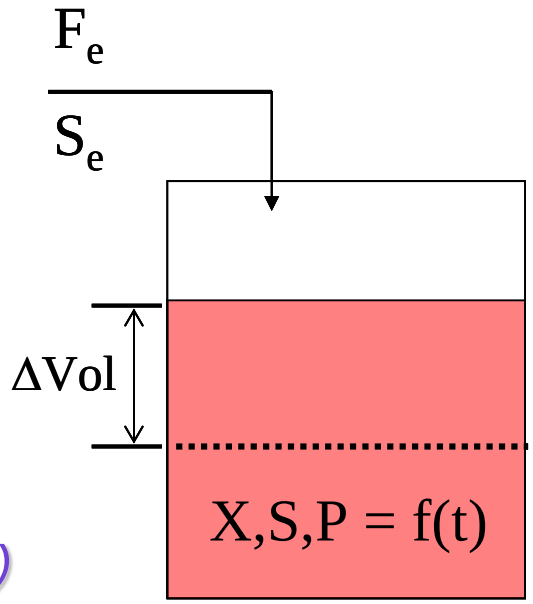
3. Parameters fitting: Definition of optimal values for parameters that minimise the discrepancies between experimental and calculated values;

4. Model validation: a comparative statistical analysis between real values (experimental) and calculated values (model simulation).

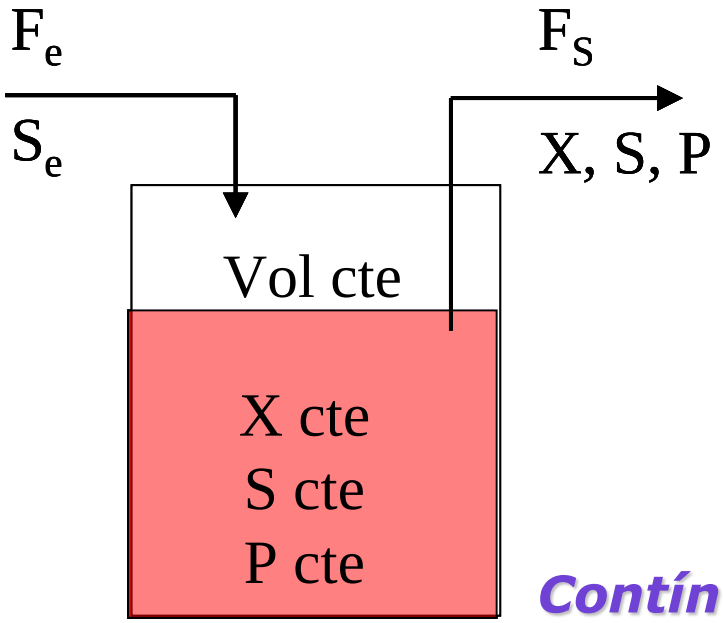
FORMAS DE OPERAR



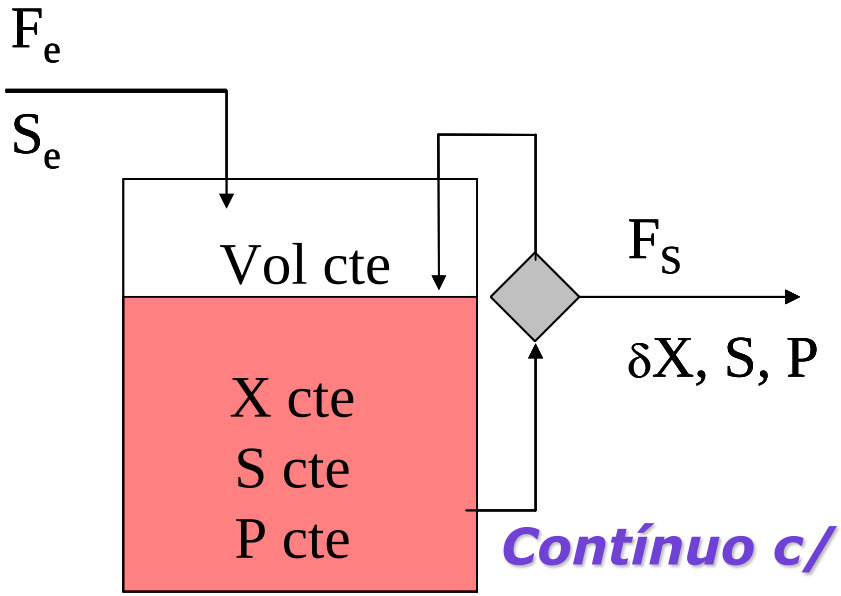
Descontínuo
(Batelada, Batch)



Descontínuo Alimentado
(Batelada alimentada, Fedbatch)

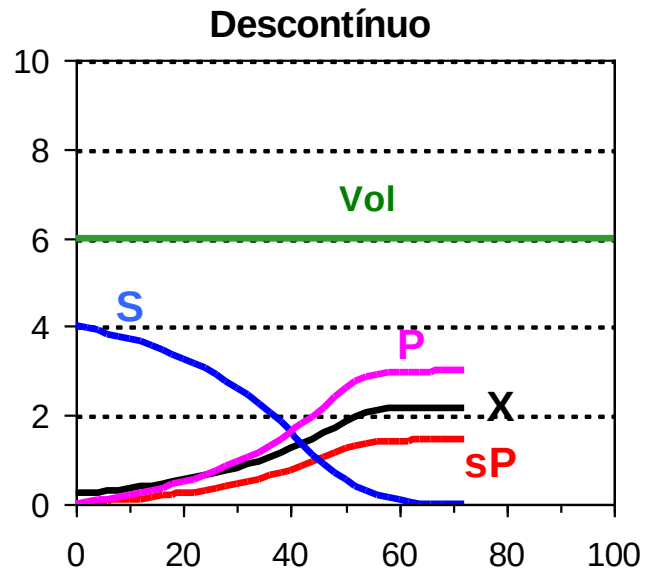


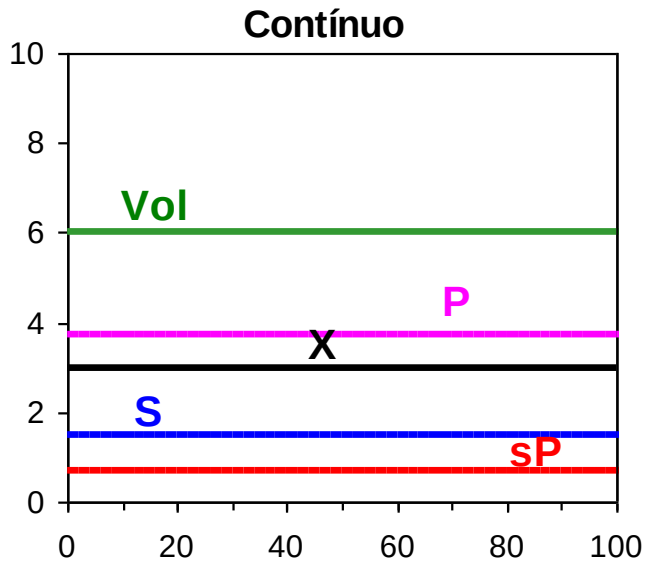
Contínuo

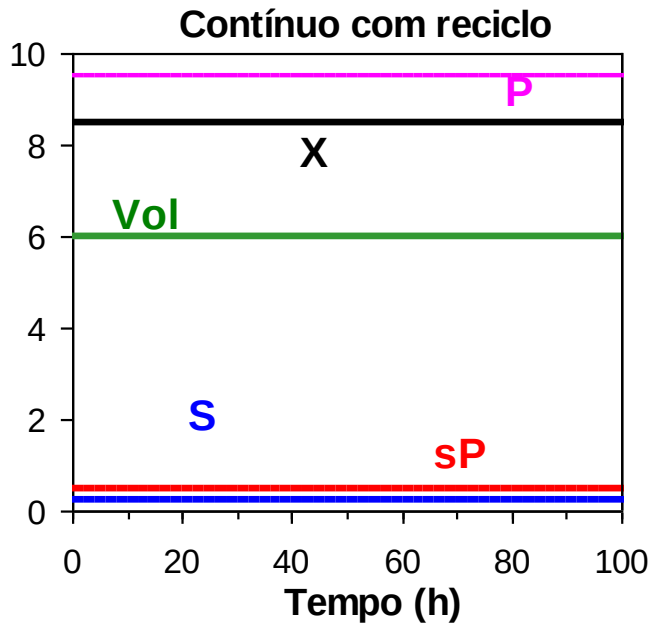


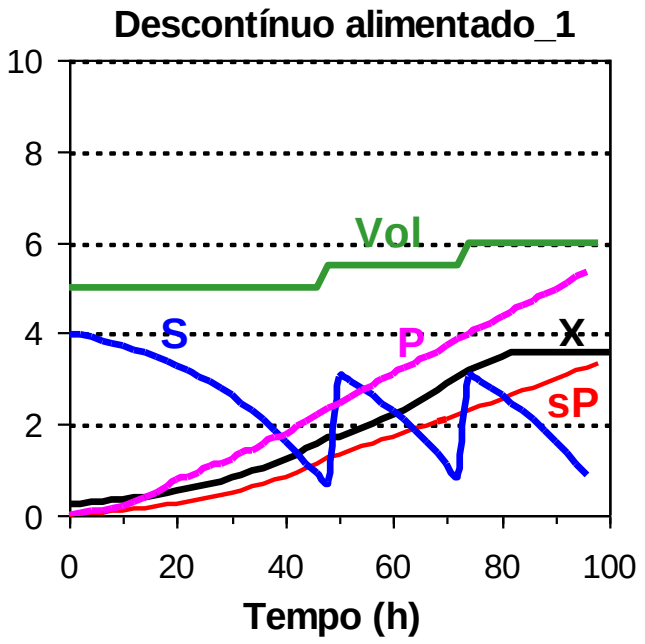
Contínuo c/ reciclo
(Perfusão)

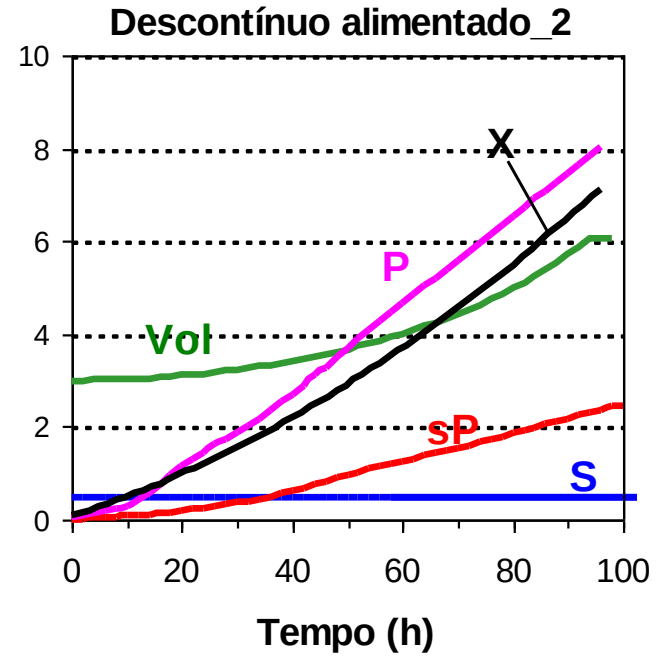
Respostas típicas





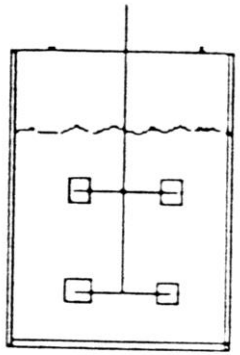




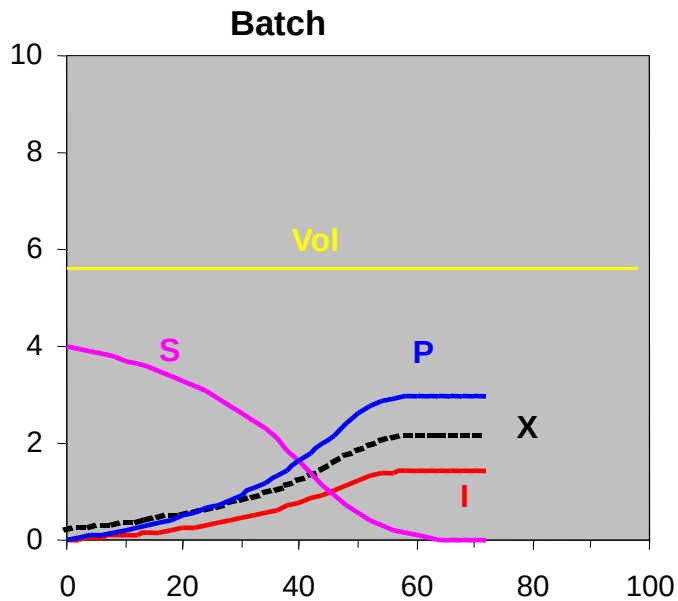


Summarizing the Mass Balance

Accumulation rate in system	=	Inlet rate in system	-	Outlet rate in system



Batch

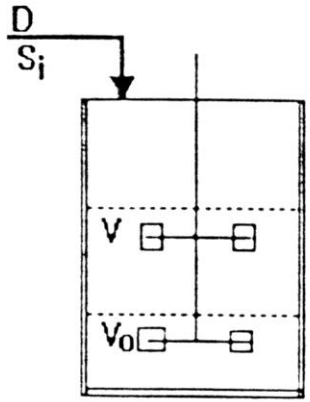


$$\frac{dX_v}{dt} = \mu X$$

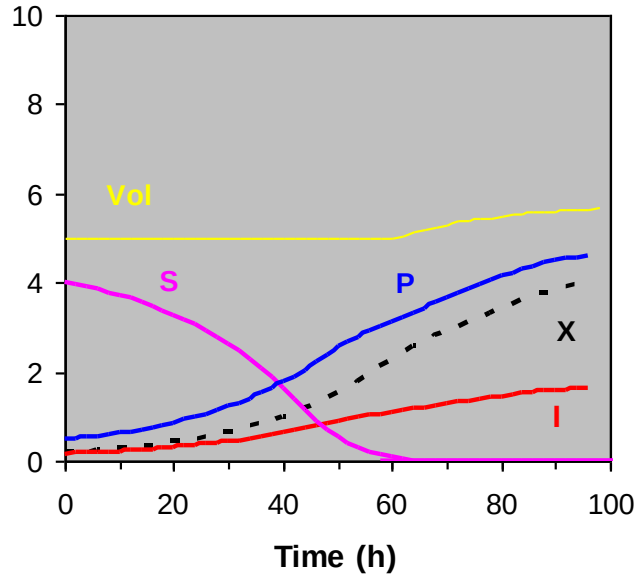
$$\frac{dS}{dt} = -\frac{1}{y_{X/S_X}}\mu X - \frac{1}{y_{P/S_P}}\alpha_1 \mu X - \frac{1}{Y_{I/S_I}}\alpha_2 \mu X$$

$$\frac{dP}{dt} = \alpha_1 \mu X + \beta_1 X$$

$$\frac{dI}{dt} = \alpha_2 \mu X + \beta_2 X$$



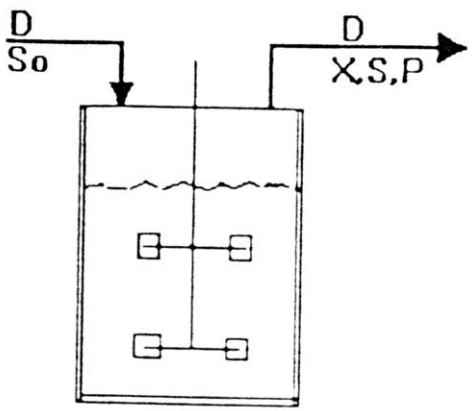
Fed batch



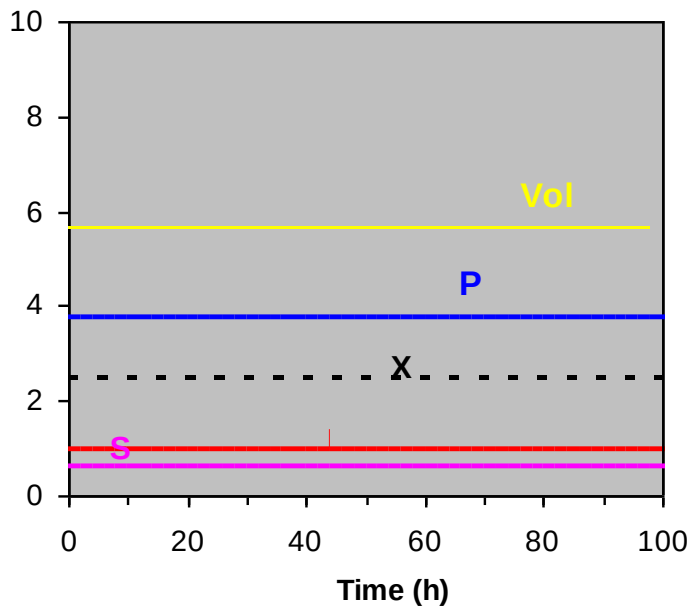
$$\frac{dx}{dt} = \mu_X x$$

$$\frac{ds}{dt} = \phi s_i - \frac{1}{Y_{X/S}} \mu x$$

$$\frac{dp}{dt} = \mu_P x$$



Continuous



$$\frac{dX}{dt} = (\mu_X - D)X$$

$$\frac{dS}{dt} = D(S_i - S) - \frac{1}{Y_{X/S}} \mu X$$

$$\frac{dP}{dt} = \mu_P X - DP$$

Steps for model definition:

~~**1. Model formulation:** establishes state variables, operational variables and parameters relevant to the process, related in a set of kinetic equations;~~

~~**2. Mass balances:** for each component, considering the state and operational variable selected~~

3. Parameters fitting: Definition of optimal values for parameters that minimise the discrepancies between experimental and calculated values;

4. Model validation: a comparative statistical analysis between real values (experimental) and calculated values (model simulation).

3. Parameters fitting

The initial parameters values can be estimated in different ways:

a) By utilizing parameter values available in the literature;

b) From the stoichiometric relationship, utilizing experimental or theoretical data (for e.g. $Y_{X/S}$, $Y_{S/P}$);

c) By linearization and, sometimes, simplification of kinetic equations and by fitting the experimental data by linear regression (for e.g. kinetic parameters, global yields).

d) Optimal parameter fitting, i.e., to minimize the difference between experimental data and that generated by the model, i.e., optimization of a objective function (OF).

Examples of simplification and linearization methods

Cálculo de K_{GLC}, μ_{max}

$$\mu_X = \mu_{X,max}\left(\frac{GLC}{K_{GLC} + GLC}\right)$$

Linearizing the equation

$$\frac{1}{\mu_X} = \frac{K_{GLC}}{\mu_{max}}\frac{1}{GLC} + \frac{1}{\mu_{X,max}}$$

Linear regression

$(1/\mu_X)$ *vs* $(1/GLC)$

$$\begin{cases} (1/\mu_{X,max}) = \text{intercept} \\ (K_{GLC}/\mu_{X,max}) = \text{slope} \end{cases}$$

Examples of simplification and linearization methods

K_{GLC}, μ_{max}

$$\mu_X = \mu_{X,max}\left(\frac{GLC}{K_{GLC}+GLC}\right)\left(\frac{GLN}{K_{GLN}+GLN}\right)\left(\frac{K_{NH4}}{K_{NH4}+NH_4}\right)\left(\frac{K_{LAC}}{K_{LAC}+LAC}\right)$$

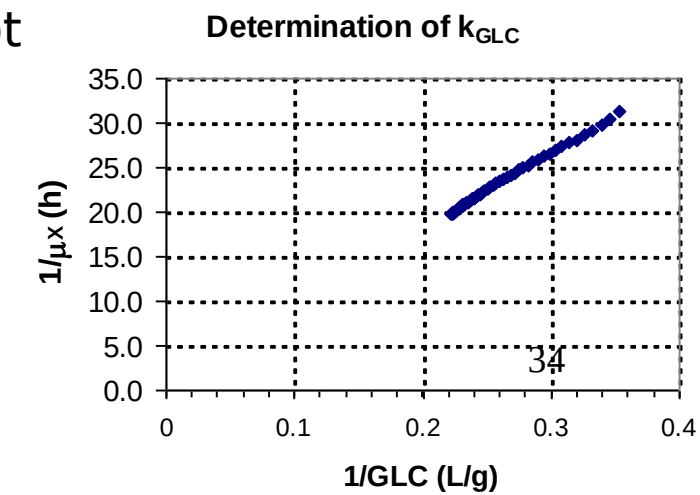
<u>Selection of a subset of data</u>

GLN-high concn.; **NH_3**-low; **LAC**-low ➡ $\mu_X = \mu_{X,max}\left(\frac{GLC}{K_{GLC}+GLC}\right)$

Linearising the equation ➡ $\frac{1}{\mu_X} = \frac{K_{GLC}}{\mu_{max}}\frac{1}{GLC} + \frac{1}{\mu_{X,max}}$

Linear regression ➡ $(1/\mu_{X,max})$ = intercept; $(K_{GLC}/\mu_{X,max})$ = slope

$(1/\mu_X)$ *vs* (1/GLC)



Etc.

Exemplos da simplificação e métodos de linearização (Bonomi & Schmidell, 2000)

α_1 ...

$$\frac{dLAC}{dt} = \alpha_1 \mu X_v = \alpha_1 \frac{dX_t}{dt}$$

➡ **Regressão linear** ➡ **α_1=inclinação**

α_2; β_m ...

$$\frac{dNH_4}{dt} = \gamma k GLN + \alpha_2 \mu_X X_v + \frac{\beta_m GLN}{K_{\beta,GLN} + GLN} X_v$$

<u>Seleção de uma parte dos dados</u>

p/ GLN >> $K_{\beta,GLN}$ ➡

$$\frac{dNH_4}{dt} = \gamma k GLN + \alpha_2 \mu X_v + \beta_m X_v$$

<u>Degradação de GLN</u>

$$GLN_{,deg} = GLN_0 e^{(-kt)}$$

➡ $(NH_4)_{GLN,deg}$

$$(NH_4)_X = NH_4 - (NH_4)_{GLN,deg}$$

$$\boxed{(\mu_{NH4})_X = \alpha_2 \mu_X + \beta_m}$$

➡ **Regressão linear** ➡

β_m=intersecção

α_2=inclinação

Ajuste de parâmetros otimizado

Objetivo: Minimizar a diferenção entre os dados experimetais e aqueles gerados pelo modelo, *i.e.*, otimização da função objetivo (FO).

Solução das equações diferenciais do modelo: aplicação de algorítmos de valores iniciais:

a) **Métodos de integração implícitos (Euler, Crank-Nicolson, Adams-Moulton),**

b) **Métodos de predição-correção (Runge-Kutta, Michelsen, Gear)**

c) **Algorítmo para problemas de valores de contorno (Ex. métodos de diferenças finitas, polinômio de Jacobi ou método de colocação ortogononal)**

Métodos de otimização *de ajuste de parâmetros*:

a) **Linear**

b) **Regressão não-linear**

- **Método sem uso de derivadas parciais da função objetivo: Por ex. Método de busca direta (Simplex ou Poliedros flexíveis)**
- **Métodos com uso de derivadas parciais da função objetivo: Ex. Métodos de busca indireta (Gauss-Seidel or Newton), Método do gradiente, método de Marquardt.**

Formulações para funções objetivo

$$FO = \sum_i (\tilde{y}_i - y_i)^2$$

$$FO = \sum_i \left(\frac{\tilde{y}_i}{(y_i)_m} - \frac{y_i}{(y_i)_m} \right)^2$$

$$FO = \sum_i \left(\frac{\tilde{y}_i - y_i}{\tilde{y}_i} \right)^2$$

$$FO = \sum_i \left[\frac{\tilde{y}_i - y_i}{\left(\frac{\tilde{y}_i - y_i}{2} \right)} \right]^2$$

FO – função objetivo

y_i- valor experimental da variável "i"

$\tilde{y}_i$ – Valor calculado da variável "i"

$(y_i)_m$- valor máximo da variável "i"

$$FO = \sum |r - 1| + \sum |a - 1|$$

$$FO = |r - 1| + |a - 1|$$

r = coeficiente de correlação da regressão linear (Variáveis experimental *vs* calculada*);*

a = inclinação da curva definida pelas variáveis experimental *vs* calculadas.

1. Introdução
2. Tratamento dos dados
   - Variáveis características
   - Identificação de fenômenos
3. Formulação de modelos para células animais
4. Balanço material
5. Ajuste de parâmetros
6. **Validação**
7. **Estudo de caso**

Validação do modelo

A análise estatística é empregada para,

a) **Verificação do erro experimental;**

b) **Determinação do melhor modelo;**

c) **Identificação do desvio sistemático entre modelo ajustado e dados experimentais;**

d) **Verificação da consistência do modelo, comparado ao erro experimental.**

F teste é o teste estatístico mais amplamente empregado na validação de modelos

$$F_c = \frac{s_c^2}{s_e^2} \begin{cases} s_c^2 = \dfrac{\sum_{i=1}^{v}\sum_{j=1}^{n}(\tilde{y}_{ij} - y_{ij})^2}{(nv)_c - p} & \text{Variância do erro do modelo} \\ s_e^2 = \dfrac{\sum_{i=1}^{v}\sum_{j=1}^{n}(y_{ij} - \bar{y}_i)^2}{(nv)_e - v} & \text{Variância do erro experimental} \end{cases} \Rightarrow \begin{cases} F_c < 1 \\ \text{or} \\ s_c^2 < s_e^2 \end{cases}$$

n = número de pontos; v = número de variáveis de estado; $(nv)_c$ = número de pontos ajustados; $(nv)_e$ = número de pontos experimentais; p = número de parâmetros

1. Introdução
2. Tratamento dos dados
   - Variáveis características
   - Identificação de fenômenos
3. Formulação de modelos para células animais
4. Balanço material
5. Ajuste de parâmetros
6. Validação
7. **Estudo de caso**

Estudo de caso: Produção de anticorpo monoclonal a-TNP tipo IgG1 (Suarez, 2002)

Célula - Hibridoma: 1B2.1B6 (IB - Unicamp)

- Mieloma murino Sp2 Ag14/0;
- Linfócito de Balb/C, imunizado por TNP-OVA (Trinitrofenil/ ovalbumina);

Applicação

— Reagente para kits diagnóstico tipo ELISA;

— Anticorpo irrelevante.

Meio de cultura

—DMEM (Sigma D7777) acrescido por 10% FBS, 6g/L HEPES, 3,7g/L $NaHCO_3$ e 2μL/L de β-mercaptoetanol.

Sistema de cultivo

— Frascos Spinner (250 e 500mL);

— Biorreator agitado sem bolhas (*bubble free*) com controle automático de T, pH and pO_2.

Condições experimentais

— Processo operado em batelada;

— Variáveis operacionais,

— Oxigênio dissolvido: $5 \leq pO2 \leq 50\%$ (*Spinners – sem controle*);

— Glicose: $5 \leq (GLC_0/GLC_{DMEM}) \leq 150\%$;

— Glutamina: $5 \leq (GLN_0/GLN_{DMEM}) \leq 150\%$;

—Variáveis de estado: X (X_t, X_V, X_d), GLC, GLN, A, LAC;

— Números de ensaios (*Spinner* + biorreator): 31;

— Ensaios de ”Referência” (*Spinner*) para avaliar erro experimental: 13;

— Ensaios para ajuste do modelo: 22;

— Ensaios para validação do modelo: 9.

Identificação de fenômenos

—Limitação do crescimento por GLN e GLC;

— O metabolismo da glicose e da glutamina e sua regulação são relacionadas;

— Inibição do crescimento por NH_4 e LAC

— Decréscimo da viabilidade como conseqüência de condições limitantes e inibitórias;

— Fase *lag* esporádica;

— Decomposição térmica da glutamina;

— Crescimento celular e produção de AcMo são independentes da concentração de oxigênio dissolvido na faixa de $5 \leq pO_2 \leq 50\%$

Equações de balanço de massa em processos operados em batelada

$$\frac{dX_v}{dt} = \mu X_v - \mu_d . X_v \quad \text{.....} \quad (1)$$

$$\frac{dX_d}{dt} = \mu_d X_v \quad \text{.....} \quad (2)$$

$$\frac{dGLC}{dt} = -\frac{1}{y_{(X_v+X_d)/GLC_X}} \mu X_v - \frac{1}{y_{LAC/GLC_{LAC}}} \boxed{\alpha_1} \mu X_v \quad \text{.....} \quad (3)$$

$$\frac{dGLN}{dt} = -\boxed{k} GLN - \frac{1}{y_{(X_v+X_d)/GLN_X}} \mu X_v - \frac{1}{y_{A/GLN_A}} \frac{\beta_m GLN}{K_{\beta,GLN} + GLN} X_v \quad (4)$$

$$\frac{dLAC}{dt} = \alpha_1 \mu X_v \quad \text{.....} \quad (5)$$

$$\frac{dNH4}{dt} = \boxed{\gamma} kGLN + \boxed{\alpha_2} \mu X_v + \frac{\boxed{\beta_m} GLN}{\boxed{K_{\beta,GLN}} + GLN} X_v \quad \text{.....} \quad (6)$$

Equações cinéticas

$$\mu = \boxed{\mu_{max}} \left(\frac{GLC}{\boxed{K_{GLC}} + GLC} \right) \left(\frac{GLN}{\boxed{K_{GLN}} + GLN} \right) \left(\frac{K_{NH4}}{\boxed{K_{NH4}} + NH_4} \right) \left(\frac{K_{LAC}}{\boxed{K_{LAC}} + LAC} \right) \quad ..... \quad (7)$$

$$\mu_d = \boxed{K_d} \left(\frac{NH_4}{\boxed{K_{d,NH4}} + NH_4} \right) \left(\frac{LAC}{\boxed{K_{d,LAC}} + LAC} \right) \left(\frac{K_{d,GLN}}{\boxed{K_{d,GLN}} + GLN} \right) \quad ..... \quad (8)$$

6 variáveis de estado: X_V; X_d; GLC; GLN; LAC; A;

16 parâmetros ajustáveis: marcados nesta e na página precedente;

4 parâmetros fixos: $Y_{LAC/GLC}$, K, γ, $K_{\beta,GLN}$

Equações cinéticas– 2ª Proposição

Modificações em relação ao 1º modelo:

$$\frac{dGLN}{dt} = -kGLN - \frac{1}{Y_{X_t/GLN_X}}\mu X_v - \frac{\alpha_2}{y_{NH4/GLN_{NH4}}}\mu X_v \quad \text{.....} \quad (9)$$

$$\frac{dNH_4}{dt} = \gamma k GLN + \alpha_2 \mu X_v \quad \text{.....} \quad (10)$$

Ajuste de parâmetros otimizados

—Método: Poliedros flexíveis de Nelder e Mead (Himmelblau, 1972);

—Solução das equações diferenciais pela subrotine SDRIV2 – com método de Gear (Kahaner et al., 1988);

— Estimativa do resíduo

$$\mathbf{R} = \sum \left[\frac{\left(\overline{\mathbf{Y_i}} - \mathbf{Y_i}\right)}{\left(\frac{\overline{\mathbf{Y_i}} + \mathbf{Y_i}}{\mathbf{2}}\right)} \right]^{\mathbf{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

onde

$\mathbf{R}$ = Resíduo

$\overline{\mathbf{Y_i}}$ = Valores da variável calculada

$\mathbf{Y_i}$ = Valores da variável experimental

— Restrição $\mathbf{Y_i} \rangle \varepsilon(\mathbf{Y_i})_{\mathbf{m}}$ ..... (12)

onde: $\varepsilon(\mathbf{Y_i})_{\mathbf{m}}$ = Valor mínimo pré-definido

Procedimento para ajuste de parâmetro;

— Separar ensaios em conjuntos que apresentam o mesmo fenômeno;

— Ajustar os parâmetros para o 1º conjunto, fixando os parâmetros que pertencem ao 2º conjunto;

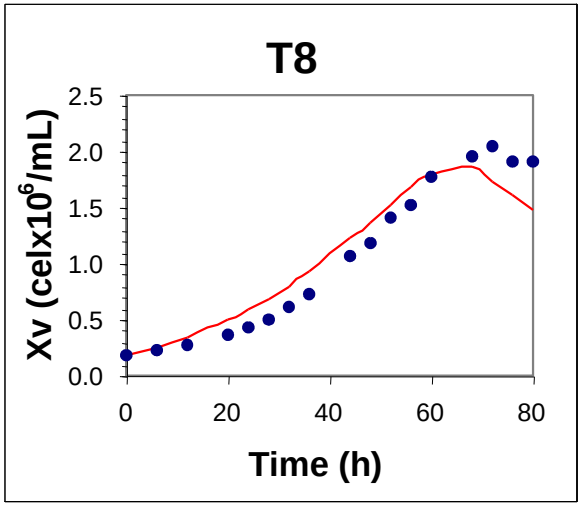
— Ajustar os parâmetros para o 2º conjunto, fixando os parâmetros que pertencem ao 1º conjunto;

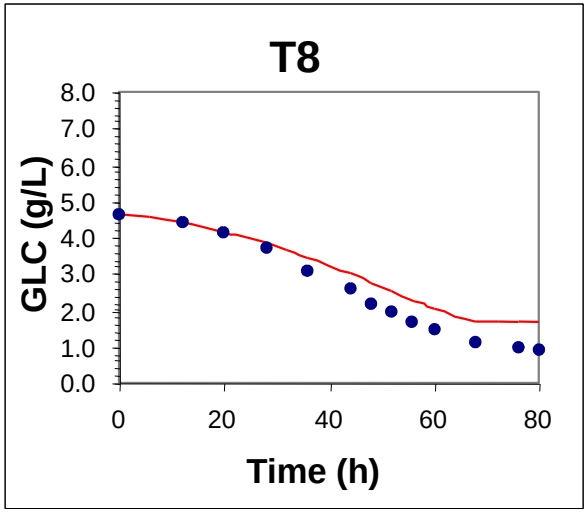
— Repetir ao ajuste para o 1º conjunto de parâmetros, usando os valores previamente calculado para os parâmetros do conjunto 2

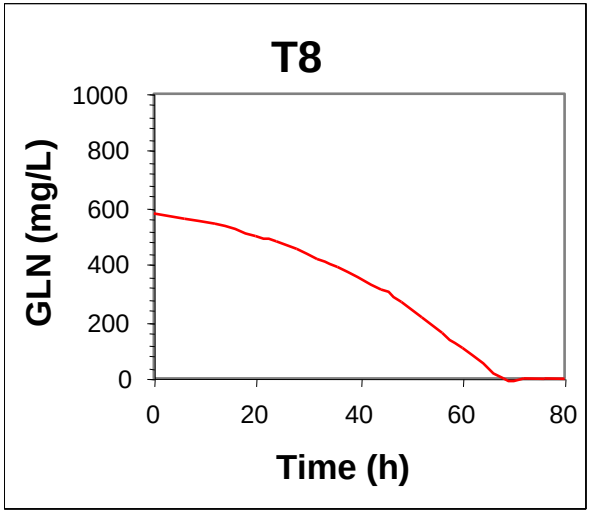
— Repetir esse procedimento até que a variação do resíduo entre 2

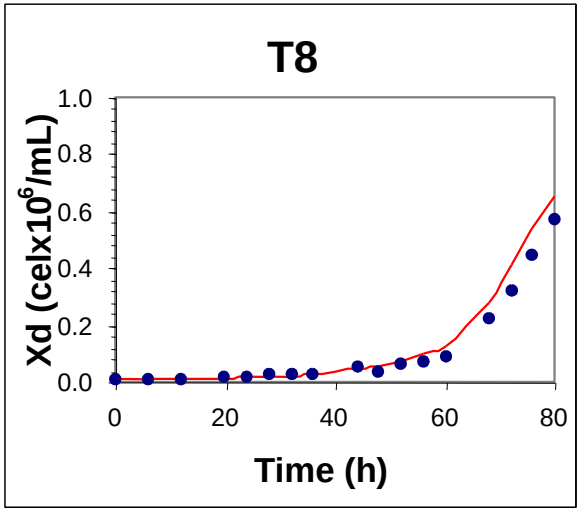
Conjunto de ensaios	Objetivos	Parâmetros
1 (14 runs)	Growth limitation and death by GLC and GLN. Growth inhibition and death by ammonia	β_m; $Y_{A/GLN}$; $K_{A,GLC}$; K_{GLN}; K_A; $K_{d,A}$; $K_{d,GLN}$; $K_{d,GLC}$
2 (22 runs)	Estimation of conversion parameters. Growth inhibition and death by lactate	α_1; α_2; $Y_{Xt/GLC}$; $K_{Xt/GLN}$; X_{MAX}; μ; K_{LAC}; $K_{d,LAC}$

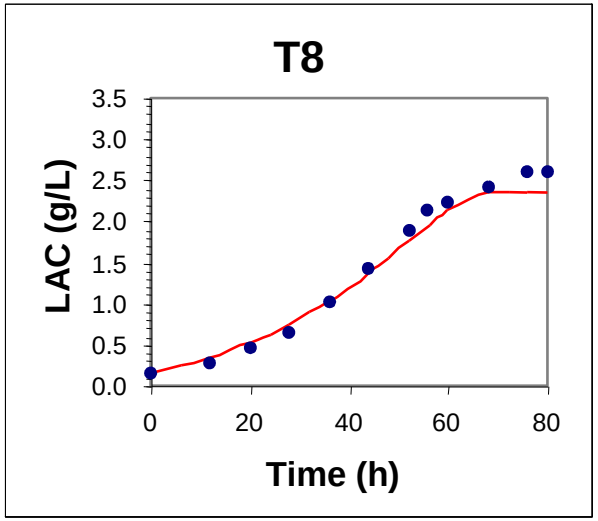
Parâmetros	Estimativa preliminar	Estimativa final		Unidade
	Modelo_1	Modelo_1	Modelo_2	
α_1	1,205	1,267	1,276	10^{-3} g LAC/10^6cel
α_2	7,74	19,44	13,713	10^{-3} mg A/10^6cel
β_m	0,0013	0,00202		10^{-3} mg A/10^6cel.h
Y_{Xt / GLC_X}	2,794	3,0755	3,22	10^6 cel/10^{-3} g GLC
Y_{Xt / GLN_X}	0,0057	0,0298	0,0379	10^6 cel/10^{-3} mg GLN
Y_{A / GLN_A}	0,00068	0,000165	0,0424	mg A/mg GLN
μ_{max}	0,0421	0,0514	0,0548	Cel/cel.h
K_{GLC}	0,083	0,149	0,157	g GLC/L
K_{GLN}	13,3	6,455	19,425	mg GLN/L
K_A	115,7	249,8	211,9	mg A/L
K_{LAC}	12,37	12,80	11,09	g LAC/L
K_d	0,1	0,0293	0,0253	Cel_d/ cel_V.h
$K_{d,A}$	30,93	0,805	0,509	mg A/L
$K_{d,LAC}$	1,36	0,741	0,102	g LAC/L
$K_{d,GLN}$	64,4	199,3	141,74	mg GLN/L
$K_{d,GLC}$	0,3	1,202	0,842	g GLC/L
Nºinterations	-	29	38	
Global residue	-	83,15	79,43	

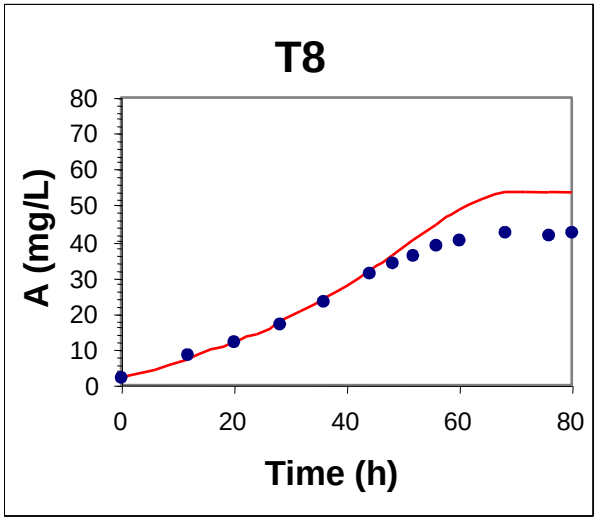












Resultado p/ simulação do modelo : GLN_o = 584 mg/L; GLC_o = 4,5 g/L

Validação do modelo através da análise estatística

— Estimativa do erro experimental usando 13 ensaios de "referência";

— fase lag esporádica;

— Coeficiente de variância;

CV_{Xv}=17%; CV_{GLC}=12.06; CV_{LAC}=14.8%;

CV_{Xd}=39.8%; CV_{GLN}=16.42%; CV_{A}=19.5%;

—Teste de χ^2 de Bartlett para discriminação do modelo,

➡ Modelo_2 é o melhor (considerando α=0.1);

— Teste F para verificar a qualidade do ajuste,

➡ Modelos 1 e 2 ajustam dados adequadamente.

Imprecisão do modelo

O modelo não representa adequadamente os seguintes fenômenos,

— Inibição do crescimento pelo lactato;

— Variabilidade dos fatores de conversão em função da mudança de concentração de substrato;

— Metabolismo celular em condição de excesso de GLC e GLN.

References

AUGUSTO EFP; BARRAL MF; PICCOLI RAM. Mathematical models for growth and product synthesis in animal cell culture. In: Castilho LR; Moraes AM; Augusto EFP; Butler M (Eds) *Animal cell technology: From Biopharmaceuticals to Gene Therapy*. Taylor & Francis , 2008, 518p.

PÖRTNER, R.; SCHÄFER, T. Modelling hybridoma cell growth and metabolism – a comparison of selected models and data. *J. Biotechnol.*, v.49, p. 119-135, 1996.

SIDOLI,F.R.;MANTALARIS, A.; ASPREY, S.P. Modelling of mammalian cells and culture processes. *Cytotechnology*, v.44, p.27-46,2004.