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# Modelling phenol biodegradation by activated sludges evaluated through respirometric techniques

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**Abstract**

In this paper respirometric techniques were used to study the effect of pH, phenol and dissolved oxygen (DO) concentrations on the phenol biodegradation kinetics by activated sludges. In addition, a mathematical model was developed to interpret the obtained respirometric curves.

Closed respirometer experiments showed that phenol inhibited the respiration rate of unacclimated sludges. On the contrary, oxygen uptake rate (OUR) of phenol acclimated sludges exhibiting the typical Haldane's substrate inhibition curve. The Monod equation adequately represented the relation between OUR of acclimated biomass and DO concentration. Within the tested pH range (4–12) the oxygen saturation coefficient was independent of pH. On the contrary, the maximum OUR was strongly affected by the pH, being its maximum between 9.5 and 10.5. Open respirometer experiments shows that as pH decreased from 10.2 to 5.8, the maximum OUR also decreased, in accordance with the trend observed in the closed respirometer experiments. Although the respiration rate of phenol degrading bacteria was strongly affected by pH, a constant phenol oxidation coefficient was observed within the studied pH interval. A mathematical model was proposed to interpret the open respirometry curves. The coefficients of the model were estimated using both pseudo steady state and dynamic conditions for different biomass concentrations. The model adequately predicted the whole OUR and DO profiles as a function of time during the biodegradation of phenol under different DO conditions. The mathematical model proposed in the present work is useful for predicting transient responses such as substrate concentration and DO concentrations as a function of time in bioreactors treating phenolic wastewaters under an overload of phenolic compounds.


*Keywords:* Oxygen uptake rate; Phenol; Biodegradation; Kinetic models; Respirometry

## 1. Introduction

Phenol, phenolic compounds and its derivatives are major environmental pollutants from industrial processes such as petroleum processing plants, oil refineries, coke oven and pharmaceutical industries. The largest use of phenol is as an intermediate in the production of phenolic resins, synthetic fibers and for epoxy resin precursors. Besides, phenol is naturally present in some foods, in human and animal wastes, in decomposing organic material, and is produced endogenously from the metabolism of protein [1].

The presence of phenols in the environment poses a significant risk to aquatic biota; phenol is lethal to fish at low concentrations of 5–25 mg $L^{-1}$, for example. As environmental regulations become more restrictive, innovative treatments of wastewaters containing phenols must be considered. Phenols can be removed from industrial effluents by physicochemical methods such as ozonation, Fenton's reagent, UV or hydrogen peroxide [2,3], but these treatments are usually complex and expensive. For these reasons, the interest in the use of biological methods is increasing. Many aerobic bacteria are capable to use aromatic compounds as the sole carbon and energy source. Although both aerobic and anaerobic microorganisms are able to degrade phenol, aerobic processes are preferred. A typical pathway for metabolizing phenol is to hydroxylate the ring to form catechol and then to open the ring through

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*ortho* or *meta* oxidation [4]. Batch and semicontinuous [5,6] processes employing both suspended and immobilized cultures [7–9] were used to study the phenol degradation kinetics. Most of these studies were focused on the effect of the initial phenol concentration on the aerobic phenol degradation kinetics [5,10–12]. Only a few studies cover other environmental factors such as temperature, additional carbon sources, and salinity [13,14].

Since the aerobic phenol biodegradation is preferred to the anaerobic pathway, the dissolved oxygen (DO) concentration may be a limiting factor of the treatment process [15]. Melo et al. [16] studied the phenol degradation in an aerobic rotating biological contactor. Those authors found that the phenol degradation rate improved by increasing the rotation speed due to the increase of the oxygen mass transfer coefficient; thus, the process efficiency was limited by the DO availability. In addition, other environmental factors, such as pH, may decrease the efficiency of the biotreatment. For example, Alva and Peyton [17] reported that although *Halomonas campisalis* can completely degrade phenol and catechol when the pH range between 8 and 11, the metabolic intermediates *cis,cis*-muconate and (+)-mucunolactone are accumulated at pH 8 and 9. Magbanua and Bowers [18] studied the production of soluble microbial products (SMPs) by activated sludge receiving a mixed feed of phenol and glucose; those authors found that at pH ranging between 6.5 and 7.5, about 10–20% of the phenol carbon appeared in the SMPs. Thus, from an environmental perspective, the accumulation of soluble microbial products may decrease the overall degradation process due to the increase of the soluble COD of the effluent.

The utilization of biological processes for purification of wastewaters containing toxic organic compounds emphasizes the practical requirement for developing adequate mathematical models to be used for the design and operation of these processes. The knowledge of microbial substrate utilization kinetics is important for the accurate prediction of the quality of the treatment process effluent. Accurate kinetic parameters also help engineers to optimize operational conditions such as pH, substrate and dissolved oxygen concentrations, in order to meet discharge requirements minimizing the operational costs.

In this paper phenol was chosen to represent organic toxic wastewaters. The objectives of the present work were (i) to study the effect of the initial phenol concentration, pH, and dissolved oxygen concentration on the phenol biodegradation kinetics of phenol acclimated and unacclimated mixed cultures from activated sludge reactors, and (ii) to propose a mathematical model to interpret the obtained results.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Phenol (loose crystals, >99%) was obtained from Sigma (St. Louis, MO, USA). All inorganic salts were commercial products of reagent grade from Anedra (San Fernando, Argentina). Dehydrated cheese whey was from Food S.A. (Villa Maipú, Argentina).

### 2.2. Activated sludge and culture conditions

Activated sludges used in this study were cultured in two laboratory scale (2.5 L) cylindrical semicontinuous reactors. Hydraulic retention time was 24 h; a solid retention time of 60 days was maintained by direct wastage. Aeration was provided at the bottom of the reactor through an air-stone using an air pump at 2 L $min^{-1}$; in both cases the DO concentration was maintained above 5 mg $L^{-1}$. One reactor was fed with a synthetic wastewater with the following composition [19]: dehydrated cheese whey 1500 mg, $(NH_4)_2SO_4$ 940 mg, and $NaHCO_3$ 1030 mg; all the components were diluted in 1 L of tap water. Phenol acclimated biomass was obtained feeding the other reactor with a culture medium with phenol as the sole carbon-limiting source; its composition was the following [5]: $(NH_4)_2SO_4$ 227 mg, $MgSO_4{\cdot}7H_2O$ 25 mg, $MnSO_4{\cdot}H_2O$ 2.5 mg, $FeCl_3$ 2 mg, $CaCl_2{\cdot}2H_2O$ 1.2 mg, $KH_2PO_4$ 250 mg, $K_2HPO_4$ 500 mg, phenol 300 mg; all the components were diluted in 1 L of tap water. The final pH was adjusted to $7.0 \pm 0.05$ by adding a few drops of concentrated solutions of NaOH or ClH. The acclimated biomass was obtained by replacing gradually the cheese whey medium by the phenol medium to reach 300 mgPhenol $L^{-1}$. The reactor performance was monitored periodically by measurements of total and soluble chemical oxygen demand (COD) and phenol concentrations. The biomass was considered acclimated when phenol and biomass concentrations at the end of each fill and withdraw cycle was constant at least for 1 week.

### 2.3. Analytical procedures

Biomass concentration was estimated as the difference between total and soluble COD [20]. COD measurements were performed using a commercial test (Hach Cat. No. 21259). Samples digestion (2 h, 150 °C) were performed in a Hach COD Reactor 45600; a Hach DR 2000 photometer was used for the absorbance determination step. Phenol concentration was measured using the Folin–Ciocalteu reactive [8]. When a steady state was reached, mixed liquor samples were used as a source of biomass used in all the experiments.

### 2.4. Closed respirometer experiments

The effects of initial phenol concentration, pH and DO limitation on the oxygen uptake rate (OUR) of both phenol acclimated and unacclimated activated sludges were analyzed using a closed respirometer [21]. The closed respirometer allows studying a large number of experimental conditions because this technique consumes small sample volumes and the assay requires less than 10 min.

The closed respirometer consisted in a 30 mL glass vessel maintained at $25 \pm 0.5$ °C by means of a water bath. The vessel was filled with the tested sample and air was supplied until oxygen saturation level was reached; then, the vessel was sealed with

the insertion of a polarographic DO probe (YSI model 5739). The sample was continuously stirred with a magnetic stir-bar and the decay of the DO concentration as a function of time was recorded. Data were acquired with a computer interfaced to the DO monitor (YSI model 58) at 1 data/s.

Ten millilitres of both acclimated or unacclimated activated sludge samples were adjusted to a desired pH level by adding a few drops of concentrated NaOH or $H_2SO_4$ solutions. A pulse of a concentrated phenol solution (oxidizable substrate) was added and a phosphate buffer (1 mM) with the desired pH level was used to complete 25 mL; then, the mixture was placed in the closed respirometer vessel. In the experiments performed to study the effect of initial phenol concentration on the OUR, a pH 7.0 was selected and the tested initial phenol concentrations ranged between 0 and 4000 mgPhenol $L^{-1}$. In addition, to avoid oxygen limitations on OUR, all the measurements were performed at a DO level ranging between 6 and 8 mg$O_2$ $L^{-1}$. When the effect of pH on OUR was studied, pH ranged from 4 to 12; in this case the initial phenol concentration was 150 mgPhenol $L^{-1}$. The tested mixtures were aerated for 10 min to reach DO saturation, then the aeration was turned off and the drop of DO was monitored. The total OUR ($OUR_T$) was calculated using a moving regression window over five data using the software Sigma Plot 9.0.

### 2.5. Open respirometer experiments

The effects of pH and DO concentration on the phenol degradation kinetics using acclimated biomass were assessed using an open (flowing gas/static liquid) respirometer [22]. This technique allows obtaining the kinetic and stoichiometric coefficients that describe the aerobic biodegradation of the tested compounds. However, larger sample volumes and longer assay times were required for this technique in comparison to the closed respirometer.

The open respirometer consisted in a 500-mL working volume reactor with temperature control (25 ± 0.5 °C) in which the sample was placed. The sample was stirred with a magnetic stir-bar and it was continuously aerated at the bottom of the respirometer using an air pump. Air was set to a stable flow rate (1.0 L $min^{-1}$) using a high-precision rotameter (Bruno Schilling model MB 60 V, Argentina). The DO concentration ($C$) as a function of time ($t$) was recorded at 12 data/min by means of the DO probe and monitor as was described in the previous section. Before each phenol pulse, the oxygen mass transfer coefficient ($K_La$) and the endogenous OUR ($OUR_e$) were obtained using a non-steady state procedure [23]. The exogenous OUR associated to the phenol oxidation ($OUR_H$) was calculated from the DO mass balance in the reactor:

$$OUR_H = K_La(C_S - C) - OUR_e - \frac{dC}{dt} \quad (1)$$

where $C_S$ and $C$ are the saturation and the instantaneous DO concentrations, respectively.

The phenol oxidation coefficient ($Y_{O/S}$), which represents the amount of oxygen consumed per unit of substrate (phenol) oxidized, was calculated as follows:

$$Y_{O/S} = \frac{\int OUR_H \, dt}{S_0} \quad (2)$$

where $S_0$ is the initial phenol concentration (mgPhenol $L^{-1}$).

Five hundred milliliters of activated sludge samples were poured into the open respirometer. Then, the pH was adjusted to a desired value by adding a few drops of concentrated NaOH or $H_2SO_4$ solutions. When a stable DO concentration was observed, a pulse of phenol was added and the respirogram was recorded. In these experiments the phenol concentration ($S_0$) was 50 mgPhenol $L^{-1}$ and biomass concentration ($X$) ranged between 400 and 5200 mgCOD $L^{-1}$.

### 2.6. Numerical simulations and coefficient estimation

All numerical simulations and coefficients estimation were performed using the software package Gepasi 3 [24]. The optimization method used was the Multistart (with Levenberg–Marquardt local optimization) algorithm. Multistart is a hybrid stochastic–deterministic optimization method. Rather than running a simple local optimization (like the gradient descent method, for example), multistart method runs several of them, each time starting from a different initial guess. The first run takes as the initial guess the parameter values entered by the user. Then, the initial guesses for the subsequent runs are generated randomly within the boundaries of the adjustable parameters. The local optimizer used is the Levenberg–Marquardt method as this has proved to be the most efficient gradient optimizer used in Gepasi [24].

## 3. Results and discussion

### 3.1. Closed respirometer experiments

#### 3.1.1. Effect of initial phenol concentration on the OUR of unacclimated and acclimated biomass

Closed respirometer experiments were performed to evaluate the effect of the initial phenol concentration on the total OUR of phenol unacclimated and acclimated activated sludges. The obtained total OUR values ($OUR_T$) represented the sum of the exogenous ($OUR_H$) and endogenous ($OUR_e$) respiration rates. However, within the experimental conditions used in this work the values corresponding to the endogenous OUR (2–15 mg$O_2$ $L^{-1}$ $h^{-1}$) were much lower than the exogenous OUR (100–170 mg$O_2$ $L^{-1}$ $h^{-1}$). Fig. 1 shows the effect of the initial phenol concentration on the $OUR_T$ of unacclimated and acclimated activated sludges. Phenol was inhibitory in the case of unacclimated biomass; the $OUR_T$ values decreased from 12 mg$O_2$ $L^{-1}$ $h^{-1}$ (endogenous level, without phenol) to 3 mg$O_2$ $L^{-1}$ $h^{-1}$ as the phenol concentration increased. On the contrary, the respiration of acclimated biomass increased with respect to the initial value (endogenous OUR) within the tested phenol concentrations.

The plot of $OUR_T$ of acclimated biomass as a function of the initial phenol concentration exhibited the typical substrate inhi-

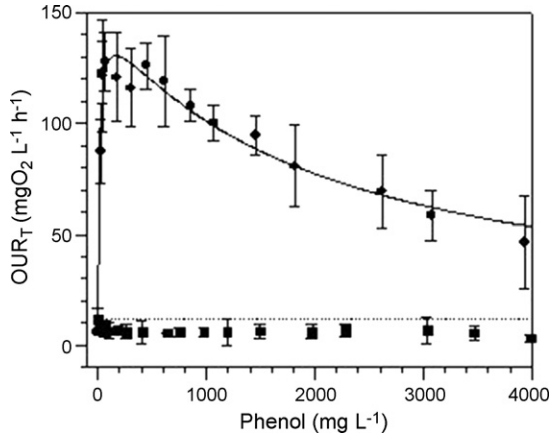


Fig. 1. Effect of the initial phenol concentration on the total OUR corresponding to unacclimated (■), and phenol acclimated (●) activated sludges. Data were obtained with the closed respirometer. Continuous line indicates the Haldane's model (Eq. (3)). Dotted line indicates the initial OUR value. In these tests pH 7.0. Bars indicate the standard deviation corresponding to two replicates.

bition curve (Fig. 1). The inhibition of phenol biodegradation in pure and mixed cultures at high phenol concentrations is well known [11] and it was associated with the hydrophobic perturbation of the microorganisms' membrane [25]. Among the various substrate inhibition models, the Haldane's equation has been used extensively to describe phenol biodegradation. Assuming that $OUR_e$ is constant with respect to the phenol concentration ($S$), $OUR_T$ of phenol acclimated biomass can be represented as follows:

$$OUR_T = OUR_e + OUR_{Hm}\frac{S}{K_S + S + S^2/K_i} \quad (3)$$

where $OUR_{Hm}$ is the maximum exogenous respiration rate, $K_S$ the half-saturation coefficient, and $K_i$ the inhibition coefficient. When $K_i$ is very large the Haldane's equation simplifies to the Monod equation; in this context, $K_S$ is usually defined as the substrate concentration at which the measured activity (OUR in the present work) is equal to half of the maximum activity. If the substrate is inhibitory then it is not possible to observe the maximum $OUR_T$; thus, $K_S$ takes on an hypothetical meaning. It can be demonstrated that the substrate concentration ($S^*$) corresponding to the actual $OUR_T$ maximum can be calculated as follows:

$$S^* = \sqrt{K_S K_i} \quad (4)$$

Eq. (3) was fitted to the experimental data using a non-linear regression (Sigma Plot 9.0) to obtain the following values: $OUR_{Hm} = 146 \pm 8\ mgO_2\ L^{-1}\ h^{-1}$, $K_S = 14 \pm 4\ mgPhenol\ L^{-1}$, and $K_i = 1900 \pm 300\ mgPhenol\ L^{-1}$; from Eq. (4), $S^*$ was computed as $164 \pm 27\ mgPhenol\ L^{-1}$.

The obtained coefficients were in the range of those reported by other authors; however, there is a broad range of these biokinetic constants reported in the literature, even when pure cultures were studied. For example, Nuhoglu and Yalcin [5] investigated the phenol biodegradation in a batch reactor using mixed cultures; those authors reported the following kinetic constants of the Haldane equation: $K_S = 87\ mgPhenol\ L^{-1}$, and $K_i = 107\ mgPhenol\ L^{-1}$; from these data $S^*$ was computed as $97\ mgPhenol\ L^{-1}$. The biodegradation of phenol using *Bacillus brevis* was studied by Arutchelvan et al. [12]; in this case, the reported kinetic constants were $K_S = 2.2–29.31\ mgPhenol\ L^{-1}$, and $K_i = 868–2434.7\ mgPhenol\ L^{-1}$. Kumar et al. [11] studied the biodegradation of phenol by *Pseudomonas putida* (MTCC 1194) finding the following kinetic coefficients: $K_S = 36\ mgPhenol\ L^{-1}$, $K_i = 130\ mgPhenol\ L^{-1}$, and $S^* = 68\ mgPhenol\ L^{-1}$. In addition, those authors reviewed several studies related to phenol biodegradation demonstrating that reported $K_S$ and $K_i$ values in the literature were in the range 0.015–53.9 $mgPhenol\ L^{-1}$, and 106–934 $mgPhenol\ L^{-1}$, respectively. The variability of the reported coefficients may be due to several factors such as inoculum history (acclimation), the culture system used in the study (batch or continuous), changes in the predominating microbial species during the assays, techniques used to evaluate the inhibition (microbial growth or respirometry), and environmental factors (initial phenol concentration, pH, temperature).

### 3.1.2. *Effect of pH on the oxygen uptake kinetics of phenol acclimated biomass*

Closed respirometer experiments were performed to evaluate the effect of pH on the oxygen uptake kinetics of the phenol acclimated biomass. Fig. 2 shows a typical curve of the oxygen consumption (Fig. 2a) and the corresponding total oxygen

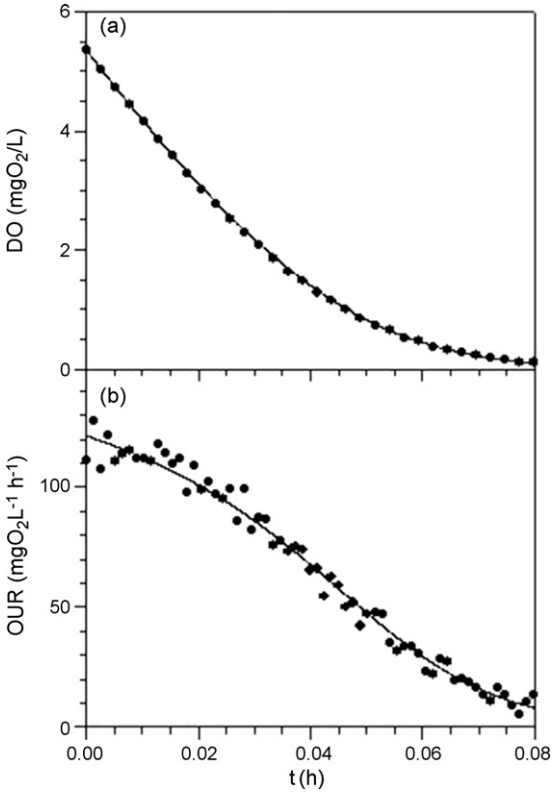


Fig. 2. (a) Dissolved oxygen (DO) concentration and (b) the corresponding oxygen uptake rate (OUR) as a function of time in a typical closed respirometer experiment of phenol ($S_0 = 150\ mgPhenol\ L^{-1}$) biodegradation using acclimated biomass ($X = 765\ mgCOD\ L^{-1}$); in this example pH 7.1.

Table 1
Oxygen saturation coefficient ($K_O$) and phenol oxidation coefficient ($Y_{O/S}$) corresponding to phenol acclimated biomass measured at different pH values

| pH | $K_O$ ($mgO_2\,L^{-1}$)[a] | $Y_{O/S}$ ($mgO_2\,mgPhenol\,L^{-1}$)[b] |
|---|---|---|
| 4.85 | $1.33 \pm 0.59$ | n.d. |
| 5.80 | $1.49 \pm 0.37$ | $0.78 \pm 0.03$ |
| 6.40 | $2.24 \pm 0.10$ | $0.79 \pm 0.01$ |
| 7.00 | $2.10 \pm 0.06$ | $0.76 \pm 0.03$ |
| 7.80 | $1.76 \pm 0.02$ | $0.75 \pm 0.05$ |
| 8.50 | $1.71 \pm 0.49$ | n.d. |
| 9.60 | $2.28 \pm 0.85$ | n.d. |
| 10.20 | n.d. | $0.75 \pm 0.01$ |
| Overall | $1.84 \pm 0.34$ | $0.77 \pm 0.02$ |

Coefficient values are expressed as mean ± 95% confidence interval. n.d.: not determined.
[a] Obtained from closed respirometer experiments.
[b] Obtained from open respirometer experiments.

uptake rate (OURT) (Fig. 2b) as a function of time. In these experiments the initial phenol concentration was $150\,mg\,L^{-1}$, close to the optimum concentration determined in the previous section ($S^* = 164\,mg\,L^{-1}$). For phenol concentrations that were neither inhibitory nor limiting to the oxygen uptake rate, such as those used in the above mentioned experiments, it was accepted a Monod-type relationship between $OUR_T$ and the dissolved oxygen concentration ($C$) [7]:

$$OUR_T = OUR_m \left( \frac{C}{K_O + C} \right) \quad (5)$$

where $OUR_m$ ($mgO_2\,L^{-1}\,h^{-1}$) is the maximum total oxygen uptake rate, and $K_O$ ($mgO_2\,L^{-1}$) the oxygen saturation coefficient. Eq. (5) was rearranged to obtain the following expression:

$$\frac{C}{OUR_T} = \frac{K_O}{OUR_m} + \frac{C}{OUR_m} \quad (6)$$

Eq. (6) was used to estimate the coefficients $OUR_m$ and $K_O$ corresponding to each experimental condition. Table 1 shows that the obtained values corresponding to the oxygen saturation coefficient ($K_O$) were independent of pH within the tested range; thus, an overall mean $K_O$ value of $1.84 \pm 0.34\,mgO_2\,L^{-1}$ was calculated. Literature data with respect to the value of $K_O$ of phenol degrading bacteria are scarce. Etzensperger et al. [15] studied the phenol degradation in a three-phase biofilm fluidized sand bed reactor and these authors reported a value for $K_O = 0.5\,mgO_2\,L^{-1}$.

The effect of pH on the $OUR_m$ is shown in Fig. 3; in this case the results were expressed as a fraction of the obtained maximum value (normalized $OUR_m$) to compare independent experiments. Fig. 3 shows that the respiration rate was strongly affected by the pH, being the maximum OUR value at pH values ranging between 9.5 and 10.5. When acidic conditions were tested the respiration rate decreased due to the toxicity of the protonated phenol species; in addition, a sharp decrease of the respiration rate was observed when the pH was higher than 10.5. These results are in agreement with other authors. Alva and Peyton [17] reported that *H. campisalis* degraded phenol and catechol when the pH ranged between 8 and 11. Kulkarni and Chaudhari [26] reported that the toxicity of *p*-nitrophenol on pure cultures

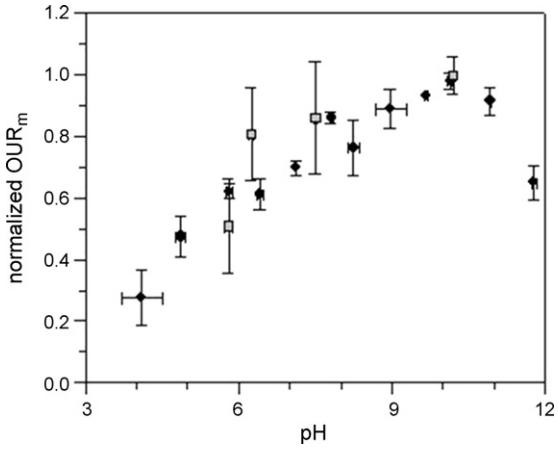


Fig. 3. Effect of pH on the respiration rate of phenol degrading bacteria. (●) Closed respirometer data and (■) open respirometer data. Bars indicate the standard deviation corresponding to two replicates at least.

of *P. putida* decreased in alkaline conditions. Arutchelvan et al. [12] found that the most favorable pH for *B. brevis* to achieve the maximum rate of phenol degradation was around 8.0.

### 3.2. Open respirometer experiments

#### 3.2.1. Effect of pH on the oxygen uptake kinetics of phenol acclimated biomass

Open respirometer experiments were carried out to study the effect of pH on the maximum respiration rate and phenol oxidation coefficient ($Y_{O/S}$). Fig. 4 shows several examples of DO curves (Fig. 4a) and the corresponding $OUR_H$ values (Fig. 4b) as a function of time obtained at different pH values. For pH 10.3, when a pulse of $50\,mgPhenol\,L^{-1}$ was added ($t = 0$), the DO dropped to quite low values due to the increase of the respiration rate. The $OUR_H$ increased progressively towards its maximum value; this transient period corresponded to the phenomenon called biological start-up [27]. After the transient period, the $OUR_H$ reached a maximum value and then it decreased to a pseudo steady-state value due to a DO limitation condition. This peak in the OUR profile due to an oxygen limitation was also reported by Guisasola et al. [21] with respect to the respiration rate of nitrifying bacteria. Fig. 4 shows that as the pH decreased from 10.2 (considered close to the optimum pH) to 5.8, the maximum $OUR_H$ decreased. Thus, the minimum concentration of DO increased (non-limited DO conditions were obtained) and the peak in the $OUR_H$ became smaller. In addition, the normalized OUR obtained using the open respirometer decreased as the pH decreased, in accordance with the results obtained previously in the closed respirometer experiments (Fig. 3).

For each pH studied, the phenol oxidation coefficient ($Y_{O/S}$) was calculated using Eq. (2). Although the respiration rate of phenol degrading bacteria was strongly affected by pH (Figs. 3 and 4), $Y_{O/S}$ was almost constant within the studied pH interval (Table 1). The overall mean $Y_{O/S}$ value = $0.77 \pm 0.02\,mgO_2\,L^{-1}$ was in accordance with those reported by other authors that employed respirometry to study the aerobic phenol biodegradation. For example, Chudoba et

al. [28] reported a substrate oxidation coefficient ($Y_{O/S}$) of $0.88 \pm 0.08\ mgO_2\ mgPhenol^{-1}$. Watanabe et al. [29] reported a value for $Y_{O/S}$ close to $0.68\ mgO_2\ mgPhenol^{-1}$. Orupold et al. [7] studied an activated sludge acclimated to phenolic compounds finding $Y_{O/S} = 0.87 \pm 0.12\ mgO_2\ mgPhenol^{-1}$. However, it should be noted that $Y_{O/S}$ values obtained by using other techniques, such as carbon and reduction degree balances applied to the biomass growth and phenol consumption, ranging between 1.02 and $1.80\ mgO_2\ mgPhenol^{-1}$ [11,14,17,30]. Taking into account that typical time scales for such experiments are about a few days and respirometric tests last only a few hours, these differences suggest that estimations of $Y_{O/S}$ using respirometric techniques may reflect a partial oxidation of the phenol.

### 3.2.2. Effect of DO limitation on the oxygen uptake kinetics of phenol acclimated biomass

The obtained results demonstrated that pH affected the kinetics of the biological phenol oxidation via the $OUR_m$. However, $K_O$ and $Y_{O/S}$ did not depend on pH values within the tested range. In order to study the effect of the DO on the aerobic phenol biodegradation, different biomass concentrations were tested to obtain different conditions of DO limitation. Fig. 5 shows several DO curves (Fig. 5a) and the corresponding $OUR_H$ values (Fig. 5b) obtained at pH 7.5 with different concentrations of acclimated biomass ($X$). Fig. 5 shows that in the case of a high biomass concentration ($X = 2900\ mgCOD\ L^{-1}$) the $OUR_H$ exhibited the above-mentioned sharp peak due to a DO limitation; however, the peak tended to disappear with lower biomass concentrations. This effect can be explained considering that in these experiments $K_La$ was maintained as a constant; thus, the higher the biomass concentration, the higher the DO limitation.

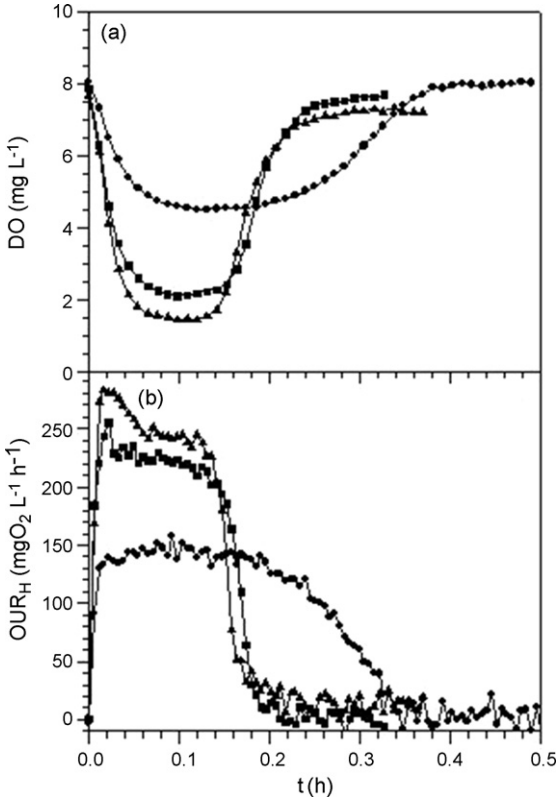


Fig. 4. Effect of pH on the open respirometry curves: (a) dissolved oxygen (DO) concentration; (b) heterotrophic oxygen uptake rate ($OUR_H$). (●) pH 5.8, (■) pH 7.5, and (▲) pH 10.3. In all cases biomass concentration = 1800 mgCOD $L^{-1}$, initial phenol concentration = 50 mgPhenol $L^{-1}$ and $K_La = 39\ h^{-1}$.

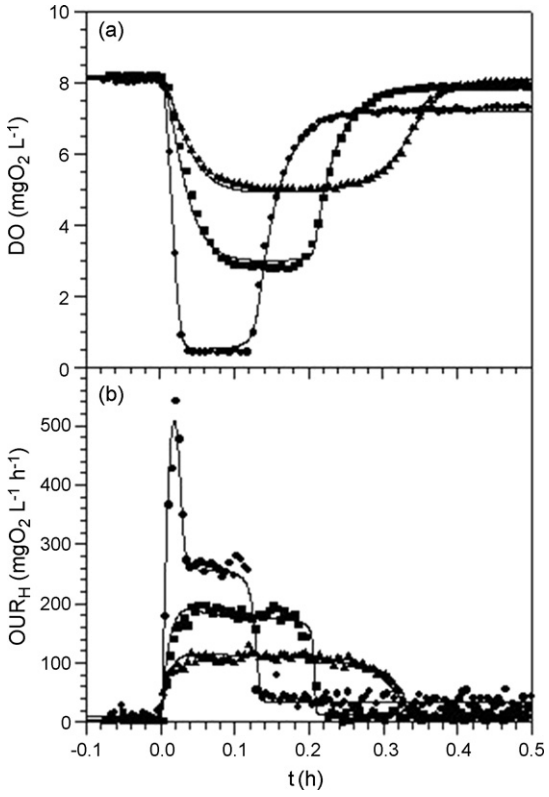


Fig. 5. Effect of biomass concentration ($X$) on the open respirometry curves: (a) dissolved oxygen (DO) concentration; (b) heterotrophic oxygen uptake rate ($OUR_H$). (●) $X = 2900\ mgCOD\ L^{-1}$; (■) $X = 780\ mgCOD\ L^{-1}$; (▲) $X = 460\ mgCOD\ L^{-1}$. In all cases pH 7.5 and $K_La = 33\ h^{-1}$. Lines indicate the proposed model (Eqs. (7) and (8)).

## 3.3. Modeling the biological phenol oxidation by acclimated biomass as affected by DO and phenol concentrations

### 3.3.1. Model development

In general, the Haldane's equation is used to represent kinetics of biological phenol degradation [10,11,31]. However, phenol can be considered as a non-inhibitory compound for a phenol acclimated activated sludge [12]. Besides, Fig. 1 shows that at low initial phenol concentration such as the tested in the open respirometry experiments (50 mgPhenol $L^{-1}$) the inhibition term in the Haldane's model can be neglected obtaining the Monod equation. In these conditions, the respirometric curves can be modeled using the following expressions:

$$\frac{dC}{dt} = K_La(C_S - C) - \left[q_e + q_{Hm}\left(\frac{S}{K_S + S}\right)\right]\left(\frac{C}{K_O + C}\right) X\left(1 - e^{-t/tau}\right) - \Delta OUR\left(\frac{K_S}{K_S + S}\right) \quad (7)$$

$$\frac{dS}{dt} = -\frac{q_{Hm}}{Y_{O/S}}\left(\frac{S}{K_S + S}\right)\left(\frac{C}{K_O + C}\right)X\left(1 - e^{-t/tau}\right) \quad (8)$$

where $C_S$ and $C$ are the saturation and the instantaneous DO concentrations respectively ($mgO_2\,L^{-1}$), $S$ is the phenol concentration ($mgPhenol\,L^{-1}$), $q_e$ is the specific endogenous OUR ($mgO_2\,mgCOD^{-1}\,h^{-1}$), $q_{Hm}$ is the specific maximum oxygen uptake rate corresponding to the phenol oxidation ($mgO_2\,mgCOD^{-1}\,h^{-1}$), $Y_{O/S}$ is the substrate oxidation coefficient ($mgO_2\,mgPhenol^{-1}$), $K_O$ is the DO saturation coefficient ($mgO_2\,L^{-1}$), $K_S$ is the phenol saturation coefficient ($mgPhenol\,L^{-1}$), and $X$ is the biomass concentration ($mgCOD\,L^{-1}$). The last term in the oxygen mass balance (Eq. (7)) was introduced to take into account that OUR value after the complete substrate depletion was slightly higher than the OUR value after the pulse of substrate ($OUR_e$); thus, $\Delta OUR$ represents the difference between $OUR_e$ and the final OUR level [32]. This effect might reflect the oxidation of metabolic intermediates such as catechol, 2-hydroxymuconic semialdehyde or *cis,cis*-muconate, for example [5,17]. Assuming that the oxidation of the metabolic intermediates starts when the phenol concentration is low, the switching function $K_S/(K_S + S)$ proposed by Pratt et al. [32] was included. To describe the biological start-up phenomenon, a first order correction factor ($1 - e^{-t/tau}$) as proposed by Coen et al. [33] was included; in all the calculations of the present paper tau = 42 s was used. This correction factor represents the active biomass fraction that is capable of consuming oxygen.

### 3.3.2. Pseudo steady-state (PSS) approximation for the estimation of model coefficients

Kinetic and stoichiometric coefficients of the proposed model can be obtained fitting Eqs. (7) and (8) to the DO profiles. However, this procedure yielded high errors in some of the estimated coefficients. Thus, in order to reduce these errors a pseudo steady-state approximation was used. Fig. 5 shows that after the transient start-up period ($t \gg$ tau), DO concentrations and OUR values remained stable until the substrate depletion; within this interval a pseudo steady-state with no substrate limitation ($S \gg K_S$) was reached. In this condition Eq. (7) yields the following:

$$\frac{dC}{dt} = K_L a(C_S - \bar{C}) - [q_e + q_{Hm}]\left(\frac{\bar{C}}{K_O + \bar{C}}\right)X = 0 \quad (9)$$

where $\bar{C}$ is the pseudo steady-state DO concentration. The Eq. (9) was rearranged to obtain the following expression:

$$H = \frac{X\bar{C}}{K_L a(C_S - \bar{C})} = \frac{K_O}{q_{O_2}} + \frac{\bar{C}}{q_{O_2}} \quad (10)$$

where $q_{O_2}$ is the total ($q_e + q_{Hm}$) specific oxygen uptake rate, and $H$ is a combination of variables. Measuring the pseudo steady-state DO concentration ($\bar{C}$) for different $K_L a$ and/or $X$ values, the coefficients $K_O$ and $q_{O_2}$ were obtained by plotting the combined variable $H$ as a function $\bar{C}$. In this work constant agitation and aeration conditions (constant $K_L a$) were used and the different $\bar{C}$ values were obtained by testing different biomass concentrations (Fig. 6). However, for each run $K_L a$ was measured in order to take into account the effect of the biomass concentration.

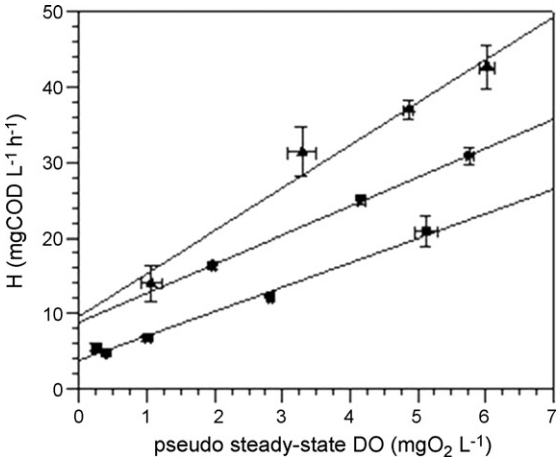


Fig. 6. The combined variable $H$ defined in Eq. (10) as a function of the pseudo steady-state dissolved oxygen (DO) concentration for three different independent experiments (●) A, (■) B, (▲) C, to obtain the total specific oxygen uptake rate ($q_{O_2}$) and the oxygen saturation coefficient ($K_O$). Regression results are shown in Table 2. Bars indicate the standard deviation corresponding to two replicates.

Table 2 shows that the values of $K_O$ obtained using the PSS approximation were in agreement with the values estimated in the closed respirometer experiments (Table 1). With regard to the total specific oxygen uptake rate ($q_{O_2}$), there is a broad range of reported values in the literature. Yoong et al. [6] found $q_{O_2}$ values ranged from 0.02 to 0.04 $mgO_2\,mgCOD^{-1}\,h^{-1}$ corresponding to activated sludge samples grown in a sequencing batch reactor. Orupold et al. [7] studied the bio-oxidation of phenol on activated sludge using OUR measurements finding $q_{O_2}$ values around 0.045 $mgO_2\,mgCOD^{-1}\,h^{-1}$. Feitkenhauer et al. [30] studied the phenol degradation by pure cultures of *Bacillus thermoleovorans*; based on the reported kinetics and carbon balance equations, the estimation for the $q_{O_2}$ value ranged from 0.79 to 2.40 $mgO_2\,mgCOD^{-1}\,h^{-1}$. Peyton et al. [14] studied the kinetics of phenol biodegradation by halophilic mixed cultures; based on the reported kinetic and stoichiometric data, the calculated $q_{O_2}$ values ranged between 0.25 and 0.80 $mgO_2\,mgCOD^{-1}\,h^{-1}$. It should be noted that up to the present there are no techniques to evaluate the fraction of biomass that actually is involved in the degradation of a specific compound. Several authors assume that all bacteria in the activated sludge sample are capable of degrading all compounds [7]. This assumption was adopted in this work; consequently, our results underestimate the values of $q_{O_2}$ because of the overestimation of the phenol-consuming bacteria that are present in the tested activated sludge samples.

### 3.3.3. Kinetic and stoichiometric coefficient determination using dynamic conditions (Dyn)

The software package Gepasi 3 [24] was used to estimate the kinetic coefficients $K_S$, $q_{Hm}$ and $Y_{O/S}$ of the proposed model. In order to reduce computational effort and fitting errors, $K_O$ values obtained by the PSS approximation (Table 2) were introduced in the model. Eqs. (7) and (8) were fitted to DO concentration values as a function of time for the different initial biomass concentrations. Fig. 5 shows several examples demonstrating

Table 2
Phenol biodegradation kinetic coefficients of the proposed model obtained for three independent open respirometer experiments (A, B, C)

| Method | Coefficients | Experiment | | |
|---|---|---|---|---|
| | | A | B | C |
| PSS | $K_O$ ($mgO_2\,L^{-1}$) | $2.30 \pm 0.20$ | $1.34 \pm 0.11$ | $1.41 \pm 0.33$ |
| | ($q_{O_2}$) ($mgO_2\,mgCOD^{-1}\,h^{-1}$) | $0.26 \pm 0.01$ | $0.32 \pm 0.02$ | $0.17 \pm 0.01$ |
| | $r^2$ | 0.9917 | 0.9854 | 0.9589 |
| Dyn | $K_S$ ($mgPhenol\,L^{-1}$) | $1.09 \pm 0.04$ | $0.43 \pm 0.11$ | $0.96 \pm 0.01$ |
| | $q_{Hm}$ ($mgO_2\,mgCOD^{-1}\,h^{-1}$) | $0.23 \pm 0.01$ | $0.28 \pm 0.02$ | $0.17 \pm 0.01$ |
| | $Y_{O/S}$ ($mgO_2\,mgPhenol^{-1}$) | $0.74 \pm 0.03$ | $0.68 \pm 0.03$ | $0.70 \pm 0.08$ |
| | $r^2$ | 0.9700 | 0.9948 | 0.9937 |
| | RMSE | 0.2825 | 0.1780 | 0.1428 |
| Number of runs per experiment | | 9 | 10 | 8 |

Coefficient values are expressed as mean ± 95% confidence interval. PSS: pseudo steady-state method (linear regression of Eq. (10)); Dyn: dynamic method (fitting Eqs. (7) and (8) to DO vs. time using the software Gepasi 3); RMSE: root mean squared error.

that the proposed model adequately predicts the DO concentration and the corresponding $OUR_H$ profile as a function of time for the different tested biomass concentrations. The sharp $OUR_H$ peak obtained when a high biomass concentration was tested tended to disappear as the biomass decreased. Table 2 shows the coefficients obtained under dynamic conditions for three independent experiments (A, B, C), with 8–10 runs per experiment. Phenol oxidation coefficients ($Y_{O/S}$) estimated from these experiments (Table 2) were close to the overall mean $Y_{O/S}$ value previously obtained (Table 1). In addition, the phenol saturation constant ($K_S$) was in accordance with values reported by other authors that studied the phenol degradation using respirometric techniques. Chudoba et al. [28] reported a $K_S$ of $1.3 \pm 0.25\,mgPhenol\,L^{-1}$; Orupold et al. [7] found $K_S$ values ranging from 0.8 to 1.6 $mgPhenol\,L^{-1}$, corresponding to an adapted activated sludge on phenolic compounds. It should be noted that $K_S$ obtained using other techniques than respirometry, such as phenol consumption or biomass production, yield higher values. For example, Kumar et al. [11] studied the phenol degradation by *P. putida* batch cultures. Based on biomass growth studies, those authors reported a $K_S$ value ranging between 20.6 and 36.3 $mgPhenol\,L^{-1}$. Besides, Arutchelvan et al. [12] found $K_S$ values ranging from 8.5 to 82 $mgPhenol\,L^{-1}$ corresponding to *B. brevis* growing on a phenol-limited medium.

The differences reported in the literature between the coefficients obtained using respirometry and batch growth techniques could be due to the different features of the biooxidation process that are actually tested. It may be assumed that respirometry measures enzyme activities related to the first oxidation steps of the tested compound, especially when oxygenases are involved such as in the aerobic phenol oxidation pathway [4]. However, in batch growth techniques, tested compounds are generally utilized as a carbon and/or energy source for the growth of microorganisms; thus, a higher oxidation degree of the tested compound is usually achieved. For this reason, a careful analysis of the coefficients has to be performed prior to extrapolate respirometric results to design phenolic compounds removal facilities. Although respirometry is one of the most used technique to study the aerobic biodegradation of toxic compounds, this technique yields information about the short-term dynamic response of bioreactors under an overload of the tested compound. Thus, the mathematical model proposed in the present work is useful for predicting transient responses such as substrate concentration and DO concentrations as a function of time in bioreactors treating phenolic wastewaters under an overload of phenolic compounds.

## 4. Conclusions

In this paper respirometric techniques were used to study the effect of the initial phenol concentration, pH, and dissolved oxygen (DO) concentration on the phenol biodegradation kinetics using phenol acclimated and unacclimated mixed cultures from activated sludge reactors.

Closed respirometer experiments showed that phenol inhibited the respiration rate of unacclimated sludges. On the contrary, respiration rate of acclimated sludges showed the typical Haldane's substrate inhibition curve within the tested phenol concentrations. The Monod equation adequately represented the relation between the oxygen uptake rate (OUR) of acclimated biomass and the DO concentration. Within the tested pH range (4–12) the oxygen saturation coefficient ($K_O$) was independent of pH levels. On the contrary, the maximum respiration rate ($OUR_m$) was strongly affected by the pH, being its maximum value between 9.5 and 10.5.

Open respirometer experiments showed that as pH decreased from 10.2 to 5.8, the observed maximum OUR decreased and the time to obtain total substrate depletion increased. The normalized OUR obtained using the open respirometer decreased as pH decreased, in accordance with the trend observed in the closed respirometer experiments. Although the respiration rate of phenol degrading bacteria was strongly affected by pH, the phenol oxidation coefficient ($Y_{O/S}$) was constant within the studied pH interval. Thus, pH affected the kinetics (via the $OUR_m$) but neither the oxygen saturation coefficient ($K_O$) nor the stoichiometric coefficient $Y_{O/S}$ of the biological phenol oxidation.

A mathematical model was proposed to interpret the open respirometric curves. The coefficients of the model were estimated using both pseudo steady state and dynamic conditions for different biomass concentrations. The proposed model ade-

quately predicts the whole OUR and DO profiles as a function of time during the biodegradation of phenol under limiting and non-limiting DO conditions.

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