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A NEW MODEL OF PHENOL BIODEGRADATION AND ACTIVATED SLUDGE GROWTH IN FEDBATCH CULTURES

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Abstract: Phenol biodegradation using acclimated activated sludge was investigated in batch and fedbatch cultures with variable initial concentrations of phenol and biomass. The batch experimental data show a cell growth after phenol exhaustion, making the use of a conventional Haldane model, for which specific growth rate $\mu = 0$ when substrate $S = 0$, inadequate to describe biomass growth profiles. On the other hand, the fedbatch experiments have shown enhanced inhibitory effects on phenol degradation when compared to batch cultures. Both phenomena were attributed to the metabolic intermediates accumulation and to their later consumption during phenol degradation. Consequently, a new Haldane-based model that explicitly integrates the kinetic evolution of a main intermediate was designed. The model allowed accurate predictions of both phenol and biomass concentration courses in both operating modes over a wide range of initial conditions.

Keywords: Batch, Fedbatch, Phenol biodegradation, Substrate inhibition, Activated sludge, Mathematical modelling

1. INTRODUCTION

Phenol is a pollutant commonly found in industrial wastewaters and the aquatic environment due mainly to its numerous applications as intermediate in chemical processes. This molecule is toxic to several biochemical functions and to fish life and causes severe odor and taste problems in drinking water even at low concentrations. Since regulatory agencies impose strict limits on the phenol content in industrial discharges, the development and application of efficient treatment technologies are necessary.

Phenol has usually been removed by costly physicochemical methods, as adsorption, ion exchange or chemical oxidation. However, biological treatments, mostly based on activated sludge systems, are well-suited decontamination techniques because they have shown to be economical and lead to complete mineralization of phenol to innocuous products (CO_2 and H_2O). The major drawback of these processes is their sensitivity to variations in the pollutant load, as at certain concentrations phenol is an inhibitory substrate even for the bacterial species able of using it as an energy and carbon source. In this paper, the attention is focused on *fedbatch* reactors, in which substrates are fed either intermittently or continuously during the process. This operating mode offers many advantages –at least from an industrial viewpoint– over batch and continuous cultures. The main advantage is concretely economical, since improved pro-

ductivity may be obtained by providing controlled conditions in the supply of inhibitory substrates (Shioya, 1992).

Mathematical modelling is a powerful tool for understanding the behavior of biological processes and optimizing their efficiency through the design of model-based control strategies. Although a simple Monod equation has been successfully applied to describe phenol degradation (Reardon *et al.*, 2000), the Haldane substrate-inhibition model has been the most frequently used in the various operating modes (batch, fedbatch and continuous) for both mixed and pure cultures (Leonard *et al.*, 1999; Spigno *et al.*, 2004).

However, as it has been often described in the literature, the Haldane model predictions may present large discrepancies between data generated by batch runs and their application to fedbatch or continuous cultures (Garcia-Sanchez *et al.*, 1998). Even in batch cultures, evident disagreements between measured and estimated phenol concentration profiles were observed when using higher initial concentrations of phenol. Explanations of these discrepancies have often been attributed to the non-stationary behavior of the microorganisms, resulting in changing values of the model's kinetic parameters, including the biomass-to-substrate yield coefficient.

Other authors have pointed out the fact that inhibition parameters can only be estimated at higher phenol concentrations and that the Haldane model does not take into account the effects due to the production and consumption of several metabolic intermediates during phenol degradation. Among these, the major intermediate, the 2-hydroxymuconic acid semialdehyde, has been reported to be associated with the quantity of phenol consumed during the culture, likewise its accumulation has been correlated to the appearance of a yellow color in the culture medium (Leonard *et al.*, 1999; Mörsen and Rehm, 1990). However, the quantification of each of the intermediates is obviously a highly difficult task to carry on, especially in the case of mixed populations.

In terms of mathematical modelling, some authors have proposed modified Haldane models without explicit integration of the inhibitory intermediate effects on the model structure (Nuhoglu and Yalcin, 2005). A very few studies reported a dynamic model that explicitly integrates the intermediate production kinetics, e.g. the work of (Garcia-Sanchez *et al.*, 1998) for chemostat cultures with a pure culture of *P. putida* and none have been applied in the case of activated sludge in fedbatch cultures.

The aim of this work is to develop a new model of phenol biodegradation and biomass growth suited to design fedbatch flow rates strategies able to optimize the phenol consumption and minimize the cell growth. In this study, various batch and fedbatch experiments regarding the growth of an acclimatized activated sludge culture on phenol were carried out, and a new model able to efficiently fit the corresponding experimental data and that explicitly takes into account the inhibitory effects of the metabolic intermediates on phenol biodegradation was proposed.

2. PROCESS DESCRIPTION

2.1 Reactor system

A laboratory scale reactor system that could operate in batch and fedbatch modes was used for the study. A diagram of the bioreactor system is shown in Fig. 1. The plant was linked to a monitoring PC through a NI-PCI-6024E data acquisition board from the National Instrument family. The Labview 7 Express was used as programming language and development tool for data acquisition and storage, graphic display, digital implementation of the feed flow rate profiles in fedbatch cultures, control of the steered speed, pH, aeration and peristaltic pumps.

2.2 Materials and methods

Phenol (99.5% purity) was purchased from Sigma-Aldrich (Germany); 4-amino antipyrine and other chemicals were purchased from J. T. Baker (U.S.A.). Samples of activated sludge were obtained from a plant treating both municipal and industrial wastewater (San Juan Ixhuatepec, Mexico). Raw activated sludge was acclimated to increasing phenol concentrations in daily semi-continuous cycles. Each day, a liter of activated sludge was mixed with the same volume of settled domestic wastewater sampled from the University sewer ($340 - 400$ mg DBO_5/l) and a variable volume of concentrate phenol solution (20 g/l). After 23 hours of aeration and 0.75 hours of sedimentation, the supernatant was drained off and new sewage added. The phenol concentrations were $5 - 700$ mg/l starting and finishing the acclimation period, respectively. The solids content in the reactor was maintained at 4–5 gTSS/l. The medium used had the following composition (mg/l): K_2HPO_4 (404), KH_2PO_4 (220), $(NH_4)_2SO_4$ (50), $MgSO_47H_2O$ (10), $CaCl_22H_2O$ (1.85), $MnCl_24H_2O$ (1.5), $FeCl_36H_2O$ (0.3). Batch experiments were conducted at ambient temperature (21^0C) in 1 l glass bottles containing 600 ml of medium and variable volumes of a phenol solution (20 g/l) and acclimatized activated sludge, in order to provide different initial contents of

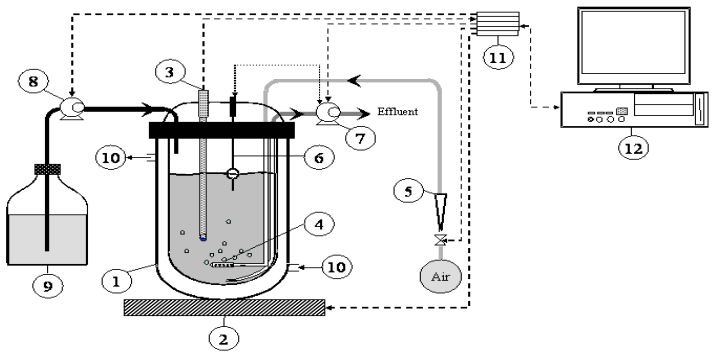

Fig. 1. Diagram of the laboratory bioreactor system; 1 = bioreactor; 2 = magnetic stirrer; 3 = pH meter; 4 = air sprinkler; 5 = air flux meter; 6 = level controller; 7 = controlled peristaltic pump; 8 = controlled peristaltic pump; 9 = phenol feed tank (1.036 g/l); 10 = thermostated water jacket; 11 = data acquisition card (stirred speed, pH, aeration, pumps); 12 = monitoring computer

substrate and biomass. 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 concentrations. For phenol analysis, samples were first centrifugated at 3500 rpm for 10 minutes. Phenol was determined colorimetrically by using the 4-aminoantipyrine method (Woolard and Irvine, 1995). 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 minutes. 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 6 N NaOH and finally diluting to 1 l. The content of the vial was mixed and allowed to react for 20 minutes. The absorbance of the mixture at 506 nm was measured in a Perkin Elmer Lambda 40 UV-vis spectrophotometer within the next 30 minutes, in order to avoid a decrease in the assay response. The calibration curves were made and found linear up to a concentration of 25 mg/l ($r^2 = 0.999$). This method had a detection threshold limit of 0.07 mg/l and a variation coefficient of 1.06% of the measured values ($n = 9$). For measuring biomass, 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 hours at 105 ^{0}C. The detection threshold limit of the method was 0.284 gTSS/l, with a variation coefficient of 2.68% of the measured values ($n = 9$).

3. KINETIC MODELLING CONSIDERING THE ROLE OF A METABOLIC INTERMEDIATE

3.1 Model structure

In this work, a mathematical model that explicitly takes into account the main intermediate effect on both phenol degradation and cell growth is proposed. The process is assumed to be described by the following simplified reaction scheme:

$$S_1 \overset{\curvearrowleft}{\rightarrow} X + S_2 \quad (1)$$

$$S_2 \overset{\curvearrowleft}{\rightarrow} X \quad (2)$$

where S_1 is the phenol concentration, S_2 is the main metabolic intermediate concentration, X is the total microbial population concentration growing on either S_1 and S_2. Since the main intermediate S_2 was not identified, its true concentrations are unknown, so S_2 is actually expressed in pseudo-concentration. The mass-balance equations for the various constituents of phenol biodegradation gives the following first-order set of differential equations:

$$\frac{dX}{dt} = \mu X - \frac{Q_{in}}{V} X \quad (3)$$

$$\frac{dS_1}{dt} = -q_{s_1} X + \frac{Q_{in}}{V}\left(S_1^{in} - S_1\right) \quad (4)$$

$$\frac{dS_2}{dt} = \nu_{s_2} X - q_{s_2} X - \frac{Q_{in}}{V} S_2 \quad (5)$$

$$\frac{dV}{dt} = Q_{in} - Q_{out} \quad (6)$$

where μ is the specific biomass growth rate, q_{s_1} and q_{s_1} are, respectively, the specific consumption rate of phenol and the intermediate; ν_{s_2} is the specific intermediate production rate. The different

modes of culture can be directly coupled to this general set of mathematical equations by setting ($Q_{in} = Q_{out}$) in continuous cultures, ($Q_{in} = Q_{out} = 0$) in batch cultures and ($Q_{out} = 0$) in fedbatch cultures.

3.2 Modelling of the specific reaction rates

In this work, since it was assumed that the activated sludge is growing on both phenol and the metabolic intermediate, the global specific growth rate μ may be expressed as:

$$\mu = \mu_1 + \mu_2 \tag{7}$$

where μ_1 is modelled according to a modified (in order to integrate the inhibitory effect of intermediate accumulation on phenol consumption) Haldane-type equation whilst a modified Monod-type one is used for μ_2:

$$\mu_1 = \frac{\mu_{\max_1} S_1}{K_{s_1} + S_1 + S_1^2/K_{i_1}} \cdot \frac{K_2}{K_2 + S_2} \tag{8}$$

$$\mu_2 = \frac{\mu_{\max_2} S_2}{K_{s_2} + S_2} \cdot \frac{K_1}{K_1 + S_1} \tag{9}$$

One may note from (8) and (9) the dual-substrate type of structure of the phenol and intermediate kinetics. Constant values of biomass-to-phenol Y_1 and biomass-to-intermediate Y_2 yield coefficients were obtained, so the specific growth and consumption rates where correlated by the following linear relationships:

$$q_{s_1} = \frac{\mu_1}{Y_1} \tag{10}$$

$$q_{s_2} = \frac{\mu_2}{Y_2} \tag{11}$$

The specific production rate of the metabolic intermediate was linearly correlated to the specific growth rate of biomass on phenol as follows:

$$\nu_{s_2} = \alpha \mu_1 \tag{12}$$

Table 1. Parameter values used for model simulation

Symbol	Batch	Fedbatch	SI Units
$\mu_{\max_1}$	0.39	0.4	1/h
Y_1	0.57	0.67	mg/mg
K_{s_1}	30	2	mg/l
K_{i_1}	170	17	mg/l
K_2	160	91	mg/l
$\mu_{\max_2}$	0.028	0.3	1/h
Y_2	0.67	0.75	mg/mg
K_{s_2}	350	75	mg/l
K_1	-	66	mg/l
α	1.6	6.7	mg/l

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 a direct search method (Hooke and Jeeves, 1961). This common method is often used for models with large numbers of variables and parameters, e.g. the proposed phenol degradation model has 10 parameters.

Two sets of experiments, experiments (A_1, A_2) and (B_1, B_2, B_3), corresponding to two generation of acclimatized activated sludge were achieved (the A-generation of activated sludge was accidently lost). The difference between the acclimation period, i.e. 6 months for the A-generation and 2 months for the B-generation, explains the difference between the kinetic parameters values used for model simulation (see Table 1). The fact that the kinetic parameters vary according to the *history* of the microorganisms has already been pointed out (Sokol, 1988).

Simulations were obtained by using the general dynamical model given by (3-6) together with the kinetic expressions given by (7-12) and a fourth-order Runge-Kutta algorithm for numerical integration of the ordinary differential equations.

4. MODEL VALIDATION

4.1 Batch cultures

The experimental and model predicted data of phenol (S_1), the metabolic intermediate (S_2) and biomass (X) concentrations in batch cultures (A_1 and A_2 experiments) are illustrated in Figs. 2 to 3. From these results, it can be noticed that the fitting between the model-simulated and the experimental data is satisfactory. In particular, the phenomenon of biomass growth after phenol exhaustion is clearly observed, i.e. after $t = 6$ h for the A_1 experiment (Fig. 2) and after $t = 9$ h for the A_2 experiment (Fig. 3). When phenol is totally removed, the main contribution to the total biomass growth is due to the intermediate degradation as shown by the simulated profiles of this variable. This confirms the choice of a model based on the reaction scheme given by Eqs. (1-2). Notice that the effect of the K_1 inhibitory constant in (9) was not included in the model for the A-batch cultures (see Table 1).

4.2 Fedbatch cultures

An exponential feed profile was chosen for the fedbatch experiments (Ben-Youssef *et al.*, 2004).

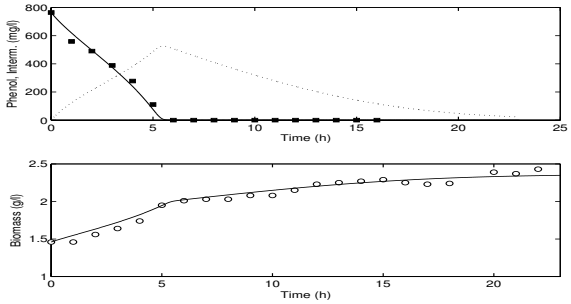

Fig. 2. Batch A_1 experimental and model-simulated phenol, biomass and metabolic intermediate (dotted line) concentrations versus time.

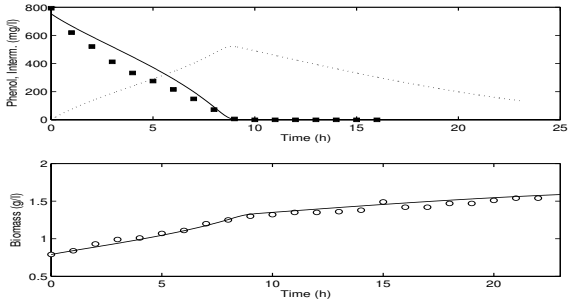

Fig. 3. Batch A_2 experimental and model-simulated phenol, biomass and metabolic intermediate (dotted line) concentrations versus time.

The general expression of the feed flow rate profile is given by:

$$Q_{in}(t) = \begin{cases} 0 & 0 \leq t < t_1 \\ Q_0 e^{a(t-t_1)} & t_1 \leq t \leq t_2 \\ 0 & t_2 < t \leq t_f \end{cases} \qquad (13)$$

Two fedbatch experiments were carried out with two different feeding profiles. The corresponding input parameters of (13) are summarized in Table 2.

Table 2. Parameter values of the fedbatch feed flow rate

Experiment	a	Q_0	t_1	t_2	t_f
Fedbatch F_1	0	350	0	3	6.5
Fedbatch F_2	0.0617	335	0.38	3	6.5

A preliminary batch experiment was necessary to adjust the new set of kinetic parameters and to tune the model (see Fig. 4) for the B-generation of acclimated activated sludge.

The experimental and model-predicted data of phenol, the intermediate and biomass concentrations in fedbatch cultures are illustrated in Fig. 5 and Fig. 6.

The first fedbatch experiment (B_2) was arbitrarily designed with a constant feed flow rate $Q_{in} = 350$ ml/h during 3 hours at a feeding phenol concentration $S_1^{in} = 1036$ mg/h in order to test the ability of the activated sludge to consume approximatively 1 g of phenol. As illustrated by the experimental data plotted in Fig. 5, total phenol degradation was observed whatever the time culture, which is indicative of the substrate-limiting mode of the experiment. The proposed model was able to predict adequately both biomass and phenol concentration evolutions.

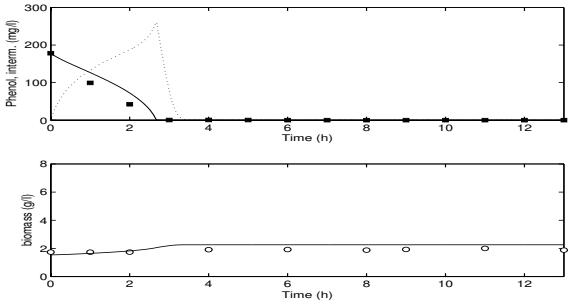

Fig. 4. Batch B_1 experimental and model-simulated phenol, biomass and metabolic intermediate (dotted line) concentrations versus time.

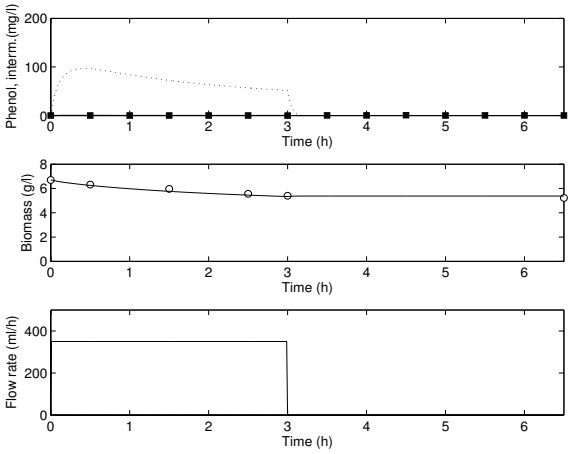

Fig. 5. Fedbatch B_2 experimental and model-simulated evolution: phenol, metabolic intermediate (dotted line) and biomass concentrations; feed flow rate.

The second fedbatch experiment (B_3) was then designed in order to try to switch from phenol degradation under substrate-limitation, as realized in the B_2 experiment, to phenol degradation under substrate-inhibition. However, the principal objective was not only to create inhibitory conditions, which may be easily obtained under high phenol initial and/or high feed flow rate operating conditions, but at the same time to try to perform an inhibitory experiment close to the unstable operating point corresponding to the transition between the limitation and inhibition branches. The *a priori* estimation of the B_3 experiment operating conditions that may satisfy our objective was made by a predictive simulation study using the available proposed phenol degradation model.

The chosen strategy consisted in increasing the initial phenol concentration up to 125 mg/l, then starting a short culture in batch mode during 23 min. (e.g. until the intermediate accumulates enough) and finally activating the fedbatch mode with a slightly different feed flow profile than that used in the B_2 experiment.

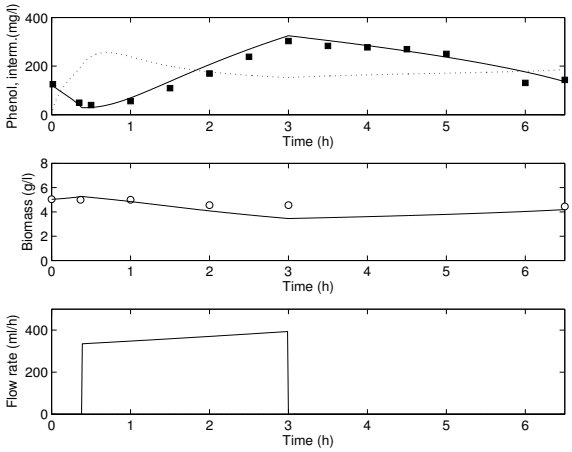

Fig. 6. Fedbatch B_3 experimental and model-simulated evolution: phenol, metabolic intermediate (dotted line) and biomass concentrations; feed flow rate.

The model predictions were accurate as shown in Fig. 6 for both phenol and biomass concentrations. From these figures, it is interesting to note how, despite the slight difference between the two fedbatch policy, higher levels of intermediate concentrations were reached for the B_3 fedbatch experiment. This is in complete accordance with the proposed model structure, which predicts a higher inhibition effect on phenol biodegradation due to the intermediate accumulation.

5. CONCLUSIONS

The main scope of this paper was to derive a reliable new kinetic model for a phenol biodegradation process using acclimatized activated sludge in batch and fedbatch cultures. While the conventional Haldane model appeared to be inaccurate, the explicit integration of the main metabolic intermediate production kinetics in the new model allowed the obtention of adequate fitting results between the experimental and simulated data. The complete model has been experimentally validated for the entire set of cultures under different initial concentrations of biomass and phenol. The model predictions allowed the design of substrate-limitation and substrate-inhibition fedbatch experiments that may be useful to design fedbatch strategies with improved productivity for phenol biodegradation systems.

6. ACKNOWLEDGMENTS

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