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Biodegradation dynamics and cell maintenance for the treatment of resorcinol and p-cresol by filamentous fungus *Gliomastix indicus*

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ABSTRACT

Biodegradation of resorcinol and p-cresol using fungus *Gliomastix indicus* MTCC 3869 was investigated in batch culture experiments at 28 °C temperature and pH of 6 in the medium up to the initial concentration of 1300 mg/L and 700 mg/L for resorcinol and p-cresol, respectively. Five specific growth kinetic models and five specific degradation rate models were fitted to the experimental data in order to get best fitted kinetic models. The variation of observed growth yield and maintenance energy requirement with the initial substrate concentration was also studied. The model for maintenance energy coefficient was fitted to the experimental data. The model parameters were: $m_1 = 0.0135\,h^{-1}$, $k = 0.054$, $\mu_{max} = 0.132\,h^{-1}$ for resorcinol and $m_1 = 0.0229\,h^{-1}$, $k = 0.011$, $\mu_{max} = 0.102\,h^{-1}$ for p-cresol. Two mathematical models comprising of two sets of ODE were solved simultaneously to get degradation profiles with time. The model with varying growth yield and maintenance energy was found to be most appropriate biodegradation model.

1. Introduction

Resorcinol and p-cresol are the pollutants in wastewater which remain present in the effluents of industries such as pulp and paper mills, textile mills, coal gasification units, herbicides, fungicides and synthetic coal fuel conversion processes. These pollutants result in the hazardous effects on environment and on human being as well as other living organisms [1–4]. Due to toxic properties, the removal of resorcinol and p-cresol by biodegradation technique is of great importance as it has potential to mineralize the toxic compound completely at relatively low cost.

In majority of biodegradation studies authors have frequently reported biomass growth kinetics and biodegradation kinetics without taking into account the changes in biomass growth yield and maintenance energy requirement of the culture [5–7]. But the cells under biodegradation studies require a minimum, constant and continuous amount of maintenance energy for their survival at each growth phase. This amount of maintenance energy is consumed by the cells for activities such as maintenance of membrane potential, internal pH maintenance, repair and turnover of cellular components and cell motility [8]. Further, when substrate inhibition takes place in the medium, the degree of toxicity of substrate including various intermediates and the extracellular products, decreases the biomass growth yield and leads to the higher maintenance energy requirement to overcome the substrate inhibition effect [9,10]. This finding suggests that the biomass growth yield and the substrate degradation are not dependent on each other. Substrate degradation takes place even though the biomass growth yield is low, because the consumed substrate is utilized for high maintenance of microbial cells at higher initial concentrations of toxic substrates like resorcinol, p-cresol that cause the inhibition to biomass growth and to its own biodegradation. Therefore, the quantification of maintenance energy expenditure is needed to provide proper description of biodegradation dynamics. The knowledge of biodegradation dynamics is important to design the biodegradation unit and to predict the component concentration in the wastewater. To the best of our knowledge the biodegradation dynamics with incorporation of variable maintenance energy and growth yield have not yet been investigated in the studies available in literature. There is a lot of information available about bacterial biodegradation of phenol derivatives, but only a few studies are available on its biodegradation using fungi [11–19].

In our previous study [20] on biodegradation of p-cresol by fungal strain *Gliomastix indicus,* the specific growth rate was investigated at constant biomass yield and maintenance energy coefficient. Therefore, in the present study we focus on the biodegradation dynamics of p-cresol and resorcinol by fungal strain *G. indicus*, incorporating the maintenance energy and observed growth yield variation. A quantitative discussion on variable maintenance energy coefficient and observed biomass growth yield has been presented. In particular, the effect of initial substrate

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Nomenclature

X	biomass concentration (mg/L)
X_0	initial biomass concentration (mg/L)
K_S	saturation constant or half velocity constant (mg/L)
K_i	inhibition constant (mg/L)
t	time (h)
S	substrate concentration (mg/L)
S_0	initial substrate concentration (mg/L)
$(Y_{X/S})_O$	observed growth yield coefficient (g/g)
$(Y_{X/S})_T$	true growth yield coefficient (g/g)
m_s	maintenance energy coefficient (h^{-1})
m_1	constant component of maintenance energy coefficient (h^{-1}).
m_2	growth dependant component of maintenance energy coefficient (h^{-1}).
q_S	specific degradation rate constant (h^{-1})
$q_{S_{max}}$	maximum specific degradation rate constant (h^{-1})
Greek letters	
μ_g	specific growth rate coefficient (h^{-1})
μ_{max}	maximum specific growth rate coefficient (h^{-1})

concentration on maintenance energy expenditure has been modeled for a wide range of p-cresol and resorcinol concentrations. Besides, the substrate degradation profiles have been modeled and presented for resorcinol and p-cresol.

2. Kinetic modeling

Biomass growth rate in exponential growth phase is expressed as:

$$\frac{dX}{dt} = \mu_g X \tag{1}$$

The mass balance on biomass in terms of biomass growth yield

$$\frac{dX}{dt} = (Y_{X/S})_T \left(-\frac{dS}{dt}\right) \tag{2}$$

Eq. (2) on integration with boundary condition $X = X_0$ at $S = S_0$ gives

$$X - X_0 = (Y_{X/S})_T (S_0 - S) \tag{3}$$

Rate of substrate consumption ($-dS/dt$), analogous to biomass growth rate (Eq. (1)) as a function of biomass concentration is:

$$-\frac{dS}{dt} = q_S X \tag{4}$$

The rate of substrate consumption as a function of biomass concentration using true growth yield and maintenance coefficient is expressed as

$$-\frac{dS}{dt} = \frac{dX}{dt}\frac{1}{(Y_{X/S})_T} + m_s X \tag{5}$$

m_s gives the rate of substrate consumption for cell maintenance and $(Y_{X/S})_T$ is also maximum growth yield. For the survival of cells and to overcome the growth inhibition effect of substrate, a significantly higher amount of maintenance energy is required in comparison to other cultures where energy providing substrate is non-toxic substance like glucose, fructose, molasses, etc. [8]. Therefore, during the biodegradation kinetic studies of a microorganism, estimation of maintenance energy is a crucial step.

During the exponential growth phase, the energy is required for the cell growth and multiplication in addition to that minimum constant amount of maintenance energy. This amount of energy decreases with the increase in the specific growth rate and becomes zero at maximum specific growth rate value. As the substrate concentration is increased in the medium, this portion of maintenance energy keeps on decreasing along with the increasing specific growth rate, till the specific growth rate achieves its maximum value. In view of this, there are two components of maintenance energy, one is constant and is required during the whole cultivation period, starting from the lag phase to the death phase, and the other component is growth dependent [21–23]. Thus,

$$m_s = m_1 + m_2$$

where m_1 is constant component of energy and m_2 is growth dependent component of energy.

Pirt [24] gave the following equation for the growth dependent component of maintenance coefficient

$$m_2 = k\left(1 - \frac{\mu_g}{\mu_{max}}\right) \tag{6}$$

where k is a positive quantity that depends on the substrate–microorganism system. For the same concept, Neijssel and Tempest [23] suggested that the growth dependent component of maintenance energy is proportional to the specific growth rate μ_g

$$m_2 = k\mu_g$$

where k is constant and greater than zero.

The experimental observations show that the dependence of maintenance energy expenditure for the growth only on the specific growth rate is not possible. Maintenance energy expenditure into the cells, is affected by the temperature and the salt concentrations in the medium [21,24–26]. But Tsai and Lee [27] reported that the environmental conditions do not affect the maintenance energy requirement of the cells. However, the experimental observations prove that the maintenance energy expenditure varies from one to the other substrate–microorganism system along with the culture conditions such as temperature, pH, salt concentrations and substrate toxicity in the nutrient medium. Minkevich et al. [9] reported that in the medium, there are limited mineral and substrate concentration. Therefore, the experimental data deviate from the straight line models [24]. This observation indicates that above Eq. (6) given by Pirt is not always applicable. In the present study, there was no condition of minerals and the substrate concentration limitation into the medium and all the experimental data were taken during the exponential growth phase only. Therefore, Pirt's Eq. (6) has been used for further study.

The relation between specific degradation rate q_S and specific growth rate μ_g that describes the minimum substrate consumption for the cell maintenance is as follows [21,28,29]:

$$q_S = m_1 + \frac{\mu_g}{(Y_{X/S})_T} \tag{7}$$

On keeping $q_S = (\mu_g/(Y_{X/S})_O)$ in the above equation one gets

$$\frac{1}{(Y_{X/S})_O} = \frac{1}{(Y_{X/S})_T} + \frac{m_1}{\mu_g} \tag{8}$$

The estimation of maintenance energy consumption by Eq. (8) does not include the maintenance energy expenditure at the time of cell growth, cell multiplication and endogenous metabolism at stationary phase, while the specific growth rate is assumed to be the net relative growth rate. On incorporating the growth dependent component of maintenance energy in Eq. (6), one gets

$$q_S = \frac{\mu_g}{(Y_{X/S})_T} + m_1 + k\left(1 - \frac{\mu_g}{\mu_{max}}\right) \tag{9}$$

Since $(Y_{X/S})_T$, k, μ_m and m_1 are constants, Eq. (9) can be reduced to

$$q_S = A\mu_g + B \quad (10)$$

where $A = ((1/(Y_{X/S})_T) - (k/\mu_{max}))$ and $B = (k + m_1)$

Further, Pirt [30] reported that the Eq. (9) is not applicable during the condition of very low specific growth rates, caused by the formation of dormant cells in the medium. In the current study, since there is no dormant cell formation observed during the experimentation, Eq. (9) is used for the estimation of the maintenance energy expenditure during biodegradation.

3. Materials and methods

3.1. Microorganism

The microorganism *G. indicus* MTCC 3869 was procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. This fungal strain was maintained on the potato dextrose agar (PDA) medium containing: potatoes (200 g/L), dextrose (20 g/L) and agar (15 g/L), at pH 6 using 1 N NaOH by serial transfer on the medium at temperature 28 °C after every 2 weeks.

3.2. Acclimatization and inoculum development

Modified czapeck medium was used for the biodegradation experiments. The medium composition was K_2HPO_4 (1 g/L), $FeSO_4{\cdot}7H_2O$ (0.01 g/L), and NH_4NO_3 (3 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), KCl (0.5 g/L). The medium was also supplemented with the resorcinol and p-cresol at pH 6 and temperature 28 °C. Cultures were acclimatized by exposing them to the substrate in a series of conical flasks (250 mL). Initially glucose (2%) was added to the medium for the purpose of acclimatization, thereafter as the fungus started to consume the substrate, the amount of glucose was decreased along with the gradual increase in the substrate concentration. Process of acclimatization took place over 2 months. In this way, the inoculum was developed for all batch culture experiments. The observed lag phase period was 15 h for resorcinol and 24 h for p-cresol.

3.3. Chemicals

All the chemicals used in the experimentation including resorcinol and p-cresol were of AR grade with more than 99% purity. These chemicals were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, Loba Chemie Pvt. Ltd. Mumbai, Ranbaxy Fine Chemicals Ltd. New Delhi, Reidel Chemicals, Hapur.

3.4. Determination of biomass and substrate concentrations in the sample

For the determination of biomass growth in the sample, dry weight of biomass was measured, subjecting the samples to centrifugation at 8000 × *g* for 15 min at 25 °C. Biomass was found in the form of pellet on the side wall of centrifuge tube. The supernatant was separated out for the analysis of substrate concentration. The biomass pellet was taken out on the filter paper and kept in the oven at 75 °C for 24 h for drying. The dried biomass was weighed to find out the biomass concentration in the sample. Analysis of residual substrate concentration was done by high performance liquid chromatography (HPLC) (Waters system, USA), with a Symmetry® C18, 5 μm (250 mm × 4.6 mm, Waters, Ireland) column. A mixture of methanol/water (400/300, v/v) was used as mobile phase, at a flow rate of 1.0 mL min^{-1}. Detection was done at 274 nm for resorcinol and at 277 nm wavelength for p-cresol by a Photodiode array detector (Waters 2998). Retention times for resorcinol and p-cresol were 2.53 and 4.54 min, respectively.

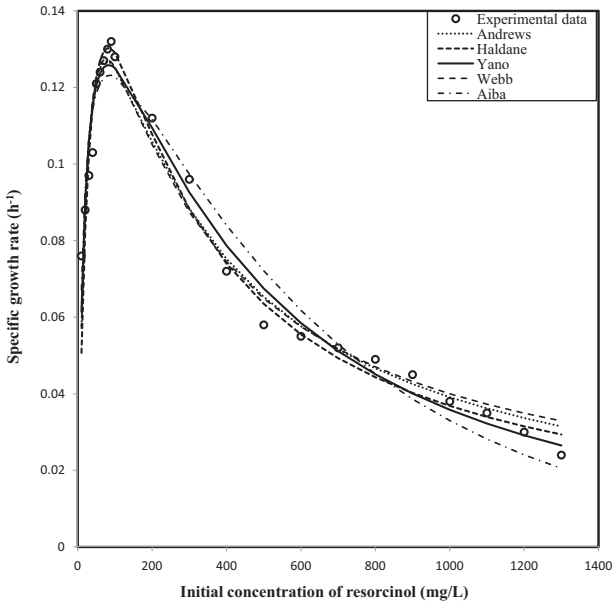

Fig. 1. Comparison between growth kinetic model predictions and experimentally determined specific growth rates at different initial concentrations of resorcinol.

5% (v/v) Inoculum was used for the whole experimentation and the inoculation step was done in aseptic conditions of UV chamber. The experimental flasks were kept in the BOD incubator-cum-orbital shaker at 28 °C and 150 rpm. Each batch experimental run was repeated two times under identical conditions and the values were averaged to get true experimental value.

4. Results and discussion

4.1. Growth kinetics

Fungal strain *G. indicus* was cultured in the medium containing either resorcinol or p-cresol as the sole energy and carbon source. Experimentally, the biodegradation time for resorcinol at initial concentration of 700 mg/L and 1300 mg/L was observed as 69 h and 183 h, respectively. While complete biodegradation of p-cresol at initial concentration of 700 mg/L was achieved in 122 h, which is much higher than that for resorcinol at initial concentration of 700 mg/L. The dependency of specific growth rate on the initial substrate concentration is shown in Figs. 1 and 2 for resorcinol and p-cresol, respectively. It can be seen that the specific growth rate μ_g increases with the initial substrate concentration; reaches to its maximum value and finally decreases. The maximum specific growth rate is achieved at the initial concentration of about 90 mg/L and 50 mg/L for resorcinol and p-cresol, respectively. The decline trend of μ_g beyond the inhibitory concentrations of 90 mg/L and 50 mg/L indicates that both resorcinol and p-cresol are inhibitory type substrates. In order to assess the specific growth rate of *G. indicus* for resorcinol and p-cresol, five single substrate inhibition growth kinetic models are selected from the literature as listed in Table 1 [20]. In this study, parameters of different growth models are estimated iteratively by non-linear least square technique using MATLAB 7.0 based on Windows XP. This software utilizes the curve fitting tool box for minimizing the sum of square of residuals. Estimated specific growth rate values by the selected models for entire experimental data range have been plotted against initial substrate concentrations in Figs. 1 and 2. The goodness of the fit of the experimental data to the proposed kinetic models is generally measured in terms of the correlation coefficient R^2 and the percent standard deviation ($\Delta\mu_g$%) between experimental and predicted

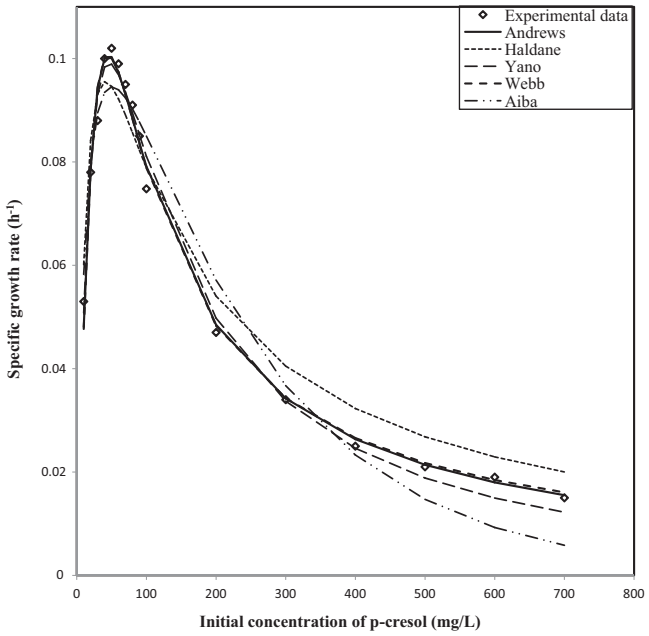

Fig. 2. Comparison between growth kinetic model predictions and experimentally determined specific growth rates at different initial concentrations of p-cresol.

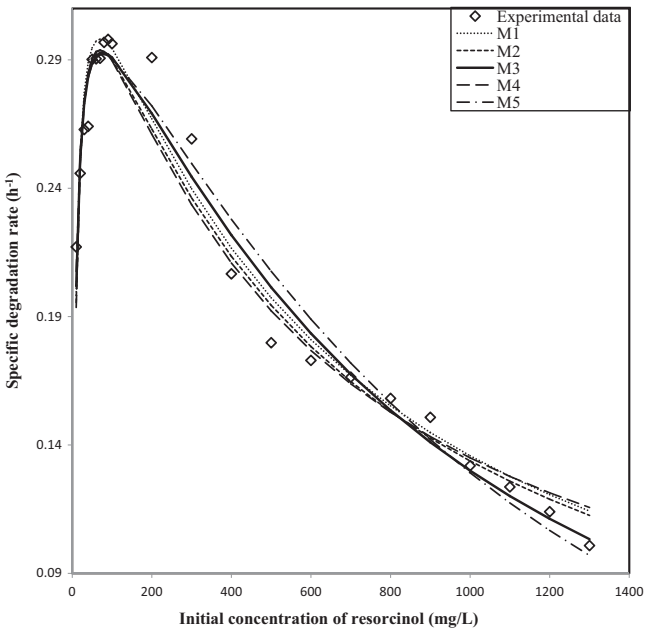

Fig. 3. Comparison between kinetic model predictions and experimentally determined specific degradation rates at different initial concentrations of resorcinol.

values of μ_g for each model. Following equation has been used for the calculation of percent standard deviation:

$$\Delta\mu_g\% \frac{100\sqrt{\sum[(\mu_{g_{exp}} - \mu_{g_{pred}})/\mu_{g_{pred}}]^2}}{N} \quad (11)$$

$\mu_{g_{exp}}$ is the experimental specific growth rate and $\mu_{g_{pred}}$ is the corresponding predicted specific growth rate according to the model under study. N is the number of measurements. It is clear that lower the value of percent standard deviation, the better is the fit of experimental data. The estimated values of kinetic parameters involved in different models along with R^2 and percent standard deviation are mentioned in Tables 2 and 3 for resorcinol and for p-cresol, respectively. For the resorcinol, Haldane, Andrews and Noack models describe the growth kinetics with equal goodness while the predictions of Webb and Aiba models differ slightly. In the case of p-cresol, the predictions by Haldane, Webb, Yano and Aiba models differ widely from the experimental data. Hence, on the basis of correlation coefficient and the percent standard deviation values in the present biodegradation study, Yano model for the resorcinol, and Andrews and Noack model for the p-cresol have been selected for further discussion of results. The exact comparison of kinetic parameters and thereby degradation efficiency with the literature results are difficult due to the different cell density, medium components, and other environmental factors [31,32].

Table 1
Single substrate growth and degradation kinetic models [20].

S. no.	Growth kinetic model	Mathematical equation
1	Andrews and Noack	$\mu_g = \frac{\mu_{max} S}{S+K_S+(S^2/K_i)}$
2	Haldane	$\mu_g = \frac{\mu_{max} S}{S+K_S+(S^2/K_i)+(S\cdot K_S/K_i)}$
3	Yano/Edward	$\mu_g = \frac{\mu_{max} S}{S+K_S+(S^2/K_i)(1+(S/K))}$
4	Webb	$\mu_g = \frac{\mu_{max} S(1+(S/K))}{S+K_S+(S^2/K_i)}$
5	Aiba	$\mu_g = \frac{\mu_{max} S \exp^{(-S/K_i)}}{S+K_S}$

	Degradation kinetic model	Mathematical equation	Analogous growth kinetic model
1	M1	$q_S = \frac{q_{S_{max}} S}{S+K'_S+(S^2/K'_i)}$	Andrews and Noack
2	M2	$q_S = \frac{q_{S_{max}} S}{S+K'_S+(S^2/K'_i)+(S.K'_S/K'_i)}$	Haldane
3	M3	$q_S = \frac{q_{S_{max}} S}{S+K'_S+(S^2/K'_i)(1+(S/K))}$	Yano/Edward
4	M4	$q_S = \frac{q_{S_{max}} S(1+(S/K'))}{S+K'_S+(S^2/K')}$	Webb
5	M5	$q_S = \frac{q_{S_{max}} S \exp^{(-S/K_i)}}{S+K'_S}$	Aiba

4.2. Biodegradation kinetics

To study the substrate biodegradation kinetics, specific degradation rate q_S was calculated using specific growth rate and observed growth yield $(Y_{X/S})_O$ data at their respective initial substrate concentrations. Figs. 3 and 4 represent the variation of

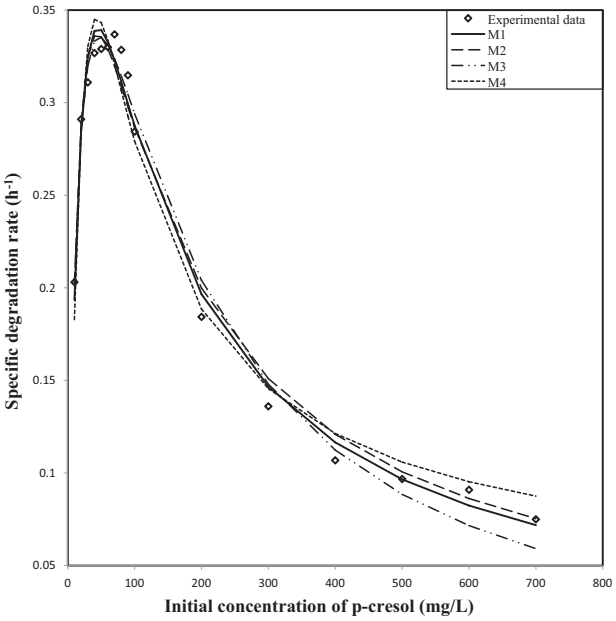

Fig. 4. Comparison between kinetic model predictions and experimentally determined specific degradation rates at different initial concentrations of p-cresol.

Table 2
Estimated values of biomass growth kinetic model parameters for resorcinol degradation.

S. no.	Model	Estimated value of resorcinol growth kinetic parameters using experimental data				R^2	Percent standard deviation ($\Delta\mu_g$%)
		μ_{max} (h^{-1})	K_S (mg/L)	K_i (mg/L)	K (mg/L)		
1	Haldane	0.640	100	67.54	–	0.963	2.17
2	Yano	0.185	19.83	376	1790	0.974	1.73
3	Andrews and Noack	0.147	12.17	9920	–	0.917	2.19
4	Webb	0.223	28.56	195.8	9944	0.966	2.47
5	Aiba	0.164	14.08	627.9	–	0.963	2.46

specific degradation rate with initial concentrations of resorcinol and p-cresol, respectively. For the resorcinol, the maximum value of specific degradation rate has been achieved at the initial concentration of 90 mg/L at which specific growth rate was found to be maximum. In the case of p-cresol, maximum value of specific degradation rate is found at the concentration of 70 mg/L while the maximum specific growth rate value was found at the initial concentration of 50 mg/L. Similar trend has also been reported by Minkevich et al. [9] in their biodegradation studies on ethanol – *Candida valida* system. They emphasized that the specific degradation rate q_S continued to increase with the substrate concentration due to the increase of cell maintenance rate whereas the observed growth yield coefficient continued to decrease which in turn decreased the specific growth rate.

In order to model specific biodegradation rate, five models (M1, M2, M3, M4 and M5) analogous to single substrate growth kinetic models, have been proposed and are listed in Table 1. The parameters of these models are estimated using a nonlinear least square regression analysis of experimental data, on MATLAB 7 based on Windows XP. The values of kinetic constants for specific degradation rate of resorcinol and p-cresol are presented in Tables 4 and 5, respectively. Predictions of models M1, M2, M3, M5 show good agreement with the experimental specific degradation rate of resorcinol with correlation coefficient R^2 value >0.97. In case of p-cresol, model M1, M2 and M3 are found to fit the experimental specific degradation rate data with the correlation coefficient (R^2) value >0.98. Therefore, for the judgment of the best fit model to the experimental specific degradation rate data, percent standard deviation (Δq_S%) values are estimated by applying the following equation:

$$\Delta q_S\% = \frac{100\sqrt{\sum[(q_{S_{exp}} - q_{S_{pred}})/q_{S_{pred}}]^2}}{N} \quad (12)$$

In Eq. (12), $q_{S_{exp}}$ is the experimental specific degradation rate and $q_{S_{pred}}$ is the corresponding predicted specific degradation rate. N is the number of measurements. The percent standard deviation value is minimum for models M3 and M1 for specific degradation rate data of resorcinol and p-cresol, respectively. Therefore, the model M3 for resorcinol and the M1 for p-cresol are selected for further biodegradation kinetic studies. Model M3 is a four-parameter model. The specific degradation rates for resorcinol and p-cresol can now be restated by Eqs. (13) and (14) respectively as given below:

$$q_S = \frac{0.347S}{S + 7.07 + (S^2/853.5)(1 + (S/2386))} \quad \text{(for resorcinol)} \quad (13)$$

$$q_S = \frac{0.759S}{27.88 + S + (S^2/73.42)} \quad \text{(for p-cresol)} \quad (14)$$

K'_S is saturation constant that indicates the substrate affinity to biomass while K'_i and K are constants indicating the degree of substrate inhibition. During experimental study, resorcinol and p-cresol have been observed as inhibitory substrates. Therefore, it is not possible to observe an actual degradation rate. The value of initial substrate concentration (S_0^*) at which degradation rate (q_S) attains its maximum value $q^*_{S_{max}}$ can be obtained by differentiating Eqs. (13) and (14) with respect to S and equating them to zero. The value of S_0^* along with the values of parameters $q_{S_{max}}$, K'_S, K'_i, and K, is substituted in Eqs. (13) and (14) to get corresponding values of $q^*_{S_{max}}$. Thus, the values of S_0^* and $q^*_{S_{max}}$ are determined by the following equations:

$$S_0^* = \sqrt{K'_S K'_i} \quad (15)$$

$$q^*_{S_{max}} = \frac{q_{S_{max}}}{2(\sqrt{(K'_S/K'_i)}) + 1} \quad (16)$$

Values of $q^*_{S_{max}}$ and S_0^* are computed as 0.294 h^{-1} and 78 mg/L for resorcinol, and as 0.339 h^{-1} and 45.24 mg/L for p-cresol. Eq. (16) reflects that at larger K'_S/K'_i value, the smaller $q^*_{S_{max}}$ value will be achieved relative to $q_{S_{max}}$, and thus greater will be the degree of inhibition.

Similar calculations are performed on the best fitted specific growth kinetic models, Yano and Andrews and Noack models (Section 4.1), for resorcinol and p-cresol, respectively to get S_0^* and μ^*_{max} values. These equations are as follows:

$$\mu_g = \frac{0.185S}{S + 19.83 + (S^2/376)(1 + (S/1790))} \quad \text{(for resorcinol)} \quad (17)$$

$$\mu_g = \frac{0.512S}{S + 91.87 + (S^2/21.99)} \quad \text{(for p-cresol)} \quad (18)$$

Finally computed values of S_0^* and μ^*_{max} are 86.35 mg/L and 0.127 h^{-1} for resorcinol, and 44.94 mg/L and 0.101 h^{-1} for p-cresol, respectively. Here also, degree of inhibition is determined by K_S/K_i ratio as μ^*_{max} becomes closer to μ_{max} at lower value of K_S/K_i.

Table 3
Estimated values of biomass growth kinetic model parameters for p-cresol degradation.

S. no.	Model	Estimated value of p-cresol growth kinetic parameters using experimental data				R^2	Percent standard deviation ($\Delta\mu_g$%)
		μ_{max} (h^{-1})	K_S (mg/L)	K_i (mg/L)	K (mg/L)		
1	Haldane	0.382	41.04	41.15	–	0.966	4.07
2	Yano	0.279	43.12	54.63	997.8	0.992	2.05
3	Andrews and Noack	0.512	91.87	21.99	–	0.993	1.03
4	Webb	0.577	105.8	18.75	9914	0.993	1.13
5	Aiba	0.157	15.7	214	–	0.962	5.68

Table 4
Estimated values of kinetic parameters for resorcinol degradation.

S. no.	Model	Estimated values of resorcinol degradation kinetic parameters using experimental data				R^2	Percent standard deviation (Δq_S%)
		$q_{S_{max}}$ (h^{-1})	K'_S (mg/L)	K'_i (mg/L)	K (mg/L)		
1	M1	0.366	8.48	582.6	–	0.97	1.23
2	M2	0.37	8.6	573.9	–	0.97	1.15
3	M3	0.347	7.07	853.5	2386	0.976	1.04
4	M4	0.372	8.93	498.5	1000	0.967	1.27
5	M5	0.338	6.22	1040		0.972	1.22

Table 5
Estimated values of kinetic parameters for p-cresol degradation.

S. no.	Model	Estimated values of p-cresol degradation kinetic parameters using experimental data				R^2	Percent standard deviation (Δq_S%)
		$q_{S_{max}}$ (h^{-1})	K'_S (mg/L)	K'_i (mg/L)	K (mg/L)		
1	M1	0.759	27.88	73.42	–	0.989	1.30
2	M2	1.344	44.14	44.36	–	0.987	1.43
3	M3	0.594	18.64	132.2	1000	0.987	2.22
4	M4	1.168	51.43	32.38	1000	0.979	1.91

4.3. Maintenance energy and growth yield coefficient

As the initial substrate concentration is enhanced in the medium gradually, beyond the inhibitory initial concentration of substrate, the observed growth yield value tends to decrease while there is a simultaneous increase in the required maintenance energy value at each initial concentration of substrate, along with the increasing inhibition effect. The maximum specific growth rate value was found at initial resorcinol concentration of 90 mg/L and initial p-cresol concentration of 50 mg/L. Following equation is used to estimate the maintenance energy expenditure in the cells (Section 2) at each initial concentration of substrate.

$$m_s = m_1 + k\left(1 - \frac{\mu_g}{\mu_{max}}\right) \quad (19)$$

The estimated values of observed growth yield coefficient $(Y_{X/S})_O$ and maintenance energy coefficient (m_s) are shown in Figs. 5 and 6 for resorcinol and p-cresol, respectively. Maintenance energy coefficients are found in the range of 0.0135–0.0572 h^{-1} for resorcinol and 0.0229–0.0324 h^{-1} for p-cresol. In Fig. 5 the value of maintenance energy coefficient m_s is 0.0135 h^{-1} at initial resorcinol concentration of 90 mg/L that is minimum required maintenance energy and 0.0573 h^{-1} at initial concentration of 1300 mg/L which is the maximum value of maintenance energy. Similarly it is clear from Fig. 6 that for the p-cresol the minimum value of maintenance energy coefficient is 0.0229 h^{-1} at inhibitory initial concentration of 50 mg/L, while the value of maximum maintenance energy coefficient, 0.0324 h^{-1} is at initial p-cresol concentration of 700 mg/L. Thus, after estimating the values of k, the maintenance energy model equation (Eq. (16)) can be restated as follows:

$$m_s = 0.0135 + 0.054\left(1 - \frac{\mu_g}{0.132}\right) \quad (\text{for resorcinol}) \quad (20)$$

$$m_s = 0.0229 + 0.011\left(1 - \frac{\mu_g}{0.102}\right) \quad (\text{for p-cresol}) \quad (21)$$

Eqs. (20) and (21) represent maintenance energy requirement for cells during resorcinol and p-cresol biodegradation, respectively.

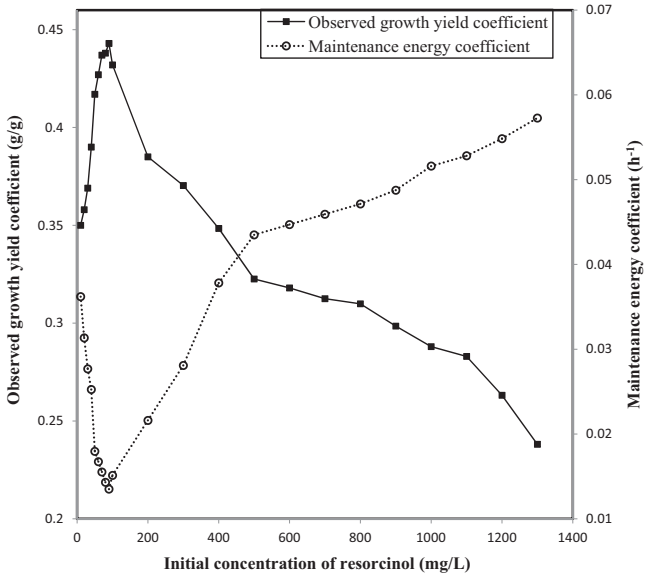

Fig. 5. Effect of initial concentration of resorcinol on biomass growth yield and maintenance energy coefficient.

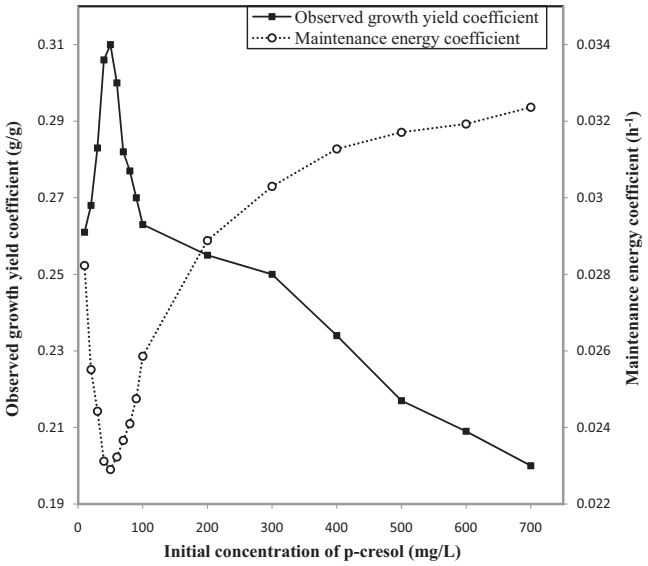

Fig. 6. Effect of initial concentration of p-cresol on biomass growth yield and maintenance energy coefficient.

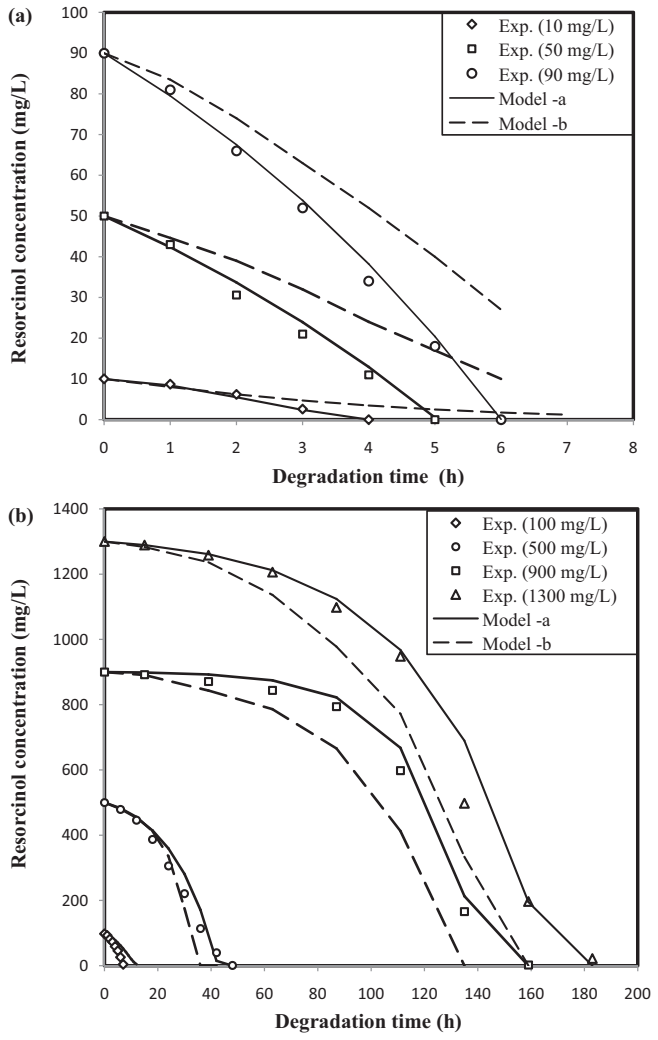

Fig. 7. Resorcinol degradation profiles at different initial concentrations with model predictions in (a) lower concentration range, and (b) higher concentration range.

At each initial substrate concentration, the observed growth yield coefficient is determined by liniarizing biomass growth with substrate degradation. Figs. 5 and 6 show observed growth yield coefficient profiles as a function of initial substrate concentration. It indicates that observed growth yield is not constant but varies with the initial substrate concentration. Maximum value of observed yield coefficient is 0.443 g/g at initial resorcinol concentration of 90 mg/L and then it decreases with the increase in initial concentration up to 1300 mg/L. In case of p-cresol, observed yield coefficient value 0.31 g/g is maximum at initial concentration of 50 mg/L but beyond this initial concentration, the observed yield coefficient value starts decreasing as the value of maintenance coefficient increases and thereby the substrate utilization increases with the increase in initial p-cresol concentration. It is noticeable that the similar trend has been observed in the case of specific growth rate of both the substrates. The decreasing trends of specific growth rate and increasing maintenance energy coefficient (m_s) beyond the inhibitory initial substrate concentration results in the reduction in the observed growth yield. This study concludes that the substrate inhibition reduces the specific growth rate as well as biomass growth yield due to the increase in the value of maintenance energy coefficient for resorcinol (or p-cresol) – *G. indicus* system.

Table 6
Set of equations used for the prediction of substrate degradation profiles.

S. No.	Model		Set of equation
1	Model – a	(i)	$\frac{dX}{dt} = \mu_g X$
		(ii)	$\frac{dS}{dt} = -q_S X$
		(iii)	$\mu_g = \frac{\mu_m S}{S+K_S+(S^2/K_i)(1+(S/K))}$ for resorcinol or $\mu_g = \frac{\mu_{max} S}{S+K_S+(S^2/K_i)}$ for p-cresol
		(iv)	$q_S = \left(\frac{1}{(Y_{X/S})_T} - \frac{k}{\mu_m}\right)\mu_g + k + m_1$
2	Model – b	(i)	$\frac{dX}{dt} = \mu_g X$
		(ii)	$\frac{dS}{dt} = -q_S X$
		(iii)	$\mu_g = \frac{\mu_m S}{S+K_S+(S^2/K_i)(1+(S/K))}$ for resorcinol or $\mu_g = \frac{\mu_{max} S}{S+K_S+(S^2/K_i)}$ for p-cresol
		(iv)	$q_S = \frac{\mu_g}{(Y_{X/S})_T}$

4.4. Computed substrate degradation profiles

In the present study, the substrate degradation profiles with time have been computed. For this purpose, two models, model – a and model – b (Table 6), have been proposed. These two

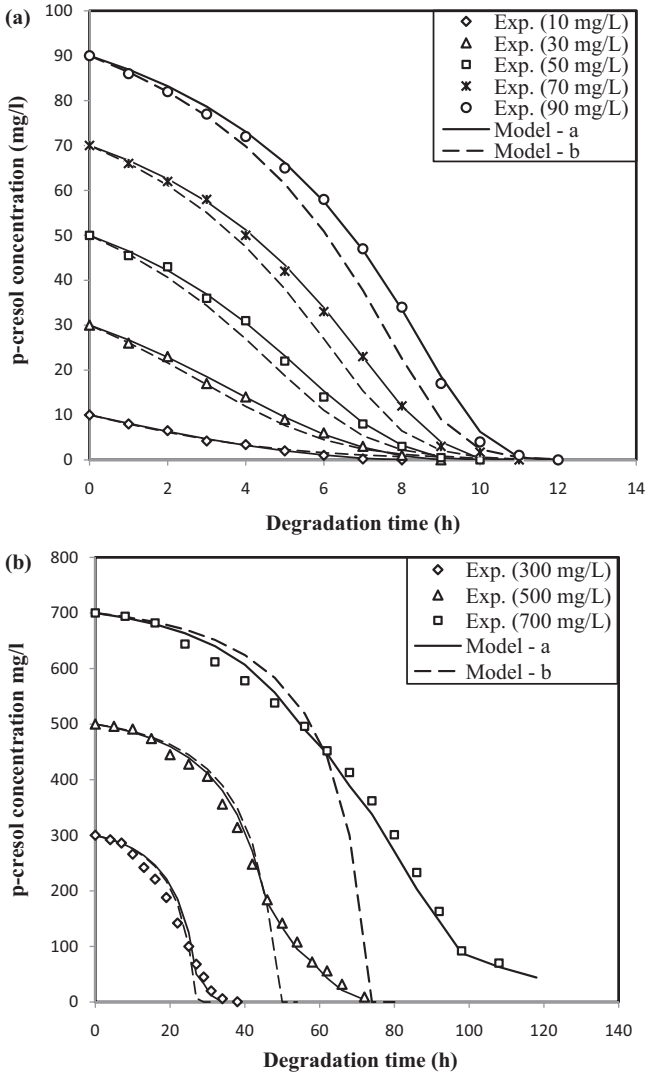

Fig. 8. p-Cresol degradation profiles at different initial concentrations with model predictions at (a) lower concentration range, and (b) higher concentration range.

models differ in the equations of specific degradation rate q_S. To obtain degradation profiles with time, two sets of model equations corresponding to the selected growth and degradation kinetic models for resorcinol and p-cresol have been solved by ordinary differential equation solver tool of MATLAB 7. The solution is represented in Fig. 7a and b for resorcinol, and Fig. 8a and b for p-cresol. The simulations are performed for different initial substrate concentrations in the range of 10–1300 mg/L and 10–700 mg/L for resorcinol and p-cresol, respectively. It can be seen that the simulation predictions by model – a are in good agreement with the experimental data in the full initial substrate concentration range for both the substrates resorcinol and p-cresol. The model – a involves variable observed growth yield and maintenance energy coefficient. Predictions by the model – b do not fit to the experimental data of resorcinol properly. However, model – b predictions are close to p-cresol experimental biodegradation data at lower concentrations only. The reason may be that the model – b does not consider maintenance energy expenditure and variation in growth yield as is shown in the expression of q_S in the model. Thus, these results indicate that the proposed model – a is quite adequate and useful to predict the substrate degradation over the entire range of substrate concentration 10–1300 mg/L and 10–700 mg/L for resorcinol and p-cresol, respectively.

5. Conclusion

Resorcinol and p-cresol were degraded by *G. indicus* over a wide concentration range of 10–1300 mg/L and 10–700 mg/L, respectively, by conducting the batch experiments at 28 °C and initial pH of 6. The kinetic models for specific growth rate and specific degradation rate were proposed. Growth model by Yano rendered better understanding for resorcinol whereas Andrews and Noack model was found best for p-cresol to fit the experimental growth data. Likewise, the degradation models analogous to Yano, Andrews and Noack models described well the kinetics for the degradation of resorcinol and p-cresol, respectively. Further, the variation of observed growth yield with initial substrate concentration was investigated. A conceptual model to describe the variation of maintenance energy expenditure with substrate concentration during biodegradation process was included. Two models were proposed to simulate the resorcinol and p-cresol degradation profiles with time. Out of these two, the model – a incorporated the variation of observed growth yield and maintenance energy expenditure with initial substrate concentration to describe the substrate degradation rate. The simulated results by model – a agreed well with experimental results for entire concentration range of resorcinol and p-cresol. Thus, the inclusion of substrate inhibition effect on maintenance and observed growth yield seems to be promising for description of degradation rate.

This modeling study revealed that the filamentous fungus *G. indicus* has potential to be used in wastewater treatment as for the bioremediation of soil contaminated with resorcinol and p-cresol. Further, the proposed modeling study would be useful for optimal design and operation of aerobic biological treatment units.

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References

[1] N.S. Gad, A.S. Saad, Effect of environmental pollution by phenol on some physiological parameters of *Oreochromis niloticus*, Global Vet. 2 (2008) 312–319.
[2] P. Kumaran, Y.L. Paruchuri, Kinetics of phenol biotransformation, Water Res. 31 (1997) 11–22.
[3] S.K. Pardeshi, A.B. Patil, Solar photocatalytic degradation of resorcinol a model endocrine disrupter in water using zinc oxide, J. Hazard. Mater. 163 (2009) 403–409.
[4] P. Saravanan, K. Pakshirajan, P. Saha, Batch growth kinetics of an indigenous mixed microbial culture utilizing *m*-cresol as the sole carbon source, J. Hazard. Mater. 162 (2009) 476–481.
[5] Y. Jiang, J. Wen, J. Bai, X. Jia, Z. Hu, Biodegradation of phenol at high initial concentration by *Alcaligenes faecalis*, J. Hazard. Mater. 147 (2007) 672–676.
[6] I. Banerjee, J.M. Modak, K. Bandopadhyay, D. Das, B.R. Maiti, Mathematical model for evaluation of mass transfer limitations in phenol biodegradation by immobilized *Pseudomonas putida*, J. Biotechnol. 87 (2001) 211–223.
[7] O.J. Hao, M.H. Kim, E.A. Seagren, H. Kim, Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species, Chemosphere 46 (2002) 797–807.
[8] M.L. Shuler, F. Kargi, Bioprocess Engineering—Basic Concepts, second ed., Pearson Education Pvt. Ltd., Singapore, 2003.
[9] I.G. Minkevich, S.V. Andreyev, V.K. Eroshin, The effect of two inhibiting substrates on growth kinetics and cell maintenance of *Candida valida*, Process Biochem. 36 (2000) 209–217.
[10] P.J. Allsop, Y. Chisti, M.M. Young, G.R. Sullivan, Dynamics of phenol degradation by *Pseudomonas putida*, Biotechnol. Bioeng. 41 (1993) 572–580.
[11] G.Y. Wang, J.P. Wen, G.H. Yu, H.M. Li, Anaerobic biodegradation of phenol by *Candida albicans* PDY-07 in the presence of 4-chlorophenol, World J. Microb. Biotechnol. 24 (2008) 2685–2691.
[12] K.M. Khleifat, Biodegradation of phenol by *Ewingella americana*: effect of carbon starvation and some growth conditions, Process Biochem. 41 (2006) 2010–2016.
[13] Y. Jiang, J.P. Wen, H.M. Li, S.L. Yang, Z.D. Hu, The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*, Biochem. Eng. J. 24 (2005) 243–247.
[14] M. Bajaj, C. Gallert, J. Winter, Phenol degradation kinetics of an aerobic mixed culture, Biochem. Eng. J. 46 (2009) 205–209.
[15] J. Bai, J.P. Wen, H.M. Li, Y. Jiang, Kinetic modeling of growth and biodegradation of phenol and m-cresol using *Alcaligenes faecalis*, Process Biochem. 42 (2007) 510–517.
[16] Z. Alexievaa, M. Gerginova, P. Zlateva, Comparison of growth kinetics and phenol metabolizing enzymes of *Trichosporon cutaneum* R57 and mutants with modified degradation abilities, Enzyme Microb. Technol. 34 (2004) 242–247.
[17] V. Arutchelvan, V. Kanakasabai, S. Nagarajan, V. Muralikrishnan, Isolation and identification of novel high strength phenol degrading bacterial strains from phenol–formaldehyde resin manufacturing industrial wastewater, J. Hazard. Mater. B127 (2005) 238–243.
[18] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, fourth ed., Metcalf & Eddy Inc., Tata McGraw-Hill Publishing Company Limited, New Delhi, 2003.
[19] I. Stoilova, A. Krastanov, H. Yemendzhiev, Z. Alexieva, Influence of concentration of conidia of *Aspergillus Awamori* NRRL 3112 on the catabolism of aromatic hydrocarbons and their mixtures, EJEAFChe 7 (2008) 2833–2843.
[20] R.K. Singh, S. Kumar, S. Kumar, A. Kumar, Biodegradation kinetic studies for the removal of p-cresol from wastewater using *Gliomastix indicus* MTCC 3869, Biochem. Eng. J. 40 (2008) 293–303.
[21] S.J. Pirt, Principles of Microbe and Cell Cultivation, Blackwell Scientific Publications, Oxford, 1975.
[22] K. Kovarova, A.J.B. Zehnder, T. Egli, Temperature-dependent growth kinetics of *Escherichia coli* ML 30 in glucose-limited continuous culture, J. Bacteriol. 178 (1996) 4530–4539.
[23] O.M. Neijssel, D.W. Tempest, Bioenergetic aspects of aerobic growth of *Klebsiella aerogenes* NCTC 418 in carbon-limited and carbon-sufficient chemostat culture, Arch. Microbiol. 107 (1976) 215–221.
[24] S.J. Pirt, Maintenance energy: a general model for energy limited and energy sufficient growth, Arch. Microbiol. 133 (1982) 300–302.
[25] S.E. Mainzer, W.P. Hempfling, Effects of growth temperature on yield and maintenance during glucose-limited continuous culture of *Escherichia coli*, J. Bacteriol. 126 (1976) 251–256.
[26] R.J. Wallace, W.H. Holms, Maintenance coefficients and rates of turnover of cell material in *Escherichia coli* ML 308 at different growth temperatures, FEMS Microbiol. Lett. 37 (1986) 317–320.
[27] S.P. Tsai, Y.H. Lee, A model for energy-sufficient culture growth, Biotechnol. Bioeng. 35 (1990) 138–145.
[28] J.L. Parks, M. Edwards, Boron in the environment, Crit. Rev. Environ. Sci. Technol. 35 (2005) 81–114.
[29] P.V. Bodegom, Microbial maintenance: a critical review on its quantification, Microb. Ecol. 53 (2007) 513–523.
[30] S.J. Pirt, The energetics of microbes at slow growth rates: maintenance energies and dormant organisms, J. Ferment. Technol. 65 (1987) 173–177.
[31] R.S. Juang, S. Tsai, Growth kinetics of *Pseudomonas putida* in the biodegradation of single and mixed phenol and sodium salicylate, Biochem. Eng. J. 31 (2006) 133–140.
[32] J. Shen, R. He, L. Wang, J. Zhang, Y. Zuo, Y. Li, X. Sun, J. Li, W. Han, Biodegradation kinetics of picric acid by *Rhodococcus* sp. NJUST16 in batch reactors, J. Hazard. Mater. 167 (2009) 193–198.