INHIBITION KINETICS DURING THE OXIDATION OF BINARY MIXTURES OF PHENOL WITH CATECHOL, RESORCINOL AND HYDROQUINONE BY PHENOL ACCLIMATED ACTIVATED SLUDGE

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Abstract - In this work the aerobic degradation of phenol (PH), catechol (CA), resorcinol (RE), hydroquinone (HY) and of the binary mixtures PH+CA, PH+RE, PH+HY by phenol-acclimated activated sludge was studied. Single substrate experiments show a Haldane-type dependence of the respiration rate on PH, RE and HY, while CA corresponded to the Monod model. Binary substrate experiments demonstrated that the presence of a second substrate only affected the kinetics, but not the stoichiometry of the oxidation of the compounds tested. While CA inhibited the oxidation of PH, PH inhibited the oxidation of RE and HY. A mathematical model was developed to represent the aerobic biodegradation of the phenolic compounds tested. The agreement between the proposed model and the experimental data indicates that the proposed model can be useful for predicting substrate and dissolved oxygen concentrations in bioreactors treating phenolic wastewaters.
Keywords: Phenolic compounds; Biodegradation; Respirometry; Activated sludge; Mathematical model.

INTRODUCTION

Due to the wide use of phenol and its derivatives, phenolic compounds are widespread in the environment. Several physico-chemical methods, such as adsorption, Fenton, photo Fenton, or combinations of these techniques can be used to remove phenolic compounds from industrial wastewater (Houari *et al*., 2014; Wang *et al*., 2014). However, in some cases these treatments utilize expensive reagents and generate chemical sludges that are frequently toxic

(Inchaurrondo *et al*., 2014). Conversely, most biological methods are simple to manage, utilize innocuous reagents (e.g., atmospheric oxygen), and produce biodegradable sludges. For these reasons, biological methods are widely used (Busca *et al*., 2008).

Wastewaters generated by chemical facilities are often extremely variable in terms of flowrate, composition and concentration of organic compounds, causing operational problems in continuous flow treatment systems (Edwards, 1995). Thus, despite the

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presence of equalizer tanks, actual facilities operate under transient conditions. Because steady-state conditions are assumed when sizing continuous flow bioreactors, wastewater treatment plants are generally designed with excess capacity to ensure the degradation under overload conditions. The utilization of biological processes for purification of toxic wastewaters emphasizes the practical requirement of developing adequate mathematical models to be used for the design and operation of these processes under transient conditions. The knowledge of microbial substrate utilization kinetics is important for the accurate prediction of the quality of the treatment process effluent. One key feature is the relationship between the concentrations of substrate and oxygen and their consumption rates (Arutchelvan *et al*., 2006; Kumar and Kumar, 2005; Nuhoglu and Yalcin, 2005). While the actual substrate consumption rate determines the size of bioreactors, knowledge of the oxygen uptake rate (OUR) is crucial for an appropriate design of aeration devices. For example, according to Vanrollehem and Gillot (2002), aeration costs represent about 45% of the total operational cost (e.g., aeration, pumping and sludge treatment) in a typical activated sludge wastewater treatment plant. Therefore, accurate kinetic parameters help engineers to optimize operational conditions in order to meet discharge requirements while minimizing costs.

Batch and semicontinuous processes employing suspended or immobilized biomass were used to study the phenol degradation kinetics (Karigar *et al*., 2006; Nuhoglu and Yalcin, 2005; Orupõld *et al*., 2001; Tziotzios *et al*., 2005; Yoong *et al*., 2000). Several studies have also focused attention on the aerobic biodegradation of single phenolic compounds and their mixtures by activated sludge (Bajaj *et al*., 2008; Lepik and Tenno, 2011; Pramparo *et al*., 2012). Considering that the knowledge of OUR is crucial for an appropriate design of aeration devices, the aim of this work was a study of the aerobic degradation of phenol (PH), catechol (CA), resorcinol (RE), hydroquinone (HY) and the binary mixtures PH+CA, PH+RE, PH+HY by phenol-acclimated activated sludge. A mathematical model was developed to describe the aerobic degradation of binary mixtures of PH with CA, RE and HY by phenol-acclimated activated sludge. The model was calibrated using respirometric profiles corresponding to single solutions of phenol (PH), catechol (CA), resorcinol (RE), and hydroquinone (HY). Then, the proposed model was validated using respirometric profiles corresponding to the binary mixtures of phenolic compounds.

MATERIALS AND METHODS

Chemicals and Reagents

Phenol (PH) (loose crystals, > 99%) and resorcinol (RE) (ACS reagent, > 99%) were obtained from Sigma (St. Louis, MO, USA). Catechol (CA) and hydroquinone (HY) were analytical grade from Biomed Inc. (Aurora, Ohio). Dehydrated cheese whey was from Food S.A. (Villa Maipú, Argentina). All inorganic salts were commercial products of reagent grade from Anedra (San Fernando, Argentina).

Acclimation Procedure and Culture Conditions

Phenol-acclimated activated sludge was obtained from a laboratory scale (2.5 L) cylindrical semicontinuous fill and draw reactor. Aeration was provided at the bottom of the reactor through an air-stone using two air pumps at 2 L min^{-1}; the dissolved oxygen (DO) concentration was maintained above 4 mgO$_2$ L^{-1}. The reactor was fed with the following culture medium with PH as the sole carbon-limiting source (Nuhoglu and Yalcin, 2005): $(NH_4)_2SO_4$ 226 mg L^{-1}, K$_2$HPO$_4$ 500 mg L^{-1}, KH$_2$PO$_4$ 250 mg L^{-1} MgSO$_4$.7H$_2$O 25.2 mg L^{-1}, MnSO$_4$.H$_2$O 2.52 mg L^{-1}, CaCl$_2$ 2 mg L^{-1}, FeCl$_3$ 1.2 mg L^{-1}, phenol 300 mg L^{-1}. The pH was adjusted to 7.0 ± 0.05 by adding a few drops of concentrated solutions of NaOH or HCl. During this phase the solids retention time was 45 days to prevent the biomass washout. The reactor was monitored periodically by measurements of total suspended solids (TSS), soluble chemical oxygen demand (COD) consumption rate, and total phenols (TPh) consumption rate. The biomass was considered to be acclimated to phenol when the specific consumption rates of COD and TPh were constant for at least two weeks. This phenol-acclimated activated sludge was used in all further experiments.

Respirometric Assays

The aerobic degradation kinetics of the tested compounds (phenol, resorcinol, catechol, and hydroquinone) by phenol-acclimated activated sludge was assessed using an open (flowing gas/static liquid) respirometer. Activated sludge samples (500 mL) were washed three times using a phosphate buffer (15 mM, pH = 7) and resuspended using 500 mL of the above mentioned buffer. Then, 0.5 mL of micronutrient solutions M1 and M2 were added. The composition of M1 was (g/100 mL): FeSO$_4$.7H$_2$O 1.5, ZnSO$_4$.7H$_2$O 0.5, MnSO$_4$.H$_2$O 0.3, CuSO$_4$.5H$_2$O 0.075, CoCl$_2$.6H$_2$O 0.015, and citric acid 0.6. Micronutrient

solution M2 contained the following (g/100 mL): $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.05, BO_3H_3 0.01, KI 0.01 (Lobo *et al.*, 2013).

500 mL of washed activated sludge were poured into the respirometer. Agitation was provided by a magnetic stir-bar; the respirometer was continuously aerated by 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 60V, Argentina). The operation temperature was 20 ± 1 °C. Before the addition of the tested compound, the oxygen mass transfer coefficient of the respirometer (k_La) and the endogenous oxygen uptake rate (OUR_{end}) were obtained using a non-steady state procedure (Lobo *et al.*, 2014). According to the experimental conditions, such as biomass concentration and the compound tested, the k_La values employed ranged between 10 and 40 h^{-1}. When a stable DO concentration was obtained, the respirometer was spiked with the tested compound and the dissolved oxygen concentration (C) was recorded as a function of time (t). The oxygen uptake rate associated with the substrate oxidation (OUR_{ex}) was calculated from the DO mass balance in the respirometer:

$$\frac{dC}{dt} = k_L a \left(C_e - C \right) - OUR_{ex} \qquad (1)$$

where C_e is the DO concentration in the absence of an oxidizable substrate and C is the instantaneous DO concentration. From the DO profile as a function of time, OUR_{ex} values can be obtained by solving Eq. (1) for OUR_{ex}. Then the oxygen consumed (OC) during the oxidation of the tested compound can be calculated as follows:

$$OC = \int_0^t OUR_{ex} dt \qquad (2)$$

At a given time t = t_f the substrate was depleted and OUR_{ex} values returned to zero. Thus, the OC value at time t_f corresponded to the total oxygen consumed (OC_T) during the oxidation of the tested compound. In the case of repeated additions of one or more substrates, the cumulative oxygen consumption (Sum(OC)) was calculated as the sum over the individual OC_T values. For all the tested compounds, abiotic control experiments (e.g., without biomass) were negative with regard to the oxygen consumption (data not shown).

Analytical Procedures

Total suspended solids (TSS, mg/L) were used as a measure of the biomass concentration in the respi-rometer. Known sample volumes (8 mL in this work) were poured into pre-weighed centrifuge tubes, centrifuged and washed twice with distilled water, and incubated at 105 °C for 24h; the TSS of each sample was calculated as the difference between the final weight (dry sample + tube) and initial weight (tube alone) divided by the sample volume. Duplicate biomass measurements were performed to reduce experimental errors; average and maximum relative errors for TSS were 4% and 13%, respectively. The DO concentration was measured using a YSI (model 5739) polarographic DO probe; data were acquired on a personal computer interfaced to a YSI (model 58) DO monitor at 1 measurement/sec.

Estimation of the Model Coefficients and Dynamic Simulations

Calculation of the coefficients of the mathematical model proposed in this work and the dynamic simulations were performed using the software package Gepasi 3 (Mendes, 1993). The routine LSODA (Livermore Solver of Ordinary Differential Equations) was selected to integrate the system of differential equations of the proposed model. The Multistart Optimization algorithm (with Levenberg-Marquardt local optimization) was selected to fit the proposed model to the experimental data. To describe the biological start-up phenomenon, a first order correction factor was included (Contreras *et al.*, 2008).

RESULTS AND DISCUSSION

Oxidation of Single Phenolic Compounds by Phenol-Acclimated Activated Sludge

Figures 1 and 2 show typical examples of the DO profile and the corresponding exogenous oxygen uptake rate (OUR_{ex}) as a function of time (t) during the biodegradation of phenol (PH), catechol (CA), resorcinol (RE) and hydroquinone (HY) by phenol-acclimated activated sludge. As a general rule, substrate and oxygen uptake rates corresponding to the biodegradation of PH and CA (Fig. 1) were about 4 to 6 times higher than those corresponding to RE or HY (Fig. 2). During the biodegradation of PH and CA, OUR_{ex} values rapidly increased towards a given maximum. Then, OUR_{ex} decreased to a new pseudo steady-state value (Fig. 1b,d) caused by DO concentrations lower than 0.1 mM (Fig. 1a,c), which led to a DO limitation condition. The presence of these peaks in the OUR_{ex} profiles due to an oxygen limitation of the external substrate oxidation was also reported by several authors (Contreras *et al.*, 2008;
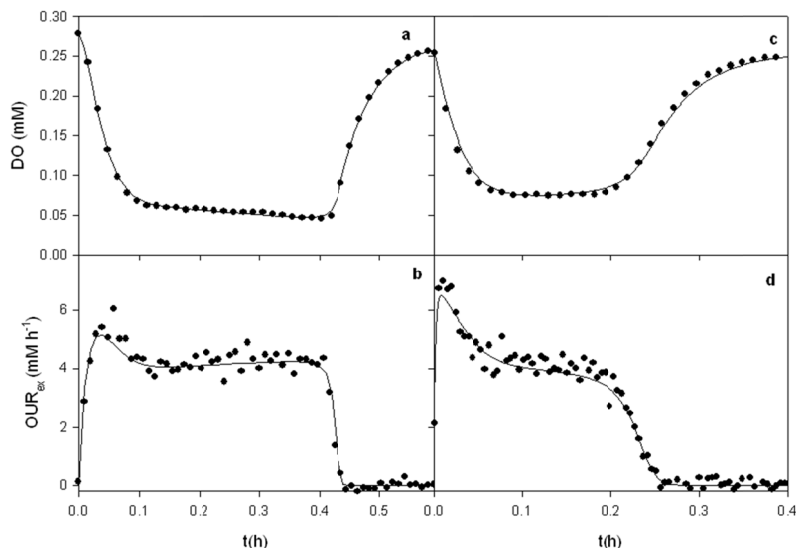
Figure 1: Typical examples of DO profiles (a,c) and the corresponding OUR_{ex} (b,d) during the biodegradation of phenol (a,b) and catechol (c,d) by phenol-acclimated activated sludge. Lines indicate the proposed model (Eqs. (3) to (6)). Experimental conditions: (a,b) $X = 3.1$ gTSS L^{-1}, $k_L a = 19$ h^{-1}, $S_0 = 1.06$ mM; (c,d) $X = 1.3$ gTSS L^{-1}, $k_L a = 23$ h^{-1}, $S_0 = 0.91$ mM.
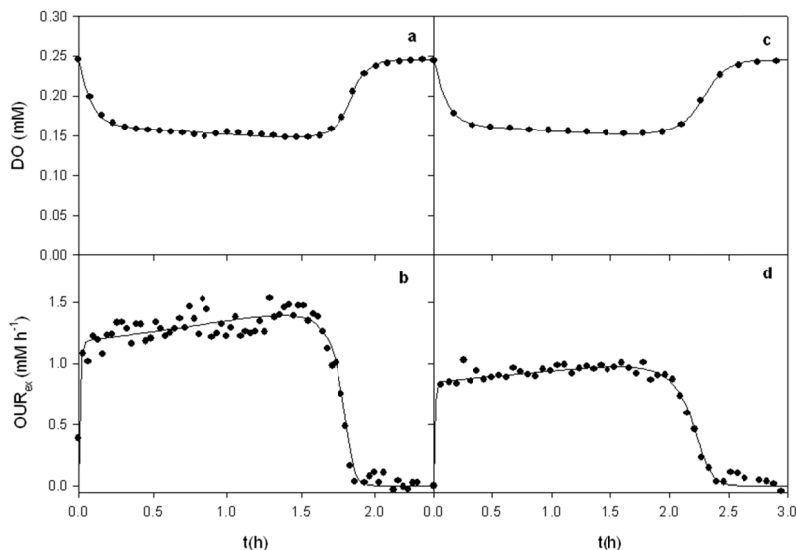

Figure 2: Typical examples of DO profiles (a,c) and the corresponding OUR_{ex} (b,d) during the biodegradation of resorcinol (a,b) and hydroquinone (c,d) by phenol-acclimated activated sludge. Lines indicate the fitted kinetic model (Eqs. (3) to (6)). Experimental conditions: (a,b) $X = 2.1$ gTSS L^{-1}, $k_L a = 14$ h^{-1}, $S_0 = 0.91$ mM; (c,d) $X = 2.7$ gTSS L^{-1}, $k_L a = 10$ h^{-1}, $S_0 = 0.91$ mM.

Guisasola *et al.*, 2006). Because of the low OUR_{ex} obtained during the biodegradation of RE and HY (Fig. 2b,d), in all cases the DO values were higher than 0.15 mM (Fig. 2a,c). The absence of the above mentioned OUR_{ex} peaks when RE and HY were spiked in the respirometer suggests that, within the tested conditions, the effect of the DO concentration on RE and HY biodegradation kinetics was negligible.

Oxidation of Binary Mixtures of Phenolic Compounds by Phenol-Acclimated Activated Sludge

Figures 3 to 5 show DO and the corresponding OUR_{ex} profiles during the degradation of binary mixtures of the tested compounds. For all the tested compounds, respiration rates of phenol-acclimated activated sludge in the presence of PH, CA, RE and HY

were approximately constant as a function of the test number. In a previous work, Lobo *et al.* (2013) demonstrated that repeated additions of PH and HY had a negligible effect on PH and HY consumption rates. Those authors also reported that the cumulative substrate (CA, RE) concentrations necessary to reduce by 50% the biodegradation rate (IC_{50}) of CA or RE were 7.5 and 8.0 mM, respectively. Although repeated additions of CA or RE were performed in the present work (Figs. 3 and 4), maximum cumulative substrate concentrations were 5.4 mM, which was lower than IC_{50} corresponding to these compounds (Lobo *et al.*, 2013). Moreover, in the present work, every addition of CA or RE was followed by the addition of PH, which could have a protective effect against the toxicity of these compounds. For these reasons, in the present work the effects of repeated additions of CA or RE on their consumption rates were negligible.
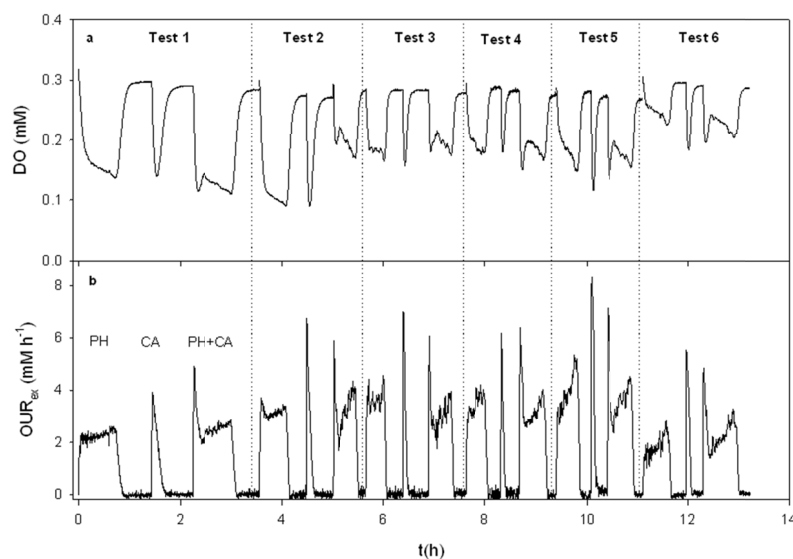

Figure 3: DO profile (a) and the corresponding OUR_{ex} (b) during the biodegradation of phenol (PH, 1.06 mM), catechol (CA, 0.45 mM), and the mixture of phenol (1.06 mM) and catechol (0.45 mM) (PH+CA) by phenol-acclimated activated sludge. Dotted lines indicate each cycle of addition of substrates.
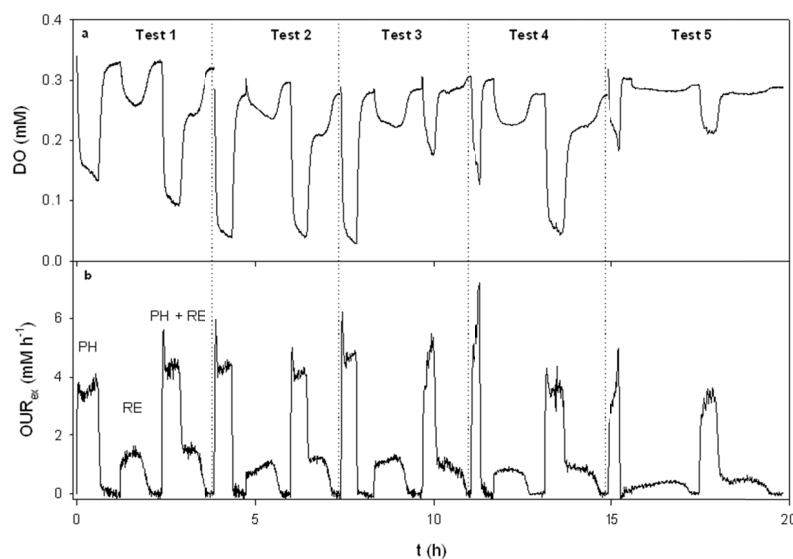

Figure 4: DO profile (a) and the corresponding OUR_{ex} (b) during the biodegradation of phenol (PH, 1.06 mM), resorcinol (RE, 0.45 mM), and the mixture of phenol (1.06 mM) and resorcinol (0.45 mM) (PH+RE) by phenol-acclimated activated sludge. Dotted lines indicate each cycle of addition of substrates.
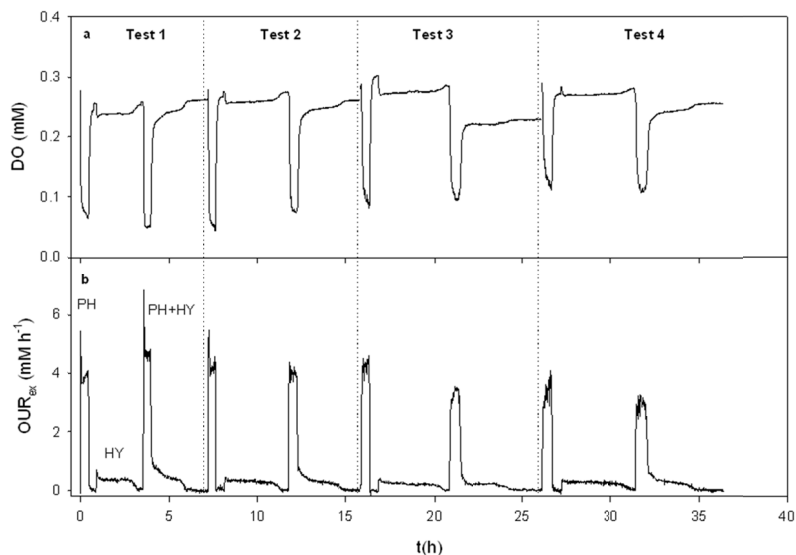
Figure 5: DO profile (a) and the corresponding OUR_{ex} (b) during the biodegradation of phenol (PH, 1.06 mM), hydroquinone (HY, 0.45 mM), and the mixture of phenol (1.06 mM) and hydroquinone (0.45 mM) (PH+HY) by phenol-acclimated activated sludge. Dotted lines indicate each cycle of addition of substrates.

Figure 6a shows that the maximum OUR_{ex} values corresponding to CA were higher than those corresponding to PH. However, the maximum OUR_{ex} value corresponding to the mixture PH+CA was close to that obtained during the addition of CA alone. This result indicates that the OUR_{ex} profile corresponding to the binary mixture of PH and CA cannot be represented by the sum of profiles corresponding to each single compound. Moreover, the results suggest that, during the first phase of the respirogram corresponding to the mixture PH+CA, CA inhibited the metabolism of PH; for this reason, the maximum OUR_{ex} values corresponding to the mixture PH+CA were close to those obtained during the addition of CA alone.

However, because CA was actively oxidized, its concentration diminished below a certain value, allowing the oxidation of PH; under this condition, the second phase of the respirogram represents the oxidation of PH. Figure 6b,c shows that the initial parts of the OUR_{ex} profiles corresponding to the mixtures PH+RE and PH+HY were also close to those obtained during the addition of PH alone. Similarly to the respirograms corresponding to the mixture PH+CA (Fig. 6a), Figure 6b,c shows that the respirograms obtained during the oxidation of PH+RE or PH+HY can also be divided into two phases. However, in these cases, the first part represents the oxidation of PH, while the second phase corresponds to the oxidation of RE or HY.
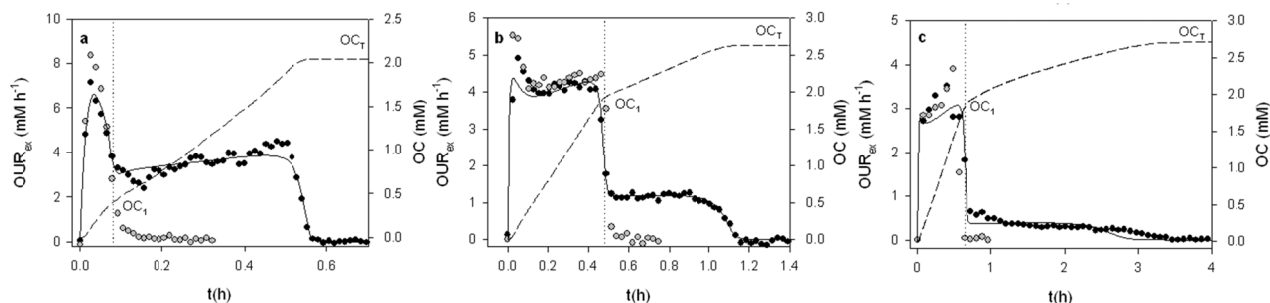
Figure 6: Examples of oxygen uptake rate (OUR_{ex}, circles) and oxygen consumed (OC, dashed lines) during the oxidation of: a) PH+CA (black) and CA alone (grey), b) PH+RE (black) and PH alone (grey), c) PH+HY (black) and PH alone (grey). Dotted lines indicate the time corresponding to the end of the first phase of the respirogram. OC_1 = OC of the first phase, OC_T = total OC. Continuous lines represent the proposed model (Eqs. (3) to (6))

Modelling the Oxidation of Single Substrates and Binary Mixtures of Phenolic Compounds

Among the various substrate inhibition models, the Haldane equation has been widely used to describe the aerobic degradation kinetics of phenol and phenolic compounds (Christen *et al*., 2012; Lepik and Tenno, 2011; Pramparo *et al*., 2012). In the case of CA, Figure 1b suggests that the biodegradation rate obeyed a Monod-type equation with respect to CA within the tested concentrations. In addition, a similar expression is usually included to take into account the effect of the dissolved oxygen concentration (C) on substrate and oxygen consumption rates (Contreras *et al*., 2008; Guisasola *et al*., 2006). However, due to the low OUR_{ex} values obtained when RE or HY were tested (Fig. 2), the DO concentration had a negligible effect on RE and HY biodegradation rates. To represent the effect of an inhibitor on a given bioprocess, the noncompetitive inhibition model is one of the most used. For example, in the Activated Sludge Model No. 1 (ASM1) and its progeny (ASM2, ASM3), the inhibition of denitrification by dissolved oxygen is modelled using this approach (Gujer *et al*., 1999; Henze *et al*., 1987; Henze *et al*., 1999). Finally, it was assumed that, in the presence of several substrates, the oxygen consumption rate can be represented by the sum of OUR corresponding to each substrate; moreover, the presence of a second substrate only affects the kinetics, but not the stoichiometry of the oxidation of the tested compounds.

Based on these considerations, the following expressions for the consumption rates corresponding to PH (R_{PH}), CA (R_{CA}), RE and HY (R_Z) and oxygen (OUR_{ex}) were proposed:

$$R_{PH} = q_{SmPH} \left(\frac{PH}{K_{SPH} + PH + PH^2/K_{iPH}} \right)$$
$$\left(\frac{C}{K_{O2PH} + C} \right) \left(\frac{K_{inPH/CA}}{K_{inPH/CA} + CA} \right) X \quad (3)$$

$$R_{CA} = q_{SmCA} \left(\frac{CA}{K_{SCA} + CA} \right) \left(\frac{C}{K_{O2CA} + C} \right) X \quad (4)$$

$$R_Z = q_{SmZ} \left(\frac{Z}{K_S + Z + Z^2/K_{iZ}} \right) \left(\frac{K_{inZ/PH}}{K_{inZ/PH} + PH} \right) X \quad (5)$$

$$OUR_{ex} = Y_{O/PH} R_{PH} + Y_{O/CA} R_{CA} + Y_{O/Z} R_Z \quad (6)$$

where,

Z represents RE or HY. The term $\left(\dfrac{K_{inPH/CA}}{K_{inPH/CA} + CA} \right)$ in Eq. (3) corresponds to the inhibition of the PH oxidation due to the presence of CA, $K_{inPH/CA}$ being the inhibition constant. For a given CA concentration, the lower the $K_{inPH/CA}$ value, the higher the inhibition; thus, a low $K_{inPH/CA}$ value indicates a strong inhibition of the oxidation of PH by CA. According to Figure 6, PH is oxidized first and then RE of HY. For this reason, the term $\left(\dfrac{K_{inZ/PH}}{K_{inZ/PH} + PH} \right)$ that represents the inhibition of the oxidation of Z (e.g., RE or HY) by PH was included in Eq. (5), $K_{inZ/PH}$ being the inhibition constant. Although the proposed model (Eqs. (3) to (6)) has 20 coefficients, 17 of them can be estimated based on the OUR_{ex} profiles corresponding to the single compounds (Figs. 1 and 2). However, binary substrate respirometric experiments (Figs. 3 to 5) were necessary to evaluate the inhibition constants $K_{inPH/CA}$, $K_{inRE/PH}$ and $K_{inHY/PH}$.

In a first step, the model proposed in the present work was fitted to the respirometric profiles (e.g., DO concentration as a function of time) corresponding to the single phenolic compounds. Figures 1 and 2 show that, using the coefficients depicted in Table 1, the proposed model adequately represented DO and OUR_{ex} profiles during the degradation of all the tested compounds. Table 1 shows that the maximum specific substrate consumption rate (q_{Sm}) corresponding to PH was 7- and 9-fold greater than the values corresponding to RE or HY, respectively. Moreover, the q_{Sm} value corresponding to CA was about 4 times the value corresponding to PH, suggesting that during the metabolism of PH there was no accumulation of CA. Based on the values of q_{Sm} (Table 1), the tested phenolic compounds can be ordered as follows: CA > PH >> RE > HY. This order of metabolism rates agrees with other authors that studied the oxygen consumption rate in the presence of phenolic compounds by intact cells, and enzyme (cell-free) preparations (Ahuatzi-Chacón *et al*., 2004; Lepik and Tenno, 2011; Lobo *et al*., 2013, 2014; Orupõld *et al*., 2001). Table 1 shows that the values corresponding to the coefficients K_S, and K_i had relative errors ranging from 30 to 53%. However, it is known that the sensitivity of OUR with respect to K_i is rather low (e.g., a high variation of K_i has a small effect on OUR). For this reason, large variations in the estimation of K_i are frequently obtained when OUR measurements are used to evaluate this parameter (Guisasola *et al*., 2006).

Table 1: Kinetic coefficients corresponding to the aerobic degradation of phenol (PH), catechol (CA), resorcinol (RE) and hydroquinone (HY) by phenol-acclimated activated sludge (mean ± confidence interval at a 95% confidence level are reported)

	q_{Sm} (mmol gTSS^{-1} h^{-1})	K_S (mM)	K_i (mM)	K_{O2} (mM)	$Y_{O/S}$ (mol/mol)
PH	1.82 ± 0.26	0.021 ± 0.004	2.1 ± 0.6	0.06*	1.52 ± 0.07
CA	7.40 ± 1.21	0.045 ± 0.030	n.a.	0.087 ± 0.018	0.87 ± 0.13
RE	0.26 ± 0.11	0.013 ± 0.004	3.6 ± 1.4	n.a.	2.04 ± 0.11
HY	0.20 ± 0.04	0.029 ± 0.010	1.7 ± 0.5	n.a.	2.05 ± 0.41

n.a.: not applicable
* from Contreras *et al.* (2008)

The $Y_{O/S}$ values obtained (Table 1) are in accordance with those reported by other authors (Divari *et al.*, 2003; Lepik and Tenno, 2011; Lobo *et al.*, 2013; Orupõld *et al.*, 2001). Table 1 shows that, within the experimental error, 1.5 mol of oxygen is consumed by phenol-acclimated activated sludge during the oxidation of one mol of PH under the tested conditions. Taking into account that the activated sludge studied also readily oxidized CA, and that about 1 mol of oxygen is consumed per mol of CA oxidized (Table 1), it is concluded that, during the first oxidation step of PH to CA, only 0.5 mol of oxygen is consumed per mol of PH oxidized under the tested conditions.

Several authors report that the stoichiometry of PH oxidation by *in vitro* preparations of PHMO in the presence of NADPH is one mol of oxygen consumed per mol of PH oxidized, producing one mol of CA (Detmer and Massey, 1985; Enroth *et al.*, 1998; Sejlitz and Neujahr, 1987). It must be pointed out that most of these studies were performed using a PHMO isolated from an eukaryote, the soil yeast *Trichosporon cutaneum.* However, activated sludge is mainly comprised of bacteria (Henze *et al.*, 2002); thus, this difference could be the reason that the stoichiometric coefficient corresponding to the oxygen consumption during the oxidation of phenol to catechol obtained in this work was one half the value reported by other authors.

To represent the oxygen uptake rate (OUR) in the presence of more than one phenolic compound (Eq. (6)), it was assumed that the observed oxygen consumption rate is the sum of the OUR corresponding to each substrate. This hypothesis was tested as follows. According to Lobo *et al.* (2013), in the case of repeated additions of a single substrate, the cumulative oxygen consumption (Sum(OC)) can be calculated as the product between the cumulative substrate concentration (Sum(S)) and the corresponding oxidation coefficient ($Y_{O/S}$). In the case of binary mixtures, if both oxidation coefficients were not affected by the presence of the other substrate, the cumulative

oxygen consumption (Sum(OC)) corresponding to a binary mixture composed of PH and a given compound S can be calculated as follows:

$$Sum(OC)_{calc} = Y_{O/PH}Sum(PH) + Y_{O/S}Sum(S) \quad (7)$$

where S represents CA, RE or HY, $Y_{O/PH}$ is the PH oxidation coefficient, and $Y_{O/S}$ corresponds to the oxidation coefficient of CA, RE or HY. Figure 7 shows that using the oxidation coefficients depicted in Table 1, Eq. (7) satisfactorily predicts the oxygen consumed due to the oxidation of the tested phenolic compounds and of the binary mixtures composed of PH and CA, RE or HY. Additionally, 78% of the calculated Sum(OC) values using Eq. (7) were within ±15% of the experimental values, with -19% and +26% being the minimum and maximum relative deviations, respectively. These results indicate that, within the experimental errors, oxidation coefficients corresponding to PH, CA, RE, and HY can be considered to be independent with respect to the presence of another phenolic compound in a binary mixture, confirming the validity of Eq. (6).

Figure 6a suggests that, for the mixture PH+CA, CA was metabolized first and then PH. For this reason, an inhibition term of PH consumption due to the presence of CA was included in Eq. (3). To evaluate the inhibition constant $K_{inPH/CA}$, the fraction of PH that was consumed during the first phase of the respirogram corresponding to the binary mixture PH+CA was calculated as follows. According to Figure 7, the oxidation coefficients corresponding to the single compounds were not affected by the presence of a second phenolic compound. Thus, the oxygen consumed (OC) during the oxidation of the binary mixture PH+CA can be calculated as follows:

$$OC = Y_{O/PH}(PH_0 - PH) + Y_{O/CA}(CA_0 - CA) \quad (8)$$

where PH_0 and CA_0 represent the initial PH and CA concentrations, respectively. The total OC (OC_T) is

reached when both compounds are completely consumed:

$$OC_T = Y_{O/PH}PH_0 + Y_{O/S}CA_0 \qquad (9)$$

The ratio between Eqs. (8) and (9) yields the following expression:

$$\frac{OC}{OC_T} = \frac{Y_{O/PH}(PH_0 - PH) + Y_{O/CA}(CA_0 - CA)}{Y_{O/PH}PH_0 + Y_{O/CA}CA_0} \quad (10)$$

Figure 6a suggests that the first phase of the respirogram represents the oxidation of CA; when the first phase ended at $t = t_1$, it can be assumed that CA was completely consumed (i.e., $CA_1 \cong 0$). Thus, according to Eq. (10), the ratio between the oxygen consumption corresponding to the first phase (OC_1) and OC_T is:

$$\frac{OC_1}{OC_T} = \frac{Y_{O/PH}(PH_0 - PH_1) + Y_{O/CA}CA_0}{Y_{O/PH}PH_0 + Y_{O/CA}CA_0} \quad (11)$$

where PH_1 represents the concentration of PH at the end of the first phase of the respirogram.
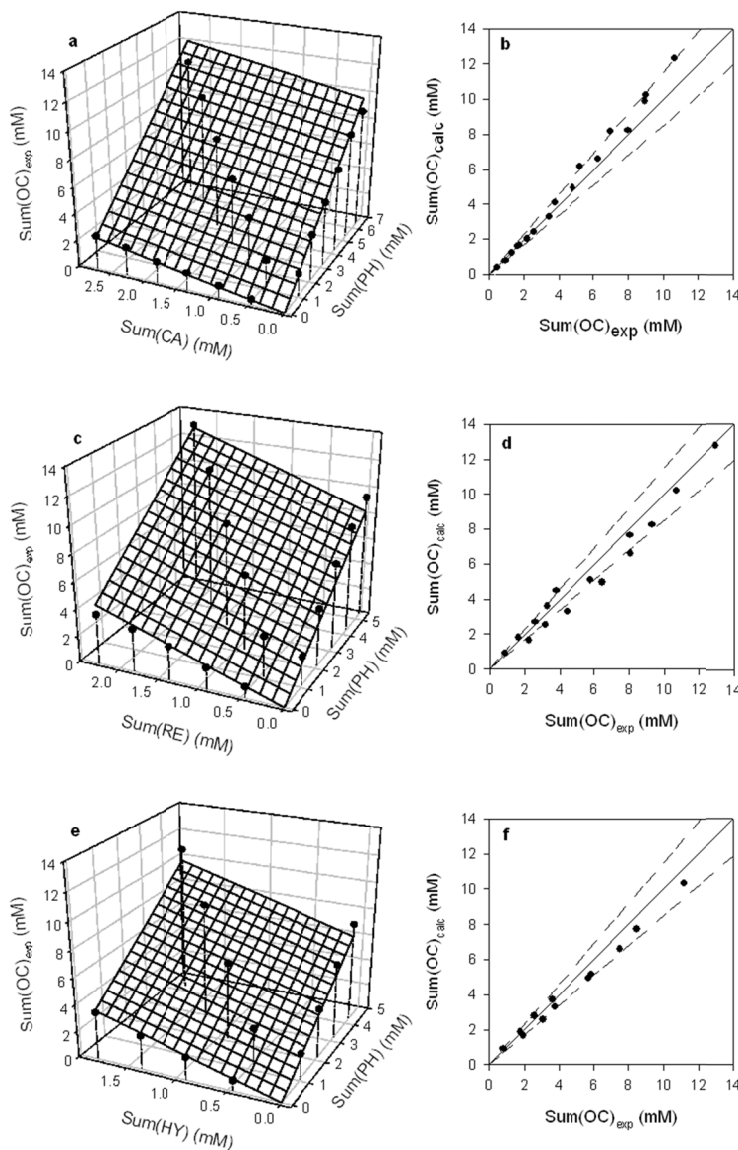

Figure 7: Cumulative oxygen consumption (Sum(OC)) as a function of the cumulative substrate concentration (Sum(S)) corresponding to the mixtures a) PH+CA, b) PH+RE and c) PH+HY. Grey planes in a, c and e represent Eq. (7) using the oxidation coefficients depicted in Table 1. Continuous lines in b, d and e represent the identity line; dashed lines represent ±15% of the experimental value.

Rearranging Eq. (11), the fraction of PH consumed during the first phase of the respirogram (f_{PH1}) during the oxidation of the binary mixture PH+CA can be calculated as follows:

$$f_{PH1} = \frac{PH_0 - PH_1}{PH_0} = \frac{OC_1}{OC_T}$$
$$+ \left(\frac{Y_{O/CA} CA_0}{Y_{O/PH} PH_0} \right) \left(\frac{OC_1}{OC_T} - 1 \right) \quad (12)$$

With regard to the mixtures PH+RE and PH+HY, Figure 6b,c suggests that the first phase of these respirograms corresponded to the oxidation of PH; thus, it can be assumed that at $t = t_1$ (i.e., the end of the first phase), PH was completely consumed ($PH_1 \cong 0$). Using a similar procedure, it can be demonstrated that the fraction of RE or HY consumed during the first phase of the respirogram (f_{Z1}) corresponding to the oxidation of the binary mixture PH+RE or PH+HY is:

$$f_{Z1} = \frac{Z_0 - Z_1}{Z_0} = \frac{OC_1}{OC_T} + \left(\frac{Y_{O/PH} PH_0}{Y_{O/Z} Z_0} \right) \left(\frac{OC_1}{OC_T} - 1 \right) (13)$$

where Z_0 represents the initial concentration of RE or HY, and Z_1 is the concentration of the compound Z (RE or HY) at the end of the first phase of the respirogram.

Based on the respirograms shown in Figures 3 to 5, the OC_1 and OC_T values corresponding to the tested binary mixtures were obtained. Then, using Eqs. (12) and (13) with the stoichiometric coefficients depicted in Table 1, the fractions of PH and RE (or HY) con

sumed during the first phase of the respirogram (f_{PH1}, f_{Z1}) were calculated. Table 2 shows that, in the case of the mixture PH+CA, the consumption of PH during the first phase of the respirogram (f_{PH1}) was about 5% of the initial concentration, confirming that this part mainly reflected the oxidation of CA. In the case of the mixtures PH+RE and PH+HY, the fractions of RE and HY degraded during the first phase of the respirograms (f_{Z1}) were 27% and 21%, respectively.

Once these fractions (f_{PH1}, f_{Z1}) were obtained (Table 2), the inhibition constants $K_{inPH/CA}$, $K_{inRE/PH}$ and $K_{inHY/PH}$ were estimated as follows. For example, in the case of the binary mixture PH+CA, a set of simulations was performed using the proposed model (Eqs. (3) to (6)) with the coefficients depicted in Table 1 and assuming different $K_{inPH/CA}$ values. Then, for each assumed $K_{inPH/CA}$ value, the PH consumed during the first part of the simulated respirograms (PH_1) was evaluated. Finally, the obtained PH_1 values were plotted as a function of $K_{inPH/CA}$ (Fig. 8a). According to the experimental results depicted in Table 2, PH_1 was about 5%. From the comparison between the experimental and simulated PH_1 values, it was concluded that the $K_{inPH/CA}$ value was about 0.03 mM (Fig. 8a). Based on a similar procedure, the estimated $K_{inRE/PH}$ and $K_{inHY/PH}$ values were 0.21 and 0.18 mM, respectively (Fig. 8b,c). Using these inhibition constants along with the kinetic coefficients shown in Table 1, Eqs. (3) to (6) were used to simulate the OUR_{ex} profiles corresponding to the tested binary mixtures. Figure 6 shows that the model proposed in the present work adequately describes the oxygen consumption during the oxidation of binary mixtures of phenolic compounds by phenol-acclimated activated sludge.

Table 2: Oxygen consumption during the first phase of the respirogram (OC_1), total oxygen consumption (OC_T) and fraction of PH and RE (or HY) consumed during the first phase of the respirograms shown in Figures 3 to 5 corresponding to the binary mixtures studied.

Test number	PH+CA			PH+RE			PH+HY		
	OC_1 (mM)	OC_T (mM)	$f_{PH1}(\%)$*	OC_1 (mM)	OC_T (mM)	$f_{RE1}(\%)$**	OC_1 (mM)	OC_T (mM)	$f_{HY1}(\%)$**
1	0.49	2.15	4.0	2.33	3.16	27.6	2.16	3.08	17.9
2	0.39	1.61	5.8	2.00	2.63	34.0	2.00	2.84	18.7
3	0.31	1.49	1.6	1.64	2.29	21.8	1.99	2.69	28.5
4	0.44	1.75	6.9	1.94	2.67	24.7	1.93	2.73	19.5
5	0.56	2.04	9.8	1.62	2.21	26.4			
6	0.37	1.64	3.7						
Mean	0.42	1.78	5.3	1.91	2.59	26.9	2.02	2.84	21.2
Confidence interval (95%)	0.10	0.27	3.0	0.36	0.47	5.6	0.16	0.28	7.8

* Eq. (12) using $Y_{O/PH} = 1.52$, $Y_{O/CA} = 0.87$, $PH_0 = 1.06$ mM, $CA_0 = 0.45$ mM
** Eq. (13) using $Y_{O/PH} = 1.52$, $Y_{O/RE} = 2.04$, $Y_{O/HY} = 2.05$, $PH_0 = 1.06$ mM, $RE_0 = 0.45$ mM, $HY_0 = 0.45$ mM
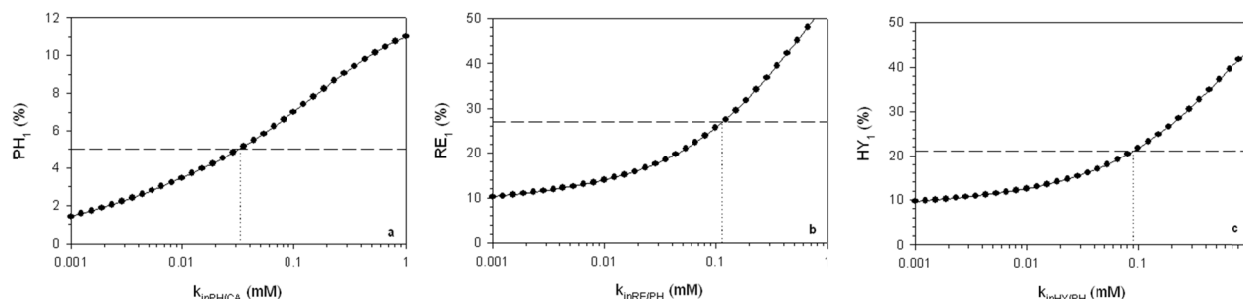
Figure 8: Effect of the inhibition constant ($K_{inA/B}$) on the oxidation of the substrate A (PH, a; RE, b; HY, c) due to the presence of B (CA, a; PH, b, c). Continuous lines represent the percentage of A consumed during the first part of the simulated respirogram (A_1) using the proposed model (Eqs. (3) to (6)) with the coefficients depicted in Table 1; dashed lines indicate experimental A_1 values (Table 2). Dotted lines indicate $K_{inA/B}$ values for which the experimental and simulated A_1 values were similar.

CONCLUSIONS

This work addresses the aerobic degradation of phenol (PH), catechol (CA), resorcinol (RE) hydroquinone (HY) and the binary mixtures PH+CA, PH+RE, PH+HY by phenol-acclimated activated sludge. Based on single substrate experiments, the phenolic compounds tested can be ordered according to their substrate and oxygen consumption rates as follows: CA > PH >> RE > HY. Binary substrate experiments demonstrate that the presence of a second substrate only affected the kinetics, but not the stoichiometry of the oxidation of the tested compounds. CA inhibited the oxidation of PH, for this reason, maximum OUR_{ex} values corresponding to the mixture PH+CA were close to those obtained during the addition of CA alone. While PH inhibited the oxidation of RE and HY, in these cases the first phase of the respirogram represented the oxidation of PH.

A mathematical model was developed to represent the aerobic degradation of the tested phenolic compounds by phenol-acclimated activated sludge. The proposed model adequately represented the oxidation of the tested single phenolic compounds and their binary mixtures. The proposed model can be useful for predicting transient responses such as substrates and DO concentrations as a function of time in bioreactors treating phenolic wastewaters. The model could also help in designing the aeration devices of removal facilities treating phenolic wastewaters.

NOMENCLATURE

C	instantaneous dissolved oxygen (DO) concentration (mM)
CA_0	initial catechol concentrations (mM)
C_e	DO concentration in the absence of an oxidizable substrate (mM)
DO	Dissolved oxygen (mM)
Ki	substrate inhibition coefficient (mM)
$K_{inA/B}$	inhibition constant for the consumption of A due to the presence of B (mM)
$K_{inPH/CA}$	inhibition constant for phenol in the presence of catechol (mM h^{-1})
$K_{inZ/PH}$	inhibition constant for Z (RE or HY) in the presence of phenol (mM h^{-1})
k_La	oxygen mass transfer coefficient (h^{-1})
K_{O2}	oxygen saturation coefficient (mM)
K_S	substrate saturation coefficient (mM)
OC_1	oxygen consumption during the first phase of the respirogram (mM)
OC_T	total oxygen consumed (mM)
OUR_{ex}	oxygen uptake rate associated with the substrate oxidation (mM h^{-1})
PH_0	Initial phenol concentrations (mM)
qO_{2m}	maximum specific oxygen consumption rate (mmol gTSS^{-1} h^{-1})
q_{Sm}	maximum specific substrate consumption rate (mmol gTSS^{-1} h^{-1})
R_{CA}	consumption rate corresponding to catechol (mM h^{-1})
R_{PH}	consumption rate corresponding to phenol (mM h^{-1})
R_S	substrate consumption rate (mM h^{-1})
R_Z	consumption rate corresponding to Z, where Z is resorcinol or hydroquinone (mM h^{-1})
S	substrate concentration (mM)
Sum(OC)	cumulative oxygen consumption (mM)
Sum(S)	cumulative substrate concentration (mM)
t	time (h)
$Y_{O/CA}$	oxidation coefficient for catechol (mol/mol)

$Y_{O/PH}$ oxidation coefficient for phenol (mol/mol)
$Y_{O/S}$ oxidation coefficient for S, where S
              represents CA, RE or HY (mol/mol)
Z_0 initial concentration of RE or HY (mM)

ACKNOWLEDGEMENTS

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