Aspects Regarding the Kinetics of Phenols Degradation by *Pseudomonas Putida*

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The treatment of phenol waters resulted from a petrochemical platform was studied. The purpose of this work was to determine if the Haldane model fit to the real process. The biodegradation time was determined by Haldane model in different conditions of initial phenols concentration and was compared with the experimental biodegradation time. The Haldane equation $\mu=\mu_m c_S/((K_S + c_S + (c_S^2/K_i))$ *with kinetics constants* $K_S=6.19\ mg\cdot L^{-1}$, $K_i=54.1\ mg\cdot L^{-1}$ *and* $\mu_m=0.436h^{-1}$ *provided good results compared to the real case.*

Keywords: biodegradation time, Haldane equation, Pseudomonas putida

Organic chemical mixtures are present in wastewaters from industrial sources. Phenolic compounds are common pollutants in many industrial wastewaters from oil refineries, chemical plants, explosives, resins and coke manufacture, coal conversion, pesticides and textile industries. Wastewaters containing phenol in a range of 5-500 mgL^{-1} are considered suitable for treatment by biological processes. The effluents containing such compounds are usually treated in activated sludge processes which are known to be sensitive to fluctuations in the phenolics load. The treatment of phenols waters due to the restricted limits imposed at the evacuation into the effluents, less than 7 ppm still represents an open problem. The bioremediation of these substances has become an alternative to the traditional physical and chemical methods that can be costly and produce hazardous products [1- 5].

To describe substrate biodegradation, it is necessary to evaluate the relationship between the specific growth rate μ and the phenol concentration c_S. Phenol biodegradation by microbes has generally been known to be inhibited by phenol itself. The Haldane equation has been frequently used to describe the phenols degradation in pure or mixed cultures [4 - 15]:

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

The aim of this study was to investigate the possibility of biodegradation of phenols by *Pseudomonas putida* like main bacteria, released from a petrochemical platform, at high initial concentrations under Haldane model, to simulate the real process varying the operating parameters. The study provided also a view of the microorganism growth kinetics using Haldane equation during biodegradation of phenols.

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 $mg\cdot L^{-1}$ and a final imposed phenols concentrations less than 7 ppm. During 3 years of 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 $m^3\cdot h^{-1}$.

Table 1
EXPERIMENTAL DATA FROM THE INDUSTRIAL PETROCHEMICAL PROCESS

Crt. No.	Phenols concentration		
	Initial phenols concentration ($mg\cdot l^{-1}$)	Intermediate phenols concentration ($mg\cdot l^{-1}$)	Final phenols concentration (mg/l)
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
22.	23.4	17.480	0.0270
23.	33.6	21.000	0.0520
24.	9.7	12.450	0.0190

The real petrochemical wastewaters biodegradation process used an active sludge recycling bioreactor type basin composed from 2 bioreactors working in series. The main bacteria was the *Pseudomonas putida*. The nutrient agents 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.

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Kinetics models

The present study considers that kinetics of phenols biodegradation process is described by Haldane equation (1).

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 are expressed by the equations below:

$$D(t,c) := \begin{pmatrix} -v_{rs}(c_0, c_1, c_2) \\ v_{rp}(c_2) \\ v_{rx}(c_0, c_1, c_2) \end{pmatrix} \quad (2)$$

$$c_{in} := \begin{pmatrix} 23.4 \\ 0 \\ 2 \end{pmatrix} \quad (3)$$

$$v_{rx}(c_S, c_P, c_x) = \mu(c_S, c_P, c_x) \cdot c_x ; \quad (4)$$

$$\mu(c_s, c_p, c_x) := \frac{\mu_m \cdot (c_s)}{Ks + c_s + \frac{c_s^2}{Ki}} \quad (5)$$

$$v_{rs}(c_S, c_P, c_x) = q(c_S, c_P, c_x) \cdot c_x ; \quad (6)$$

$$q(c_s, c_p, c_x) := \left(\frac{\mu(c_s, c_p, c_x)}{Y_{XS}} \right) + m_s \quad (7)$$

$$v_{rp}(c_x) = v(c_x) \cdot c_x ; \quad (8)$$

$$v(c_x) := v_m \cdot e^{-k \cdot c_x} \quad (9)$$

Results and discussion

The parameters values used in Haldane model were as follows:

- K_S=6.19 mg/L, K_i=54.1 mg/L and μ_m=0.436h^{-1} [5]
- the initial phenols concentration, c_{S0}=23.4; 18.5; 12.7; 8.5 and 1.7 mg·L^{-1}
- the final phenols concentration c_S=0 mg·L^{-1}
- the initial biomass concentration c_{X0}=2 mg·L^{-1}
- the initial product (clean water) concentration c_{P0}=0 mg·L^{-1}
- Y_{XS}=0.5 g· g^{-1}(biomass growth resulted from the substrate coefficient)
- v_m = 0.55 g· g^{-1}· h^{-1}(maximum specific product grow rate)
- m_S=0.27 g· g^{-1}· h^{-1}(maintenance coefficient)
- k = 0.006 l · g^{-1}(constant).

The values of the parameters K_S, K_i and μ_m are specific for pure culture of bacteria, for *Pseudomonas putida*.[5]

Table 2
THE MODELLING RESULTS OBTAINED USING HALDANE MODEL FOR CULTURES OF *PSEUDOMONAS PUTIDA*

z	t	c_S (mg·l^{-1})	c_P (mg·l^{-1})	c_x (mg·l^{-1})
0	0	23.400	0	2.000
1	0.4	22.739	0.458	2.217
2	0.8	22.005	0.964	2.458
3	1.2	21.190	1.525	2.726
4	1.6	20.284	2.145	3.023
5	2.0	19.278	2.833	3.354
6	2.4	18.161	3.594	3.722
7	2.8	16.921	4.436	4.130
8	3.2	15.549	5.369	4.581
9	3.6	14.033	6.400	5.079
10	4.0	12.367	7.538	5.623
11	4.4	10.552	8.793	6.211
12	4.8	8.602	10.172	6.834
13	5.2	6.557	11.678	7.47
14	5.6	4.502	13.310	8.078
15	6.0	2.578	15.054	8.589
16	6.4	0.974	16.884	8.917
17	6.8	-0.161	18.754	9.000
18	7.2	-0.830	20.617	8.851
19	7.6	-1.171	22.434	8.551

Using the Haldane model presented above results the variation in time of phenols concentration presented in table 2.

The results presented in table 2 represent program's iterations and the program ends when the concentration became negative. Further to all mentioned above, running the program based on Haldane model, results a biodegradation time of 6.5 h.

In the real case, the biodegradation time is 7.2 h for the both reactors working in series and for a constant flow rate of 800 $m^3 \cdot h^{-1}$. In the first bioreactor the waters are kept about 6h and in the second reactor the residence time is 1.2 h.

The biodegradation time obtained using Haldane model shows that normally we need less time for the biodegradation of the phenols and maybe only one bioreactor. Anyhow the difference between the model and the real case is not very big and the result obtained using Haldane model could be explained by the fact that during the simulation was considered that the biodegradation of phenols is realized with a pure culture of *Pseudomonas putida* while in the real industrial case the *Pseudomonas putida* is only the main bacteria. However the literature indicates results within the interval 10-15h for the biodegradation of phenols using pure culture of bacteria. [8-15]

Alvaro and all [5] obtained 6.05 h only for the duration of the lag phase. They 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 was *Pseudomonas putida* DSM 548 and the purpose of their 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 Alvaro and all for the input μ_m=0.436h^{-1}, K_S=6.19 mg·L^{-1}, Ki=54.1 mg·L^{-1}, Y= 0.0017 was 14 h for the same phenol concentration, S_0=23.4 mg·L^{-1}.

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^{-1} phenol and /or 545.5 mg·L^{-1} glucose.

Similar results regarding the biodegradation time obtained Pawlowski and Howell [12] that used for the biodegradation of phenol mixed culture of the bacteria as follows: *Pseudomonas various, Pseudomonas aeruginosa.*

The next step of the simulation was to vary the initial phenols concentration in order to see what biodegradation time results. Thus the concentration was varied within the interval 20 – less than 2 mg·L^{-1} and the process was simulated for a decreasing initial phenols concentration as follows: 18.5; 12.7; 8.5 and 1.7 mg·L^{-1}.

The biodegradation time decreases with the decreasing of initial phenols concentration.

Alvaro and all showed that the biodegradation time increases with phenol concentration at phenol concentrations between 5-100 mg·L^{-1}. High concentrations of phenol have an inhibitory effect on microbial growth and thus the biodegradation time increases [12 – 16].

Table 3 presents the results of Haldane model regarding the biodegradation time for the above mentioned concentrations selected from the experimental data.

Table 3
BIODEGRADATION TIME RESULTS BASED ON HALDANE MODEL FOR DIFFERENT INITIAL PHENOLS COMNCENTRATION

Crt. No.	c_{S0} (mg·l^{-1})	t (h)
1.	23.4	6.5
2.	18.5	6.2
3.	12.7	5.4
4.	8.5	4.7
5.	1.7	2.0

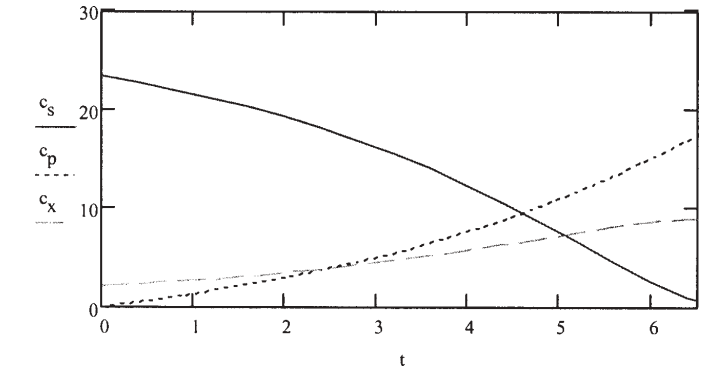

Fig.1. The variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 23.4 (mg·L^{-1})

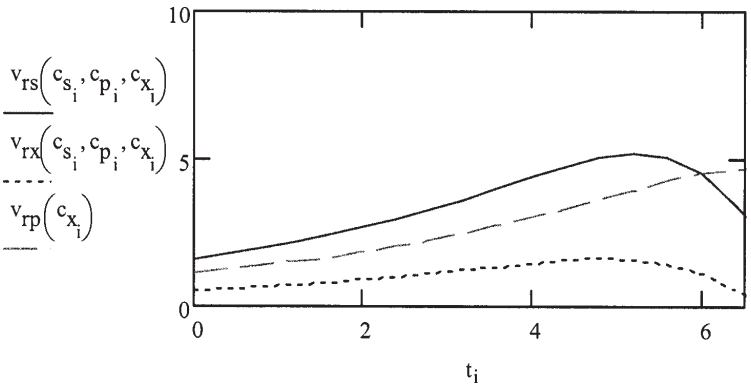

Fig. 2. The variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 23.4 (mg·L^{-1})

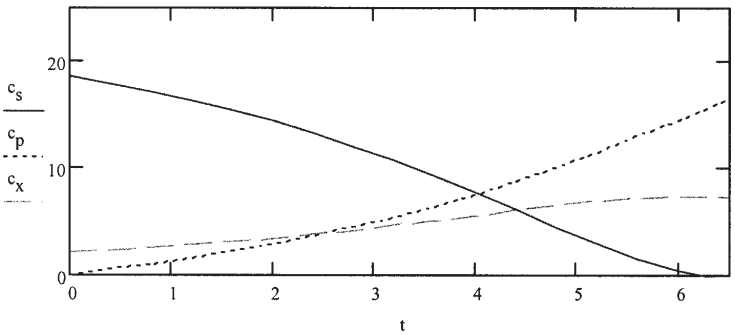

Fig. 3.The variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 18.5 (mg·L^{-1}).

In the industrial process, as we already said, the biodegradation time is 7.2 h, irrespective of initial phenols concentration. The wastewaters are kept into the basin 7.2 h, the time necessary for waters to traverse the basin at a constant flow rate of 800 m^3·h^{-1}. The solution adopted in the real case covers the high initial phenols concentrations. The disadvantage is when the initial phenols concentrations record low values and when the oxygen consumption is up than necessary.

The Haldane model provides also an evaluation of the kinetics of biodegradation process shown in figures below presented.

The figure 1 presents the variation in time of substrate concentration, biomass concentration and water concentration for the biodegradation of phenolic waters with a phenols content of 23.4 mg·L^{-1}.

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}) in the same conditions.

In the figures 3-10 are presented the variation of the same parameters as in the figures 1 and 2 at the following concentrations: 18.5; 12.7; 8.5 and 1.7 mg·L^{-1}.

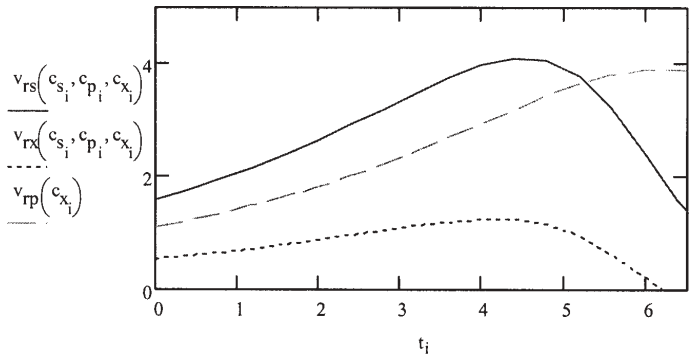

Fig. 4. The variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 18.5 (mg·L^{-1}).

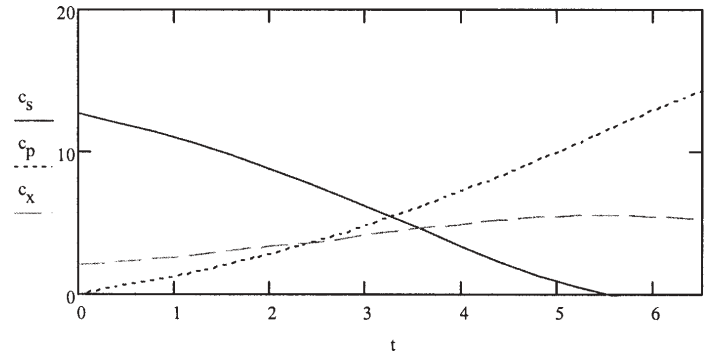

Fig. 5. The variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 12.7 (mg·L^{-1}).

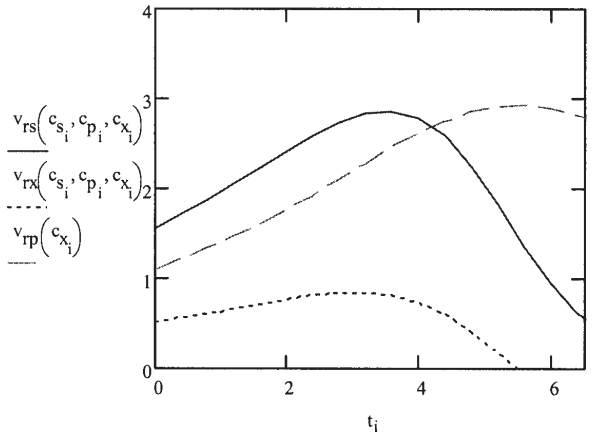

Fig. 6. The variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 12.7 (mg·L^{-1}).

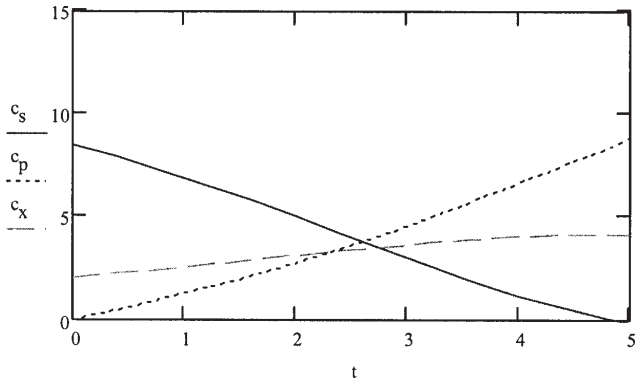

Fig. 7. The variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 8.5 (mg·L^{-1}).

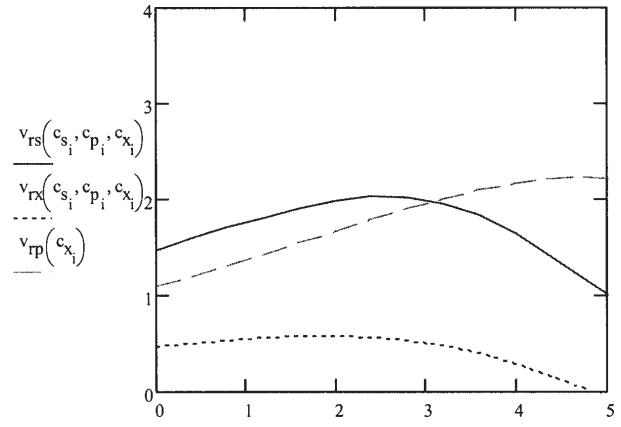

Fig. 8. The variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 8.5 (mg·L^{-1}).

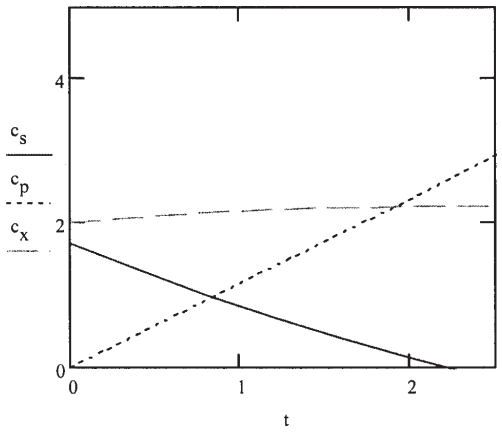

Fig. 9. The variation in time of substrate (phenols) concentration, product (clean water) concentration and cells (biomass) concentration at an initial phenols concentration of 1.7 ($mg \cdot L^{-1}$).

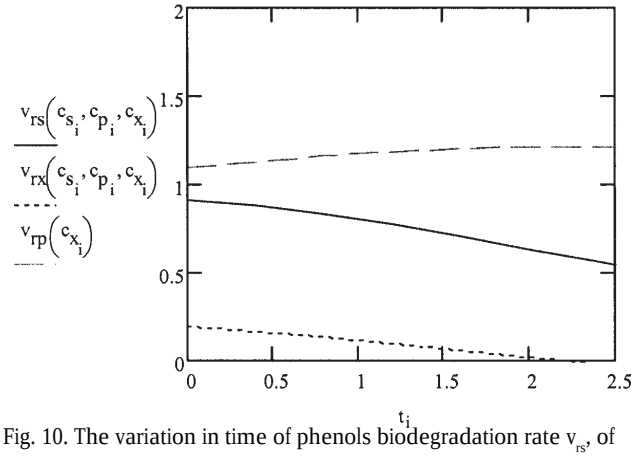

Fig. 10. The variation in time of phenols biodegradation rate v_{rs}, of clean water rate v_{rp} and biomass growth rate v_{rx} at an initial phenols concentration of 1.7 ($mg \cdot L^{-1}$)

Conclusions

The biodegradation time resulted from the Haldane model was in accordance with the industrial process time, justifying geometrical dimensions of industrial reactor for high phenols concentrations. For lower phenols concentrations only one bioreactor could be enough for the biodegradation of wastewaters.

The Haldane model permits the process simulation of the bioreactor in other operating conditions.

The Haldane model provides data related to the process kinetics that in the real case was difficult to measure.

The model supposed the cells were able to consume phenol completely. Microorganism growth kinetics was adjusted to the Haldane equation, which included inhibition terms. The model provided also an excellent prediction of the microorganism growth kinetics.

The real industrial process was simulated in the same operating conditions and using the same kinetics parameters, specific for pure culture of *Pseudomonas putida*, under the Monod model based on the equation Monod-Jarzebski. Similar results were obtained and will be presented in a future work.

Nomenclature

c_p - product (clean water) concentration ($mg \cdot L^{-1}$)
c_{P0} - initial product (clean water) concentration ($mg \cdot L^{-1}$)
c_S – phenol concentration ($mg \cdot L^{-1}$)
c_{S0} – initial phenol concentration ($mg \cdot L^{-1}$)
c_x – biomass concentration ($mg \cdot L^{-1}$)
c_{X0} - initial biomass concentration ($mg \cdot L^{-1}$)
D – duration of the process, biodegradation time (h)
k - growth rate constant (g^{-1})
K_i – inhibition coefficient for phenol ($mg \cdot L^{-1}$)
K_S – half – saturation coefficient for phenol ($mg \cdot L^{-1}$)
m_S - maintenance coefficient ($g \cdot g^{-1} \cdot h^{-1}$)
v_{rx} - biomass growth rate ($g \cdot g^{-1} \cdot h^{-1}$)
v_{rs} - phenols biodegradation rate ($g \cdot g^{-1} \cdot h^{-1}$)$v_{rp}$ - clean water rate ($g \cdot g^{-1} \cdot h^{-1}$)
v_m - maximum specific product grow rate ($g \cdot g^{-1} \cdot h^{-1}$)
Y_{XS} – biomass growth resulted from the substrate coefficient ($g \cdot g^{-1}$)
z – iterations of the program
Greek letters
μ - specific growth rate of biomass (h^{-1})
μ_m – maximum specific growth rate of biomass (h^{-1})

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