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Two-step modeling of the biodegradation of phenol by an acclimated activated sludge

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Abstract

Phenol biodegradation by an acclimated activated sludge was investigated in batch cultures with variable initial conditions of substrate and biomass ($0.10 \leq S_0/X_0 \leq 1$ g phenol g TSS^{-1}). As conventional Haldane model could not explain the biomass growth observed after phenol exhaustion, a model based on the production and later consumption of an inhibitory metabolic intermediate was developed to describe the phenol biodegradation and the biomass growth profiles. This two-step model considers that biodegradation is accomplished by two microbial populations constituting the whole biomass. The new model depicts successfully both biomass and phenol courses by using a single set of kinetic parameters, and support the notion that the production of metabolic intermediates has a determinant role in phenol biodegradation kinetics.

Keywords: Phenol; Biodegradation; Modeling; Activated sludge; Inhibition; Metabolic intermediates

1. Introduction

Phenol and its higher molecular homologues are important environmental pollutants. Due to their toxic character, these molecules tend to accumulate in water and soil after being discharged without an adequate treatment. For this reason, several decontamination techniques of phenolic compounds have been extensively studied and optimized [1].

Biological treatment, particularly by the activated sludge process, is a suitable technology for the removal of phenol in water. This cost-effective treatment permits the phenol degradation to innocuous, low-molecular-weight compounds (mainly CO_2 and H_2O). However, the inhibition of the microorganisms carrying out the degradation by high phenol concentrations remains as the major drawback of the process.

Dynamic modeling is a powerful tool in predicting and optimizing the performance of wastewater treatment processes. A dynamic model depicting the behavior of both biomass and substrate concentrations allows the process response under various operational conditions to be estimated quantitatively and then the optimization of the process control scheme. Although the Monod expression has been used for describing the phenol consumption kinetics [2], the Haldane model is preferred in the case of operation conditions inhibiting the biodegradation. It has been used to model the phenol specific degradation rate in cultures of *Pseudomonas putida* [3,4], *Ralstonia eutropha* [5] and activated sludges [6–9]. Nevertheless, some authors have reported that the production of metabolic intermediates of the phenol degradation, such as the 2-hydroxymuconic acid semialdehyde (2-hmas), makes inadequate the use of this model [6,10].

In this work, the kinetics of phenol biodegradation by activated sludge was studied. To this end, the sludge was acclimated to 0.70 g l^{-1} of phenol by a fill-and-draw procedure. The acclimated biomass was then cultivated in batch mode on phenol at various initial substrate to biomass ratios (S_0/X_0), in order to obtain different biodegradation patterns. In fact, this ratio is the main control parameter in batch cultures, since it determines the biodegradation kinetics, the lag time and the degree of intermediates formation [11]. Based on the experimental results, a new kinetic model representing both phenol and biomass evolution is developed.

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Nomenclature

K_i	inhibition constant ($g\,l^{-1}$)
K_S	half-saturation constant ($g\,l^{-1}$)
P	reaction product
q_S	specific consumption rate of substrate ($g\,g^{-1}\,h^{-1}$)
S	substrate concentration ($g\,l^{-1}$)
T	time (h)
X	biomass concentration ($g\,TSS\,l^{-1}$)
Y	observed yield coefficient ($g\,g^{-1}$)

Greek symbols

α	growth-associated constant ($g\,g^{-1}$)
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

Subscript

0	initial value
1	parameter related to the biomass metabolizing phenol (X_1)
2	parameter related to the biomass metabolizing the intermediate (X_2)

2. Materials and methods

2.1. Organisms and materials

Samples of activated sludge were obtained from a plant treating both municipal and industrial wastewater (San Juan Ixhuatepec, Mexico). Phenol with a purity of 99% was used (Sigma–Aldrich, Germany); 4-aminoantipyrine and other chemicals were purchased from J.T. Baker (U.S.A.).

2.2. Acclimation of activated sludge

Raw activated sludge was gradually acclimated to incremental phenol concentrations by daily semi-continuous cycles. Each day, 1 l of settled domestic wastewater sampled from the University sewer (340–400 mg $DBO_5\,l^{-1}$) was added to the same volume of activated sludge, as well as a variable volume of concentrated phenol solution ($20\,g\,l^{-1}$). After 23 h of aeration and 0.75 h of sedimentation, the supernatant was drained off and fresh sewage added. The phenol concentrations were 0.005–0.7 $g\,l^{-1}$ starting and finishing the acclimation period, respectively. The total suspended solids (TSS) content in the reactor was maintained at 4–5 $g\,l^{-1}$.

2.3. Batch culture medium and experimental conditions

Batch cultures were conducted on a minimum medium prepared with distilled water (Table 1). The growth medium contained phenol as the sole carbon and energy source.

The experiments were carried out at different initial conditions, namely at various initial substrate to biomass ratios (S_0/X_0). For this, the cultures were grown in 1 l glass bottles containing 600 ml of growth medium and variable volumes of a concentrated phenol solution ($20\,g\,l^{-1}$) and of acclimated activated sludge ($4.5\,g\,TSS\,l^{-1}$). Prior inoculation, the sludge from the acclimation reactor was washed twice with growth medium lacking in phenol, in order to avoid the presence of other carbonaceous substrates. Air was introduced to the bottles by means of aquarium sprinklers, which assured also a good mixture of the cultures. During the experiments, samples were obtained at different times to assess the phenol and biomass contents. For phenol determination, the samples were first centrifuged at 3500 rpm for 10 min and then frozen until analysis. The experiments were performed simultaneously at ambient temperature (21 °C).

Table 1
Composition of the growth medium

Component	Concentration ($mg\,l^{-1}$)
Phenol	Variable (180–800)
K_2HPO_4	404
KH_2PO_4	220
$(NH_4)_2SO_4$	50
$MgSO_4{\cdot}7H_2O$	10
$CaCl_2{\cdot}2H_2O$	1.85
$MnCl_2{\cdot}4H_2O$	1.5
$FeCl_3{\cdot}6H_2O$	0.3

2.4. Analytical methods

Phenol was determined colorimetrically by using the 4-aminoantipyrine method [12]. In short, 0.2 ml of a 0.1 M glycine solution containing 5% (w/v) $K_3Fe(CN)_6$ was added to 2 ml of centrifuged sample in a 10 ml vial. After mixing, the content was allowed to react for 5 min. Then, 2 ml of glycine buffer containing 0.25% (w/v) 4-aminoantipyrine was added. The glycine buffer was prepared by mixing 5.58 g of glycine hydrochloride, 3.75 g of glycine and 900 ml of distilled water, and by adjusting the pH to 9.7 with 6N NaOH and finally diluting to 1 l. The content of the vial was mixed and allowed to react for 20 min. The absorbance of the mixture at 506 nm was measured in a Perkin-Elmer Lambda 40 UV–vis spectrophotometer within the next 30 min, in order to avoid a decrease in the assay response. The calibration curves were made and found linear up to a concentration of $0.025\,g\,l^{-1}$ ($r^2 = 0.999$). This method had a detection threshold limit of $0.07\,mg\,l^{-1}$ and a variation coefficient of 1.06% of the measured values ($n = 9$).

The total suspended solids (TSS) concentration was determined gravimetrically by filtering 10 ml-samples through a 0.2 μm-pore-size membrane and drying for 24 h at 105 °C.

3. Results and discussion

3.1. Conventional modeling of the growth and biodegradation kinetics

For batch cultures, a mass balance gives the following first-order differential equations often used for describing biomass

(X) growth when cell decay is negligible:

$$\frac{dX}{dt} = \mu X \quad (1)$$

and substrate (S) consumption:

$$\frac{dS}{dt} = -q_S X = -\frac{\mu}{Y} X \quad (2)$$

where μ and q_S correspond to the specific growth and substrate consumption rates, respectively. The observed yield coefficient Y is usually assumed as constant.

In the case of phenol biodegradation processes, the specific growth rate μ, and hence the specific phenol consumption rate q_S in Eq. (2), is typically modeled by the conventional Haldane equation [3–9]:

$$\mu = \frac{\mu_{max} S}{K_S + S + (S^2/K_i)} \quad (3)$$

Then, the modeling task consists in the determination of the values of the kinetic constants of Eq. (3), namely μ_{max}, K_S and K_i (either experimentally or usually by using an algorithm based on the nonlinear least-square technique) and the estimation of the yield coefficient Y if biomass concentration measurements are available. A summary of these kinetic constants as reported in other studies is shown in Table 2.

3.2. 3.2 Growth of the acclimated activated sludge on phenol

Four cultures were conducted at different initial concentrations of phenol (0.18–0.80 g l^{-1}) and biomass (0.74–1.74 g TSS l^{-1}). The following S_0/X_0 ratios resulted: 0.1, 0.52, 0.79 and 1 g phenol g TSS^{-1}. Phenol depletion was followed during 15 h and the corresponding biodegradation levels were calculated. The results are presented in Fig. 1.

As indicated in Fig. 1, the S_0/X_0 ratio influenced the phenol biodegradation kinetics. Complete biodegradation was first observed for the lower S_0/X_0 ratio after only 3 h of culture, whereas the assay carried out at the highest ratio needed about 9 h for the total phenol removal. Since the biomass was acclimated to a phenol concentration of 0.70 g l^{-1} and the S_0/X_0 ratios were short enough, no lag phases were observed in any culture.

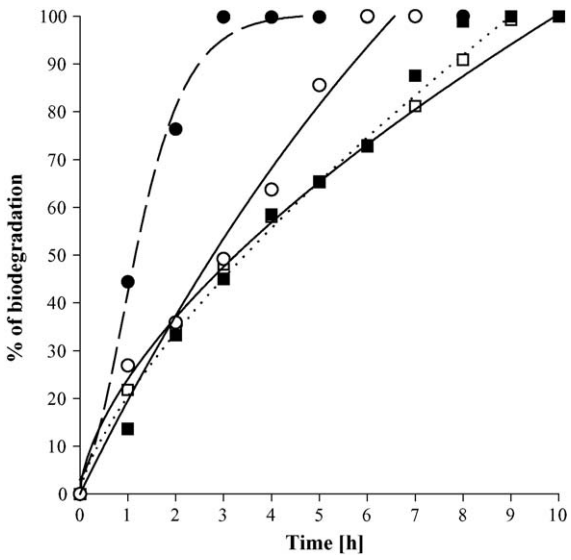

Fig. 1. Biodegradation of phenol at different S_0/X_0 ratios (g phenol g TSS^{-1}): (●) 0.10; (◯) 0.52; (■) 0.79; (□) 1.00.

Concerning the biomass evolution, the experimental data presented a behavior scarcely reported. In the four cultures, the biomass showed a residual growth after the phenol exhaustion. Fig. 2 presents the biomass and phenol courses corresponding to the culture for which this fact was more markedly observed. This residual growth contradicts the conventional Haldane model, which predicts a non-growing phase in absence of substrate, i.e. $\mu = 0$ when $S = 0$.

For *P. putida* F1 cultures, a biomass production has been observed 10 h after phenol was depleted, which was attributed to the production of an intermediate being later consumed [2]. This phenomenon has also been pointed out during BTX biodegradation by the same bacterial strain [13]. According to these authors, the biomass growth even after the original substrate is consumed indicates that the growth is controlled by the intermediates rather than this original substrate. In our experiments, the acclimation to phenol was made in the presence of domestic wastewater, in order to maintain a high microbial diversity. Complex interactions occur in such a population, and hence, the behavior of the acclimated sludge could be explained by a predominant strain biodegrading phenol in a similar way to *P. putida* F1 or by several populations using consecutively phenol

Table 2
Summary of kinetic constant values obtained for phenol biodegradation

Microorganism	Haldane-model constants			Y (g g^{-1})	Ref.
	μ_{max} (h^{-1})	K_S (g l^{-1})	K_i (g l^{-1})		
P. putida F1[a]	0.11 ± 0.01	0.032	–	0.80 ± 0.07	[2]
P. putida MTCC 1194	0.305	0.036	0.129	0.65	[3]
P. putida ATCC 17484	0.534	<0.001	0.470	0.52 ± 0.08	[4]
Ralstonia eutropha	0.410	0.002	0.350	0.68	[5]
Activated sludge	0.143	0.087	0.107	0.60	[6]
Activated sludge	0.246	0.027	0.524	–	[7]
Activated sludge	0.131–0.363	0.005–0.266	0.142–1.199	–	[8]
Activated sludge	0.260	0.025	0.173	0.545–0.616	[9]

[a] The biodegradation kinetics was modeled by using the Monod equation.

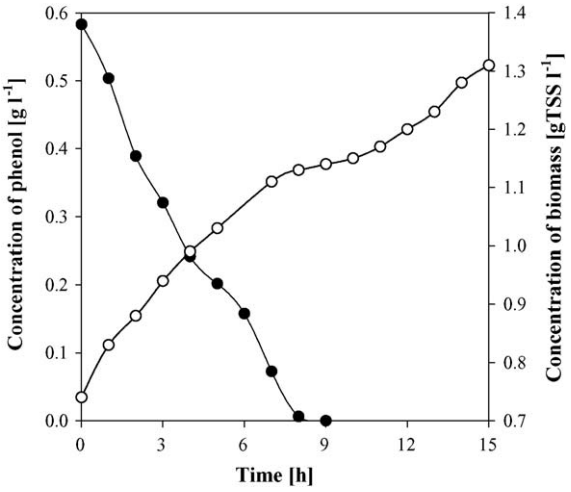

Fig. 2. Kinetics of growth and substrate consumption of activated sludge ($S_0/X_0 = 0.79\ g\ g^{-1}$) (●) phenol; (◯) biomass.

and its metabolic intermediates as substrates and presenting the same overall behavior.

3.3. Two-step modeling of the phenol biodegradation

The evident disagreement between the experimental data and the conventional Haldane-predicted profile may be explained by the fact that this model does not take into account the effects due to the production of several inhibitory metabolic intermediates and to their accumulation and consumption during phenol biodegradation.

As reported in several studies [5,10,14], the *meta*-cleavage of phenol, which is a widespread catabolic pathway of aromatic compounds, is often characterized by the accumulation of 2-hydroxymuconic acid semialdehyde (denoted 2-hmas). This accumulation has been correlated to the quantity of phenol consumed during the culture and to the apparition of a yellow color in the culture medium [14]. The 2-hmas concentration has been estimated by a linear correlation with the yellow color intensity as measured spectrophotometrically, allowing the control of the metabolic activity of *R. eutropha* during a fedbatch phenol biodegradation process [5]. Nuhoglu and Yalcin [6] and Wang and Loh [10] proposed modified Haldane models based on the direct integration of the initial phenol concentration (S_0) in the specific growth rate expression equation (3). These models depict properly the kinetics of phenol biodegradation for wide ranges of phenol concentrations, but do not consider the growth of the biomass (activated sludge and *P. putida*, respectively).

As the biomass growth is not considered in these models and the production of metabolic intermediates was suggested during the batch cultures previously described, a new mathematical model that explicitly takes into account the effect of metabolites on both phenol biodegradation and cell growth is proposed. To this end, the phenol biodegradation has been assumed to be a two-step process. In the first step phenol is degraded by a fraction of the total biomass, which grows and produces one or several metabolic intermediates (Eq. (4)). During this step the biodegradation of phenol is inhibited by phenol itself, as described formerly. This assumption allows the accumulation of metabolic intermediates to be explained. In the second step, the intermediate is mineralized by another microbial population through a reaction yielding carbon and energy for its growth (Eq. (5)). The utilization of this intermediate as a substrate explains the residual growth of the biomass. The following reaction scheme summarizes this process:

$$S_1 \rightarrow X_1 + S_2 + P_1 \tag{4}$$

$$S_2 \rightarrow X_2 + P_2 \tag{5}$$

where S_1 is the phenol concentration; X_1 is the portion of the total biomass concentration (X_t) growing on S_1; S_2 is the concentration of the major metabolic intermediate; X_2 is the fraction of X_t growing on S_2, and P_1 and P_2 are unknown products or other minor intermediates. It is important to note that only the total biomass X_t is measurable ($X_t = X_1 + X_2$).

The dynamical model of the batch biodegradation process has the following form:

$$\frac{dX_1}{dt} = \mu_1 X_1 \tag{6}$$

$$\frac{dS_1}{dt} = -\frac{\mu_1}{Y_1} X_1 \tag{7}$$

$$\frac{dX_2}{dt} = \mu_2 X_2 \tag{8}$$

$$\frac{dS_2}{dt} = \alpha \mu_1 X_1 - \frac{\mu_2}{Y_2} X_2 \tag{9}$$

where α is a growth-associated constant relying the production of metabolic intermediates to the phenol consumption. From Eq. (9), it can be noticed that the production of the intermediate S_2 depends on the conversion of phenol S_1 by the biomass X_1.

Since both phenol and the intermediates are considered as inhibitory substrates, the specific growth rates μ_1 and μ_2 are modeled according to a Haldane-type equation (Eqs. (10)–(11)):

$$\mu_1 = \mu_{\max_1} \frac{S_1}{K_{S_1} + S_1 + (S_1^2/K_{i_1})} \tag{10}$$

$$\mu_2 = \mu_{\max_2} \frac{S_2}{K_{S_2} + S_2 + (S_2^2/K_{i_2})} \tag{11}$$

There are several methods of parameter estimation for model tuning. A model may be fitted either *via* a numerical method, such as least squares or a quadratic estimation method, or via a heuristic method. In this work, the kinetic parameters were estimated using the direct search method of Hooke and Jeeves [15], which is commonly used for models with large numbers of variables and parameters (e.g. the proposed phenol biodegradation model has nine parameters). The kinetic parameter values are listed in Table 3. Although a direct comparison between these values and others reported by the literature (Table 2) is not possible, it can be noted that they lay in the same order of magnitude. Simulations were made by using Eqs. (6)–(11) and a fourth-order Runge-Kutta algorithm for numerical integration of the ordinary differential equations.

Table 3
Kinetic parameters used for the simulation of the proposed model

Symbol	Value
μ_{max_1}	0.25
K_{S_1}	0.3
K_{i_1}	0.45
Y_1	0.67
μ_{max_2}	0.1
K_{S_2}	0.07
K_{i_2}	0.09
α	0.95
Y_2	0.80

The experimental and model-predicted data of the phenol concentration (S_1) and the total biomass concentration (X_t) are illustrated in Figs. 3–6, as well as the simulated concentration of inhibitory metabolic intermediates. It is seen that the proposed two-step model tracks well the biomass and phenol experimental profiles, above all those obtained at lower S_0/X_0 ratios (Figs. 3–5).

In the experiments carried out with the highest S_0/X_0 ratios (Figs. 4–6), the biomass growth after phenol exhaustion is clearly observed, which is attributed to the higher accumulation of biodegradation intermediates. This observation confirms that the phenol biodegradation kinetics is controlled by the metabolic intermediate production rate and thus the choice of a model based on the reaction scheme given by Eqs. (4)–(5).

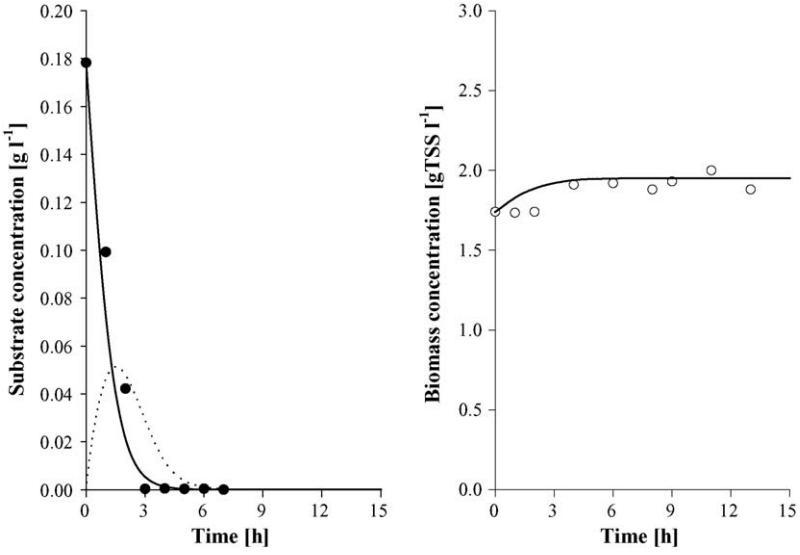

Fig. 3. Measured and estimated phenol (●) and biomass (◯) concentrations ($S_0/X_0 = 0.10$ g g^{-1}). The symbols represent the experimental results and the continuous lines, the data resulting from the simulated kinetic model. The dotted line simulates the behavior of the metabolic intermediate (S_2).

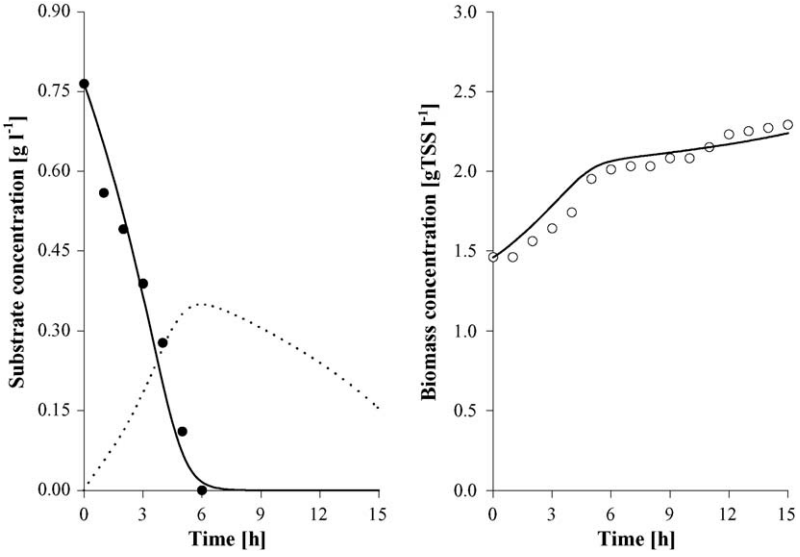

Fig. 4. Measured and estimated phenol (●) and biomass (◯) concentrations ($S_0/X_0 = 0.52$ g g^{-1}). The symbols represent the experimental results and the continuous lines, the data resulting from the simulated kinetic model. The dotted line simulates the behavior of the metabolic intermediate (S_2).

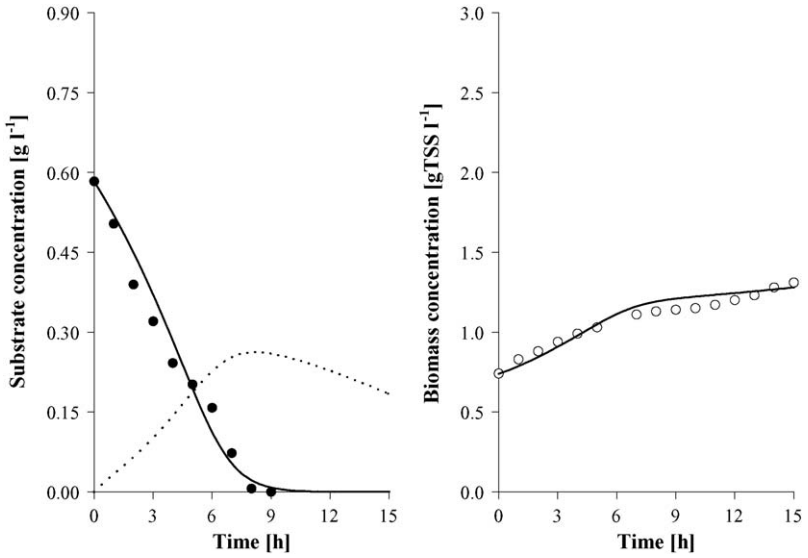

Fig. 5. Measured and estimated phenol (●) and biomass (◯) concentrations ($S_0/X_0 = 0.79$ g g^{-1}). The symbols represent the experimental results and the continuous lines, the data resulting from the simulated kinetic model. The dotted line simulates the behavior of the metabolic intermediate (S_2).

In addition, the simulated production of metabolic intermediates is consistent with the literature, which establishes that the levels of metabolites are higher as the S_0/X_0 increases [16]. However, the unavailability of direct measurements of the intermediate concentrations does not permit more realistic conclusions.

The simulated profiles of the intermediate show that its degradation is slower than that of phenol. This fact is also evidenced by the biomass growth rate observed after total phenol consumption, and consequently the inhibition constant value of the metabolic intermediate K_{i_2} is smaller than the K_{i_1} value. This is in agreement with Wang and Loh [10], who found that the inhibition effect of the intermediates (which is inversely proportional to the inhibition constant) was very strong respecting to the inhibition of phenol.

Table 4
Relative average error (in percent) between the values of phenol and biomass given by the model and the experimental data

	S_0/X_0 (g g^{-1})			
	0.10	0.52	0.79	1.0
S	9.7	8.4	33.9	24.2
X	3.1	5.4	5.0	6.5

Table 4 presents the relative average error between the values given by the model and the experimental data for each S_0/X_0 ratio. In the case of total biomass concentration, the relative average error was less than 10% for all the S_0/X_0 ratios. Regarding to the phenol concentrations, the error was more important for the highest S_0/X_0 ratios. However, the predicted values are seen

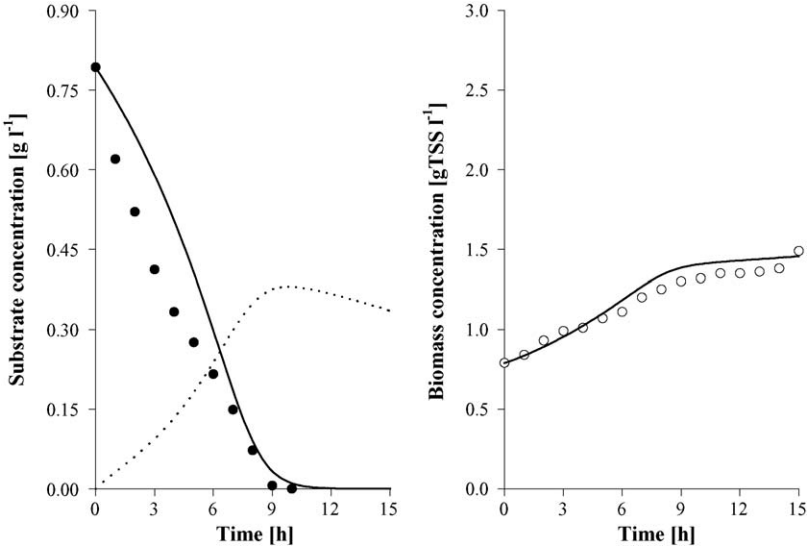

Fig. 6. Measured and estimated phenol (●) and biomass (◯) concentrations ($S_0/X_0 = 1.0$ g g^{-1}). The symbols represent the experimental results and the continuous lines, the data resulting from the simulated kinetic model. The dotted line simulates the behavior of the metabolic intermediate (S_2).

Table 5
Results of the parameter sensitivity analysis

Parameter	Nominal value	Value range	Mean absolute error, X_t ($g\,l^{-1}$)	Mean absolute error, S_1 ($g\,l^{-1}$)
μ_{max_1}	0.25	0.05/0.50	0.324/0.060	0.417/0.068
K_{S_1}	0.30	0.02/0.40	0.028/0.010	0.032/0.011
K_{i_1}	0.45	0.05/1.00	0.317/0.026	0.409/0.030
Y_1	0.67	0.30/0.90	0.208/0.090	0.070/0.039
μ_{max_2}	0.10	0.05/0.50	0.074/0.176	0.000/0.000
K_{S_2}	0.07	0.02/0.40	0.046/0.052	0.000/0.000
K_{i_2}	0.09	0.05/1.00	0.048/0.123	0.000/0.000
α	0.95	0.50/1.50	0.022/0.038	0.000/0.000
Y_2	0.80	0.30/0.90	0.030/0.004	0.000/0.000

to be in reasonable agreement with the experimental data for all the tested S_0/X_0 ratios, particularly if it is considered that the model depicts both the biomass and substrate concentrations by using only a set of kinetic parameters.

3.4. Parameter sensitivity analysis

The parameter sensitivity analysis indicates which kinetic parameters most affect the ability of the model to predict phenol degradation and microbial growth. Such an analysis was performed for the kinetic parameters of the model, whose results are presented in Table 5. The model ability to fit biomass and phenol concentrations with 1.46 g TSS l^{-1} and 0.76 g l^{-1} as initial conditions, respectively, was investigated for varying values of the model parameters (μ_{max_1}, K_{S_1}, K_{i_1}, Y_1, μ_{max_2}, K_{S_2}, K_{i_2}, α, and Y_2). The minimum and maximum levels of the kinetic parameters of phenol biodegradation were established by considering the values listed in Table 2. For the metabolic intermediate these parameters are unknown, therefore we used the same values as in the case of phenol biodegradation. Simulations were carried out by varying one parameter at a time, as the remaining parameters were held constant at the nominal values presented in Table 5. Sensitivity was assessed by computing the mean of the absolute errors between the simulation with the nominal value and with one modified parameter, for both biomass and phenol concentrations. These absolute errors were calculated for each of the 15-h simulation period, and then the mean was obtained.

As shown in Table 5, μ_{max_1} and K_{i_1} are the parameters having the most notable impact, which reflects the relative importance of the population X_1 in the overall biodegradation process. For predicting the total biomass growth, the model is also sensitive to changes of the parameter Y_1 and, to a lesser extent, μ_{max_2}. Concerning the prediction of the phenol concentration, the model is less sensitive to changes of K_{S_1} and Y_1, whilst no influence was found for those parameters related to X_2 (μ_{max_2}, K_{S_2}, K_{i_2}, α and Y_2) since phenol consumption is unaffected by the kinetic behavior of this microbial population. The sensitivity of the model to the inhibition-related parameters (e.g., K_{i_1}) appears to be overestimated in Table 5, due to the wide range of values reported in the literature for these parameters.

4. Conclusions

The main scope of this work was to derive a reliable kinetic model for a phenol biodegradation process using acclimated activated sludge. Since the conventional Haldane expression appeared to be inadequate to describe the microbial growth observed after the exhaustion of phenol, a new model considering the phenol biodegradation as a two-step process was developed. In the first step, phenol is considered to be degraded by a fraction of the total biomass, having one or several metabolic intermediates as products. It was also assumed that the intermediates are degraded during the second step, by another portion of the biomass. The proposed model is capable of describing adequately both phenol degradation and biomass growth profiles at various initial conditions (S_0/X_0) with only one set of model parameters. Therefore, by considering explicitly the inhibitory effect of metabolic intermediates, this dynamical model may be used as a prediction tool in the design of control strategies improving the biological treatment of phenolic wastewaters.

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