On the mathematical modelling of a batch fermentation process using interval data and verification methods

Svetoslav Markov

September 21, 2014

Co-authors: R. Alt, V. Beschkov, S. Dimitrov and M. Kamburova

Contents

Microbial growth (cell growth)

Microorganisms — procariotic, eucariotic

Procariotic — bacterial cells

Cells transform nutrient substrates (via their enzymes) into products

Products = intermediate metabolic subtances + final excrements

In addition, (some) cells divide and the cell population grows

Microbial growth—Malthusian growth model

Malthusian growth model, Malthus 1810–1825:

$dx/dt = kx, \quad x(0) = x_0 \qquad x(t) = x_0 e^{kt}$

$X \xrightarrow{k} X + X$ reproduction

More realistic assumption: $S + X \xrightarrow{k} X + X$ leading to

$dx/dt = ksx$

exponential unlimited growth

Microbial growth—Verhulst-Pearl model

Verhulst-Pearl model (1838):

$$dx/dt = kx - (k/a)x^2 = (k/a)x(a-x), \quad x(0) = x_0$$

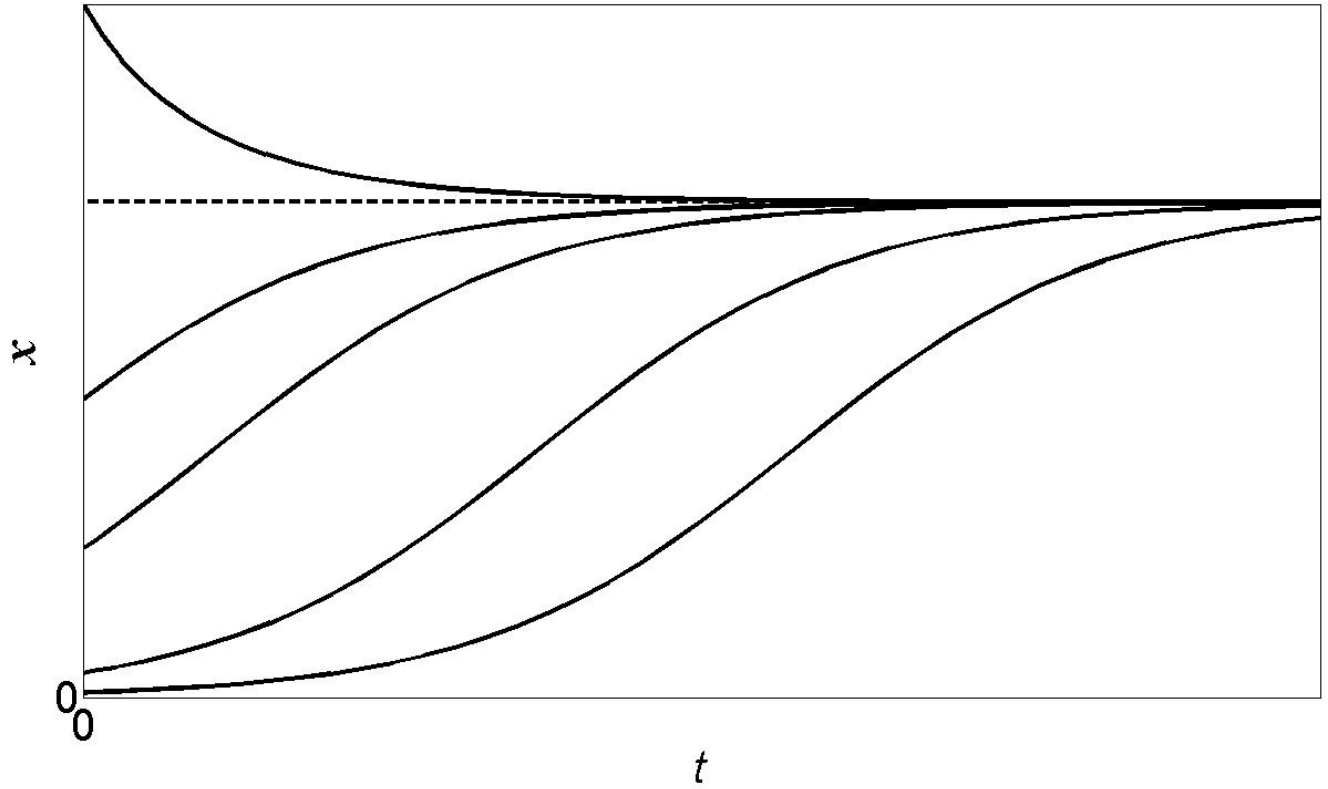
$$x(t) = \frac{a}{1 + be^{-kt}}, \qquad b = (a - x_0)/x_0$$

$$S + X \underset{k_{-1}}{\overset{k}{\rightleftarrows}} X + X \qquad \text{reproduction !}$$

$$dx/dt = ksx - k_{-1}x^2, \quad x(0) = x_0$$

if $s = \text{const}$ then same as above

Microbial growth - phases



Microbial growth - phases

(A) **lag phase**: During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

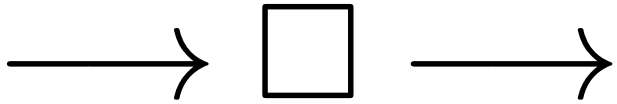
(B) **log phase**: Exponential or log phase is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant growth rate so both the number of cells and the rate of population increase doubles with each consecutive time period.

Microbial growth - phases

(C) **stationary phase**: During stationary phase, the growth rate slows as a result of nutrient depletion and accumulation of toxic products. This phase is reached as the bacteria begin to exhaust the resources that are available to them. This phase is a constant value as the rate of bacterial growth is equal to the rate of bacterial death.

From: `http://en.wikipedia.org/wiki/Bacterial_growth`

Bio-reactors: three types



$[S + X \longrightarrow P + X]$ batch

$S \longrightarrow [S + X \longrightarrow P + X]$ fed-batch

$S \longrightarrow [S + X \longrightarrow P + X] \longrightarrow P, X$ continuous

Dynamics of S needed

Bio-reactors: Monod model—functiom μ

$$\begin{aligned} ds/dt &= -\alpha\mu(s)x \quad [+F(s_{in}-s)] \\ dx/dt &= \mu(s)x - k_d x \quad [-Fx] \end{aligned}$$

$x = x(t)$ — the biomass

$s = s(t)$ — substrate concentration

k_d — decay constant (death rate)

Monod model—functiom μ

$$\mu(s) = \mu_{max} \frac{s}{K_s + s}$$

$$\mu(s) = \mu_{max} \frac{s}{K_s + s + s^2/K_i}$$

over 30 expressions for μ collected in :

M. Gerber, R. Span: An Analysis of Available Mathematical Models for Anaerobic Digestion of Organic Substances for Production of Biogas, proc. IGRC, Paris 2008.

Classical cell growth models

A batch mode chemostat/bioreactor

$$ds/dt = -\alpha\mu(s)x, \quad (1)$$

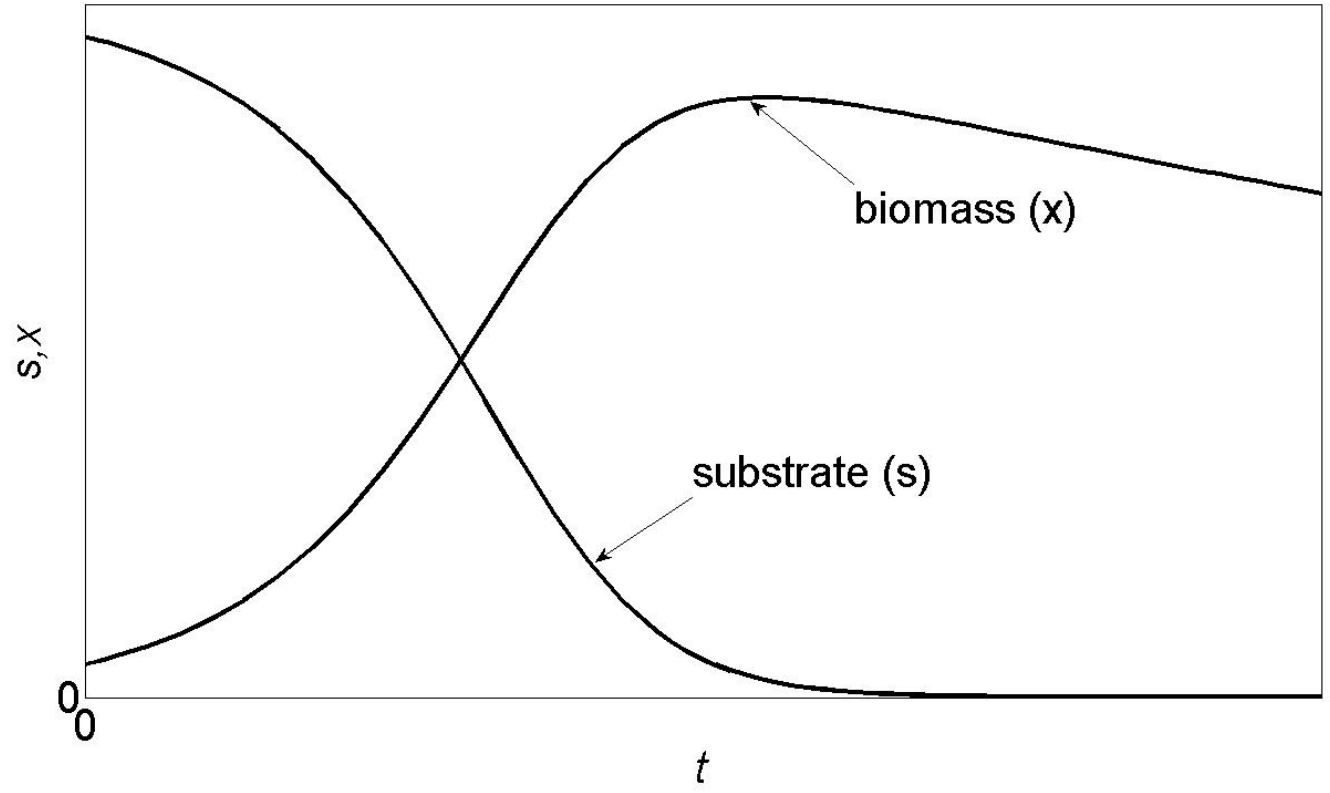
$$dx/dt = \mu(s)x - k_d x, \quad (2)$$

with $s(0) = s_0 > 0,\ x(0) = x_0 > 0$

Monod function:

$$\mu(s) = \mu_{max}\frac{s}{K_s + s}, \quad K_s \geq 0 \quad (3)$$

Classical cell growth models



Classical unstructured models with product

A classical model for batch cultivation of bacteria cells involving the dynamics of s, x and p

$$\begin{array}{rcl} ds/dt & = & -\ \delta\mu x \\ dx/dt & = & \mu x\ \ (-\gamma x) \\ dp/dt & = & (\alpha\ \mu + \beta)x \end{array} \tag{4}$$

$s(0) = s_0,\ x(0) = x_0,\ p(0) = p_0 = 0$

$\alpha,\ \beta,\ \delta$ are specific rate constants

Classical unstructured models

The parameter μ is the "specific growth rate" which is (usually) a function of s. Typical choices for μ are the Monod function

$$\mu(s) \quad = \quad \mu_{max}\ s/(K_s + s) \tag{5}$$

and the Haldane/Andrews function

$$\mu(s) \quad = \quad \mu_{max}\ s/(K_s + s + K_i s^2) \tag{6}$$

Experimental data provided

Experimental data provided:

a) for the CGTase production by bacteria *Bacteria circulans* ATCC21783 (data provided by prof. V. Beschkov from the Institute of Chemical Engineering, BAS);

b) for EPS production from thermophylic bacteria (data provided by prof. M. Kamburova from the Institute of Microbiology, BAS)

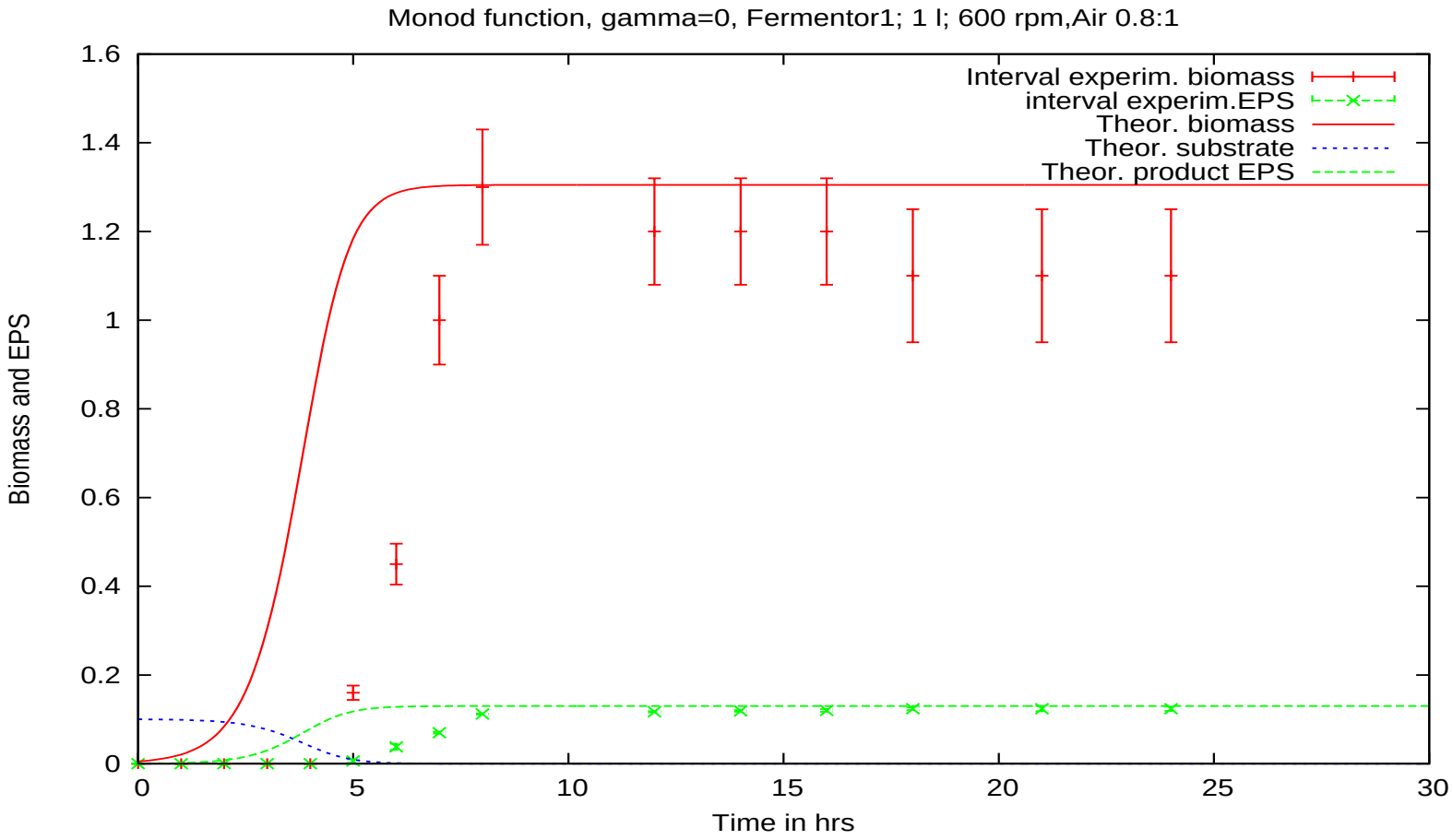
Both models provide data for the biomass x and the product p

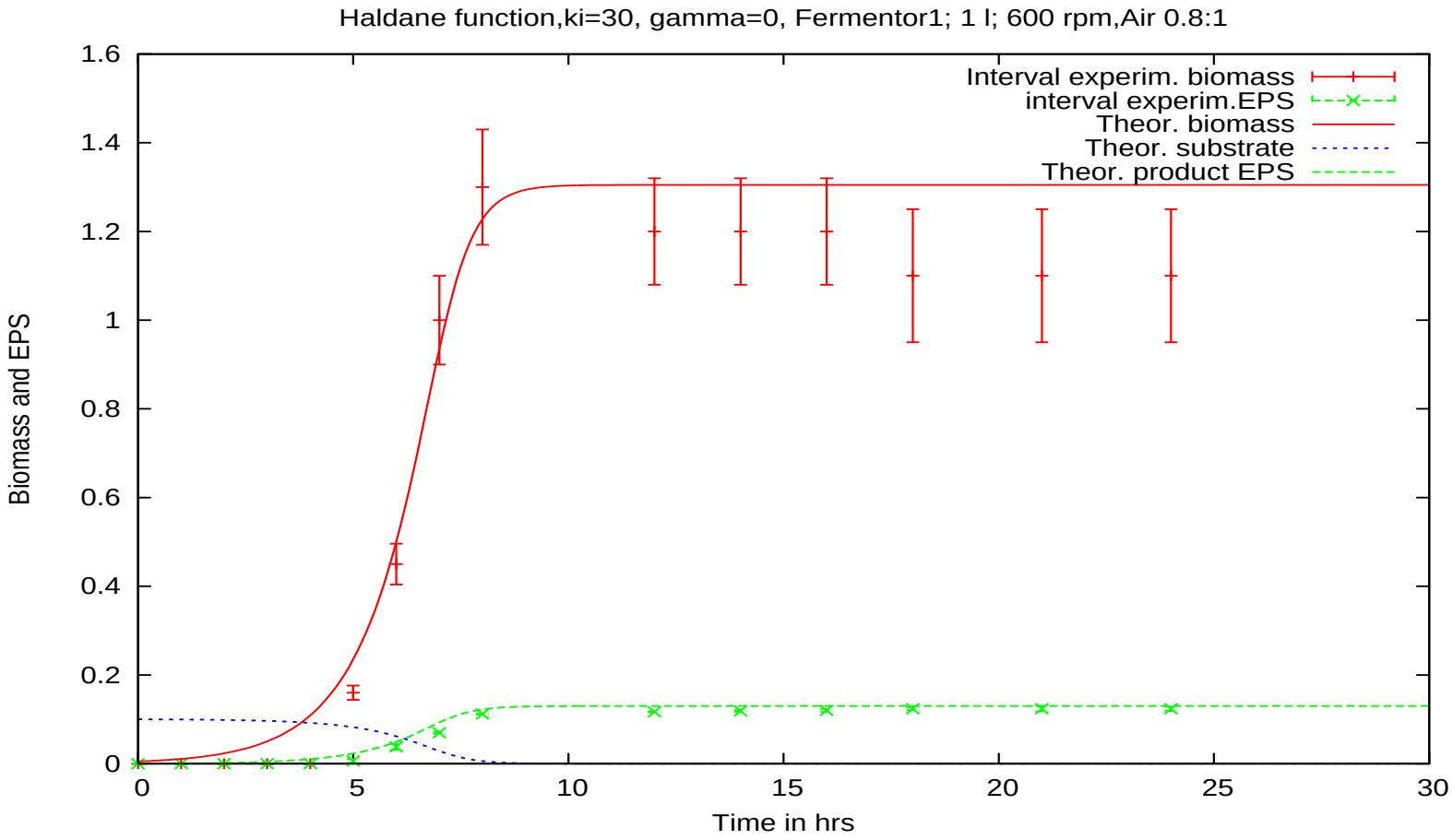
Classical Monod model

The Monod model has been applied to fit real experimental data for EPS production

using first the Monod function and then the Haldane function

$s_0 = 0.1,\ x_0 = 0.05,\ \mu_{max} = 5.,\ \alpha = 0.1,\ \beta = 0,\ \gamma = 0.005,$
$K_s = 0.25,\ K_i = 30, \delta = 13$


Monod function, gamma=0, Fermentor1; 1 l; 600 rpm,Air 0.8:1
Interval experim. biomass
interval experim.EPS
Theor. biomass
Theor. substrate
Theor. product EPS
Biomass and EPS
Time in hrs
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
5
10
15
20
25
30


Haldane function,ki=30, gamma=0, Fermentor1; 1 l; 600 rpm,Air 0.8:1
Interval experim. biomass
interval experim.EPS
Theor. biomass
Theor. substrate
Theor. product EPS
Biomass and EPS
Time in hrs
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
5
10
15
20
25
30

Modelling process: graphics via *Mathematica*

Parameter identification is performed conveniently using *Mathematica* tools

Slide keys ("sliders")

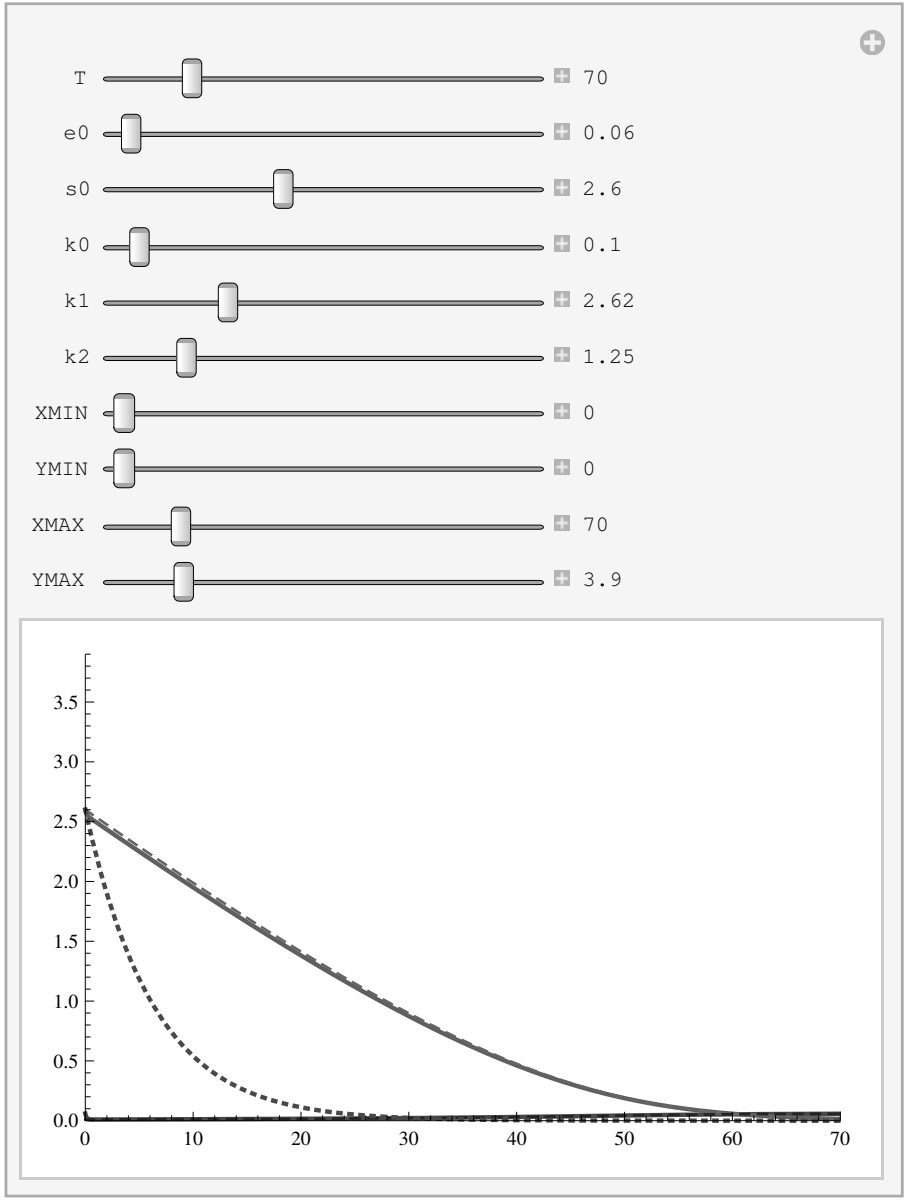
`http://reference.wolfram.com/language/ref/Slider.html`

If no solution sets passing through all intervals are found,

then we abandon the model and look for another model structure

— usually a modified one

Out[20]=


T 70
e0 0.06
s0 2.6
k0 0.1
k1 2.62
k2 1.25
XMIN 0
YMIN 0
XMAX 70
YMAX 3.9
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70

A two-phase model

Model (4) is a two-phase model proposed in CAMWA [1]

$$\begin{aligned} ds/dt &= -k_1 xs - (\alpha+\beta)ys, \\ dx/dt &= -k_1 xs + k_2 y + \alpha ys - k_x x, \\ dy/dt &= k_1 xs - k_2 y + \beta ys - k_y y \\ dp/dt &= \gamma * s * (x+y) \end{aligned} \tag{7}$$

with the initial conditions

$$s(0) = s_0,\ x(0) = x_0,\ y(0) = y_0,\ p(0) = p_0.$$

s is the substrate, p is the product

x, y are two phases of the biomass

A two-phase model

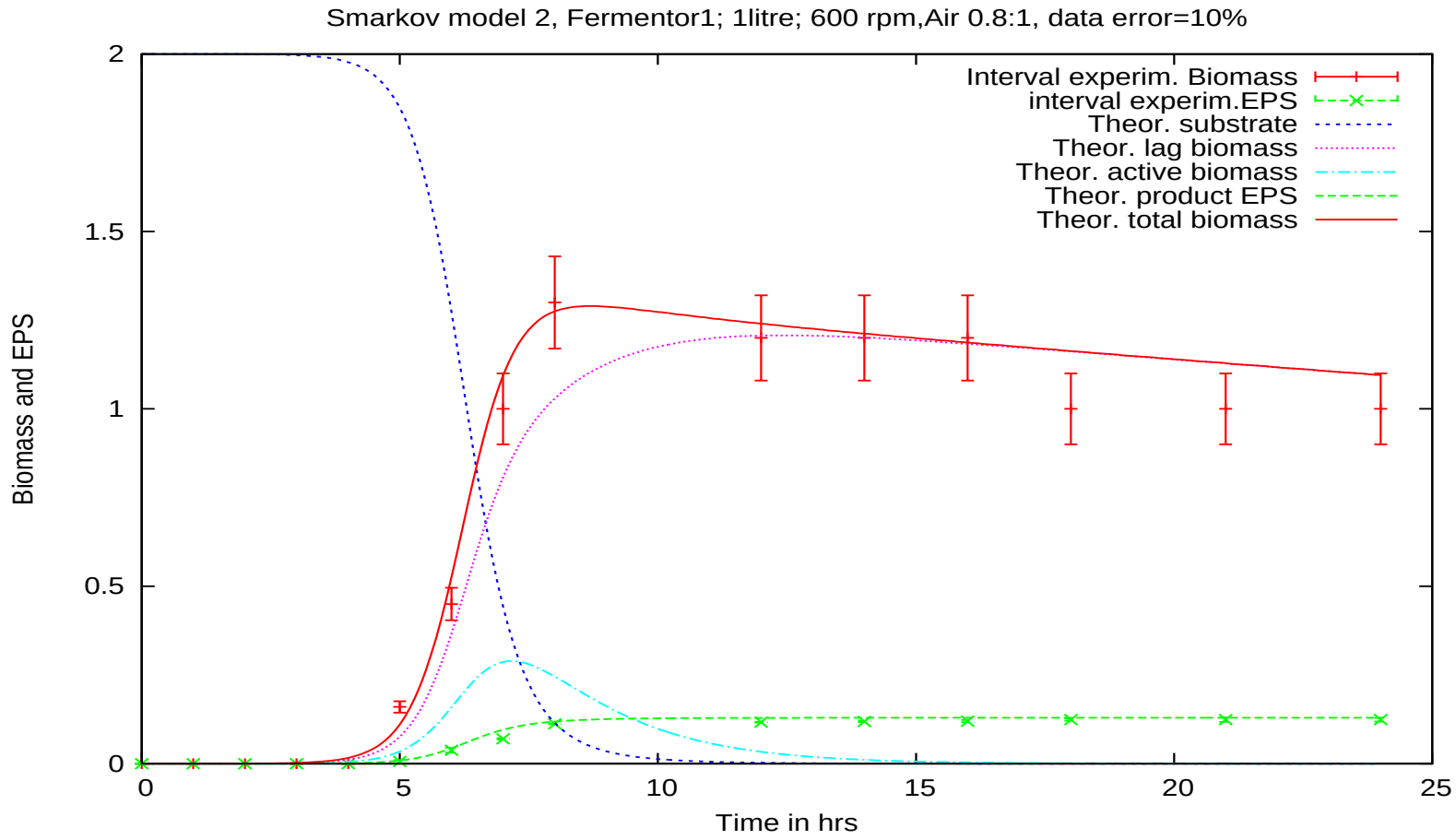
A good approximation of the experimental data using this model have been found with the following coefficients:

$k_1 = 0.5;\ k_2 = 0.5;\ k_x = 0.01;\ k_y = 0.1;\ \alpha = 3;\ \beta = 0.2$

and with initial conditions:

$s_0 = 2;\ x_0 = 5.10^{-5},\ y_0 = 0, p_0 = 0$

The above approximation is visualized on the next Figure


Smarkov model 2, Fermentor1; 1litre; 600 rpm,Air 0.8:1, data error=10%
Interval experim. Biomass
interval experim.EPS
Theor. substrate
Theor. lag biomass
Theor. active biomass
Theor. product EPS
Theor. total biomass
Biomass and EPS
Time in hrs
0
0.5
1
1.5
2
0
5
10
15
20
25

The basic two-phase model

Reproduction—simple reaction scheme

Y-cells are engaged in formation of products P, which are important components of newborn cells. Reproduction as part of the production process: a) cell growth is due to reproduction—hence to dividing Y-cells; b) Y-cells utilize product P to reproduce

$$P + Y \xrightarrow{k_3} 2Y$$

The basic two-phase model

The dividing mother Y-cells transform into daughter cells plus a newborn cell built by means of product P

$$S + X \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} Y \xrightarrow{k_2} P + X,$$

$$P + Y \xrightarrow{k_3} 2Y,$$

Many other variants (like replacing Y by X) were checked and waived

The basic two-phase model

Applying the mass action law, we obtain the model:

$$\begin{aligned} ds/dt &= -k_1 x s + k_{-1} y \\ dx/dt &= -k_1 x s + k_{-1}\ y + k_2 y \\ dy/dt &= k_1 x s - k_{-1}\ y - k_2 y + k_3 p y \\ dp/dt &= k_2 y - k_3 p y \end{aligned} \tag{8}$$

with initial conditions (corresponding to a batch cultivation process): $s(0) = s_0,\ x(0) = x_0,\ y(0) = y_0,\ p(0) = 0.$

The basic two-phase model

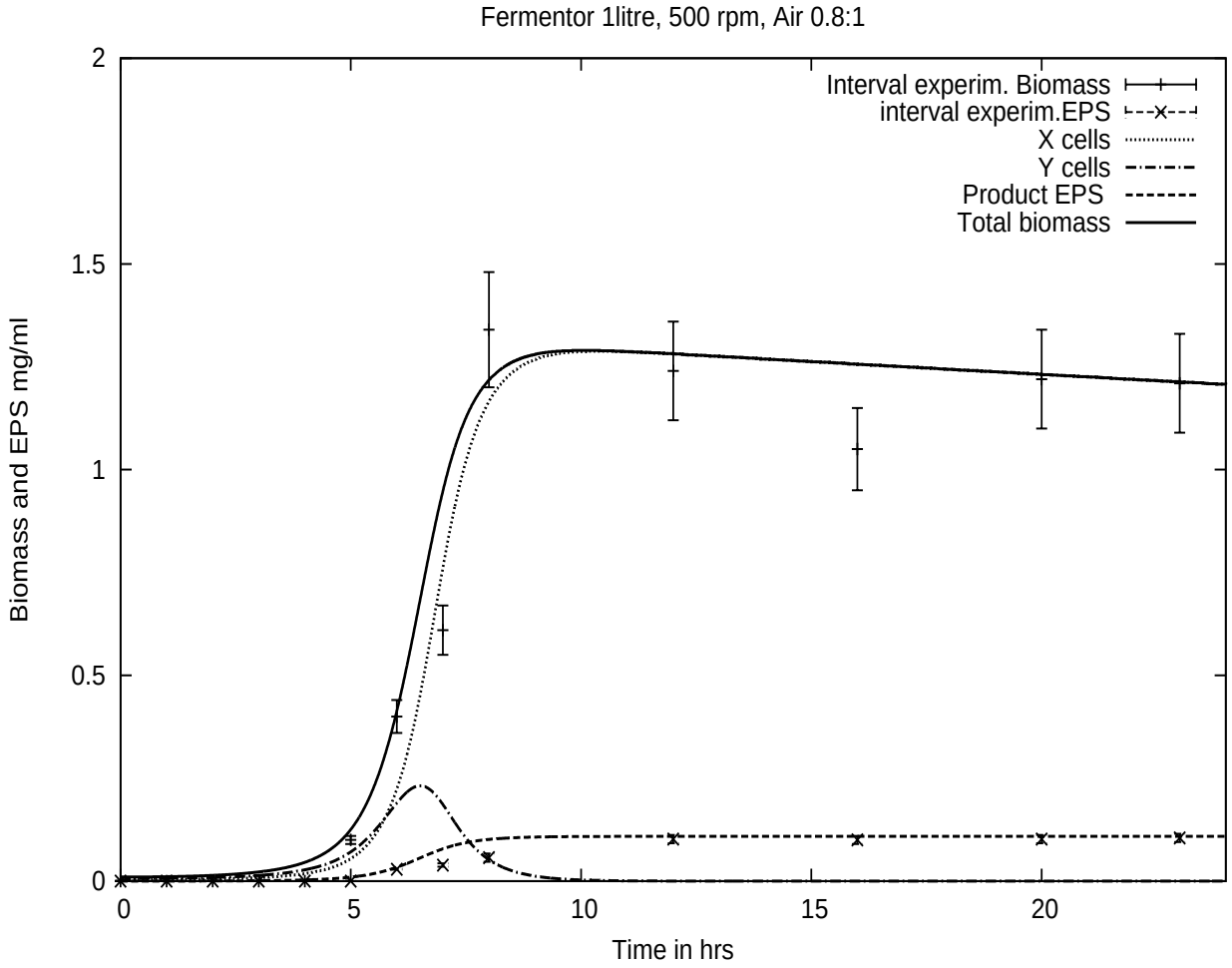
Respectively, assuming $k_{-1} = 0$ we obtain the simpler model:

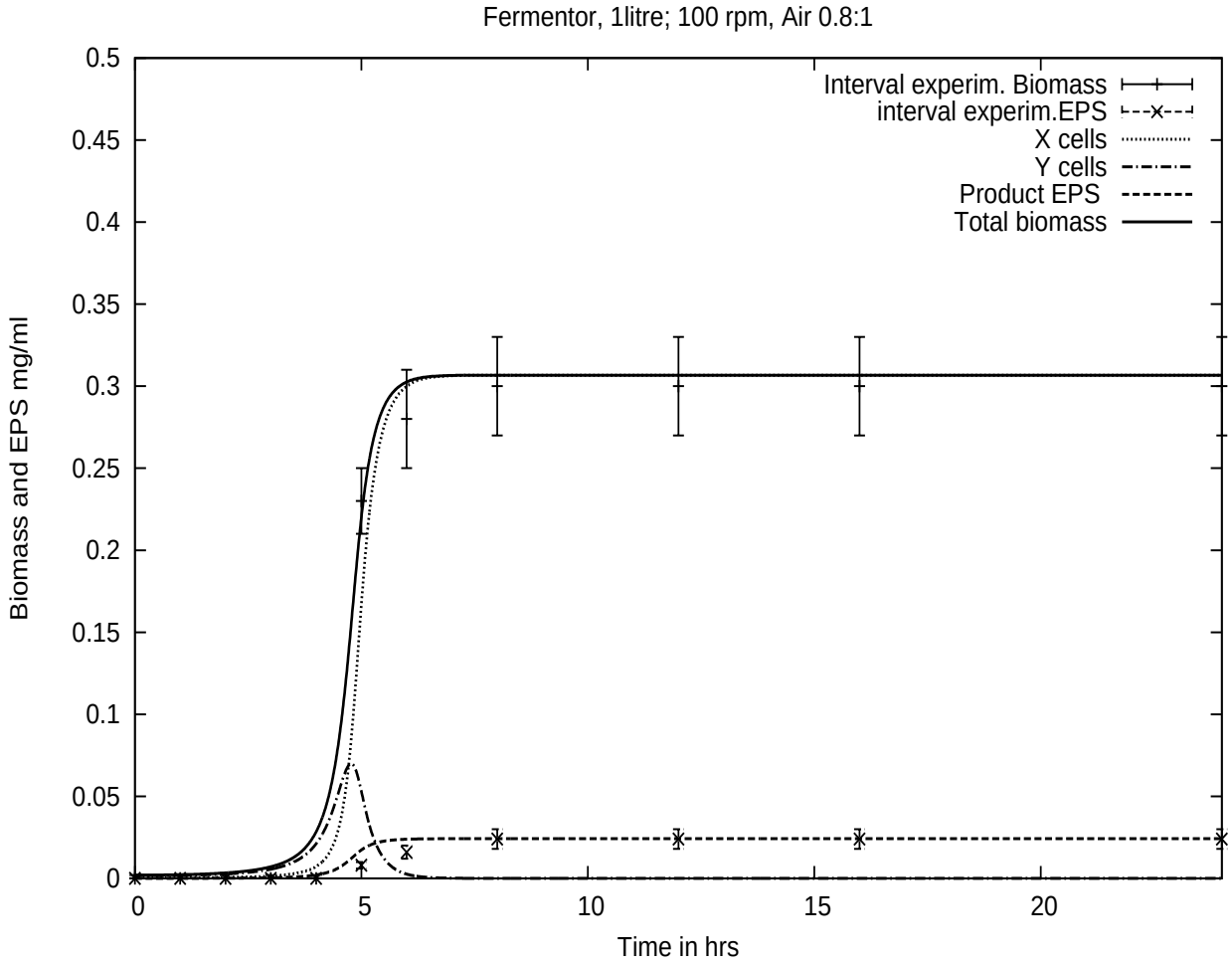
$$\begin{aligned} ds/dt &= -k_1 xs \\ dx/dt &= -k_1 xs + k_2 y \\ dy/dt &= k_1 xs - k_2 y + k_3 py \\ dp/dt &= k_2 y - k_3 py \end{aligned} \tag{9}$$

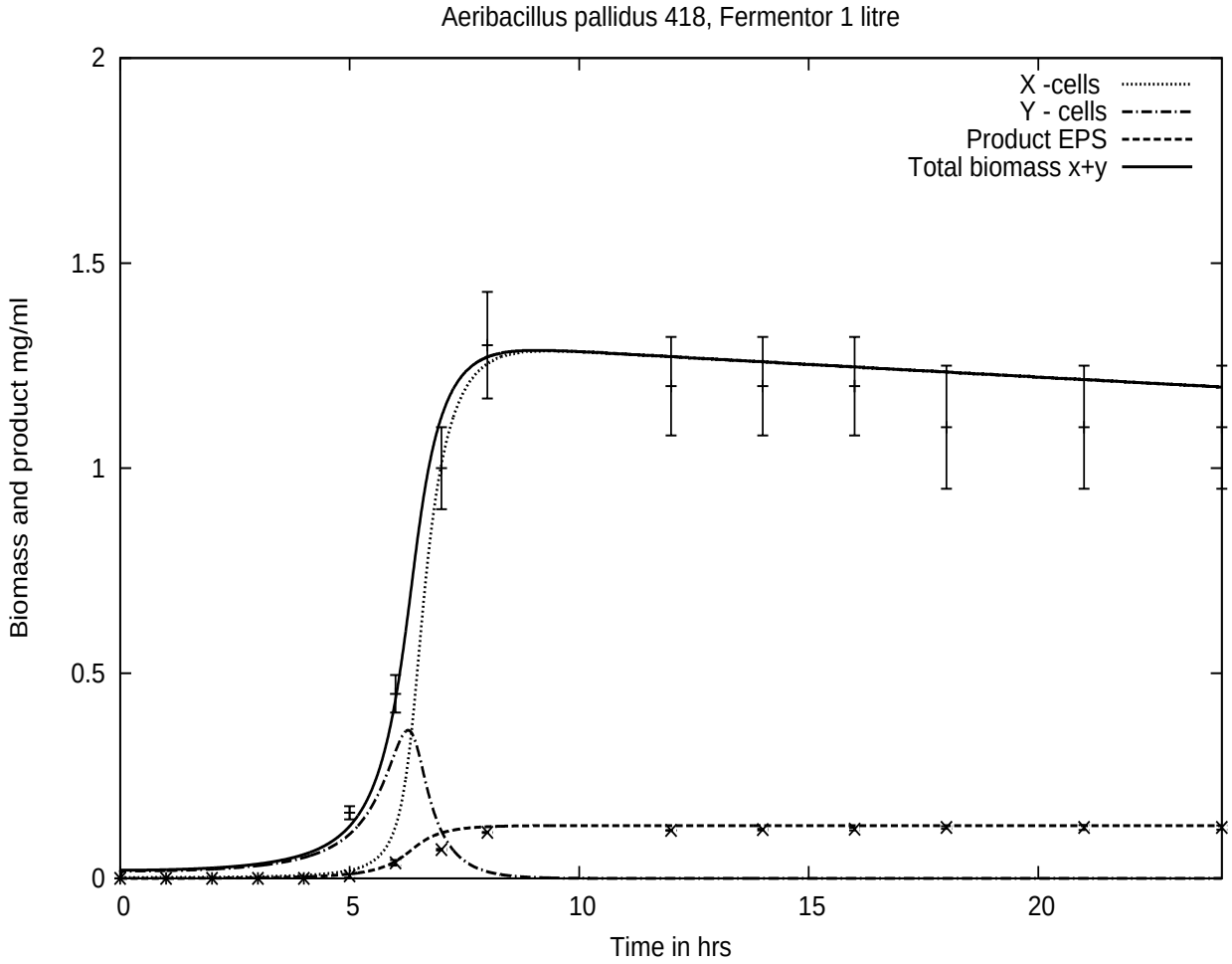
induced by the reaction scheme:

$$S + X \xrightarrow{k_1} Y \xrightarrow{k_2} P + X,$$

$$P + Y \xrightarrow{k_3} 2Y.$$


Fermentor 1litre, 500 rpm, Air 0.8:1
Interval experim. Biomass
interval experim.EPS
X cells
Y cells
Product EPS
Total biomass
Biomass and EPS mg/ml
Time in hrs
0
0.5
1
1.5
2
0
5
10
15
20


Fermentor, 1litre; 100 rpm, Air 0.8:1
Interval experim. Biomass
interval experim.EPS
X cells
Y cells
Product EPS
Total biomass
Biomass and EPS mg/ml
Time in hrs
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0
5
10
15
20


Aeribacillus pallidus 418, Fermentor 1 litre
X -cells
Y - cells
Product EPS
Total biomass x+y
Biomass and product mg/ml
Time in hrs
0
0.5
1
1.5
2
0
5
10
15
20

Comparison between Monod model and our basic model

Proposition. Monod model is a special case of our basic Model under the assumption that favourable conditions are present (the biomass x and the product p are nearly constant)

Proof — in BIOMATH 2/2 paper

Our basic model and Henri reaction scheme

Our model makes use of Victor Henri reaction scheme in enzyme kinetic, when identifying free enzymes with X-cells and bounded enzymes with Y-cells, following the familiar stoichiometric-like scheme for the kinetics of enzymes with just one active site:

$$S + X \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} Y \xrightarrow{k_2} P + X,$$

wherein k_1, k_{-1}, k_2 are rate constants.

Open math problems induced by cell growth kinetics

P1. To approximate in Hausdorff metric the interval step function by means of the X-solution (or the $X + Y$-solution) of the basic cell-growth system

P2. To approximate in Hausdorff metric the interval Dirac function by means of the y-solution of the basic cell-growth system

Conclusion remarks

Basic biological processes—such as cell growth—still need adequate models

For the model construction and parameter identification the following tools prove to be useful:

Interval approach—very useful both in model construction and parameter identification

Contemporary computational tools—CAS *Mathematica*

Reaction schemes—very helpful when establishing the model structure of the biological process

Conclusion: achievement

It has been demonstrated that classical Monod type models fail to model adequately cell growth in the transision periods (lag-log, log-stationary)

Proposed two-phase models provide more flexibility and can be very well fitted to real data

Our proposed two-phase models are built on simple principles related to enzyme kinetic, implied by reaction schemes. This contributes to more clarity in the biological meaning

All parameters in the proposed two-phase models are numeric and have clear biological meanings as specific rate constants (not depending on the concentrations involved)

Bastin-Dochain theory

Bastin-Dochain "reaction scheme"

$$S + X \xrightarrow{\nu(s)} P + X$$

"Applying MAL" we get:

$$ds/dt = -\nu(s)sx$$

putting $\nu(s) = 1/(K_s + s)$ one obtains Monod model

References

[1] Alt, R., S. Markov, Theoretical and computational studies of some bioreactor models, *Computers and Mathematics with Applications* 64 (2012), 350–360.

[2] Markov, S., Biomathematics and interval analysis: a prosperous marriage. In: M. D. Todorov, Ch. I. Christov, Eds., AIP Conf. Proc. 1301, Amitans2010, AIP, 2010, 26–36.

[3] Markov, S., Cell Growth Models Using Reaction Schemes: Batch Cultivation, *Biomath* 2/2 (2013), 1312301.

[4] Radchenkova, N., M. Kambourova, S. Vassilev, R. Alt, S. Markov, On the mathematical modelling of EPS production by a thermophilic bacterium, *BIOMATH* 3/1 (2014), 1407121

Figure 1: Rue Monod in Lion SCAN 2010