Environ Sci Pollut Res
DOI 10.1007/s11356-017-9735-5

Monitoring and modeling 4-chlorophenol biodegradation kinetics by phenol-acclimated activated sludge by using open respirometry

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Received: 16 December 2016 / Accepted: 7 July 2017

Abstract The aim of this study was to analyze the mechanisms, stoichiometry, and stability of 4-chlorophenol (4CP) biodegradation kinetics by phenol-acclimated activated sludge using open respirometry. While the removal of 4CP was higher than 98%, the removal of chemical oxygen demand (COD) ranged between 69 and 79% due to the accumulation of an intermediate metabolite. The value obtained from respirometric profiles for the stoichiometric ratio of O_2 to 4CP ($Y_{O2/4CP}$) was 1.95 ± 0.04 mol of oxygen consumed per mol of 4CP removed. This $Y_{O2/4CP}$ value reflected the action of the oxygenases responsible for the first steps of the aerobic oxidation of 4CP. The 4CP degradation activity decreased noticeably when successive pulses of 4CP were added to the respirometer. A mathematical model was developed to represent the aerobic biodegradation of 4CP. The fitted model adequately predicted the oxygen consumption rate, total phenols, and soluble COD concentrations as a function of time. The results presented could help to predict the dynamic of biodegradation of chlorophenols in a biological wastewater treatment system.

Responsible editor: Gerald Thouand

Electronic supplementary material The online version of this article (doi:10.1007/s11356-017-9735-5) contains supplementary material, which is available to authorized users.

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Keywords Acclimated activated sludge · 4-Chlorophenol · Respirometry · Biodegradation · Oxidation coefficient · Mathematical model · Kinetics

Introduction

Phenols and their chlorinated derivatives have become a worldwide concern. Due to their toxicity and ability to persist in the environment, chlorophenols have been classified as priority pollutants by the European Union (EU) and the US Environmental Protection Agency (US EPA). Chlorophenols have used mainly in agricultural activities as raw material intermediates in the production of herbicides, insecticides, and fungicides (Olaniran and Igbinosa 2011). In addition, mono-chlorophenols are used as preservatives in paints, leather, and textile goods (Kim et al. 2002).

Due to their wide use and recalcitrant nature, chlorophenols are regular contaminants of industrial wastewater. Several physical-chemical treatments such as adsorption (Nadavala et al. 2009), photocatalytic degradation (Çatalkaya et al. 2003), and combined microfiltration and catalytic wet peroxide oxidation (Rueda-Márquez et al. 2015) have been developed. As a general rule, these treatments are usually complex and often generate undesirable intermediate compounds. Conversely, biological treatments are an economic and simple alternative (Field and Sierra-Alvarez 2007).

Among chlorophenols, 4-chlorophenol (4CP) is toxic independently of the environment (Grén et al. 2012). Several attempts have been proposed to find an adequate biological method to remove 4CP from contaminated waters (Field and Sierra-Alvarez 2007; Lim et al. 2013; Sanchis et al. 2014). The aerobic and anaerobic biodegradation of chlorophenols has been studied using pure cultures as well as bacterial consortia (Konya et al. 2007; Jiang et al. 2008). Several microorganisms, such as

Published online: 20 July 2017

Norcadia, *Pseudomonas*, *Bacillus*, and *Mycobacterium*, have been tested in the aerobic biodegradation of 4CP. In addition, the key enzymes in microbial degradation have been characterized from cell extracts and biodegradation pathways have been proposed (Olaniran and Igbinosa 2011).

Different studies have focused on the production of acclimated activated sludge to degrade 4CP. However, acclimation is a time-consuming process mainly due to the long duration of the lag phase. Liu and Pacepavicius (1990) reported that during the degradation of 2-chlorophenol and 4CP under aerobic conditions, the pentachlorophenol-acclimated sludge studied had a lag phase of 25 h. Moreno-Andrade and Buitrón (2004) analyzed the changes in microbial activity during the acclimation phases in 4CP biodegradation by activated sludge and reported that the complete biodegradation of 0.77 mM of 4CP was achieved in 52, including a 20-h lag phase.

The use of a co-substrate has been proposed as an alternative to reduce the lag phase and to improve the biodegradation of chlorophenols. For example, Ye and Shen (2004) studied the effect of sucrose on the removal of chlorophenols by activated sludge and reported that the addition of sucrose enhanced the acclimation of sludge to 2-chlorophenol and 3-chlorophenol. In contrast, in the case of 4CP, the presence of a co-substrate could not improve its biodegradation. Bali and Şengül (2002) demonstrated that 4CP inhibited the utilization of glucose by activated sludge. Several works have analyzed the cometabolic biodegradation of phenol and 4CP and found that although low concentrations of phenol are able to enhance the biodegradation of 4CP and the presence of small quantities of 4CP can inhibit phenol biodegradation (Jiang et al. 2008; Monsalvo et al. 2009).

To avoid long periods of acclimation, a promising alternative is the use of a biomass with potential affinity for 4CP, such as phenol-acclimated activated sludge. According to several authors (Westmeier and Rehm 1987; Sahinkaya and Dilek 2005; Monsalvo et al. 2009), the aerobic biodegradation of 4CP is initiated by a monooxygenase to produce 4-chlorocatechol (4CC). Then, 4CC can be further oxidized via three different aerobic metabolic pathways. In the first pathway, 4CC oxidation may proceed via ortho-ring (intradiol) cleavage by a catechol-1,2-dioxygenase to produce 3-chloromuconate. Then, the release of chloride by an isomerase and the cleavage of a lactone ring by a hydrolase, maleyl acetate is obtained. In the second pathway 4CC is oxidized via meta-ring (extradiol) cleavage by a catechol-2,3-dioxygenase to produce 5-chloro-2-hydroxymuconic semialdehyde (5C2HMS). Then, chloride is released during the metabolization of 5C2HMS, forming formic, glicolic, and piruvic acids (Gao et al. 2010). Althougth some microorganisms degrade 4CC by meta cleavage, the main aerobic biodegradation pathway is the ortho cleavage (Olaniran and Igbinosa 2011). In the third pathway proposed by Nordin et al. (2005), 4CP is oxidized by a monooxygenase to obtain 4CC, which is further oxidized by the same monooxygenase to produce 1,2,4-benzenetriol (BT) with the release of chloride. During this reaction, 5-chloro-1,2,4-benzenetriol is the intermediate. 4CP can also be firstly oxidized and then dehalogenated to obtain hydroquinone, which can be oxidized to produce BT (Nordin et al. 2005). In both cases, BT can be oxidized by hydroxyquinol 1,2-dioxygenase to produce maleyl acetate. Alternatively, by the sequential action of a dehydrogenase and a reductase, BT can be converted to *p*-benzoquinone. Then, *p*-benzoquinone was reduced by a NADPH-dependent reductase to hydroquinone which is further oxidized by a dioxygenase to 4-hydroxymuconate semialdehyde. Finally, a dehydrogenase oxidized 4-hydroxymuconate semialdehyde to maleyl acetate (Gao et al. 2010). According to Zhang et al. (1996), BT can also react with molecular oxygen to produce 2-hydroxy-1,4-benzoquinone and hydrogen peroxide. During this process, highly toxic reactive oxygen species, such as superoxide and hydroxyl radicals, are produced. It must be noted that the presence of hydroxyl radicals can trigger the polymerization of phenols, producing colored compounds. This reaction competes with the abovementioned enzymatic reactions of BT, and the net result depends on several factors, such as the dissolved oxygen concentration and the presence of radical scavengers (e.g., carbonates from the microbial respiration) (Beltrán 2004).

Several mathematical models, such as Monod or Haldane equations, have been proposed to represent the removal kinetics of 4CP. Respirometry is a well-established procedure in the field of biological wastewater treatment. This technique is based on the measurement of the respiration rate of microorganisms under a controlled environment (Ros 1993). Respirometric techniques have been used to determine parameters of kinetic models that describe the aerobic biodegradation of wastewaters, single compounds, or mixtures of chemicals (Orhon et al. 2009; Lobo et al. 2013, 2014, 2016; Sanchis et al. 2014; Ferro Orozco et al. 2016a, 2016b). These techniques were also employed to monitor the acclimation process of an activated sludge to a new environment (Aktas 2012; Ferro Orozco et al. 2013). Considering the growing interest to predict the performance of activated sludge in the tratment of toxic compounds, the objective of the present work was to study the 4CP biodegradation kinetics by phenol-acclimated activated sludge by using open respirometry.

Materials and methods

Chemicals and reagents

Phenol (loose crystals, >99%) and 4CP (ACS reagent, >99%) were obtained from Sigma (St. Louis, MO, USA).

All other salts used were reagent grade from Anedra (San Fernando, Argentina).

Activated sludge

Phenol-acclimated activated sludge was obtained from a laboratory scale (2.5 L) cylindrical semi-continuous reactor. Solid retention time was maintained in 20 days, and the hydraulic retention time was 3.3 days. 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 phenol as the sole carbon-limiting source (Nuhoglu and Yalcin 2005): phenol 3.19 mM, $(NH_4)_2SO_4$ 226 mg L^{-1}, K_2HPO_4 500 mg L^{-1}, KH_2PO_4 250 mg L^{-1} $MgSO_4{\cdot}7H_2O$ 25.2 mg L^{-1}, $MnSO_4{\cdot}H_2O$ 2.52 mg L^{-1}, $CaCl_2$ 2 mg L^{-1}, and $FeCl_3$ 1.2 mg L^{-1}; the pH was adjusted to 7.0 ± 0.05 by adding concentrated solutions of NaOH or HCl. The reactor was monitored periodically by measurements of total suspended solids (TSS) and consumption rates of soluble chemical oxygen demand (COD) and total phenols (TPh). The biomass was considered acclimated to phenol when the specific consumption rates of COD and TPh were constant for at least 2 weeks. This phenol-acclimated activated sludge was used in all further experiments.

Biodegradation of 4CP by phenol-acclimated activated sludge

The kinetics of the aerobic degradation of 4CP 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 phosphate buffer (15 mM, pH = 7) and resuspended in 500 mL of this buffer. Then, 0.5 mL of the micronutrient solutions M1 and M2 was added (Lobo et al. 2013).

A volume of 500 mL of the washed activated sludge was 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 60 V, Argentina). The temperature was maintained at 30 ± 1 °C by a recirculating water bath. Before the addition of the tested compound, the oxygen mass transfer coefficient of the respirometer (k_La) was measured using a non-steady-state procedure (Lobo et al. 2014). According to the experimental conditions (e.g., biomass and tested compound concentrations), the k_La values obtained ranged between 26 and 95 h^{-1}. When a stable dissolved oxygen concentration was reached, the respirometer was spiked with 4CP and the dissolved oxygen concentration (C) was recorded as a function of time (t). In these experiments, the initial 4CP concentrations tested (S_0) in the respirometer ranged from 0.64 to 3.56 mM. The oxygen uptake rate associated with the oxidation of 4CP (OUR_{ex}) was calculated from the DO mass balance in the respirometer:

$$\mathrm{OUR_{ex}} = k_L a\,(C_e - C) - \frac{dC}{dt} \tag{1}$$

where C_e is the DO concentration in the absence of an oxidizable substrate and C is the instantaneous DO concentration. To calculate OUR_{ex} profiles, C values were plotted as a function of time. Then, a numerical procedure was used to obtain the derivative term of Eq.(1) using the Sigma Plot 10.0 software. Based on the OUR_{ex} values as a function of time, the oxygen consumed (OC) during the substrate oxidation was calculated as follows:

$$\mathrm{OC} = \int_0^t \mathrm{OUR_{ex}}\,dt \tag{2}$$

Abiotic control experiments (e.g., without biomass) showed negligible oxygen consumption (data not shown).

Stability of the biodegradation of 4CP by phenol-acclimated activated sludge

To evaluate the stability of the biodegradation of 4CP, successive pulses of 0.64 mM of 4CP were poured into the respirometer. For each pulse, the degradation time (t_d) was calculated as the time interval between the addition of 4CP to the respirometer and the time at which OUR_{ex} returned to the baseline. Then, the mean specific substrate removal rate (q_{Smean}) was calculated as follows (Lobo et al. 2013):

$$q_{Smean} = \frac{4CP_0}{t_d X} \tag{3}$$

Because the initial concentration of 4CP for all the additions was constant (0.64 mM), the relative activity of 4CP degradation (RDA) in the pulse i with respect to the degradation activity in the first pulse can be calculated as follows:

$$\mathrm{RDA}(\%) = 100\frac{t_{d1}}{t_{di}} \tag{4}$$

where t_{d1} and t_{di} are the total degradation time corresponding to the first pulse and pulse i, respectively.

Analytical procedures

Total suspended solids (TSS, g L^{-1}) were used as a measurement of the biomass concentration in the respirometer. Known sample volumes (8 mL) were poured into pre-weighed centrifuge tubes, centrifuged and washed twice with distilled water, and incubated at 105 °C for 24 h; the TSS of each sample was

calculated as the difference between the final weight (dry sample + tube) and initial weight (empty tube) 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. Soluble 4CP and COD concentrations were determined as follows: 4.5 mL of culture samples were centrifuged at 13,000 rpm for 10 min (Eppendorff 5415C). Clear supernatants were analyzed for COD and 4CP contents.

The COD of the supernatant (COD_S) was determined using a commercial test (Hach Cat. No. 21259). Digestion of the samples (2 h at 150 °C) was performed in a Hach COD Reactor 45,600; a Hach DR 2800 photometer was used for the absorbance determination of the digested samples. To determine the concentration of 4CP of the supernatant, the 4-aminoantipyrene colorimetric method was used (Greenberg et al. 1989). The method uses two reagents, 20.8 mM of 4-aminoantipyrine (Sigma-Aldrich, reagent grade) in 0.25 M $NaHCO_3$ (Ciccarelli, ACS) and 83.4 mM of $K_3Fe(CN)_6$ (Sigma-Aldrich, ACS) in 0.25 M $NaHCO_3$, as color-generating substrates when combined with phenolic compounds (Ferro Orozco et al. 2015). Colored complexes were measured at 510 nm in a Hach DR 2800 spectrophotometer.

Absorbance at 380 nm of the supernatant was also measured in a spectrophotometer Shimadzu AA-6650 to estimate the production of 5C2HMS, a known intermediate during the aerobic degradation of 4CP (Farrell and Quilty 1999).

DO concentration was measured using an Optical Dissolved Oxygen probe (YSI ProODO™); data were acquired on a personal computer at 5 s/measurement. The pH was measured using a pH meter (Hach sensION+ 3).

Estimation of the model coefficients and dynamic simulations

The kinetic coefficients of the mathematical models studied in this work and the dynamic simulations were estimated using the software package Gepasi 3 (Mendes 1993). Gepasi integrates the systems of differential equations with the routine Livermore Solver of Ordinary Differential Equations (LSODA). The LSODA algorithm measures the stiffness of the equations and switches the integration method dynamically according to this measure. For non-stiff regions, it uses the Adams integration method with variable step size and variable order up to the 12th order, whereas for stiff regions it uses the Gear (or BDF) method with variable step size and variable order up to the 5th order. Among the optimization methods available in Gepasi 3, the Multistart Optimization algorithm (with Levenberg-Marquardt local optimization) was selected (for more details concerning these procedures see Supplementary data, Item SD1). Multistart is a hybrid stochastic-deterministic optimization method. Rather than run a simple local optimization (e.g., gradient descent methods), Multistart runs several of them, each time starting from a different initial guess. The first start takes for initial guess the parameter values entered by the user. The initial guesses for the subsequent starts are generated randomly within the boundaries for the adjustable parameters. The local optimizer used is the Levenberg-Marquardt method as this has proved the most efficient gradient optimizer used in Gepasi 3. In order to reduce fitting errors of the adjustable coefficients, the initial concentrations of biomass and 4CP were adjusted within ±5 and ±2% of their measured values, respectively. This procedure takes into account the degree of uncertainty in the initial conditions due to analytical errors (Mendes and Kell 1998). The CHEAQS software (Verweij 2009) was used to calculate the pH of the medium as a function of 4CP degradation.

Results and discussion

Biodegradation kinetics of 4CP by phenol-acclimated activated sludge

Figure 1a shows the exogenous oxygen uptake rate (OUR_{ex}) as a function of time for different initial 4CP concentrations. As a general rule, OUR_{ex} increased sharply immediately after the addition of 4CP. Then, OUR_{ex} values slowly decreased, and when the substrate was exhausted, OUR_{ex} returned to a value a slightly higher than the initial one. Also, the difference between initial and final OUR_{ex} increased with the increase in the initial 4CP concentration, slow oxidation of a metabolic intermediate. In all cases, the time at which OUR_{ex} returned close to the baseline value increased with higher 4CP initial concentrations. Moreover, Fig. 1b, c demonstrates that this degradation time (t_d) was in accordance with the depletion of TPh and COD_S. It must be noted that the decrease in TPh and COD_S was observed immediately after the addition of 4CP. Hence, these results demonstrate that the degradation of 4CP by phenol-acclimated activated sludge occurred without prior acclimation to 4CP. In other words, the oxygenases involved in the phenol metabolization were also able to oxidize 4CP.

Based on the OUR_{ex} profiles depicted in Fig. 1a, the oxygen consumed (OC) as a function of time was calculated using Eq.(2). Then, these OC values were plotted as a function of the 4CP consumed (Δ4CP). Figure 2a shows that for all the 4CP initial concentrations tested, the 4CP oxidation coefficient $Y_{O2/4CP}$ was 1.95 ± 0.04 mol of oxygen consumed per mol of 4CP removed (r^2 = 0.994). So far, all the aerobic degradation pathways reported begin with the action of two oxygenases, which implies the consumption of two moles of oxygen per mol of 4CP oxidized (Arora and Bae 2014). During each oxidation step, mono and dioxygenases consume both one mol of oxygen (Frey and Hegeman 2007), which

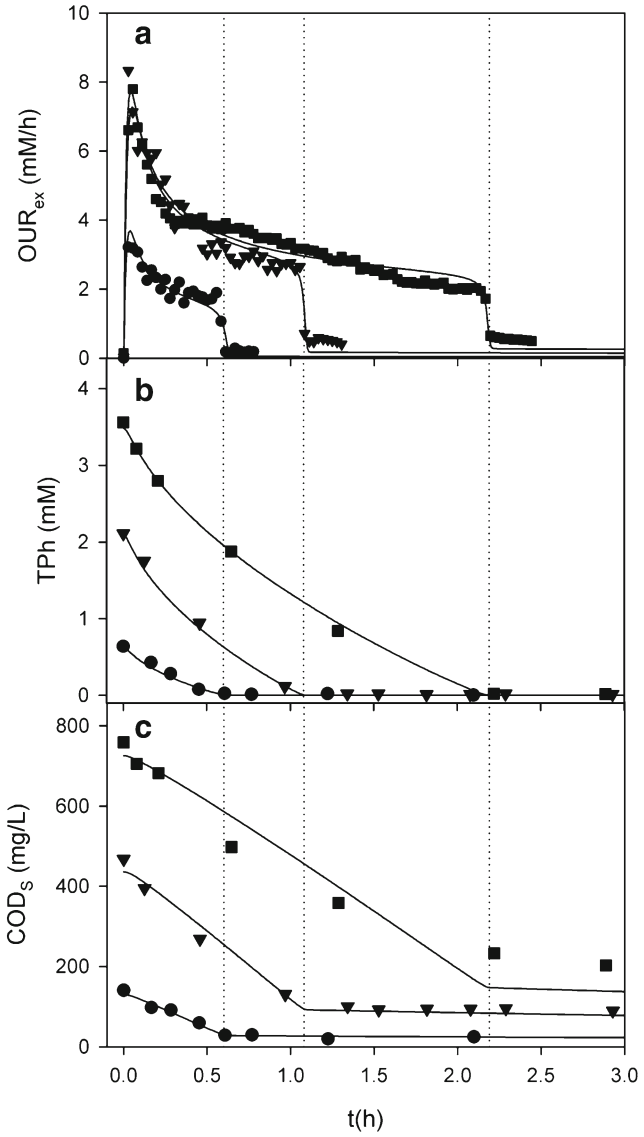

Fig. 1 **a** Exogenous oxygen uptake rate (OUR_{ex}), **b** total phenols (TPh), and **c** soluble COD concentration (COD_S) as a function of time corresponding to the following initial 4CP concentrations: (*circles*) 0.64 mM, (*triangles*) 2.10 mM, (*squares*) 3.56 mM. In all cases, lines are the results obtained using the proposed model (Model #5)

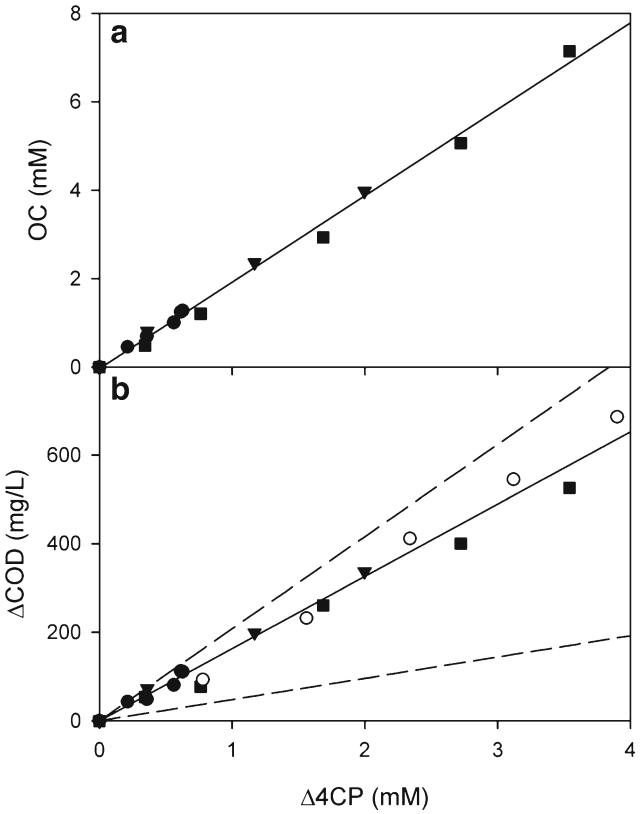

Fig. 2 **a** Oxygen consumption (OC) and **b** soluble COD consumption (ΔCOD) as a function of 4CP consumed (Δ4CP). In both cases, *hollow circles* correspond to the results obtained from successive pulses of 4CP; *different symbols* indicate independent experiments and *continuous lines* represent the linear regression of the depicted data. *Upper dashed line* in **b** represents the theoretical COD consumption assuming that only 4CP is in solution; *lower dashed line* in **b** corresponds to the theoretical COD consumption assuming that 4CP is oxidized to 5C2HMS. Details of these calculations can be found in the Supplementary Data, Item SD2

implies the consumption of two moles of oxygen per mol of 4CP oxidized (Arora and Bae 2014). According to several authors (Westmeier and Rehm 1987; Sahinkaya and Dilek 2005; Monsalvo et al. 2009), the aerobic biodegradation of 4CP is initiated by a monooxygenase to produce 4CC. Then, 4CC can be further oxidized via three different aerobic metabolic pathways. In the first pathway, 4CC oxidation may proceed via ortho-ring (intradiol) cleavage by a catechol-1,2-dioxygenase to produce 3-chloromuconate. In the second pathway, 4CC is oxidized via meta-ring (extradiol) cleavage by a catechol-2,3-dioxygenase to produce 5-chloro-2-hydroxymuconic semialdehyde (Gao et al. 2010). In the third pathway, 4CP is oxidized by a monooxygenase to obtain 4CC, which is further oxidized by the same monooxygenase to produce 1,2,4-benzenetriol (BT) with the release of chloride proposed (Nordin et al. 2005). Thus, the value of $Y_{O2/4CP}$ obtained in the present work suggests that the OUR_{ex} profiles depicted in Fig. 1a reflected the action of the oxygenases responsible for the first steps of the aerobic oxidation of 4CP.

Figure 2b shows that in all the experiments, COD_S consumption (ΔCOD) increased linearly as a function of 4CP depletion (Δ4CP). From the slope of the straight line obtained, a value of 163 ± 5 g COD per mol of 4CP removed was obtained (r^2 = 0.985). The theoretical oxygen demand (ThOD) for the complete oxidation of 4CP to CO_2 and HCl is 208 g COD per mol of 4CP oxidized. Assuming that 4CP was completely removed from the solution, Fig. 2b also shows that the calculated change in COD_S as a function of the 4CP removed (Δ4CP) (upper dashed line, 208 g COD per mol of 4CP oxidized) is higher than the experimental line (163 g COD per mol of 4CP oxidized). This difference suggested that a metabolite was released during these experiments (more

details concerning these calculations can be found in the Supplementary Data, Item SD2).

During the respirometric experiments, a yellowish color appeared in the respirometer; this yellowish color was evaluated through the absorbance at 380 nm (Farrell and Quilty 1999). Figure 3 shows that the absorbance at 380 nm reached a maximum value when OUR_{ex} returned to the baseline value and 4CP depletion was attained (Fig. 1). Several authors have reported the production of 5-chloro-2-hydroxymuconic semialdehyde (5C2HMS) during the metabolization of 4CP by 4CP-acclimated activated sludge (Moreno-Andrade and Buitrón 2004; Sahinkaya and Dilek 2005; Monsalvo et al. 2009). Considering that the ThOD of 5C2HMS is 160 g COD per mol, if this compound remained in solution, the calculated COD consumption as a function of 4CP oxidized should be much lower than the value obtained (Fig. 2b, lower dashed line). This result suggested that if this compound is actually 5C2HMS, not all the 5C2HMS produced remained in the solution, probably due to its metabolization or adsorption onto the activated sludge (Arora and Bae 2014).

Each OUR_{ex} profile depicted in Fig. 1 allowed obtaining the degradation time (t_d). Then, the mean specific substrate removal rate (q_{Smean}) was calculated using Eq.(3). Table 1 shows that for all the tested initial 4CP concentrations, the q_{Smean} obtained was about 0.74 mmol 4CP $gSST^{-1}$ h^{-1}. This value is in agreement with those found by other authors. For example, Sahinkaya and Dilek (2005) reported q_S ranging from 0.47 to 0.61 mmol 4CP $gTSS^{-1}$ h^{-11} for a 4CP-acclimated activated sludge. Conversely, Buitrón et al. (2003) studied 4CP removal by activated sludge in a sequencing batch reactor and reported q_S values from 0.23 to 0.39 mmol 4CP $gTSS^{-1}$ h^{-1}.

Table 1 also shows the COD and 4CP removal degree corresponding to the aerobic biodegradation of the tested initial 4CP concentrations by phenol-acclimated activated sludge. While in all cases depletion of 4CP was almost a complete, COD removals were about 70–80%. These values were similar to those obtained by Uysal and Turkman (2007) during the biodegradation of 4CP (150 mg L^{-1}) and glucose (1500 mg COD L^{-1}) by activated sludge. Sahinkaya and Dilek (2005) evaluated the biokinetic coefficients for the biodegradation of 4CP as the sole carbon source by activated sludge and reported COD removal degree of 40 and 46% for 0.87 and 2.13 mM of 4CP, respectively. Lim et al. (2013) studied the performance of phenol-acclimated activated sludge in the presence of various phenolic compounds and reported 4CP removal degree close to 90.0% for both 0.77 and 1.56 mM of 4CP. However, in both cases, the time required to reach this 4CP removal degree was 1.6 and 5 h, respectively. These times were higher than those obtained in the present work (Fig. 1). Zhao et al. (2016) studied the correlation between microbial diversity and toxicity in a sequencing batch reactor treating 4CP; those authors report that unacclimated activated sludge achieved a COD removal of 89% after 9 h. Although lower COD removals were reached in the present work (70–80%), these values were attained in less than 3 h.

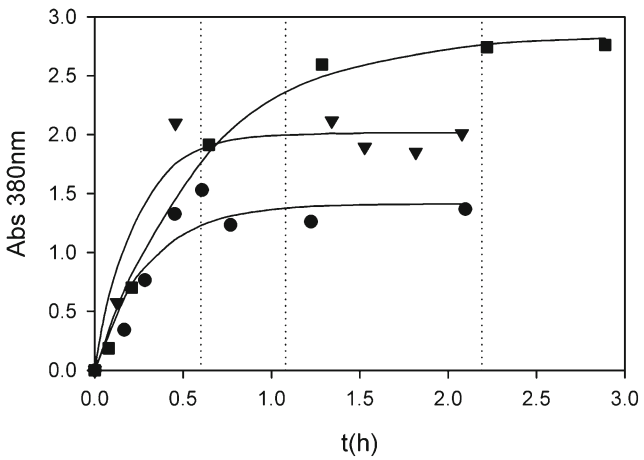

Fig. 3 Absorbance at 380 nm as a function of time corresponding to different initial 4CP concentrations: (*circles*) 0.64 mM, (*triangles*) 2.10 mM, (*squares*) 3.56 mM. In all cases, *lines* indicate a trend line

Stability of the biodegradation of 4CP by phenol-acclimated activated sludge

Successive additions of 4CP (0.64 mM) were poured into the respirometer to evaluate the stability of the biodegradation of 4CP by phenol-acclimated activated sludge. Figure 4a shows that for all additions of 4CP, an increased in the total degradation time (t_d) was clearly noticeable. These data allowed obtaining the mean specific substrate consumption rate (q_{Smean}) and the relative 4CP degradation activity (RDA) using Eqs.(3) and (4), respectively. The initial q_{Smean} value was 0.68 mmol 4CP $gTSS^{-1}$ h^{-1}, which was close to those obtained in the experiments of single pulses with different initial 4CP concentrations (Fig. 1). However, the sequential addition of 4CP pulses caused a noticeably decrease in the RDA. For example, the RDA value corresponding to the 5th pulse was 14% (Fig. 4b). Taking into account that the cumulative 4CP concentration corresponding to the 5th pulse was 3.2 mM, the loss of activity corresponding to the 5th pulse contrast with the almost constant value of q_{Smean} obtained from single additions of 4CP (Table 1). It must be pointed out that the abovementioned inhibition effect of 4CP cannot be attributed to changes in the pH of the medium. In all the experiments, the maximum decrease in pH was less than 0.5 units (Supplementary Data Item SD3, Fig. SD1). Moreover, calculations using the CHEAQS software demonstrated that the decrease observed in pH values was fully compatible with the release of only qmol of H^+ per mol of 4CP degraded. These results suggest that the concentration of intermediates such as 5C2HMS or other organic acids in the solution must be quite low. For this reason, no observable

Table 1 Effect of the initial 4CP concentration on the mean specific 4CP removal rate (q_{Smean}) and COD and 4CP removal efficiencies by phenol-acclimated activated sludge

	Initial 4-chlorophenol concentration (mM)		
	0.64	2.11	3.56
q_{Smean} (mmol4CP $gSST^{-1}$ h^{-1})	0.74 ± 0.08	0.73 ± 0.01	0.74 ± 0.02
COD removal (%)	79 ± 3	78 ± 2	69 ± 4
4CP removal (%)	96 ± 2	99 ± 1	99 ± 1

effect of these intermediates on pH values occurred. This conclusion is also in accordance with the analysis of Fig. 2b. Details of these calculations can be found in the Supplementary Data, Item SD2.

Based on the data shown in Fig. 4, the 4CP concentration corresponding to RDA = 50% was about 1.9 mM. Beltrame et al. (1984) studied the biodegradation of chloro and nitrophenols in activated sludge and found an IC50 value (e.g., the cumulative substrate concentration necessary to reduce q_S by 50% with respect to the control value) of 0.55 mM for 4CP. Sahinkaya and Dilek (2005) measured the IC50 values based on the inhibition of the specific growth rate and obtained values of 1.0 and 1.7 mM of 4CP for unacclimated and acclimated activated sludge, respectively. Using a similar approach, Lim et al. (2013) reported a IC50 value of 0.77 mM for phenol-acclimated activated sludge. Monsalvo et al. (2009) studied the simultaneous removal of phenol and 4CP in a sequencing batch reactor at different temperatures, using the Microtox Acute Test, and found a low IC50 value of 0.015 mM. However, according to Ricco et al. (2004), the Microtox Acute Test could overestimate the acute toxicity effect. On the other hand, Kargi and Konya (2006) described the biodegradation of high 4CP concentrations (0.39–10.8 mM) on 4CP-acclimated activated sludge and reported an IC50 of 3.88 mM of 4CP. These differences in the reported IC50 values can be attributed to several factors, such as the use of different tests, the source of activated sludge, and acclimation conditions.

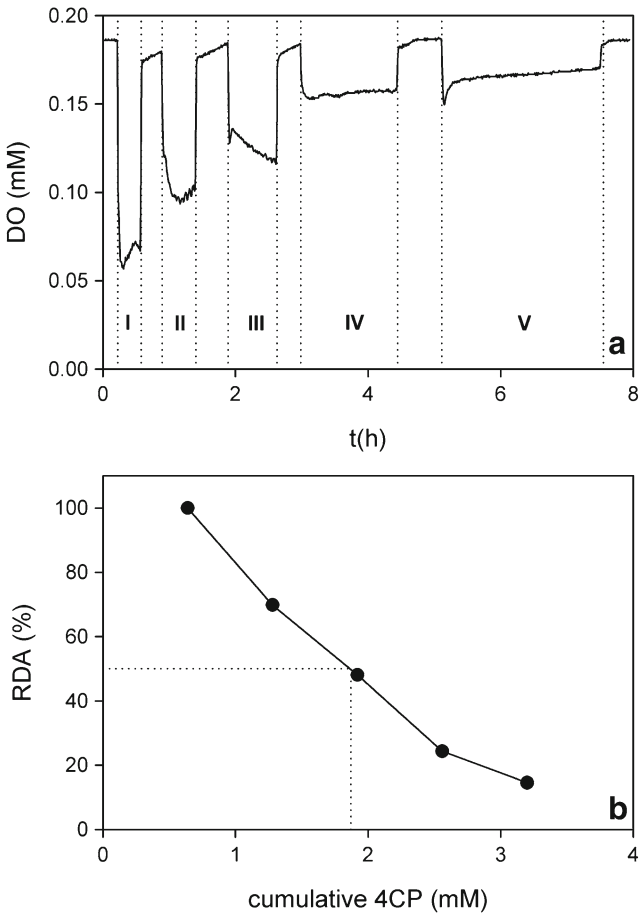

Fig. 4 **a** Dissolved oxygen concentration (DO) as a function of time, and **b** relative degradation activity (RDA) as a function of the cumulative 4CP concentration. In this experiment, five pulses (denoted I to V) of 0.64 mM of 4CP were added. *Dotted lines* in **a** represent the time interval between the addition of 4CP and its depletion. *Dotted lines* in **b** indicate the cumulative 4CP concentration that reduce 50% the initial degradation activity

Modeling the aerobic biodegradation of 4CP by phenol-acclimated activated sludge

The knowledge of the biodegradation kinetics of xenobiotic compounds is crucial to predict their biodegradation in natural and engineered environments. Monod or Haldane equations have been used to characterize the biodegradation kinetics of several phenolic compounds. However, in some cases, these models are not adequate to represent the biodegradation process under transient conditions in activated sludge systems (Hao et al. 2002; Paca et al. 2010; Sahinkaya and Dilek 2005).

According to the results presented in the previous sections, the aerobic degradation of 4CP by phenol-acclimated activated sludge was represented by the following reactions:

$$4CP + O_2 \rightarrow^{R_{4CP}} 4CC \quad (5)$$

$$4CC + O_2 \rightarrow^{R_{4CC}} P + Cl^- + H^+ \quad (6)$$

where 4CC is 4-chlorocatechol, *P* represents the product responsible for the accumulation of soluble COD at the end of

the experiments, and R_{4CP} and R_{4CC} are the oxidation rates of 4CP and 4CC, respectively. *P* represents a hypothetical (i.e., non-measured nor identified) intermediate included for modelling purposes to take into account that although a total depletion of 4CP was achieved, some soluble COD remained.

According to reactions (5) and (6), the change in 4CP, 4CC, and P as a function of time in a batch system is:

$$\frac{d[4CP]}{dt} = -R_{4CP} \tag{7}$$

$$\frac{d[4CC]}{dt} = R_{4CP} - R_{4CC} \tag{8}$$

$$\frac{d[P]}{dt} = -R_{4CC} \tag{9}$$

Because reactions (5) and (6) are expressed in mol units, the OUR is $R_{4CP} + R_{4CC}$. However, to take into account that when 4CP is exhausted, OUR_{ex} returns to a value slightly higher than the initial one (Fig. 1a); it was assumed that this increase is proportional to [P]. Based on these considerations:

$$OUR_{ex} = R_{4CP} + R_{4CC} + q_P[P] \tag{10}$$

where q_P is a proportionality constant. Besides their respective substrates (4CP, 4CC), it must be pointed out that R_{4CP} and R_{4CC} rates may be also limited by a low DO concentration. Because of the high k_La values used in the respirometer, all the experiments were performed under an excess of oxygen; for this reason, oxygen limitation is not considered in this work.

In a first attempt to represent the respirometric profiles shown in Fig. 1a, it was assumed that reactions (5) and (6) are catalyzed by different enzymes. To represent the R_{4CP} and R_{4CC} rates, Monod (M) and Haldane (H) equations were used for both reactions (Models #1 to #4). Thus, the four possible combinations of these equations were tested (Model #1 $R_{4CP\text{-}M}$, $R_{4CC\text{-}M}$; Model #2 $R_{4CP\text{-}M}$, $R_{4CC\text{-}H}$; Model #3 $R_{4CP\text{-}H}$, $R_{4CC\text{-}M}$; Model #4 $R_{4CP\text{-}H}$, $R_{4CC\text{-}H}$). The equations corresponding to each model tested can be found in Table 2. Figure 5a–d and Table 2 show that in any case the fitting results were satisfactory. Considering the complexity of the respirometric profiles and the poor fitting results obtained with standard models (Models #1 to #4), a new model was developed.

The new biokinetic model (Model #5) proposed in this work was based on the aerobic biodegradation pathway of 4CP reported by Nordin et al. (2005). According to this pathway, the same monooxygenase oxidize both 4CP and 4CC to finally produce BT with the release of chloride. Although BT can be metabolized to finally produce maleyl acetate (Gao et al. 2010), BT can also react with molecular oxygen, producing highly toxic reactive oxygen species, such as superoxide and hydroxyl radicals (Zhang et al. 1996). These reactions might explain the enhancement of the oxygen consumption rate after the 4CP depletion in the respirometer. Besides, it must be noted that the product of the first oxidation step of 4CP (4CC) is the substrate for the second one and, in both cases, a single monooxygenase catalyzes these reactions. This is a dramatic difference between this new model (Model #5) and standard models (Models #1 to #4), in which two different enzymes catalyze the two sequential oxidation steps. According to these considerations, we propose the following scheme of reactions (Model #5):

$$E + 4CP \underset{\leftarrow k2}{\overset{\rightarrow k1}{}} E4CP \rightarrow^{k3} E + 4CC \tag{11}$$

$$E + 4CC \underset{\leftarrow k5}{\overset{\rightarrow k4}{}} E4CC \rightarrow^{k6} E + P + Cl^- + H^+ \tag{12}$$

Thus, the reaction rates corresponding to 4CP, 4CC, and P are the following:

$$\frac{d[4CP]}{dt} = k2[E4CP] - k1[E][4CP] \tag{13}$$

$$\frac{d[4CC]}{dt} = k5[E4CC] - k4[E][4CC] + k3[E4CP] \tag{14}$$

$$\frac{d[P]}{dt} = k6[E4CC] \tag{15}$$

Assuming a steady stationary state for the c E4CP and E4C complexes:

$$\frac{d[E4CP]}{dt} = k1[E][4CP] - (k2 + k3)[E4CP] = 0 \tag{16}$$

$$\frac{d[E4CC]}{dt} = k4[E][4CC] - (k5 + k6)[E4CC] = 0 \tag{17}$$

Solving Eq.(16):

$$\frac{[E]}{[E4CP]} = \frac{A}{[4CP]} \tag{18}$$

where $A = \frac{k2+k3}{k1.}$

Combining Eqs.(16) and (17) and rearranging:

$$\frac{[E4CC]}{[E4CP]} = \frac{A}{B}\frac{[4CC]}{[4CP]} \tag{19}$$

where $B = \frac{k5+k6}{k4}$

The mass balance for the enzyme is:

$$E_T = [E] + [E4CP] + [E4CC] \tag{20}$$

Then, combining Eqs. (18) to (20), the following expressions can be obtained:

Table 2 Fitting results corresponding to the tested models. In all cases, the change of 4CP (mM), 4CC (mM), P (mM), and OUR (mmolO_2 L^{-1} h^{-1}) were represented by Eqs. (7) to (10). Coefficient values of Models #1 to #4 are expressed in the following units: q_{m4CP} and q_{m4CC} in mmol $gTSS^{-1}$ h^{-1}, semisaturation and inhibition constants (K_{4CP}, K_{4CC}, K_{i4CP}, K_{i4CC}) in mM. For Model #5, the units are: k_{4CP} and k_{4CC} in L $gTSS^{-1}$ h^{-1}, and semisaturation constants A and B in mM. For all Models, q_P is expressed in mmolO_2 mmolP^{-1} h^{-1}

Model	R_{4CP}	R_{4CC}	Coefficients	r^2	RMSE
#1	$q_{m4CP}X\left(\frac{[4CP]}{K_{4CP}+[4CP]}\right)$	$q_{m4CC}X\left(\frac{[4CC]}{K_{4CC}+[4CC]}\right)$	$q_{m4CP} = 8.79 \pm 0.18$ $K_{4CP} = 8.44 \pm 0.17$ $q_{m4CC} = 0.77 \pm 0.01$ $K_{4CC} = (1 \pm 2)\times10^{-4}$ $q_P = 0.130 \pm 0.006$	0.8318	0.67606
#2	$q_{m4CP}X\left(\frac{[4CP]}{K_{4CP}+[4CP]}\right)$	$q_{m4CC}X\left(\frac{[4CC]}{K_{4CC}+[4CC]+\frac{[4CC]^2}{K_{i4CC}}}\right)$	$q_{m4CP} = 1.53 \pm 0.27$ $K_{4CP} = 1.07 \pm 0.05$ $q_{m4CC} = (6.3 \pm 0.5)\times10^{3}$ $K_{4CC} = (1.4 \pm 0.3)\times10^{-5}$ $K_{i4CC} = (3.0 \pm 0.7)\times10^{-4}$ $q_P = 0.011 \pm 0.001$	0.7491	0.81324
#3	$q_{m4CP}X\left(\frac{[4CP]}{K_{4CP}+[4CP]+\frac{[4CP]^2}{K_{i4CP}}}\right)$	$q_{m4CC}X\left(\frac{[4CC]}{K_{4CC}+[4CC]}\right)$	$q_{m4CP} = 4.67 \pm 0.06$ $K_{4CP} = 3.87 \pm 0.05$ $K_{i4CP} > 10^5$ $q_{m4CC} = 0.75 \pm 0.01$ $K_{4CC} = (0.2 \pm 2)\times10^{-4}$ $q_P = 0.082 \pm 0.007$	0.8159	0.69725
#4	$q_{m4CP}X\left(\frac{[4CP]}{K_{4CP}+[4CP]+\frac{[4CP]^2}{K_{i4CP}}}\right)$	$q_{m4CC}X\left(\frac{[4CC]}{K_{4CC}+[4CC]+\frac{[4CC]^2}{K_{i4CC}}}\right)$	$q_{m4CP} = 5.35 \pm 0.11$ $K_{4CP} = 4.26 \pm 0.47$ $K_{i4CP} > 10^5$ $q_{m4CC} = 0.61 \pm 0.05$ $K_{4CC} = (0.1 \pm 1)\times10^{-4}$ $K_{i4CC} > 10^5$ $q_P = 0.100 \pm 0.013$	0.7458	0.80721
5	$k_{4CP}X\left(\frac{[4CP]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right)$	$k_{4CC}X\left(\frac{[4CC]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right)$	$k_{4CP} = 106 \pm 7$ $k_{4CC} = 82 \pm 5$ $A = 0.045 \pm 0.003$ $B = 0.011 \pm 0.001$ $q_P = 0.086 \pm 0.004$	0.96366	0.30998

RMSE root mean squared error (mmolO_2 L^{-1} h^{-1})

$$[\mathrm{E}] = \mathrm{E_T}\frac{AB}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]} \tag{21}$$

$$[E4CP] = E_T\frac{B[4\mathrm{CP}]}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]} \tag{22}$$

$$[E4CC] = E_T\frac{A[4\mathrm{CC}]}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]} \tag{23}$$

To obtain the reaction rate of 4CP, expressions (21) and (22) were combined with Eq.(13):

$$\frac{\mathrm{d}[4\mathrm{CP}]}{\mathrm{dt}} = k2\mathrm{E_T}\frac{B[4\mathrm{CP}]}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]} - k1\mathrm{E_T}\frac{AB}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]}[4\mathrm{CP}] \tag{24}$$

Rearranging Eq.(24)

$$\frac{d[4CP]}{dt} = \left(\frac{\mathrm{k2}}{A} - k1\right)E_T\frac{AB[4\mathrm{CP}]}{AB + B[4\mathrm{CP}] + A[4\mathrm{CC}]} \tag{25}$$

Taking into account that:

$$\left(\frac{k2}{A} - k1\right) = -\frac{k1k3}{k2 + k3} \tag{26}$$

Thus:

$$\frac{d[4\mathrm{CP}]}{dt} = -\left(\frac{k1k3}{k2 + k3}\right)E_T\frac{[4\mathrm{CP}]}{1 + \frac{[4\mathrm{CP}]}{A} + \frac{[4\mathrm{CC}]}{B}} \tag{27}$$

Using a similar procedure, we can obtain the expressions corresponding to the reaction rate of 4CC and P:

$$\frac{d[4\mathrm{CC}]}{dt} = \left(\frac{k1k3}{k2 + k3}\right)E_T\frac{[4\mathrm{CP}]}{1 + \frac{[4\mathrm{CP}]}{A} + \frac{[4\mathrm{CC}]}{B}} - \left(\frac{k4k6}{k5 + k6}\right)E_T\frac{[4\mathrm{CC}]}{1 + \frac{[4\mathrm{CP}]}{A} + \frac{[4\mathrm{CC}]}{B}} \tag{28}$$

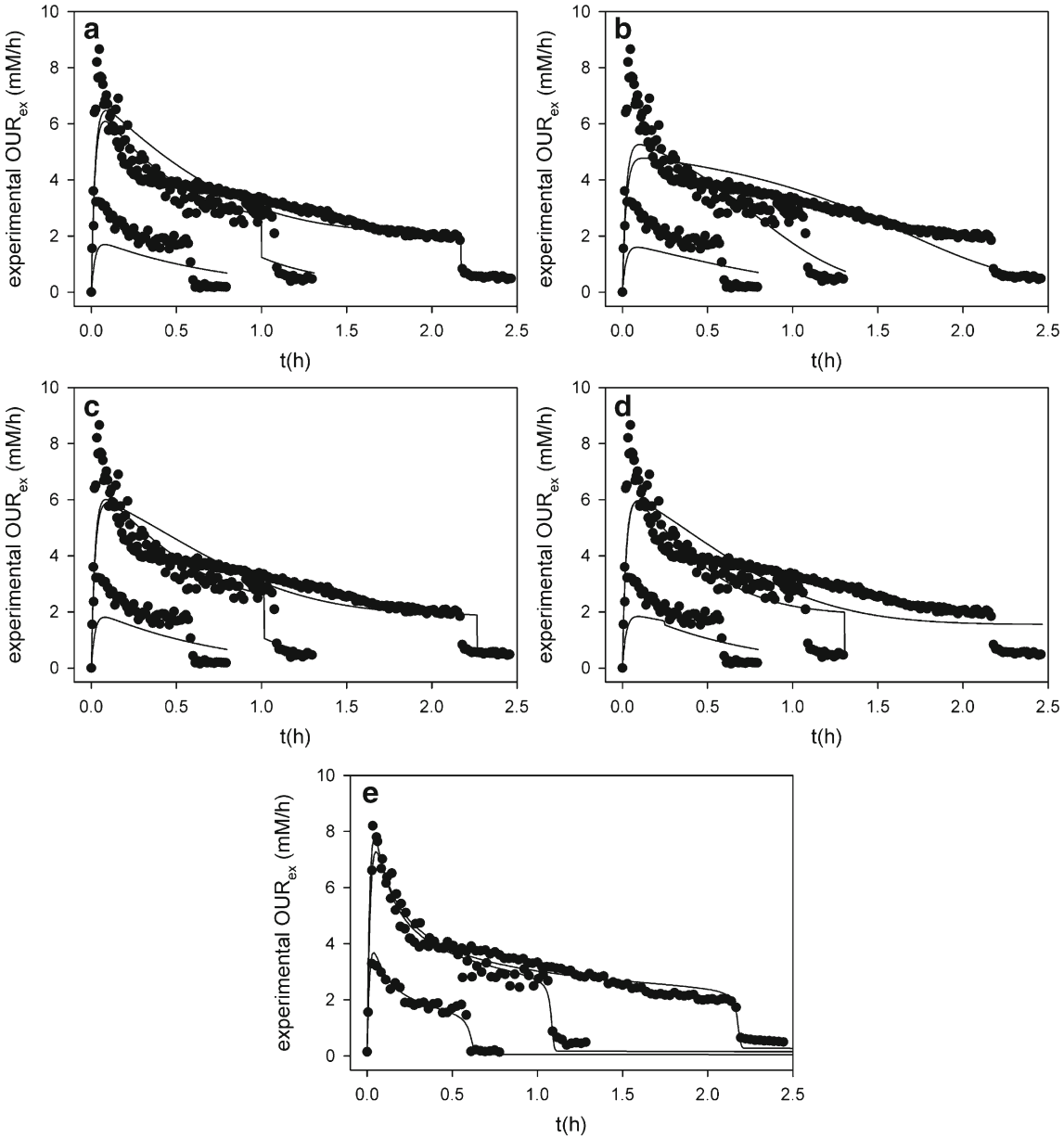

Fig. 5 OUR_{ex} as a function of time corresponding to different initial 4CP concentrations. *Continuous lines* represent the fitting results corresponding to **a** Model #1 ($R_{4CP\text{-}M}$, $R_{4CC\text{-}M}$). **b** Model #2 ($R_{4CP\text{-}M}$, $R_{4CC\text{-}H}$), **c** Model #3 ($R_{4CP\text{-}H}$, $R_{4CC\text{-}M}$), **d** Model #4 ($R_{4CP\text{-}H}$, $R_{4CC\text{-}H}$), and **e** Model #5. For more details of equations corresponding to the tested models, see Supplementary Data Item SD4

$$\frac{d[P]}{dt} = \left(\frac{k4k6}{k5+k6}\right) E_T \frac{[4CC]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}} \tag{29}$$

Assuming that E_T is proportional to the total biomass concentration (X): $E_T = q_E X$, where q_E is the specific enzyme content of the biomass, the following expressions result:

$$\frac{d[4CP]}{dt} = -R_{4CP} = -k_{4CP} X \left(\frac{[4CP]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right) \tag{30}$$

$$\frac{d[4CC]}{dt} = R_{4CP} - R_{4CC} = k_{4CP} X \left(\frac{[4CP]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right) - k_{4CC} X \left(\frac{[4CC]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right) \tag{31}$$

$$\frac{d[P]}{dt} = R_{4CP} = k_{4CC} X \left(\frac{[4CC]}{1+\frac{[4CP]}{A}+\frac{[4CC]}{B}}\right) \tag{32}$$

where $k_{4CP} = q_E\left(\frac{k1k3}{k2+k3}\right)$ and $k_{4CC} = q_E\left(\frac{k4k6}{k5+k6}\right)$

For all the tested initial 4CP concentrations, Model #5 (Eqs. 10 and 30 to 32) was fitted to the OUR_{ex} profiles as a function of time. Figure 5e shows that Model #5 reasonably represents the OUR_{ex} profiles observed. In results in Fig. 6, it allows concluding that Model #5 (Fig. 6e) represents the experimental OUR_{ex} values much better than Models #1 to #4 (Fig.6a–d). Table 2 shows that the correlation coefficient (r^2) of the predicted OUR values as a function of the experimental OUR_{ex} values ranged from 0.75 to 0.83 using Models #1 to #4 and 0.96 using Model #5. While Models #1 to #4 comprised five to seven adjustable coefficients, the good agreement between Model #5 and the respirometric profiles depicted in Fig. 1a was achieved using only five coefficients, thus reducing the fitting errors.

Konya et al. (2007) developed a mathematical model for the degradation of 4CP by activated sludge assuming that 4CP inhibits COD and 4CP removals and reported an r^2 value of 0.79 which was lower than the value obtained in the present work. Wang et al. (2015) studied the kinetic of phenol and 4CP biodegradation by *Pseudomonas putida* LY1. To this end, these authors developed a mathematical model to predict the cometabolic biodegradation of phenol and 4CP and obtained r^2 values between 0.90 and 0.96.

Model #5 and coefficients obtained from the fitting procedure (Table 2) were used to predict the TPh and COD_S

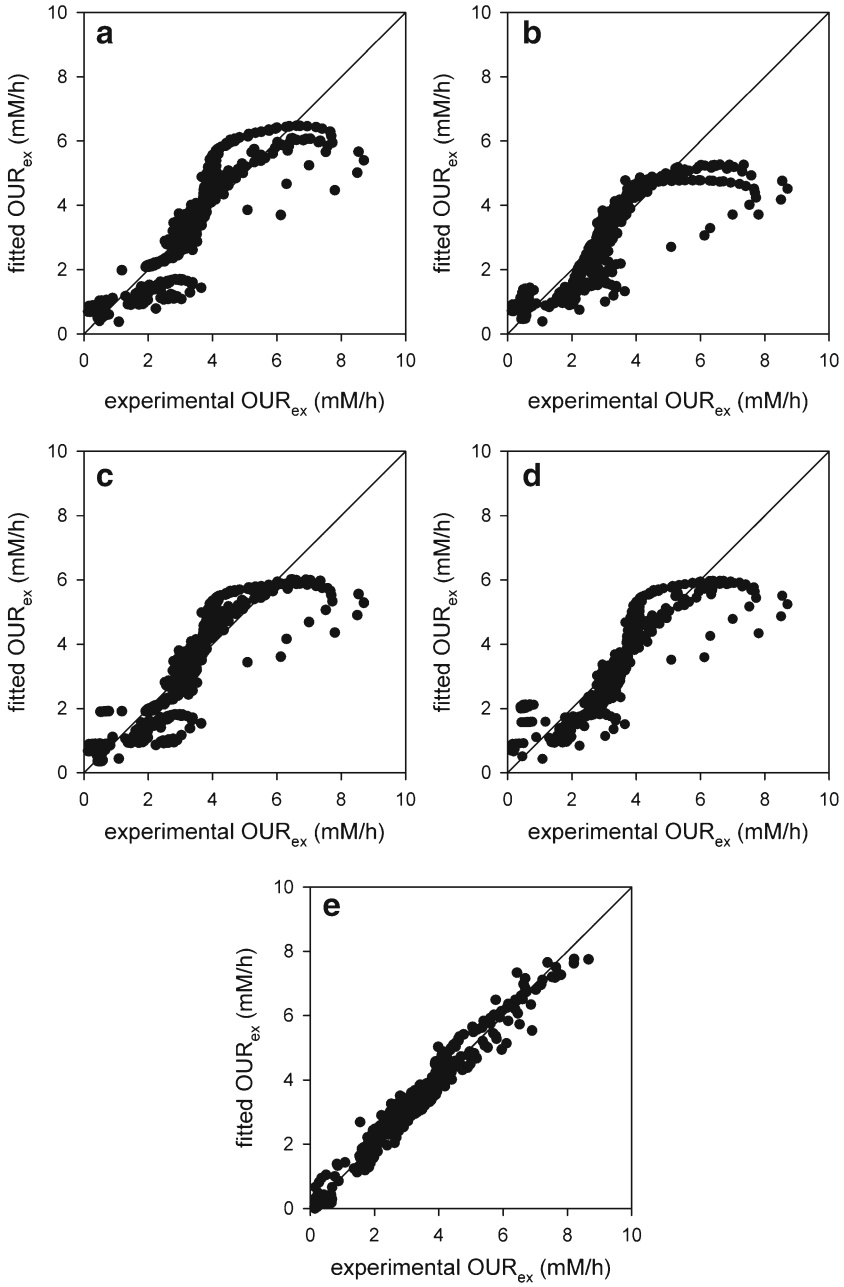

Fig. 6 Predicted as a function of experimental OUR_{ex} values using the different tested models: **a** Model #1 (R_{4CP-M}, R_{4CC-M}), **b** Model #2 (R_{4CP-M}, R_{4CC-H}), **c** Model #3 (R_{4CP-H}, R_{4CC-M}), **d** Model #4 (R_{4CP-H}, R_{4CC-H}), and **e** Model #5. The *straight line* represents the perfect correlation. For more details, see text

concentrations. Because TPh was assessed using the 4-aminoantipyrine colorimetric method, this measurement not only includes 4CP but also 4CC:

$$\mathrm{TPh} = [4\mathrm{CP}] + \mathrm{RF}\,[4\mathrm{CC}] \tag{33}$$

where RF = 0.43 is the response factor of 4CC by the 4AAP method with respect to 4CP. Similarly, the soluble COD (COD_S) is:

$$\mathrm{COD_S} = 208[4\mathrm{CP}] + 192\,[4\mathrm{CC}] + ThOD_P\,f_P[\mathrm{P}] \tag{34}$$

where 208 and 192 are the ThOD (gCOD/mol) of 4CP and 4CC, respectively. The term $ThOD_Pf_P$ represents the product between the ThOD corresponding to P ($ThOD_P$, gCOD/molP) and the amount of the P generated that is actually in the solution after the degradation of one mol of 4CP (f_P, molP/mol4CP). From the slope of the regression line depicted in Fig. 2b, a value for $ThOD_Pf_P$ = 45 gCOD/mol4CP was obtained. Details of this calculation can be found in the Supplementary Data, Item SD2. Figures 1b, c shows a reasonable agreement between the experimental values of TPh and COD_S and the ones calculated using the proposed model (Model #5). It must be pointed out that these data were not used during the fitting procedure of Model #5, but to validate the model developed. Thus, the agreement between the calculated and experimental TPh and COD_S concentrations (Fig. 1b, c) demonstrates the predictive power of the model developed concerning these two important environmental parameters.

Conclusions

The aerobic biodegradation of 4CP by activated sludge acclimated to phenol was assessed using an open respirometric technique. The results demonstrate that phenol-acclimated activated sludge can also degrade 4CP without prior acclimation to this xenobiotic. The respirometric profiles obtained reflect the action of the oxygenases responsible for the first steps of the aerobic oxidation of 4CP.

While 4CP removal was almost complete, COD removal ranged between 69 and 79% due to the accumulation of an intermediate. This intermediate had a maximum optical absorption at 380 nm, similar to that observed for 5C2HMS. However, if this compound is actually 5C2HMS, the calculations show that not all this intermediate remained in the solution, possibly due to its metabolization or adsorption onto the activated sludge. Moreover, successive additions of 4CP (0.64 mM) caused a noticeable decrease in the 4CP degradation rate.

Different kinetic models to represent the respirometric profiles during the aerobic degradation of 4CP were evaluated. Because of the poor fitting results obtained with standard models using Monod or Haldane equations, a new biokinetic model was proposed (Model #5). In this new model, it was assumed that the oxidation on 4CP and 4CC was catalyzed by the same oxygenase. Good agreement between the predicted and experimental OUR values was achieved by this new model. The fitted model also adequately predicted total phenols and soluble COD concentrations, which are two of the most relevant environmental parameters. The results obtained could help to predict the dynamics of biodegradation of chlorophenols in a biological wastewater treatment system.

4CC, 4-chlorocatechol (mM); 4CP, 4-chlorophenol (mM); 5C2HMS, 5-chloro-2-hydroxymuconic semialdehyde; A, semisaturation constants for model Model #5 (mM); B, semisaturation constants for model Model #5 (mM); BT, 1,2,4-benzenetriol; C, dissolved oxygen concentration (mM); C_e, dissolved oxygen concentration in the absence of an oxidizable substrate (mM); CODs, soluble chemical oxygen demand (mg L^{-1}); DO, dissolved oxygen; E, free enzyme; E_T, total enzyme; HQ, hydroquinone; IC50, cumulative substrate concentration necessary to reduce in 50% the specific substrate removal rate; K_{4CC}, 4-chlorocatechol semisaturation constant (mM); K_{4CP}, 4-chlorophenol semisaturation constant (mM); K_{i4CC}, 4-chlorocatechol inhibition constant (mM); K_{i4CP}, 4-chlorophenol inhibition constant (mM); k_La, oxygen mass transfer coefficient of the respirometer (h^{-1}); OC, oxygen consumed (mM); OUR_{ex}, oxygen uptake rate associated with the oxidation of 4CP (mM h^{-1}); P, intermediate oxidation product of 4-chlorophenol (mM); q_E, specific enzyme content of the biomass (mmol $gTSS^{-1}$); q_{m4CC}, maximum specific oxygen uptake rate associated with the oxidation of 4-chlorocatechol (mmol $gTSS^{-1}$ h^{-1}); q_{m4CP}, maximum specific oxygen uptake rate associated with the oxidation of 4-chlorophenol (mmol $gTSS^{-1}$ h^{-1}); q_P, specific oxygen uptake rate associated with the oxidation of product (mmolO_2 mmolP^{-1} h^{-1}); q_{Smean}, mean specific substrate removal rate (mmol $gTSS^{-1}$ h^{-1}); R_{4CC}, oxidation rate of 4-chlorocatechol (mM h^{-1}); R_{4CP}, oxidation rate of 4-chlorophenol (mM h^{-1}); RDA, relative activity of 4-chlorophenol degradation; S_0, initial 4-chlorophenol concentration (mM); t_d, observed degradation time (h); t_{d1}, observed degradation time corresponding to the first pulse (h); t_{di}, observed degradation time corresponding to the pulse I (h); ThOD, theoretical oxygen demand (gCOD/mol); TPh, total phenols (mM); TSS, total suspended solids (g L^{-1}); X, biomass concentration (g L^{-1}); $Y_{O2/4CP}$, 4-chlorophenol oxidation coefficient (mol/mol)

Acknowledgements This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), by Universidad Nacional de la Plata (UNLP), and by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina.

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