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

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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 ($\geq$ 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. 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). 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) e 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 and pharmaceutical industries) 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). Objective of this work was to formulate a kinetic model for the biodegradation of binary mixtures performed in a sequencing batch reactor (SBR)

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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.

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 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 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. To ensure the presence of required nutrients and microelements, in all cases the feed consisted of a compound 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 carbon.

2.3 Sequencing Batch Reactor

The SBR reactor is lab scale glass vessel (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. (2011).

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., 2011). Volatile Suspended Solid (VSS) concentration was determined according to standard methods (APHA, 1998) as an estimate of the biomass concentration.

Table 1: Kinetic tests plan (biomass concentration is a mean value resulting from multiple measurements during each test)

Test	Influent 4NP concentration (mg L^{-1})	Influent 2,4DMP concentration (mg L^{-1})	Influent 2,4DMP concentration (mg L^{-1})	Biomass concentration (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

2.5 Kinetic tests

Biodegradation kinetics was investigated for the single compounds and for their binary mixtures, and kinetic tests were performed in the SBR 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. 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 at different feed concentration were performed: the first one with single compounds and the 2nd with mixtures of 4NP and 2,4DMP first (MIX1) and of 4NP and 2,4DCP (MIX2).

3. Modelling

3.1 1st series: single compounds

Haldane equation, largely employed to model self-inhibitory compound 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 saturation and inhibition constants, K_S and K_I 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 run. Fitting was performed with the software package Scientist 3.0 for Windows (Micromath).

3.2 2nd series: mixture

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 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≠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 to evaluate the best-fitting parameters, then the model was validated by simulating different runs with the estimated parameters.

4. Results and discussion

4.1 1st series: single compound biodegradation kinetics

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 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. Figure 1 shows the specific substrate removal rates ($r_{sp} = r_s/X$) vs. substrate concentration for three phenols investigated; curves are obtained by simulation performed with the average values of the best-fitting parameters for each compound. From k values reported in Table 2, it is observed (as expected from the EC_{50} values) that 4NP and 2,4DMP are characterized by higher specific removal rates compared to 2,4DCP. The substrate concentration, C*, where the maximum removal rate occurs is given by:

$$C^* = \sqrt{K_S \cdot K_I} \tag{4}$$

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

4.2 2nd series: mixture biodegradation kinetics

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:

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

so Eq(2) reduces to Eq(1) for 4NP. Kinetic parameters obtained by the fitting of this 2nd test series are summarized in Table 3 while Figures 2a and 2b 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 ≥ 0.99 in all cases.

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.

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

Test	K_S (mg L^{-1})	K_I (mg L^{-1})	k (mg $mgVSS^{-1}$ d^{-1})
S 1a	50	18	2.67
S 1b	50	18	3.29
S 2a	200	18	1.58
S 2b	200	18	5.34
S 3a	18	12	0.54
S 3b	18	12	1.02

Synchronous compound 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 2b 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 3: $K_{M,2,4DCP}$ values are in the range 13 – 36.6 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 2b. 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.

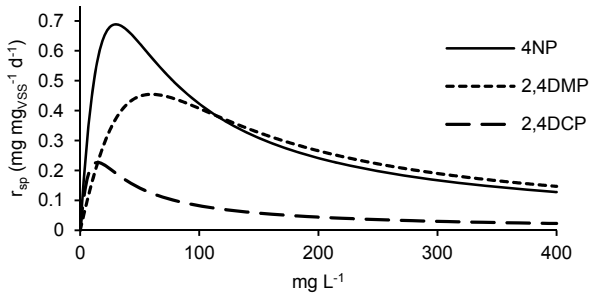

Figure 1: Specific reaction rates vs. substrate concentration for single compounds

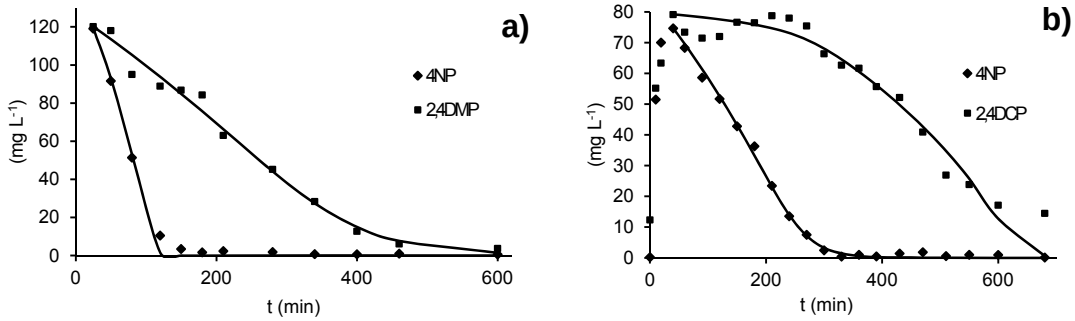

Figure 2: Experimental data and calculated profiles for M1a test (a) and M2c test (b)

Finally, the model was validated by simulating different runs with average values of the estimated parameters for each binary mixture: results are shown in Figures 3a and 3b, 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 inhibitory substrates in mixture.

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

Test	k (mg $mgVSS^{-1}$ d^{-1})			K_M (mg L^{-1})	
	4NP	2,4DMP	2,4DCP	2,4DMP	2,4DCP
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	-
M 2a	0.31	-	0.40	-	36.63
M 2b	0.44	-	0.40	-	13.59
M 2c	1.14	-	0.72	-	12.95

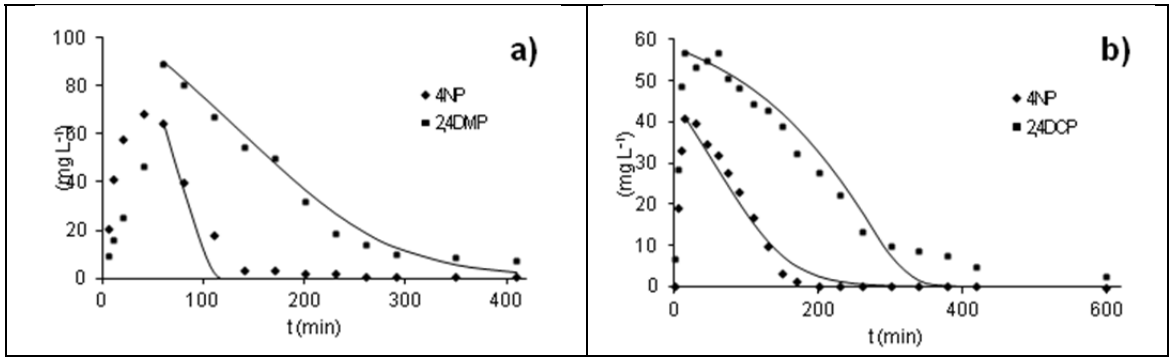

Figure 3: Experimental data and model simulations for MIX1 (a, $C_{in,4NP}$ = 200 mg L^{-1} and $C_{in,2,4DMP}$ = 200 mg L^{-1}, X = 3,260 mg_{VSS} L^{-1}) and MIX2 (b, $C_{in,4NP}$ = 90 mg L^{-1} and $C_{in,2,4DCP}$ = 120 mg L^{-1}, X = 2,760 mg_{VSS} L^{-1})

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 $\geq$ 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 mixture.

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