Biodegradation of Phenols by a Pure Bacteria of Pseudomonas Putida and by a Mixed Culture of Pseudomonas Various

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The paper presents the study of treatment of wastewaters resulted from a petrochemical platform. The purpose of this work was to simulate the industrial process under the Monod model and to determine if the model could be used for engineering working. The Monod model used to simulate the industrial process was based on equation Monod modified by Jarzebski et al. First, the biodegradation time was determined using the model at an initial phenols concentration of 23.4 mg/L and a pure culture of Pseudomonas putida for the phenols biodegradation. Then, the biodegradation time was determined in the same operating conditions, but using a mixed culture of bacteria, Pseudomonas (various species). In both cases the results were compared with the experimental time. The model provided good results compared to the industrial process when selecting a pure culture of Pseudomonas putida for phenols biodegradation.

Keywords: biodegradation time, Monod-Jarzebski equation, Pseudomonas putida, Pseudomonas various species

The effluents containing phenolic compounds are usually resulted from industrial wastewaters and are treated in activated sludge processes which are depending on the variation of the initial phenols concentration. Many operational problems are associated with the fluctuations of phenols concentrations. Despite this, the dynamics of phenol degradation in continuous aerobic systems, such as the activated sludge processes, are not well understood.

The Haldane equation has been frequently used to describe the degradation of phenol in pure or mixed culture [1-8]. Thus,

$$\mu = \frac{\mu_m c_S}{K_S + c_S + (c_S^2/K_i)} \quad (1)$$

where:

μ is the specific growth rate, c_s is the phenol concentration, K_S is the saturation coefficient for phenol, K_i is the inhibition coefficient for phenol and μ_m is the maximum specific growth rate of the biomass.

Usually, parameters derived from batch experiments have been used in equation (1) to predict the response of continuous systems. The Haldane equation predicts a global maximum specific growth rate μ at a phenol concentration c_s. Equation (1) was also used to describe the specific phenol uptake rate (q_p) of washed cells of *Pseudomonas putida* in batch system [7, 9, 10]. Notwithstanding its popularity, question remains as to the adequacy of equation (1) as a model for phenol degradation.

Biological processes are extremely complex. Cell growth and metabolite formation are the results of a very large number of cellular reactions and events like gene expression, translation of mRNA into functional proteins, further processing of proteins into functional enzymes or structural proteins, and sequences of biochemical reactions leading to building blocks needed for synthesis of cellular components.

It is clear that a complete description of all these reactions and events cannot possible be included in a mathematical model. It is also clear that a very important element in mathematical modelling of processes is defining the structure (or specifying the complexity of the model), and for this, a general rule can be stated: as simple as possible but not simpler. This rule implies that the basic mechanisms always should be included and that the model structure depends on the aim of the modelling exercise. The verbal presentation of a cell growth of a single limiting substrate can be described with many different mathematical models, but the most often applied is the Monod model, which states that [11]:

$$\mu = \frac{\mu_{max} c_S}{c_S + K_S} \quad (2)$$

where K_S is the saturation coefficient and is sometimes interpreted as the affinity of the cells towards the substrate S. The Monod model is not the only kinetic expression that has been used to describe the specific growth rate in the black box mode. Many different kinetic expressions have been used, expressions that contain adjustable parameters as in Monod model. These different expressions clearly demonstrate the empirical nature of these kinetic models, and it is therefore futile to discuss which model is to be preferred, since they are all simply data fitters, and one should simply choose the model that gives the best description of the system being studied [11].

Further to all mentioned above, it was chosen the equation Monod – Jarzebski that was applied by Jarzebski [12] in 1989 at modelling of ethanol fermentation at high substrate concentrations:

$$\mu(c_s, c_p, c_x) := \mu_m \cdot \left(\frac{c_s}{Ks + c_s}\right) \cdot \left[1 - \left(\frac{c_p}{Pm}\right)^n\right] \cdot \left[1 - \left(\frac{c_x}{X_m}\right)^m\right] \quad (3)$$

The Monod-Jarzebski equation contains the Monod equation and additionally to this, two terms that includes the maximum productivity of the system, P_m, and the maximum biomass concentration, X_m [12].

It was considered that the additionally terms could illustrate better the real industrial case.

The aim of this study was to simulate the real industrial process of biodegradation of phenols released from a petrochemical platform, at high initials phenols concentrations, up to 20 mg/L, under the Monod-Jarzebski

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model, first using *Pseudomonas putida* for the biodegradation process, and then using a mixed culture of bacteria, *Pseudomonas putida* and *Pseudomonas various*, and to compare the results obtained by both study case simulations with the data of the real industrial process and to determine which case provides better results when compared to the real process.

Experimental part

It was studied a full industrial scale process taking into consideration wastewaters from a petrochemical platform with initial phenols concentrations up to 15-20 mg/L and a final imposed phenols concentrations less than 0.3 ppm. During more than 1000 experiments, the biological phenol compounds degradation was monitored, data were recorded for parameters as follows: initial phenols concentration, temperature, pH, nutrient concentration, flow rate, sludge concentration, final phenols concentration etc. Table 1 presents a few recorded concentrations at constant flow rate of 800 m^3/h.

The real petrochemical wastewaters biodegradation process used an active sludge recycling bioreactor type basin composed of 2 units working in series. The nutrients were urea and phosphate. The oxygen was introduced directly from the atmospheric air into the basin. Inside the bioreactor, the oxygen was uniformly distributed using perforate panels arranged on the base of the basin.

The data presented in the table 1 and also the plant used for experiments were presented in a prevoius work [13].

Kinetics models

The authors intended to determine the biodegradation time using Monod-Jarzebski model and to compare the time resulted from the model with the real industrial time, with the biodegradation time obtained in a previous work under Haldane model and also with experimental data from the specific literature.

The present study considers that kinetics of phenols biodegradation process is described by Jarzebski equation, (3).

The biodegradation time results based on the biomass growth rate (v_{rx}), the phenols biodegradation rate (v_{rs}) and the clean water rate (v_{rp}) that is expressed by the equations below:

$$D(t,c) := \begin{pmatrix} -v_{rs}(c_0, c_1, c_2) \\ v_{rp}(c_2) \\ v_{rx}(c_0, c_1, c_2) \end{pmatrix} \quad (4)$$

The concentrations matrix is described by equations below:

$$c := \begin{pmatrix} 23.4 \\ 0 \\ 2 \end{pmatrix} \quad (5)$$

$$v_{rx}(c_S, c_P, c_x) = \mu(c_S, c_P, c_x) \cdot c_x \quad (6)$$

where $\mu(c_S, c_p, c_x)$ is described by ec. (3), above presented;

$$v_{rs}(c_S, c_P, c_x) = q(c_S, c_P, c_x) \cdot c_x \quad (7)$$

where

$$q(c_s, c_p, c_x) := \left(\frac{\mu(c_s, c_p, c_x)}{Y_{XS}} \right) + m_s \quad (8)$$

Table 1
A FEW EXPERIMENTAL DATA RECORDED FROM THE INDUSTRIAL PETROCHEMICAL PROCESS [13]

Experiment	Phenols concentration		
	Initial phenols concentration c_{S0} ($mg \cdot l^{-1}$)	Intermediate phenols concentration c_{Si} ($mg \cdot l^{-1}$)	Final phenols concentration c_S (mg/L)
0	1	2	3
1.	1.7	0.081	0.0290
2.	2.3	0.090	0.0200
3.	12.7	0.559	0.0019
4.	6.5	0.086	0.0097
5.	13.0	0.110	0.0240
6.	10.8	0.480	0.0290
7.	7.3	0.740	0.0290
8.	19.9	0.220	0.0200
9.	19.5	0.087	0.0192
10.	21.2	0.149	0.0126
11.	8.2	0.103	0.0132
12.	18.5	12.4	0.0240
13.	3.5	1.040	0.0290
14.	17.6	17.180	0.0830
15.	6.7	5.160	0.0360
16.	13.4	7.900	0.0170
17.	1.7	2.800	0.0070
18.	3.1	2.590	0.0105
19.	7.2	5.340	0.0420
20.	21.2	16.490	0.0200
21	18.5	10.000	0.0100
22.	23.4	13.480	0.0270
23.	33.6	21.000	0.0520
24.	9.7	12.450	0.0190

and

$$v_{rp}(c_x) = v(c_x) \cdot c_x \quad (9)$$

where

$$v(c_x) := v_m \cdot e^{-k \cdot c_x} \quad (10)$$

Results and discussions

The first step was to simulate the industrial process under Monod-Jarzebski model using a pure culture of bacteria, *Pseudomonas putida*. Further to this, the parameters values used in Monod - Jarzebski model were as follows:

-the coefficient for the half-saturation, $K_S = 6.19$ mg/L;
-the coefficient for phenol inhibition, $Ki = 54.1$ mg/L;
-the maximum specific growth rate $\mu_m = 0.436\ h^{-1}$;
-the maximum productivity $P_m = 800000\ kg/m^3$;
-the maximum biomass concentration $X_m = 900\ kg/m^3$;
-the initial phenols concentration, $c_{S0} = 23.4$ mg/L;

-the final phenol concentration, c_S = 0 mg/L;
-the initial biomass concentration, c_{X0} = 2 mg/L;
-the initial product (clean water) concentration, c_{P0} = 0 mg/L;
-biomass growth resulted from the substrate coefficient, Y_{XS} = 0.5 g/g;
-maximum specific product grow rate, v_m = 0.55 g/g·h;
-maintenance coefficient, m_S = 0.27 g·/g· h ;
-constant, k = 0.006 L/g;
-constant, m = 1.1;
-constant, n = 0.85.

Table 2
THE MODELLING RESULTS OBTAINED USING MONOD - JARZEBSKI MODEL FOR PURE CULTURES OF PSEUDOMONAS PUTIDA

Program interations z	Time t (h)	Phenols concentration c_s(mg/L)	Product concentration c_p (mg/L)	Biomass concentration c_x (mg/L)
0	0.0	23.400	0.000	2.000
1	0.2	23.004	0.225	2.142
2	0.4	22.584	0.465	2.292
3	0.6	22.137	0.722	2.452
4	0.8	21.662	0.997	2.621
5	1.0	21.159	1.290	2.799
6	1.2	20.626	1.603	2.987
7	1.4	20.062	1.936	3.186
8	1.6	19.467	2.291	3.395
9	1.8	18.849	2.668	3.615
10	2.0	18.177	3.069	3.845
11	2.2	17.482	3.494	4.085
12	2.4	16.753	3.946	4.336
13	2.6	15.989	4.424	4.597
14	2.8	15.193	4.929	4.868
15	3.0	14.363	5.464	5.147
16	3.2	13.503	6.027	5.435
17	3.4	12.612	6.620	5.729
18	3.6	11.695	7.244	6.029
19	3.8	10.754	7.899	6.333
20	4.0	9.793	8.585	6.638
21	4.2	8.818	9.301	6.942
22	4.4	7.835	10.049	7.242
23	4.6	6.852	10.826	7.534
24	4.8	5.878	11.631	7.814
25	5.0	4.924	12.464	8.077
26	5.2	4.000	13.332	8.317
27	5.4	3.121	14.202	8.529
28	5.6	2.299	15.102	8.708
29	5.8	1.546	16.018	8.847
30	6.0	0.875	16.945	8.942
31	6.2	0.291	17.879	8.992

Using the Monod-Jarzebski model presented above results the variation in time of phenols concentration presented in table 2.

The results of Monod-Jarzebski model used show a biodegradation time of 6.2 h.

In the real case the biodegradation time for the same initial phenols concentration of 23.4 mg/L is 7.2 h for the both reactors working in series. In the first bioreactor, the waters are kept during 6 h and in the second reactor, the residence time is 1 h 20 min for a constant flow rate of 800 m^3/h.

In a previous work [13] was presented the simulation of the real industrial process using Haldane model, in the same conditions of initial phenols concentration of 23,4 mg/L and at the same constant flow rate of 800 m^3/h and also using a pure culture of bacteria, *Pseudomonas putida*. The time obtained by simulation using Haldane model was 6.5 h. Both models, Haldane and Monod – Jarzebski present values for the biodegradation time smaller than the real industrial process residence time. Further to this could issue the idea of using of only one bioreactor instead of two from the industrial process to treat the residual waters. Interesting is the very appropriate results obtained by both models, even the Monod - Jarzebski model based on the Jarzebski equation includes 2 additionally terms regarding the maximum productivity of the system and the maximum biomass concentration that in Haldane equation don't exist. More interesting is the fact that in the specific literature we can find results of 10-15 h for the biodegradation time, biger than models or industrial biodegradation time [9 – 16].

Therefore, Monteiro and all [5] obtained 6.05 h only for the duration of the lag phase. It was selected also the *Pseudomonas putida* as a known representative of the aerobic degraders of aromatics, to describe the phenol biodegradation in a batch reactor. The bacteria used were *Pseudomonas putida* DSM 548 and the purpose of Monteiro and all [5] work was to determine the kinetics of biodegradation by measuring biomass growth rates and phenol concentration as a function of time in a batch reactor. The residence time obtained by Monteiro [5] for the input of $\mu_m = 0.436\ h^{-1}$, $K_S = 6.19$ mg/L, Ki = 54.1 mg/L, Y 0.0017g/g was 14 h for the same phenol concentration, $S_0 = 23.4$ mg/L.

Allsop and all [4] studied pure cultures of *Pseudomonas putida* (ATCC 17484) that were subjected to step increases in phenol feed concentration. Each test run consisted of an initial period of steady state operation followed by a step change in substrate concentration or composition. The hydraulic residence time of all runs was 11.8 – 13.6 h, with feed concentrations ranging from 200 to 2500 mg/L phenol and /or 545.5 mg/L glucose.

Monteiro and all [5] used for the study biodegradation of phenol with an initial phenol concentration of 25 mg/L a $\mu_m = 0.288\ h^{-1}$, normally used by Pawlowski and Howell [16,20] for mixed culture biodegradation of phenol.

The next step of this work was to investigate the same industrial process, under the same initial phenols concentration but using a mixed population of bacteria, *Pseudomonas various.*. The process was simulated under the Monod-Jarzebski model, using for the case of mixed population of bacteria the kinetic parameters as follows:
-K_S = 245 mg/L;
-K_i = 54.1 mg/L;
-$\mu_m = 0.288\ h^{-1}$
-Y_{XS} = 0.45 g/g;
The programme was run and the results obtained are presented in table 3.

The results of Monod-Jarzebski model using mixed cultures of bacteria for the phenols degradation show a biodegradation time of about 30 h. The value obtained for the biodegradation time when using mixed cultures of

Table 3
THE MODELLING RESULTS OBTAINED USING MONOD - JARZEBSKI MODEL FOR MIXED CULTURES OF BIOMASS: *PSEUDOMONAS VARIOUS*

Program interations z	Time t (h)	Phenols concentration, c_s(mg/L)	Product concentration, c_p (mg/L)	Biomass concentration, c_x (mg/L)
0	0.000	23.400	0.000	2.000
1	0.333	23.182	0.364	2.017
2	0.667	22.963	0.730	2.033
3	1.000	22.742	1.100	2.05
4	1.333	22.519	1.473	2.067
5	1.667	22.296	1.848	2.083
6	2.000	22.070	2.227	2.100
7	2.333	21.844	2.609	2.117
8	2.667	21.616	2.993	2.133
9	3.000	21.386	3.381	2.150
10	3.333	21.155	3.771	2.166
11	3.667	20.923	4.165	2.183
.....				
47	15.667	11.774	20.164	2.712
48	16.000	11.503	20.654	2.724
49	16.333	11.232	21.147	2.735
.....				
84	28.000	1.603	39.336	2.978
85	28.333	1.331	39.873	2.980
86	28.667	1.060	40.409	2.982
87	29.000	0.789	40.946	2.983
88	29.333	0.519	41.483	2.983
89	29.667	0.249	42.020	2.984

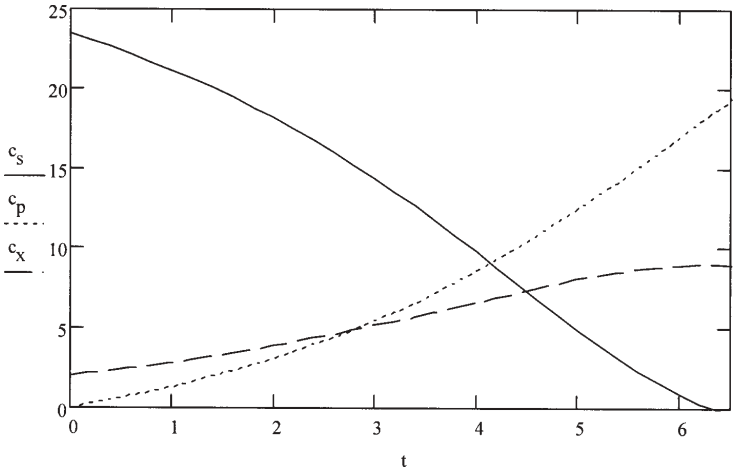

Fig. 1.Variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 23.4 (mg/L), when using a pure culture of *Pseudomonas putida*

bacteria is more than 4 time the industrial biodegradation time and almost 5 time the biodegradation time resulted from both models, Haldane and Monod-Jarzebski. Taking into consideration this we can conclude that better results consisting in smaller biodegradation times are obtained when using a pure culture of bacteria for the biodegradation of phenols compounds. Monteiro and all [5] concluded that the maximum specific growth rate of *Pseudomonas putida*, μ_m, is higher than that observed in mixed cultures. This leads to higher efficiencies when selecting a pure culture for phenol degradation.

Also, the use of pure cultures of *Pseudomonas putida* into the simulations is closer to the industrial process running and the biodegradation times and phenols concentrations resulted from both models demonstrate that we can use any model to simulate the industrial process.

The Monod-Jarzebski model provides also an evaluation of the kinetics of biodegradation process that is shown in figures below presented.

The figure 1 presents the variation in time of substrate concentration, biomass concentration and water

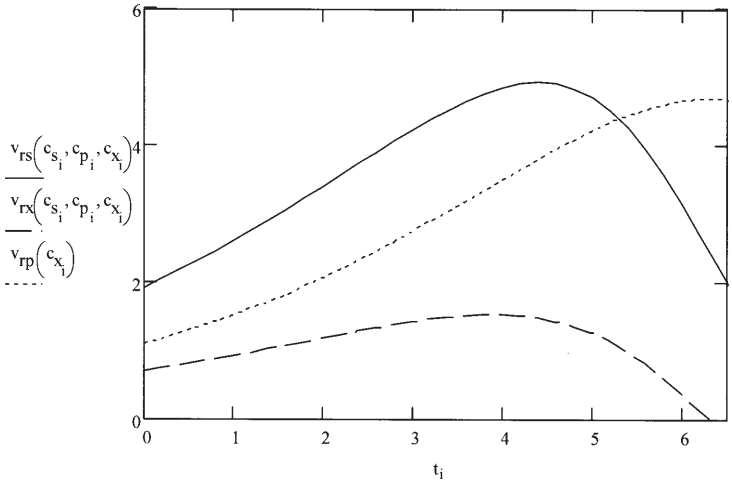

Fig. 2. Variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 23.4 (mg/L), when using a pure culture of *Pseudomonas putida*

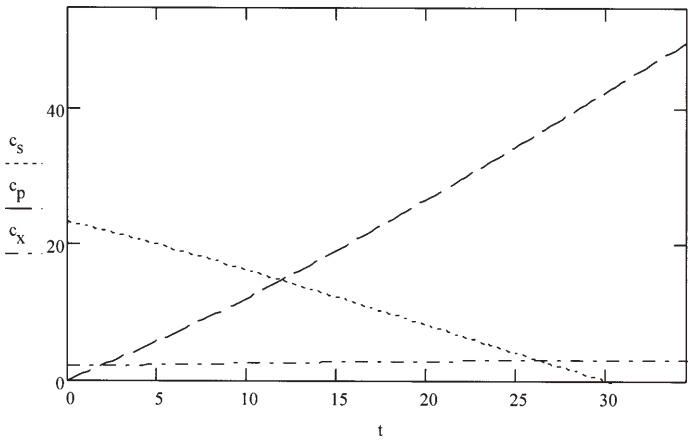

Fig. 3 Variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 23.4 (mg/L), when using a mixed culture of *Pseudomonas putida*, *Pseudomonas various* and *Pseudomonas aeruginosa*

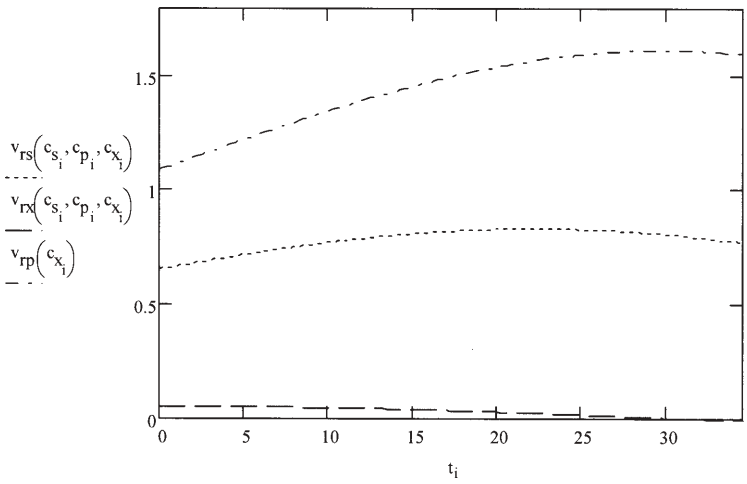

Fig. 4. Variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 23.4 (mg/L), when using a mixed culture of *Pseudomonas putida*, *Pseudomonas various* and *Pseudomonas aeruginosa*

concentration for the biodegradation of phenolic wastewaters with a phenols content of 23.4 mg/L when using a pure culture of *Pseudomonas putida*.

The figure 2 presents the variation in time of biomass growth rate (v_{rx}), the phenols biodegradation rate (v_{rs}) and the clean water rate (v_{rp}) at an initial phenols concentration of 23.4 (mg/L), when using a pure culture of *Pseudomonas putida*.

The figure 3 presents the variation in time of substrate concentration, biomass concentration and water concentration for the biodegradation of phenolic wastewaters with a phenols content of 23.4 mg/L when using a mixed culture of *Pseudomonas* putida, *Pseudomonas various* and *Pseudomonas aeruginosa*.

The figure 4 presents the variation in time of biomass growth rate (v_{rx}), the phenols biodegradation rate (v_{rs}) and the clean water rate (v_{rp}) at an initial phenols concentration of 23.4 (mg/L), when using a mixed culture of *Pseudomonas putida, Pseudomonas various* and *Pseudomonas aeruginosa*.

Conclusions

The biodegradation time resulted from the Monod-Jarzebski and Haldane models were closer to the industrial process time, when using a pure culture of bacteria, *Pseudomonas putida* for an initial phenols concentration up to 20 mg/L.

Both models, Monod-Jarzebski and Haldane, predict similar results for biodegradation time when using a mixed culture of bacteria, *Pseudomonas various*, but the biodegradation times are more than 4 times higher than the industrial process time.

Both models provided higher efficiencies when selecting a pure culture of *Pseudomoas putida* for phenols degradation instead of a mixed culture of *Pseudomonas various*.

The models supposed the cells were able to consume phenols completely. Microorganism growth kinetics was adjusted to the Monod-Jarzebski equation, which included inhibition terms.

Nomenclature

c_P - product (clean water) concentration (mg/L)
c_{P0} - initial product (clean water) concentration (mg/L)
c_S – phenol concentration (mg/L)
c_{S0} – initial phenol concentration (mg/L)
c_x – biomass concentration (mg/L)
c_{X0} - initial biomass concentration (mg/L)
D – duration of the process, biodegradation time (h)
k - growth rate constant (g^{-1})
K_i – inhibition coefficient for phenol (mg/L)
K_S – half – saturation coefficient for phenol (mg/L)
m - constant
m_S - maintenance coefficient (g/g·h)
n - constant
Pm – maximum productivity (kg/m^3)
t- time (h)
v_{rx} - biomass growth rate (g/g· h)
v_{rs} - phenols biodegradation rate (g/g· h)
v_{rp} - clean water rate (g/g·h)
v_m - maximum specific product grow rate (g/g·h)
Xm – maximum biomass concentration (kg/m)
Y_{XS} – biomass growth resulted from the substrate coefficient (g/g)
z – program iterations

Greek letters

μ - specific growth rate of biomass (h^{-1})
μ_m – maximum specific growth rate of biomass (h^{-1})

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