

Modelling of Biodegradation Kinetics for Binary Mixtures of Substituted Phenols in Sequential Bioreactors

Domenica Mosca Angelucci^{ab}, M.Cristina Annesini^b, M.Concetta Tomei^{*a}

^a Water Research Institute, C.N.R., Via Salaria km 29.300, CP 10-00016 Monterotondo Stazione (Rome) Italy

^b Department of Chemical Engineering Materials & Environment, Sapienza University of Rome, Via Eudossiana 18, 00184 Rome, Italy

tomei@irsa.cnr.it

Substituted phenols are extensively produced and utilized in chemical industry and therefore they are largely present in wastewater. In this paper we considered phenolic mixtures which are representative of industrial wastewater, usually containing multiple substrates. In these conditions, degradation process is strongly affected by the complex interactions among substrates, which include enhancement, inhibition and co-metabolism so a process model is an useful tool to explore and predict the process evolution. Objective of this work was to formulate a kinetic model for the biodegradation of binary mixtures performed in a sequencing batch reactor (SBR). Two model mixtures were investigated: a 4-nitrophenol (4NP) and 2,4-dimethylphenol (2,4DMP) mixture, and a more recalcitrant mixture of 4NP and 2,4-dichlorophenol (2,4DCP). Kinetic tests were performed at different feed concentrations, with single compounds and mixtures and each biodegradation process was kinetically characterized. Haldane equation was utilised to model the substrate inhibited kinetics for single compound while for the mixtures the model was modified with a "switching function" to account the mutual substrate interaction. The proposed model was initially calibrated with a preliminary set of data to evaluate the best-fitting parameters, then validated by simulating different runs with the estimated parameters. Satisfactory results with high correlation coefficients (≥ 0.98) and reliable predictions were obtained for the two investigated mixtures.

1. Introduction

Phenolic compounds are extensively produced and utilized in chemical industry and as a consequence they are largely present in water emissions. Their low biodegradability and strong persistence is of serious concern for the environmental protection. Application of biological processes to these xenobiotic compounds removal is a promising alternative to conventional chemical-physical treatment methods but it requires a powerful technology. Biodegradations of phenolics by anaerobic, aerobic bacteria and fungi have been reported (Karigar et al., 2006; Annachatre and Gheewala, 1996) and various technological approaches have been developed to treat phenolic effluents. Discontinuous (or semi-batch) bioreactors have been demonstrated to be effective in the biodegradation of biorefractory compounds being characterized by high operation flexibility and favourable cost/effectiveness ratio (Annesini et al., 2011). Furthermore, sequencing batch reactors (SBRs) have been investigated and proved effective also for phenolic wastewater treatment (Nakhla et al., 1993; Tomei et al., 2003; Sahinkaya and Dilek, 2006; Wang et al., 2007).

The target compounds chosen in this study are 4-nitrophenol (4NP), 2,4-dimethylphenol (2,4DMP) and 2,4-dichlorophenol (2,4DCP), all of them are toxic, included in the priority pollutant list of U.S. EPA and with EC_{50} values of 64, 190 (Volskay and Grady, 1988) and 2.3 - 40 $mg L^{-1}$ (Ren and Frymier, 2005; Erol Nalbur and Alkan, 2007), respectively. Effluents containing these compounds are originated from many industrial activities (e.g. coal conversion processes, production of herbicide and pesticide, petroleum refineries and petrochemical, textile and pharmaceutical industries, production of dyes, explosives, leather coloring...) hence binary phenolic mixtures can be considered representative of a wide spectrum of industrial wastewater.

Biodegradation process of multiple and toxic substances is strongly affected by the complex interactions that can occur among substrates, which can include enhancement, inhibition and co-metabolism. Evaluation of substrate inhibition is of strong relevance in the treatment of toxic compounds (e.g. substituted phenols) in engineered systems such as activated sludge processes (Hao et al., 2002); therefore mathematical modelling can be helpful for understanding the behaviour of biological processes and predicting the system evolution (Nuhoglu and Yalcin, 2005). Besides knowledge of the process kinetics is essential for biological wastewater treatment system design and optimization of operational conditions (Contreras et al., 2001).

The main objectives of this work were to investigate the biodegradation of binary phenolic mixtures performed in a sequencing batch reactor and to formulate a kinetic model for the biodegradation of the tested mixtures including the mutual interactions of the substrates in a system where the same mixed microbial population is catalysing the contemporary biodegradation of similar compounds characterized by different toxicity levels. Two different binary mixtures were explored: the first one constituted of 4NP and 2,4DMP (MIX1) and the second one of 4NP and 2,4DCP (MIX2). For both of them, kinetics of biodegradation in a SBR reactor was evaluated with the aim of estimating the SBR applicability to industrial wastewater treatment.

2. Materials and methods

2.1 Chemicals

4-nitrophenol (4NP) was purchased from Fluka (Germany) while 2,4-dimethylphenol (2,4DMP), 2,4-dichlorophenol (2,4DCP) (purity >99%) and sodium acetate were obtained from Sigma Aldrich (Italy). All other chemicals were commercial grade and were supplied by Carlo Erba (Italy).

2.2 Biomass

The acclimatization procedure of the mixed culture utilized in the experiments is detailed elsewhere (Tomei et al., 2004). An inoculum from this culture, previously adapted to 4NP was separately acclimatized over a 2-3 months period to 2,4DMP and 2,4DCP in mixture with sodium acetate. In this acclimatization procedure performed with the single compounds, substituted phenol concentration was gradually increased (up to 300 mg L⁻¹ for 2,4DMP and 180 mg L⁻¹ for 2,4DCP) and the sodium acetate was progressively reduced when stable performance was obtained. Finally the acetate was eliminated from the system and the bioreactor was fed with the xenobiotic as sole carbon and energy source. Once the complete removal and stable performance were achieved, the biomass was employed for the mixture biodegradation. An overview of the phenols influent concentrations during start up and the following kinetic study is given by Figures 1a and 1b for MIX1 for MIX2, respectively. To ensure the presence of required nutrients and microelements, in all cases the feed consisted of a xenobiotic solution with the addition of the mineral medium MSV (Williams and Unz, 1989). The mineral medium was formulated to ensure a C:N:P ratio in the influent equal to 100:5:1 with respect to the phenols and acetate carbon content.

2.3 Sequencing Batch Reactors

The SBR reactors are lab-scale glass vessels (working volume 0.8 L) equipped with an on line system for dissolved oxygen and temperature control and the automatic temporization of the work phases. Typical durations of the work phases are reported in the following: feed 12 - 15 min, reaction 350 - 650 min, settling 30 min and draw 15 min. A more detailed description of the SBR reactor was reported in Tomei et al. (2011a).

2.4 Analytical methods

Analysis of single phenols were performed on centrifuged (10,000 rpm for 6 min) aqueous samples; the supernatant was then analyzed by UV absorbance using a spectrophotometer (Varian, model Cary 1). For the binary mixtures a double reading of the samples was performed at two different wavelengths (320 and 280 nm for 4NP and 2,4DMP, respectively; 400 and 280 nm for 4NP and 2,4DCP, respectively) as described elsewhere (Tomei et al., 2011b). Volatile Suspended Solid (VSS) concentration was determined according to standard methods (APHA, 1998) as an estimate of the biomass concentration.

2.5 Kinetic tests

Biodegradation kinetics was investigated for the single compounds and for their binary mixtures, therefore kinetic tests were performed in SBR system by measuring the concentrations on samples of the aqueous phase taken from the reactor at predetermined time intervals (5 - 20 min) during the feed and reaction phases. VSS concentration was also monitored but at longer time intervals (hours) due to its very low variation with respect to the typical concentrations in the reactor.

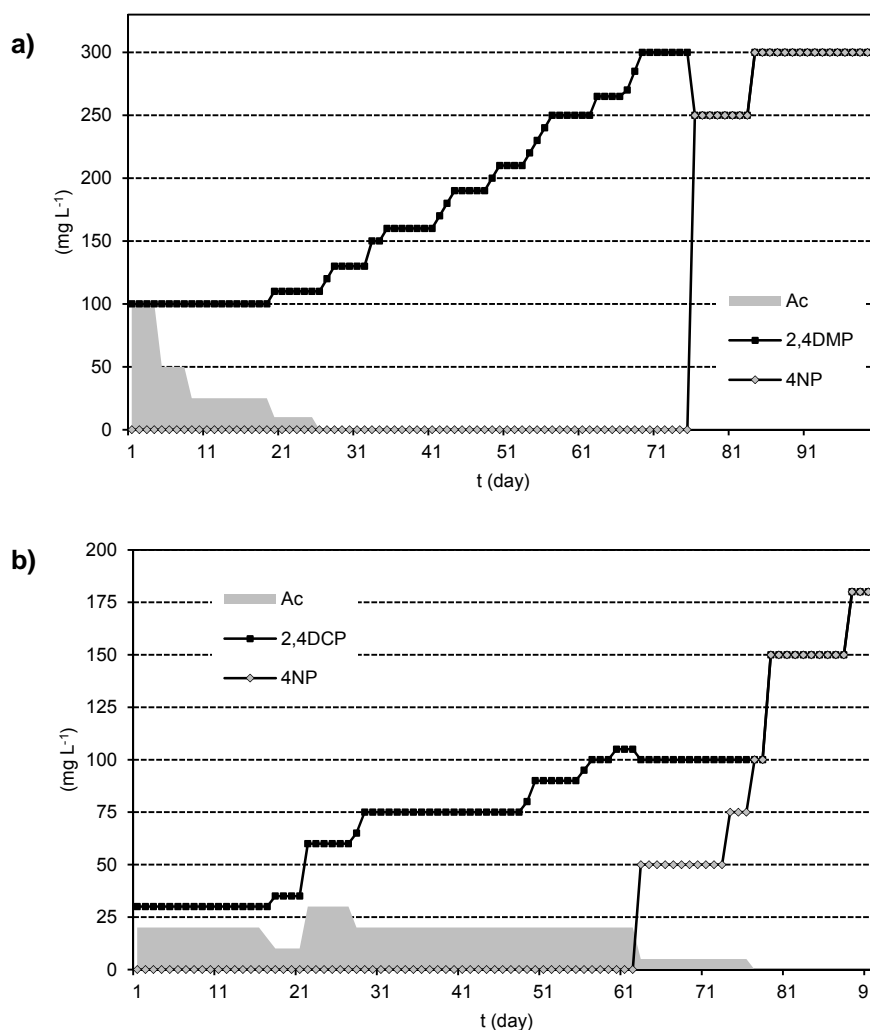


Figure 1: Overview of feeding conditions during the acclimatization procedures for (a) MIX1 and (b) MIX2 (Ac =sodium acetate)

In order to verify the reproducibility of the data, biodegradation tests were carried out in at least two replicates under the same operating conditions. Table 1 shows the test plan for the biodegradation experiments and operating conditions of the kinetic tests. Two series of tests with several feed concentration conditions were performed: the first one with single compounds and the second one with mixtures. Two different binary mixtures were utilized: the first one constituted of 4NP and 2,4DMP (MIX1) and the second one of 4NP and 2,4DCP (MIX2).

3. Modelling

Kinetic constants are evaluated by fitting the experimental data of substrate concentration vs. time in batch experiments. In this work, firstly it was investigated biodegradation kinetics of single compounds, then of the two mixtures as described below.

Table 1: Kinetic tests plan (biomass concentration is a mean value resulting from multiple measurements during each test). The two latest lines represent additional kinetic tests used for the model validation

Test	Influent concentration			Biomass concentration
	4NP (mg L ⁻¹)	2,4DMP (mg L ⁻¹)	2,4DCP (mg L ⁻¹)	(mg _{vss} L ⁻¹)
S 1a	260	-	-	1,860
S 1b	320	-	-	1,770
S 2a	-	100	-	2,065
S 2b	-	230	-	2,855
S 3a	-	-	110	1,950
S 3b	-	-	180	2,244
M 1a	300	300	-	2,290
M 1b	230	370	-	2,740
M 1c	370	230	-	2,640
M 2a	50	-	100	2,760
M 2b	100	-	100	1,940
M 2c	150	-	150	1,860
M 1d	200	200	-	3,260
M 2d	90	-	120	2,760

3.1 1st series: single compounds

Haldane equation, largely employed to model self-inhibitory compounds biodegradation (Andrews, 1968; Cooper Brown et al., 1990), was utilised to model the substrate inhibited kinetics:

$$r_s = v \frac{c}{c + K_S + \frac{c^2}{K_I}} = k \cdot X \frac{c}{c + K_S + \frac{c^2}{K_I}} \quad (1)$$

where r_s is the substrate consumption rate and X and C are the biomass and substrate concentration, respectively. This model includes three parameters: the rate constant k and the half-saturation and inhibition constants, K_S and K_I , respectively. The inhibition constant K_I , is a measure of bacterial sensitivity to inhibition exerted by self-inhibitory substances. It should be noted that for high K_I values the Haldane equation is reduced to the Monod equation (that implies that the culture is less sensitive to substrate inhibition). As a consequence, low K_I values show that the inhibition effect can be observed at low phenols concentration (Marrot et al., 2006). Kinetic parameters were determined by the fitting of substrate concentration profile with the further assumption that, in agreement with experimental results, the biomass concentration remains practically constant throughout each kinetic test. Fitting was performed with the software package Scientist[®] 3.0 for Windows (Micromath Scientific Software, USA).

3.2 2nd series: mixtures

Biodegradation data of single compounds were utilized to evaluate the intrinsic kinetic parameters but these data were not enough to predict the mutual effects on the kinetic mechanisms involved in the mixture degradation process (Tomei and Annesini, 2008). In order to model substrate inhibition as a function of also the other component concentration in the mixture, the kinetic Eq.(1) is modified with a "switching function". The concept of switching function in modeling biological processes was firstly introduced by the Task Group of the IAWPRC (International Association on Water Pollution Research Control), in their Activated Sludge Model No. 1 (Henze et al., 1987) to gradually turn process rate equations on and off as the environmental conditions were changed. The switching functions are 'Monod-like' expressions that are mathematically continuous and thereby reduce numerical instability problems during simulations. Therefore, Eq.(1) was modified as:

$$r_{S,1} = v_1 \frac{C_1}{C_1 + K_{S,1} + \frac{C_1^2}{K_{I,1}}} \cdot \frac{K_{M,1}}{K_{M,1} + C_2} = k_1 \cdot X \frac{C_1}{C_1 + K_{S,1} + \frac{C_1^2}{K_{I,1}}} \cdot \frac{K_{M,1}}{K_{M,1} + C_2} \quad (2)$$

$$r_{S,2} = v_2 \frac{C_2}{C_2 + K_{S,2} + \frac{C_2^2}{K_{I,2}}} \cdot \frac{K_{M,2}}{K_{M,2} + C_1} = k_2 \cdot X \frac{C_2}{C_2 + K_{S,2} + \frac{C_2^2}{K_{I,2}}} \cdot \frac{K_{M,2}}{K_{M,2} + C_1} \quad (3)$$

where $K_{M,i}$ represents inhibition constant accounting for the reduction on the i removal kinetics due to the presence of j ($j \neq i$) component: the higher the $K_{M,i}$ value the lowest the mutual inhibitory effect, that is not significant if $c_j \ll K_{M,i}$. The proposed model was initially calibrated with a preliminary data analysis aimed to the evaluation of the best-fitting parameters, then the model was validated by simulating different runs with the estimated parameters.

4. Results and discussion

4.1 Start up

Biomass utilized in the experiments was separately acclimatized over a 2 - 3 months period to 2,4DMP and 2,4DCP in two SBR reactors fed with the phenolic compounds and sodium acetate as biogenic substrate. After this acclimatization period, in which kinetic tests on single compounds were carried out, the SBR reactors were fed with two mixtures, as indicated in Figures 1a and 1b, for MIX1 and MIX2, respectively. Very high removal efficiencies were immediately observed for 2,4DMP: removal efficiency > 90% was observed after just one week of experimentation, therefore sodium acetate added to feed phase was quickly reduced and finally eliminated, while 2,4DMP influent concentration was progressively increased up to 300 mg L⁻¹, as showed in Figure 1a. For 2,4DCP the acclimatization procedure was more difficult, as expected by the more biorefractory nature of this compound. Removal efficiency was initially variable, in the range of 30 - 70% in the first month of experimental period. This induced to maintain 2,4DCP and sodium acetate concentration in the feed around 75 and 20 mg L⁻¹, respectively. When removal efficiencies above 80% were achieved, 2,4DCP was gradually increased to 100 mg L⁻¹ and sodium acetate reduced to 5 mg L⁻¹ and eliminated when the mixture of 2,4DCP and 4NP was fed to the reactor, as indicated in Figure 1b.

Once the complete removal and stable performance were achieved for the two reactors, the biomass were employed for mixtures kinetic biodegradation tests.

4.2 Kinetic data analysis

4.2.1. 1st series: single compounds biodegradation kinetics evaluation

Single compounds biodegradation tests on 4NP (S 1a-1b), 2,4DMP (S 2a-2b) and 2,4DCP (S 3a-3b) were performed in the first series of kinetic tests with different operating conditions summarized in Table 1. Data analysis was performed by fitting the substrate concentration values (measured during the reaction phase) vs. time. A preliminary analysis has been carried out by evaluating best fitting parameters k , K_S and K_I for each compound. Results of this first analysis indicated that we can assume a fixed pair of K_S and K_I values for each compound and evaluate a best-fitting k value for each run. This procedure was also reported in Tomei et al. (2003). This first series of data fitting gives very good correlation coefficients (> 0.99). Best-fitting parameters obtained from this first data processing are reported in Table 2. Differences in k values for the same compound can be explained with the intrinsic variability of the biological tests due to biomass adaptation to the substrate. From k values reported in Table 2, it is observed (as expected from the EC₅₀ values) that 4NP and 2,4DMP are characterized by higher specific removal rates compared to 2,4DCP.

Figure 2 shows the specific substrate removal rates ($r_{sp} = r_s/X$) vs. substrate concentration for three phenols investigated; curves are obtained by simulations performed with the average values of the best-fitting parameters for each compound.

The substrate concentration, C^* , where the maximum removal rate occurs is given by:

$$C^* = \sqrt{K_S \cdot K_I} \quad (4)$$

and calculated C^* are 30 mg_{4NP} L⁻¹, 60 mg_{2,4DMP} L⁻¹ and 14.7 mg_{2,4DCP} L⁻¹ respectively.

Table 2: Best-fitting parameters for the 1st series of kinetic tests

Test	Compound	K_S (mg L ⁻¹)	K_I (mg L ⁻¹)	k (mg mgVSS ⁻¹ d ⁻¹)
S 1a	4NP	50	18	2.67
S 1b		50	18	3.29
S 2a	2,4DMP	200	18	1.58
S 2b		200	18	5.34
S 3a	2,4DCP	18	12	0.54
S 3b		18	12	1.02

Curves reported in Figure 2 show a typical trend of self-inhibited kinetics with specific reaction rates increasing for phenols concentration lower than C^* , then the reaction rates decreases for substrate concentrations $\geq C^*$. It can be observed that the maximum of reaction rates occurs at phenol concentrations, lower than the influent concentrations utilized in the experiments: this confirms that the investigated concentrations are strongly inhibitory for the biomass.

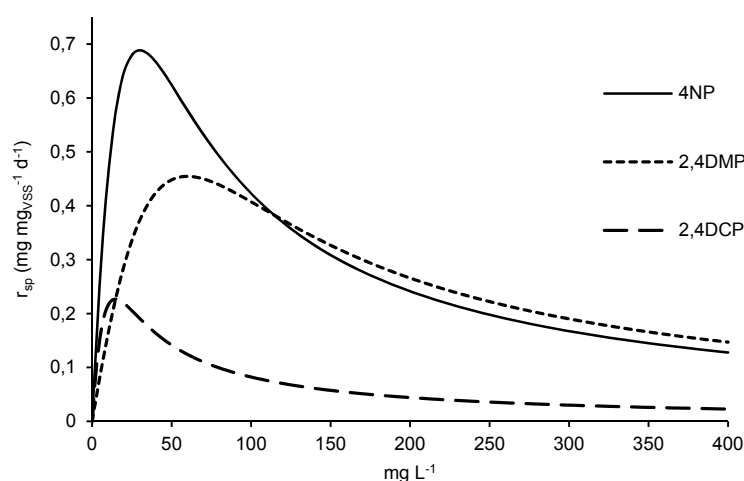


Figure 2: Specific reaction rates vs. substrate concentration for single compounds

4.2.2. 2nd series: mixtures biodegradation kinetics evaluation

In the second series of kinetic tests the biodegradation kinetics of two phenolic mixtures MIX1 and MIX2 was investigated. Several kinetic tests were carried out at different operating conditions as summarized in Table 1. Data analysis was performed by fitting the experimental data with the proposed kinetic model Eq(2) and Eq(3). In this case the same K_S and K_I values previously obtained from the first kinetic series were assumed (see values reported in Table 2), while best-fitting parameters k_1 , k_2 and $K_{M,i}$ are obtained from mixture biodegradation data. According to experimental 4NP concentration patterns, it was assumed:

$$R_{4NP} = \frac{K_{M,1}}{K_{M,1} + C_2} = 1 \quad (5)$$

so Eq.(2) reduces to Eq.(1), only for 4NP. Kinetic parameters obtained by the fitting of this 2nd test series are summarized in Tables 3 and 4 for MIX1 and MIX2, respectively, while Figures 3a and 3b show the typical patterns for the two compounds concentration profiles observed for MIX1 and MIX2, respectively, in M1a and M2c tests and the corresponding fitting curves. Satisfactory agreement between the calculated profiles and the experimental data was obtained for both M1a and M2c tests; similarly, good agreement was found for the other tests, with correlation coefficients ≥ 0.98 in all cases.

Table 3: Best-fitting parameters for the 2nd series of kinetic tests for MIX1

Test	k (mg mgVSS ⁻¹ d ⁻¹)		K _M (mg L ⁻¹)
	4NP	2,4DMP	2,4DMP
M 1a	4.82	1.53	3.70E+26
M 1b	2.53	1.22	3.58E+26
M 1c	1.66	4.26	2.74E+20

Table 4: Best-fitting parameters for the 2nd series of kinetic tests for MIX2

Test	k (mg mgVSS ⁻¹ d ⁻¹)		K _M (mg L ⁻¹)
	4NP	2,4DCP	2,4DCP
M 2a	0.31	0.40	36.63
M 2b	0.44	0.40	13.59
M 2c	1.14	0.72	12.95

For MIX1 it was observed a faster biodegradation of 4NP compared to the other substrate, thus confirming the results of single compound tests. It is also worth noting that the biodegradation of the two phenols occurs simultaneously, even if 2,4DMP degradation is characterized by a slower initial rate.

K values determined for 4NP by the fitting of the mixture data are comparable to those obtained in single compound tests with the exception of M1c test, where the higher $C_{in,4NP}$ value (370 mg L⁻¹) can justify the k decrease. Instead, for the 2,4DMP in mixture there is a reduction of about 30 % of the removal rate compared to the single compound average value. Synchronous compounds biodegradation is also highlighted by mutual inhibition constant values of MIX1: all values are significantly higher than typical feed concentrations so in the range of the investigated influent concentration values there is not a marked effect of the mutual inhibition.

The behavior of MIX2 is quite different: although degradation rate is faster for 4NP than for 2,4DCP, as in MIX1, biodegradation of the two compounds is not simultaneous. Figure 3b shows the concentration profiles of the two compounds in M2c test: 2,4DCP removal didn't start until 4NP was almost completely depleted. This pattern is more evident at higher influent concentrations (i.e. in M2b and M2c tests with respect to M2a).

The sequential degradation of the two compounds is also demonstrated by mutual inhibition constant values of MIX2 reported in Table 4: $K_{M,2,4DCP}$ values are in the range 12.95 – 36.63 mg L⁻¹ for all tests, so until 4NP concentration remains close to that range, 2,4DCP removal rate is almost negligible, as can be seen in Figure 3b.

For MIX2, kinetics of both compounds are significantly modified in comparison to single substrate tests: rate constants of both compounds are decreased and 4NP removal is strongly affected by the inhibitory action of 2,4DCP. A quantitative estimation of this effect is given by the k values: for 2,4DCP k value decreased by 35% with respect to single compound tests, while for 4NP a mean reduction of 80 % is observed. This finding suggests that 2,4DCP competitively inhibited the degradation of 4NP, as was also observed by Quan et al., 2005 and Monsalvo et al., 2009.

4.2.3. Model validation

Finally, the last step was to test the predictive capabilities of the proposed model at different phenols loading: to this aim a different data set, not utilized during the previous data fitting, was used for both mixtures. The model validation was carried out by simulating, for each binary mixture investigated, runs at different initial compounds concentrations: test M1d for MIX1 and M2d for MIX2, whose operating conditions are reported in Table 1. Kinetic parameter values used in simulations originated from those estimated from the experimental data in the second

series of kinetic tests: average values of the determined parameters for each binary mixture have been used. Figures 4a and 4b show a comparison between model simulations of phenols concentrations time-profiles and the corresponding experimental data for MIX1 and MIX2, respectively. Excellent agreement between the model predictions and the experimental data was obtained, so demonstrating that the proposed modified Haldane equation is able to model the mutual effect of self- and cross-inhibitory substrates in mixture, giving an evidently reliable description of the substrate removal. It is worth noting that the proposed model is suitable to describe both the synchronous disappearance of 4NP and DMP in MIX1 mixture and the sequential degradation obtained in the biodegradation tests of the more recalcitrant mixture, MIX2.

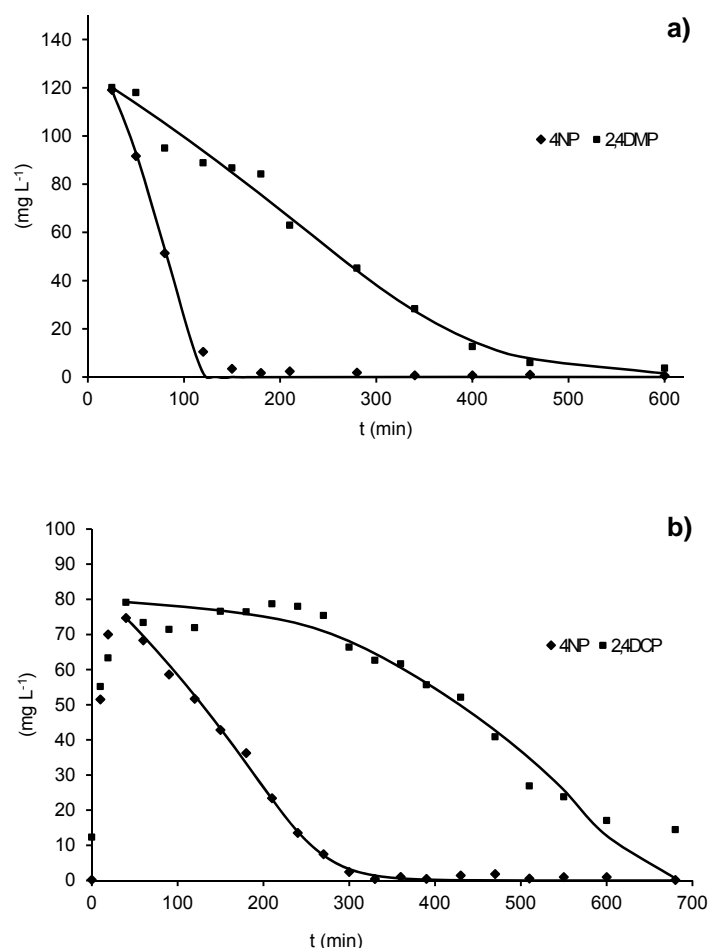


Figure 3: Phenols concentration profiles in biodegradation tests for MIX1 - test M1a (a) and MIX2 – test M2c (b): experimental data and calculated profiles

5. Conclusions

In this paper a new methodology in modeling the biodegradation kinetics of self-inhibitory compounds based on the Haldane equation modified with a switching function is proposed and applied to the biological removal of mixtures of phenolic compounds in sequential bioreactors. The proposed model is simple and easy to apply in that requires only one additional parameter with respect to the original formulation of the Haldane equation. Kinetic data of mixtures of substituted phenols characterized by different toxicity characteristics were analyzed and very good correlations (correlation coefficients always ≥ 0.98) were obtained for all the investigated mixtures. The model was calibrated and then validated with very positive results in predicting the behavior of each compound in the mixtures investigated. Model predictions indicated that the proposed modified Haldane model

successfully simulated all the experimental concentration profiles detected in biodegradation tests, such as the synchronous disappearance of MIX1 compounds and also the sequential degradation of 4NP and DCP in MIX2. A wastewater treatment plant generally receives influents consisting of a mixture of more or less recalcitrant substrates added to biogenic substances, hence evaluation of substrate inhibition is of strong relevance for understanding the behavior of biological engineered process and predicting the system evolution. Results of this study could be useful to design industrial wastewater treatment plants as well as to optimize operational conditions of existing plants.

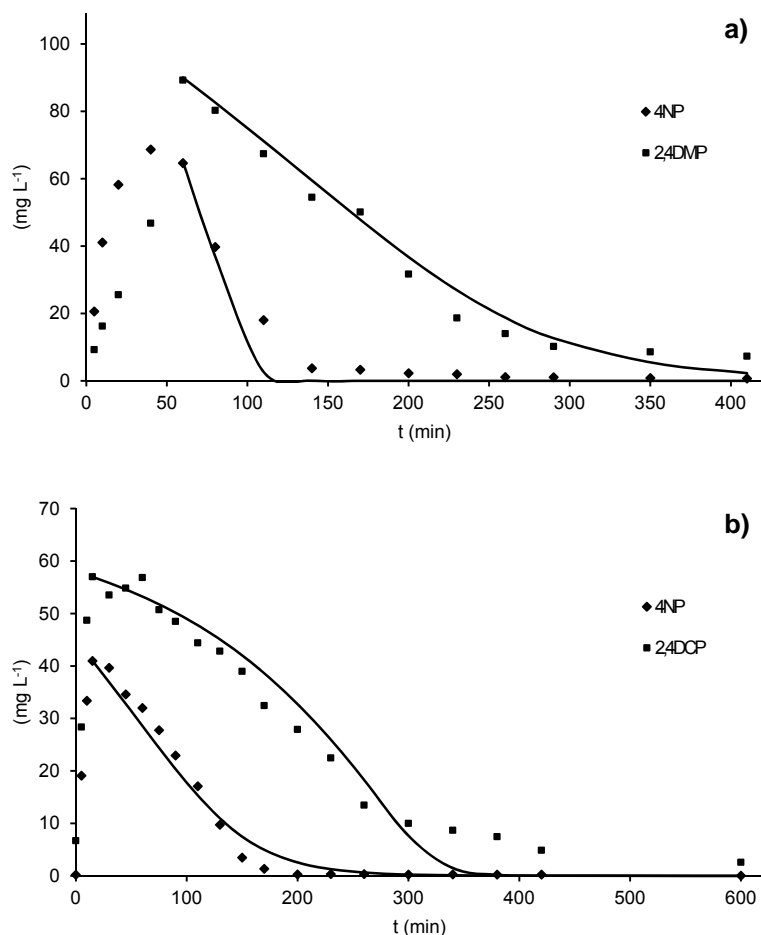


Figure 4: Model simulations and experimental data for phenols concentration in biodegradation tests for (a) MIX1 - test M1d and (b) MIX2 - test M2d

References

- APHA, 1998, Standards Methods for the Examination of Water and Wastewater, 20th edition .
- Andrews J.F., 1968, A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates, *Biotechnol. Bioeng.* 10 (6), 707-23.
- Annachatre A.P., Gheewala S.H., 1996, Biodegradation of chlorinated phenolic compounds, *Biotechnology Advances*, 14 (1), 35-56.
- Annesini M.C., Piemonte V., Tomei M.C., Daugulis A.J., 2011, SBR reactors for xenobiotic removal: dynamic simulation and operability criteria. *Chemical Engineering Transactions*, 24, 991-996.
- Contreras E., Bertola N., Zaritzky, N., 2001, The application of different techniques to determine activated sludge kinetic parameters in a food industrial wastewater. *Water SA* 27 (2), 169-176.

Cooper Brown S., Grady Jr C.P.L., Tabak H.H., 1990, Biodegradation kinetics of substituted phenolics: demonstration of a protocol based on electrolytic respirometry, *Water Res.* 24 (7), 853-61.

Erol Nalbur B., Alkan U., 2007, The inhibitory effects of 2-CP and 2,4-DCP containing effluents on sequencing batch reactors, *Int. Biodeter. Biodegr.* 60, 178–188.

Hao O.J., Kim M.H., Seagren E.A., Kim H., 2002, Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species, *Chemosphere* 46, 797-807.

Henze M., Grady C.P.L. Jr, Gujer, W., Marais G.v.R., Matsuo T., 1987, Activated Sludge Model No. 1, IAWPRC Scientific and Technical Reports No. 1, IAWPRC, London, UK.

Karigar C., Mahesh A., Nagenahalli M., Yun D.J., 2006, Phenol degradation by immobilized cells of *Arthrobacter citreus*, *Biodegradation*, 17, 47-55.

Marrot B., Barros-Martinez A., Moulin P., Roche N., 2006, Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor, *Biochem. Eng. J.* 30, 174-183.

Monsalvo V.M., Mohedano A.F., Casaa J.A., Rodriguez J.J., 2009, Cometabolic biodegradation of 4-chlorophenol by sequencing batch reactors at different temperatures, *Bioresource technology*, 100, 4572-4578.

Nakhla G.F., Al Harazi I.M., Farooq S., 1993, Organic loading effects on the treatment of phenolic wastewaters by sequencing batch reactors. *Water Environ. Res.* 65 (5), 686–689.

Nuhoglu A., Yalcin B., 2005, Modelling of phenol removal in a batch reactor, *Process Biochem.* 40, 1233-1239.

Quan X., Yang Z., Shi H., Tang Q., Qian Y., 2005, The effect of a secondary chlorophenol presence on the removal of 2,4-dichlorophenol (2,4-DCP) in activated sludge system bioaugmented with 2,4-DCP degrading special culture. *Process Biochem* 40, 3462–3467.

Ren S.J., Frymier P.D., 2005, Toxicity of metals and organic chemicals evaluated with bioluminescence assays, *Chemosphere* 58, 543-550.

Sahinkaya E., Dilek F.B., 2006, Effect of biogenic substrate concentration on the performance of sequencing batch reactor treating 4-CP and 2,4-DCP mixtures, *J. Haz. Mat.* 128, 258–264.

Tomei M.C., Annesini M.C., Luberti R., Cento G., Senia A., 2003, Kinetics of 4-nitrophenol biodegradation in a sequencing batch reactor, *Water Res.* 37, 3803-3814.

Tomei M.C., Annesini, M.C., Bussolletti S., 2004, 4-Nitrophenol biodegradation in a sequencing batch reactor: kinetic study and effect of filling time, *Water Res* 38, 375-384.

Tomei M.C., Annesini M.C., 2008, Biodegradation of phenolic mixtures in a sequencing batch reactor. A kinetic study, *Env. Sci. Pollut. Res.* 15 (3), 188-195.

Tomei M.C., Rita S., Mosca Angelucci D., Annesini M.C., Daugulis A.J., 2011a, Solid-liquid partitioning bioreactors applied to the removal of mixtures of phenolic compounds, *Chemical Engineering Transactions*, 24, 1243-1248.

Tomei M.C., Rita S., Mosca Angelucci D., Annesini M.C., Daugulis A.J., 2011b, Treatment of substituted phenol mixtures in single phase and two-phase partitioning bioreactors, *J. Haz. Mat.* 191, 190-95.

Volskay V.T., Jrand Grady C.P.L. Jr, 1988, Toxicity of selected RCRA compounds to activated sludge microorganisms, *J. Wat. Pollut. Control Fed.* 60, 1850-1856.

Wang S.G., Liu X.W., Zhang H.Y., Gong W.X., Sun X.F., Gao B.Y., 2007 Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor, *Chemosphere* 69, 769-775.

Williams T.M., Unz R.F., 1989, The nutrition of Thiothrix, Type 021N, Beggiatoa and Leucothrix strains. *Water Res.* 23, 15-22.