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Modelling cometabolic biotransformation of organic micropollutants in nitrifying reactors

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ABSTRACT

Cometabolism is the ability of microorganisms to degrade non-growth substrates in the presence of primary substrates, being the main removal mechanism behind the biotransformation of organic micropollutants in wastewater treatment plants. In this paper, a cometabolic Monod-type kinetics, linking biotransformation of micropollutants with primary substrate degradation, was applied to a highly enriched nitrifying activated sludge (NAS) reactor operated under different operational conditions (hydraulic retention time (HRT) and nitrifying activity). A dynamic model of the bioreactor was built taking into account biotransformation, sorption and volatilization. The micropollutant transformation capacity (T_c), the half-saturation constant (K_{sc}) and the solid–liquid partitioning coefficient (K_d) of several organic micropollutants were estimated at 25 °C using an optimization algorithm to fit experimental data to the proposed model with the cometabolic Monod-type biotransformation kinetics.

The cometabolic Monod-type kinetic model was validated under different HRTs (1.0–3.7 d) and nitrification rates (0.12–0.45 g N/g VSS d), describing more accurately the fate of those compounds affected by the biological activity of nitrifiers (ibuprofen, naproxen, erythromycin and roxithromycin) compared to the commonly applied pseudo-first order micropollutant biotransformation kinetics, which does not link biotransformation of micropollutants to consumption of primary substrate. Furthermore, in contrast to the pseudo-first order biotransformation constant (k_{biol}), the proposed cometabolic kinetic coefficients are independent of operational conditions such as the nitrogen loading rate applied. Also, the influence of the kinetic parameters on the biotransformation efficiency of NAS reactors, defined as the relative amount of the total inlet micropollutant load being biotransformed, was assessed considering different HRTs and nitrification rates.

1. Introduction

Macropollutants removal in activated sludge reactors has already been successfully modelled by means of Activated Sludge Models (ASM) (Henze et al., 1987), while still remains a challenge to understand the removal mechanisms of emerging organic micropollutants. A remarkable progress has been made in the past years after the publication of fate models for Pharmaceutical and Personal Care Products (PPCPs) (Urase and Kikuta, 2005; Plósz et al., 2012) or other compounds such as polycyclic aromatic hydrocarbons

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(Delgadillo-Mirquez et al., 2011) in different types of biological reactors. Accurate and well-developed models would provide decision-makers with a tool to understand the behaviour of micropollutants, to evaluate the release of these compounds into the environment and to optimize wastewater treatment plants (WWTPs) (Clouzot et al., 2013).

Biotransformation of emerging organic micropollutants in activated sludge systems is usually modelled by a pseudo-first order kinetics (Schwarzenbach et al., 2003; Joss et al., 2006), which dismisses the effect of activated sludge biological activity. However, many organic micropollutants are biotransformed by cometabolism, biochemical mechanism of microorganisms to degrade non-growth substrates in the presence of primary substrates. Different studies have modelled the biotransformation of organic compounds through cometabolism, concluding that the presence of a growth substrate is required for the biodegradation of a large amount of organic micropollutants (Alexander, 1985; Schmidt et al., 1985; Rittmann, 1992) and, therefore, it should be taken into account when predicting their removal in bioreactors (Tran et al., 2013). In fact, cometabolic kinetics has already been considered to further improve modelling the biotransformation of different organic micropollutants in aerobic and anaerobic environments (Criddle, 1993; Delgadillo-Mirquez et al., 2011). Furthermore, growth substrates and micropollutants could interact in complex manners: micropollutants could competitively inhibit growth substrate degradation (Rittmann et al., 1992; Sathyamoorthy et al., 2013); growth substrates could competitively inhibit micropollutants degradation (Chang et al., 1995; Plósz et al., 2010); and there could be no interaction between both substrates (Plósz et al., 2013). Also, the order of the kinetics is a point of discussion as microorganisms usually show a saturation in degradation rates once a certain substrate concentration has been surpassed (Alexander, 1985; Collado et al., 2012), becoming the Monod kinetics more suitable to describe the biotransformation process.

Among the main types of biomass grown in WWTPs, autotrophic biomass has been regarded as capable of transforming many organic xenobiotics (Vader et al., 2000; Batt et al., 2006; Wahman et al., 2006; Yi and Harper, 2007; Roh et al., 2009; Kocamemi and Çeçen, 2010; Khunjar et al., 2011) and, actually, micropollutants biotransformation rate has proved to be correlated to nitrification rate, assuming not only limitations in kinetics but also in the energy and electron flows generated by primary substrate degradation (Fernandez-Fontaina et al., 2012). Hence, the cometabolic model, including both aspects, seems more appropriate to describe biotransformation of organic micropollutants in nitrifying activated sludge (NAS) reactors.

This work aims at the application of a cometabolic Monod-type kinetic model for the biotransformation of organic micropollutants by nitrifying biomass, comparing its accuracy with a pseudo-first order kinetic model, which does not link micropollutants with primary substrate degradation. The kinetic parameters (the micropollutant transformation capacity (T_c) and half-saturation constant (K_{sc}) in the cometabolic kinetics, and the biodegradation kinetic constant (k_{biol}) in the pseudo-first order kinetics) were estimated applying a spike of different organic micropollutants to a nitrifying bioreactor and fitting the experimental results to two dynamic models accounting for sorption and volatilization but differing in the two different biotransformation kinetics. In order to validate the cometabolic model the concentration of micropollutants in the reactor effluent and the biotransformation rates under different operational conditions were predicted employing the estimated parameters and compared to those measured in several sampling campaigns under steady state conditions. The influence of the kinetic parameters of the cometabolic model on the performance of nitrifying reactors for micropollutant biotransformation was finally assessed, by using the estimated parameters to predict the biotransformation efficiency in nitrifying reactors, defined as the relative amount of the total inlet micropollutant load being biotransformed, under different operational conditions (hydraulic retention time (HRT) and nitrifying activity).

2. Material and methods

2.1. Nitrifying activated sludge reactor

The NAS reactor used was described elsewhere (Fernandez-Fontaina et al., 2012). Briefly, it consisted of a 30 L reactor coupled with a 5 L settler and it was fed with a solution rich in ammonium chloride (40–500 mg $N-NH_4^+/L$) and sodium bicarbonate (70–850 mg $C-HCO_3^-/L$), with stable operation for more than 3 years with a complete transformation of ammonium into nitrate.

Total and volatile suspended solids (TSS and VSS), nitrite and nitrate were determined following Standard Methods (APHA, 1999). Ammonium was determined after reaction with phenol and hypochlorite to give indophenol, whose absorbance was measured at 635 nm with a spectrophotometer (Shimadzu UV-1603, UV-visible). Total, inorganic and organic carbon (TC, IC and TOC) were determined by a Shimadzu analyser (TOC-5000). The specific nitrification rate (SNR) was calculated as the amount of ammonium nitrogen transformed into nitrate in the bioreactor per unit of biomass and time. The specific nitrifying activity (SNA), which is the maximum ammonia uptake rate per gram biomass (mg $N-NH_4^+$/g VSS d), was determined experimentally in the reactor as follows: i) interruption of feeding and saturation of the reactor with oxygen; ii) interruption of aeration; iii) addition of a non-limiting concentration of substrate (4 mg $N-NH_4^+/L$); iv) monitoring of dissolved oxygen concentration decrease.

2.2. Organic micropollutants

Two musk fragrances, galaxolide (HHCB) and tonalide (AHTN); three anti-inflammatories, ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF); four antibiotics, sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY) and roxithromycin (ROX); an antidepressant, fluoxetine (FLX); an antiepileptic, carbamazepine (CBZ); and an anxiolytic, diazepam (DZP) were selected and spiked into the feeding of the reactor in concentrations ranging from 10 to 40 μg/L. These concentrations are only slightly higher than those found in raw sewage entering WWTPs and no changes in the biotransformation mechanisms could be expected.

2.3. Modelling

2.3.1. Model development

A two phase model was considered to describe the behaviour of the micropollutants (Table 1). Biotransformation and sorption–desorption processes have been considered as well as volatilization, which was negligible for most compounds as only musk fragrances are volatile. Photodegradation was also insignificant due to the high turbidity present in activated sludge units and it was also avoided by covering the reactor with aluminium foil.

A cometabolic model (Eq. (1)) where biotransformation depends on the biomass growth rate and yield, and consequently on the uptake of primary substrate, has been applied (Criddle, 1993; Delgadillo-Mirquez et al., 2011). Also, a much simpler pseudo-first order kinetic model (Eq. (2)), widely used in the literature (Schwarzenbach et al., 2003), has been considered in order to evaluate the possible advantages of the cometabolic model. An additional term considering biotransformation in the absence of primary substrate was deemed negligible as the reactor operated at a constant NLR.

$$r_{biol} = T_C \cdot \frac{\mu}{Y} \cdot \frac{C_W}{K_{SC} + C_W} \cdot X_{VSS} \quad (1)$$

$$r_{biol} = k_{biol} \cdot X_{VSS} \cdot C_W \quad (2)$$

where r_{biol} is biotransformation rate (μg/L d), k_{biol} is biotransformation kinetic constant (L/g VSS d), X_{VSS} is biomass concentration (g VSS/L), T_C is micropollutant transformation capacity (μg/g $N-NH_4^+$), μ is specific biomass growth rate (1/d), Y is biomass yield (g VSS/g $N-NH_4^+$) and K_{SC} is micropollutant affinity constant (μg/L).

The micropollutant transformation capacity (T_C) is the maximum amount of micropollutant that can be biotransformed per gram of primary substrate consumed (μg/g $N-NH_4^+$) by NAS. It should be similar under the same conditions and with the same biomass, if the cometabolic hypothesis is correct. The higher its value is, the higher the amount of micropollutant being biodegraded at a given nitrification rate. The second parameter in the cometabolic Monod-type kinetic model is the micropollutant affinity or half-saturation constant (K_{SC}), which is the micropollutant concentration (μg/L) providing half of the maximum biotransformation rate. The lower the value of K_{SC}, the higher the rate of biotransformation is at low micropollutant concentrations. This parameter should also be fairly stable for the same biomass under the same conditions.

Table 1 – Gujer matrix representing the fate of organic micropollutants in the model.

Process	Components		Rate (μg/L d)
	Liquid phase (C_W)	Solid phase (C_S)	
Biodegradation	−1		$T_C \cdot \frac{\mu}{Y} \cdot \frac{C_W}{K_{SC}+C_W} \cdot X_{VSS}$
Sorption	−1	+1	$k_{sor} \cdot X_{TSS} \cdot C_W$
Desorption	+1	−1	$\frac{k_{sor}}{K_d} \cdot C_S$
Volatilization	−1		$\frac{H \cdot q_{air} \cdot C_W}{HRT}$

Growth of nitrifiers (g VSS/L d) could be modelled by Eq. (3) (Henze et al., 1987):

$$r_g = \mu \cdot X_{VSS} = \mu_{max} \cdot \left(\frac{C_{NH}}{K_{S,NH}+C_{NH}}\right) \cdot \left(\frac{C_{O_2}}{K_{S,O_2}+C_{O_2}}\right) \cdot X_{VSS} \quad (3)$$

where μ is growth rate (1/d), μ_{max} is maximum growth rate (1/d), C_{NH} is ammonium nitrogen concentration (g $N-NH_4^+$/L), $K_{S,NH}$ is ammonium nitrogen affinity constant (g $N-NH_4^+$/L), C_{O_2} is dissolved oxygen concentration (mg O_2/L) and K_{S,O_2} is dissolved oxygen affinity constant (mg O_2/L).

By applying the mass balance of ammonium nitrogen to the reactor under steady state conditions (Eq. (4)) and using the definition of r_g from Eq. (3), the specific nitrification rate (SNR, g $N-NH_4^+$/g VSS d), determined experimentally by means of Eq. (6), can be employed as an estimation of the primary substrate consumption rate (μ/Y), which can be introduced in the cometabolic model (Eq. (7)), avoiding the use of bibliographic values for the constants in the equation describing the growth of nitrifiers (Eq. (3)). To comply with this assumption, the reactor was kept under stable conditions during each kinetic experiment, by maintaining dissolved oxygen concentrations above 8 mg/L and a constant temperature of 25 °C.

$$\frac{dC_{NH}}{dt} = \frac{C_{NH,in} - C_{NH,out}}{HRT} - \frac{r_g}{Y} = 0 \quad (4)$$

$$\frac{dC_{NH}}{dt} = \frac{C_{NH,in} - C_{NH,out}}{HRT} - \frac{\mu}{Y} \cdot X_{VSS} = 0 \quad (5)$$

$$SNR = \frac{\mu}{Y} = \frac{C_{NH,in} - C_{NH,out}}{HRT \cdot X_{VSS}} \quad (6)$$

$$r_{biol} = T_C \cdot SNR \cdot \frac{C_W}{K_{SC}+C_W} \cdot X_{VSS} \quad (7)$$

To determine the sorption and biotransformation kinetic parameters of micropollutants, the activated sludge unit was described under transitory conditions by a Continuous Stirred Tank Reactor (CSTR) dynamic model with a differential equation for each phase.

The behaviour of the liquid phase can be described by Eq. (8) corresponding to the cometabolic model or by Eq. (9), which assumes the pseudo-first order kinetics. Eq. (10) describes the dynamics of the solid phase in both models.

Liquid phase:

$$\frac{dC_W}{dt} = -\left(\frac{1 + H \cdot q_{air}}{HRT} + T_C \cdot SNR \cdot \frac{X_{VSS}}{K_{SC} + C_W} + k_{sor} \cdot X_{TSS}\right) \cdot C_W + \frac{k_{sor}}{K_d} \cdot C_S + \frac{C_{W,i}}{HRT} \quad (8)$$

$$\frac{dC_W}{dt} = -\left(\frac{1 + H \cdot q_{air}}{HRT} + k_{biol} \cdot X_{VSS} + k_{sor} \cdot X_{TSS}\right) \cdot C_W + \frac{k_{sor}}{K_d} \cdot C_S + \frac{C_{W,i}}{HRT} \quad (9)$$

Solid phase:

$$\frac{dC_S}{dt} = k_{sor} \cdot X_{TSS} \cdot C_W - \left[\frac{k_{sor}}{K_d} + \left(\frac{X_{TSS,eff}}{X_{TSS}}\right) \cdot \frac{1}{HRT}\right] \cdot C_S \quad (10)$$

where C_W is micropollutant soluble concentration in the mixed liquor (μg/L), C_S is micropollutant sorbed concentration

(μg/L), $C_{W,i}$ is micropollutant soluble concentration in the feed, HRT is hydraulic retention time (d), X_{TSS} is total suspended solids concentration (g TSS/L), X_{VSS} is biomass concentration (g VSS/L), H is the Henry coefficient of each micropollutant (μg $L_{wastewater}$/μg L_{air}), q_{air} is specific aeration flowrate (L_{air}/$L_{wastewater}$), k_{sor} is sorption kinetic constant (L/g TSS·d), k_{des} is desorption kinetic constant (1/d), K_d is solid–liquid partitioning coefficient (L/kg TSS) and $X_{TSS,eff}$ is biomass concentration in the effluent (g TSS/L). A more detailed development of the model equations can be found in Supplementary Data.

2.3.2. Model implementation and estimation of parameters

Eqs. (8)–(10) were solved using a Runge-Kutta numerical method implemented in Matlab® with a nonlinear multidimensional optimization algorithm, with the purpose of obtaining the kinetic parameters (k_{sor}, k_{biol}, T_C and K_{SC}) which best fitted the experimental data. A pulse of 100 μg/L of micropollutants was added and the initial conditions were set from the first experimental sample taken 5 min after adding the pulse of micropollutants. First, the two phase model with the pseudo-first order biotransformation kinetics was solved (system of Eqs. (9) and (10)) by fitting the experimental soluble and sorbed concentrations (C_W and C_S) in each kinetic experiments to obtain pseudo-first order biotransformation (k_{biol}) and sorption (k_{sor}) kinetic constants for each micropollutant. The average value of the solid–liquid partitioning coefficient (K_d) in each experiment was introduced as an input. K_d was calculated according to Eq. (S3) using the experimental concentrations of micropollutants sorbed onto the sludge (μg/g TSS) and in solution (μg/L).

Secondly, the cometabolic Monod-type kinetics was considered (system of Eqs. (8) and (10)) and micropollutant transformation capacities (T_C) and affinity constants (K_{SC}) were estimated introducing the sorption kinetic coefficient (k_{sor}) obtained fitting the experimental data to the pseudo-first order model, as the sorption process must be equal in both models. It should be noted that the values of k_{sor} and k_{biol} were estimated to best fit the experimental results of each individual assay separately. On the contrary, as we aim to obtain a model describing the behaviour of micropollutants under all conditions tested, T_C and K_{SC} were estimated optimizing their values to fit the experimental data of the two experiments. Additionally, with the purpose of assessing micropollutant biodegradability, simulations of soluble and sorbed micropollutants concentrations setting biotransformation rates to zero were run (washout lines in Fig. 1).

The predictive accuracy of the models was assessed by obtaining the mean absolute error (MAE), according to the following equation:

$$MAE = \frac{1}{n_W}\sum_{i}^{n_W}\left|C_{W,measured}(t_i) - C_{W,simulated}(t_i)\right| + \frac{1}{n_S}\sum_{i}^{n_S}\left|C_{S,measured}(t_i) - C_{S,simulated}(t_i)\right| \quad (11)$$

where n_w and n_s is the number of samples taken in the liquid and solid phases, respectively, in each experiment and t_i refers to each sampling point.

2.3.3. Identifiability and sensitivity analysis

In order to test the identifiability of the model, simulations of the nitrifying reactor in the kinetic experiments were run employing the cometabolic Monod-type kinetic model for a selected compound combining different values of the parameters T_C and K_{SC}. The whole range of values obtained for all compounds after the experimental data fitting was covered. The effect of the sorption kinetic constant (k_{sor}) was tested separately.

A sensitivity analysis of the model parameters T_C and K_{SC} on the soluble concentration of a selected micropollutant was performed changing both over a ±50% range on a one-at-a-time basis. A sensitivity coefficient (σ_q) of the soluble concentration of micropollutant (C_w) to the parameter q (T_C or K_{SC}) was calculated by Eq. (12) to quantify the average spread for each parameter (Bernard et al., 2001; Delgadillo-Mirquez et al., 2011).

$$\sigma_p = \frac{1}{t_f}\int_0^{t_f}\frac{C_{w,q+\Delta q} - C_{w,q}}{C_{w,q}}\cdot dt \quad (12)$$

where t_f is the test duration, $C_{w,q}$ is the soluble concentration of micropollutant associated with the base value of the parameter q, and $C_{w,q+\Delta q}$ is the soluble concentration when the parameter q is changed an amount Δq.

2.3.4. Model validation

A different set of experimental results was used to validate the cometabolic Monod-type kinetic model. Seven steady state sampling campaigns were performed right before the kinetic experiments. In each campaign, samples of feeding, mixed liquor and effluent were withdrawn to determine micropollutant concentrations in liquid and solid phases, attaining an accurate quantification of biotransformation and sorption.

Effluent concentrations obtained under different operational steady state conditions were calculated according to the cometabolic (Eq. (13), solved numerically using Excel®) and the pseudo-first order models (Eq. (14)) in order to test the accuracy of the models to predict the behaviour of the reactor.

$$C_{W,o} = \frac{Q_i\cdot C_{W,i}}{Q_o\cdot(1+K_d\cdot X_{TSS,eff}) + Q_p\cdot(1+K_d\cdot X_{TSS}) + T_C\cdot SNR\cdot\frac{X_{VSS}}{K_{SC}+C_{W,o}}\cdot V_R + Q_i\cdot H\cdot q_{air}} \quad (13)$$

$$C_{W,o} = \frac{Q_i\cdot C_{W,i}}{Q_o\cdot(1+K_d\cdot X_{TSS,eff}) + Q_p\cdot(1+K_d\cdot X_{TSS}) + k_{biol}\cdot X_{VSS}\cdot V_R + Q_i\cdot H\cdot q_{air}} \quad (14)$$

where $C_{W,o}$ is the micropollutant soluble concentration in the effluent (μg/L) and Q_p is the wastage flowrate (L/d). The global parameters of the cometabolic model T_C and K_{SC} were used to predict the effluent concentrations in the steady state sampling campaings performed right before each of the experiments. Instead, the k_{biol} values estimated for each of the experiments (A and B) were respectively used to predict the concentrations under steady state conditions in the two experimental periods (A1-A4 and B1–B3). Average K_d values determined in the kinetic experiments (Table 3) were also used to estimate sorption equilibrium under steady state conditions, as concentration of low sorptive biodegradable compounds were below or only slightly above LOQ.

The final validation was performed by calculating the rate of micropollutants biotransformed (μg/d) in the steady state sampling campaigns considering the cometabolic Monod-type (Eq. (15)) and the pseudo-first order kinetics (Eq. (16)), and comparing the results to those obtained experimentally with the real effluent concentrations (Eq. (17)). A direct comparison between measured and predicted concentrations was deemed not appropriate due to the numerical effect of increasing relative error at low concentrations.

$$F_{biol,1} = T_C \cdot SNR \cdot \frac{C_W}{K_{SC} + C_W} \cdot X_{VSS} \cdot V_R \quad (15)$$

$$F_{biol,2} = k_{biol} \cdot C_W \cdot X_{VSS} \cdot V_R \quad (16)$$

$$F_{biol,exp} = Q_i \cdot C_{W,i} - \left[Q_o \cdot (1 + K_d \cdot X_{TSS,eff}) + Q_p \cdot (1 + K_d \cdot X_{TSS}) + Q_i \cdot H \cdot q_{air} \right] \cdot C_{W,o} \quad (17)$$

Accordingly, the biotransformation efficiency can be easily obtained with Eq. (18):

$$\text{Biodegradation efficiency} = \frac{F_{biol}}{Q_i \cdot C_{W,i}} \quad (18)$$

2.4. Estimation of parameters with kinetic experiments

In order to estimate the parameters for the biotransformation of the selected micropollutants in NAS, two experiments were performed (Table 2). Nitrogen loading rate (NLR) (0.11 g $N-NH_4^+$/L·d) and temperature (25 °C) were kept constant while different values of HRT were set for each assay. At the shortest HRT of 1 d (assay A), enrichment in autotrophic biomass led to a very high nitrifying activity of 1.29 g $N-NH_4^+$/g VSS d, with low biomass concentration of 0.27 g VSS/L and sludge retention time (SRT) of 10 d. During the second assay (assay B) at an HRT of 3.7 d, biomass concentration (0.65 g VSS/L) and SRT (70 d) increased due to the lower reactor washout and improved settler performance, decreasing the F/M ratio and, accordingly, the nitrifying activity (0.56 g N/g VSS d). In each experiment, a pulse of 100 μg/L of each substance was added to the bioreactor and samples of the mixed liquor were taken over time for at least three HRT in order to follow the response of the system until steady-state conditions were again achieved (see Table S1 of Supplementary Data for further details).

Table 2 – Operational parameters of NAS reactor during the kinetic assays.

	Kinetic assay	
	A	B
Days of operation	635–639	1150–1161
HRT (d)	1.0	3.7
Temperature (°C)	25.0	25.0
pH	7.2	7.1
X_{VSS} (g/L)	0.27	0.65
SRT (d)	10	70
NLR (g $N-NH_4^+$/L d)	0.11	0.10
SNR (g $N-NH_4^+$/g VSS d)	0.45	0.15
SNA (g $N-NH_4^+$/g VSS d)	1.29	0.56

HRT, Hydraulic Retention Time; SRT, Sludge Retention Time; NLR, Nitrogen Loading Rate; SNR, Specific Nitrification Rate; SNA, Specific Nitrifying Activity.

2.5. Analytical methods of organic micropollutants

Samples were collected in amber glass bottles and centrifuged (1200 rpm, 10 min). Solids were frozen at −20 °C and freeze-dried. Supernatants were prefiltered (AP3004705, Millipore) and stored at 2 °C prior to analysis. Solid phase extraction (SPE) was performed before 24 h. The soluble content of antiphlogistics, CBZ, DZP and musk fragrances was determined using Gas Chromatography coupled with Mass Spectrometry (GC/MS/MS) while antibiotics and fluoxetine were quantified using Liquid Chromatography coupled with Mass Spectrometry (LC/MS/MS) as described elsewhere (Serrano et al., 2011). Only parent compounds were determined. Ultrasonic solvent extraction (USE) was performed as described by Ternes et al. (2005) in order to determine the concentration of micropollutants sorbed onto solids. Three sequential extractions with methanol and acetone were performed on the lyophilized solid samples. In each extraction, samples were sonicated for 15 min and centrifuged at 3000 rpm for 5 min. All supernatants were collected and combined, filtered through glass wool and concentrated by evaporation (R-205, Büchi) under vacuum conditions at 35 °C. After dilution with distilled water, solid phase extraction and quantification by GC/MS/MS and LC/MS/MS were performed similarly to the liquid samples.

3. Results and discussion

3.1. Biotransformation and sorption kinetic parameters of organic micropollutants in NAS

The experimental results (concentrations of micropollutants in the liquid and solid phases) of one of the experiments and their fitting to the cometabolic Monod-type kinetic model are shown for IBP, HHCB, FLX and DZP in Fig. 1. These four compounds were selected as examples of the different types of behaviour observed for the 12 micropollutants considered in this work: i) highly biodegradable (k_{biol} >1 L/g VSS d) with low sorption (K_d < 100 L/kg TSS): IBP, NPX, ERY and ROX; ii) highly biodegradable with high sorption (K_d > 1000 L/kg TSS): HHCB and AHTN; iii) slowly biodegradable (k_{biol} < 1 L/g VSS d): FLX, SMX and TMP; and iv) non biodegradable (DZP, CBZ and DCF). It should be

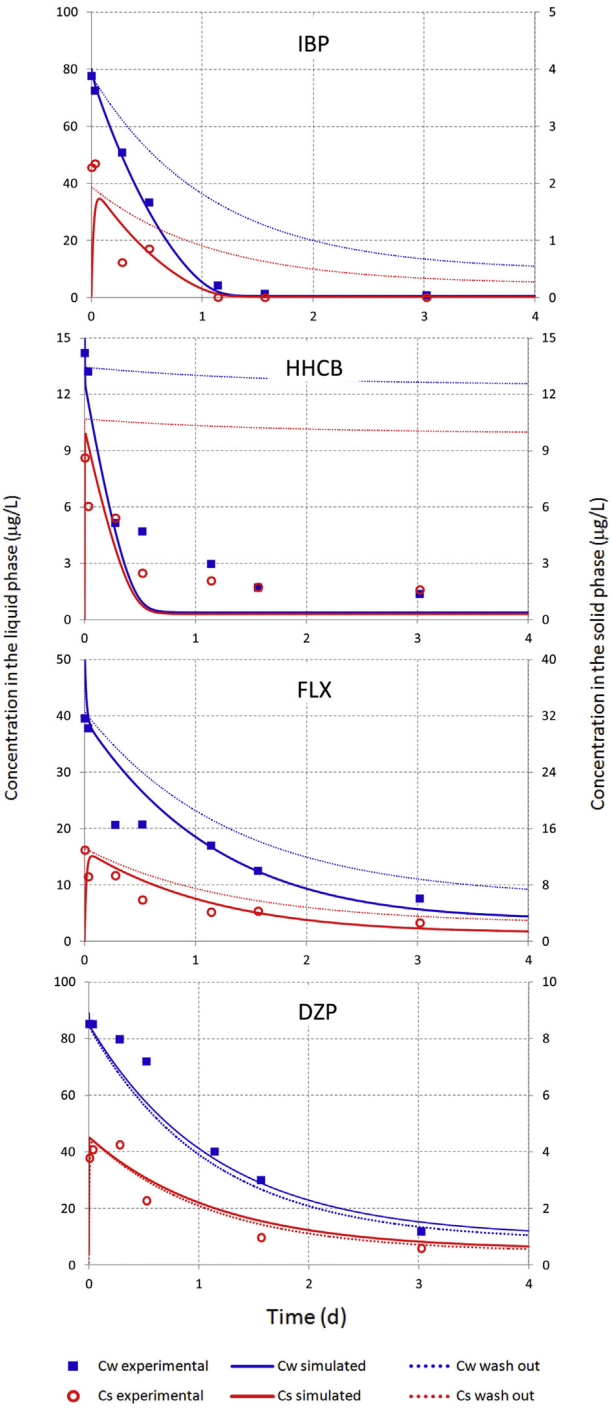

Fig. 1 – Experimental and modelled concentrations of organic micropollutants in the liquid (C_w) and solid (C_s) phases of assay A for IBP, HHCB, FLX and DZP. Washout lines show the simulated evolution of the concentrations if no biotransformation was taking place. See Table 2 for operational conditions of each assay.

clarified that these last compounds were not further considered in the following discussion as they showed no biotransformation. The data fitting of all compounds being biotransformed are available in Supplementary Data (Fig. S1). No sudden changes in the biotransformation patterns were observed regardless of

Table 3 – Biodegradation and sorption (K_d) parameters calculated for the cometabolic (T_C, K_{SC}) and the pseudo first order (k_{biol}) models. K_d, k_{sor} and k_{biol} were obtained individually for each kinetic assay A and B.

	K_d (L/kg TSS)		k_{sor} (L/g TSS d)		k_{biol}(L/g VSS d)		T_c (ug/g N)	K_{sc} (µg/L)
	A	B	A	B	A	B		
IBP	80	33	153	39	4.3	2.4	480	3.2
NPX	58	36	197	62	2.6	1.4	475	11.3
ERY	70	49	6	66	0.8	3.0	170	6.3
ROX	99	80	376	97	2.3	3.4	220	10.1
HHCB	2571	2838	1368	4705	20.9	32.9	565	1.6
AHTN	2478	2214	1122	1163	15.7	14.2	242	1.0
FLX	1043	762	3378	771	1.3	0.6	78	5.6
SMX	63	33	97	52	1.0	0.3	80	10.9
TMP	90	61	73	120	0.9	0.0	n.a.	n.a.
DCF	<6	<5	5	13	0.0	0.0	0	0.0
CBZ	20	15	13	74	0.0	0.0	0	0.0
DZP	173	78	138	107	0.0	0.0	0	0.0

using initial concentrations of 100 µg/L, one or two orders of magnitude higher than the concentrations usually found in raw sewage. The area between the washout lines and those fitting the experimental data for both phases represents the micropollutant's biodegradability. For a given value of the biotransformation parameters, this area was smaller in the case of the shorter HRT of 1.0 d (kinetic assay A) as washout played a more significant role at higher flowrates (Fig. S1). The parameters estimated in assay B were therefore less affected by the analytical error. The mean absolute error (MAE) of the experimental data fitting to the cometabolic Monod-type kinetic model in the kinetic experiments, together with the pseudo-first order kinetics, are presented in Table S2. The cometabolic model was able to fit IBP and NPX better, regardless of fitting the experimental results to different k_{biol} in each of the kinetic assays. On the contrary, ERY, ROX, HHCB, AHTN, FLX and SMX were best fitted by the pseudo-first order kinetics. However, the cometabolic Monod-type kinetics was further considered to model the behaviour of the nitrifying bioreactor under different conditions with the objective of obtaining a more general biotransformation model for micropollutants in NAS.

All the parameters estimated in the kinetic experiments are summarized in Table 3. T_C was related to the initial slope or the biotransformation rate at the highest initial concentrations in the two experiments performed at different nitrification rates. Highly biodegradable compounds under nitrifying conditions (IBP, NPX, ERY, ROX, HHCB and AHTN) displayed the highest T_C values in a range from 170 to 565 µg/g N. Slowly biodegradable compounds such as FLX and SMX showed values lower than 100 µg/g N. In the case of TMP, a change in behaviour between the two experiments did not allow the determination of the cometabolic kinetic parameters, as it was biodegraded in the first assay, with null biotransformation in the second one. A possible hypothesis explaining this change is the time elapsed between experiments (510 d), which might have allowed for changes in the catabolic capabilities of the biomass and even shifts in microbial populations. Other authors were able to isolate bacteria with the capability of biodegrading a specific micropollutant (Larcher and Yargeau, 2011; Zhang et al., 2013); therefore, shifts in the microbial ecology present in the reactor,

driven by changes in operational parameters such as SRT, might have led to the temporary biotransformation of TMP.

The micropollutant affinity or half-saturation constant (K_{SC}) ranged from 1.0 to 11.3 μg/L (Table 3). The lower the concentrations achieved at the end of the kinetic experiments, the lower the value of K_{SC} obtained. The biotransformation rates observed at different concentrations of micropollutant allowed its determination. The low range of concentrations at the end of the experiments were quite precisely fitted by the cometabolic Monod-type kinetics, probably due to the effect of an additional parameter (K_{SC}) capable of adjusting slight changes in the kinetics order (see Fig. S1 in Supplementary Data). Other authors obtained more specific evidences of this change in the kinetics order performing experiments at increasing initial concentrations of micropollutants, which rendered decreasing k_{biol} values (Collado et al., 2012).

Regarding the pseudo-first order kinetic model, the decrease in the biotransformation kinetic constant (k_{biol}) of IBP, NPX, FLX and SMX obtained in the kinetic assay B compared to assay A could be attributed to the lower specific nitrification rate (SNR). This behaviour is in accordance with the cometabolic hypothesis and could explain the high variability of k_{biol} in the literature (Pomiès et al., 2013), not only in NAS but also in conventional activated sludge (CAS), where nitrifying microorganisms are also present. On the contrary, k_{biol} increased in the cases of ERY, ROX and HHCB. The biotransformation of these compounds was not affected by the increased nitrification rates, which could be due to the lack of ability of nitrifiers to degrade them or, at least, to the better capabilities of heterotrophic bacteria, ubiquitous in any mixed culture, to degrade these compounds, due to the lower proportion of nitrifying bacteria in assay B (0.65 g VSS/L of biomass with a nitrifying activity of 0.56 g N/g VSS d) compared to assay A (0.27 g VSS/L with 1.29 g N/g VSS d). TMP and SMX decreased substantially their kinetics from assay A to B. While most bibliographic sources (Abegglen et al., 2009; Suárez et al., 2010) are in accordance with the low k_{biol} obtained in assay B for these two antibiotics, the lower accuracy of the methodology in assay A due to the higher washout rate cannot explain this divergence. The biotransformation rates observed in the steady state sampling of the reactor right before the kinetic experiment (A1-A4 in Fig. 3) confirmed the higher ability of the sludge to degrade these compounds in the first experimental period. Some studies have also reported high biotransformation efficiencies of both compounds in activated sludge developed at long SRT (Kreuzinger et al., 2004; Batt et al., 2006). As suggested earlier, it seems very likely that the biotransformation of some compounds can rely on specific microbial populations rather than on general catabolic pathways of nitrifying biomass. For the highly biodegradable compounds (IBP, NPX, ERY, ROX, HHCB, AHTN), Suárez et al. (2010) and Fernandez-Fontaina et al. (2012) reported higher values of k_{biol} (obtained by mass balances instead of performing kinetic experiments) in continuous nitrifying reactors with similar activities, probably due to the low effluent concentrations achieved under steady state conditions, leading to the overestimation of the kinetic constants. Falås et al. (2012) reported similar ranges of k_{biol} for IBP (2.2–4.8 L/g SS d) and DCF (<0.02 L/g SS d), but lower for NPX (0.3–1.1 L/g SS d) in NAS with lower nitrifying activities (0.10–0.17 g N/g SS d) performing batch experiments at initial micropollutants and ammonium concentrations of 100 μg/L and 40 mg $N-NH_4^+$/L respectively. Tran et al. (2009) reported a similar range for IBP (3.24–4.01 L/g SS d) in NAS but lower for NPX (0.39–0.93 L/g SS d) and higher for DCF (0.31–0.52 L/g SS d). In this case, the nitrifying activity (0.72 g N/g VSS d) was similar, but the determinations were performed in batch applying a single addition of 100 mg $N-NH_4^+$/L, together with 100 μg/L of micropollutants. On the contrary, the concentration of ammonium was always <0.05 mg $N-NH_4^+$/L in our continuous CSTR operating at steady state conditions regarding nitrification. Different concentrations of ammonia and micropollutants in the mixed liquor could affect biotransformation rates due to the competition between both to bind to the AMO enzyme, which was already proved for aromatic compounds in *Nitrosomonas europaea* pure cultures (Keener and Arp, 1994).

3.2. *Identifiability and sensitivity analysis*

The identifiability of the cometabolic Monod-type kinetics in the bioreactor model with the measured data was confirmed by analysing the output of the model over the whole range of all parameters estimated (k_{sor}, T_C, K_{SC}). The sorption kinetic constant (k_{sor}) of IBP was tested separately over a range of 0.1–100 L/g TSS d (Fig. S4 in Supplementary Data) assuming fixed values of T_C (480 μg/g N) and K_{SC} (3.2 μg/L). Equilibrium conditions can be assumed for k_{sor} >5 L/g TSS d, therefore for all compounds the value of k_{sor} did not affect the experimental results. The effect of a wide range of combined values of T_C (5–500 μg/g N) and K_{SC} (0.5–25 μg/L) on the evolution of the soluble and sorbed concentrations of IBP in the two kinetic assays was simulated (Fig. S5 in Supplementary Data) taking into account the experimental conditions in the two experiments. In this case, k_{sor} was given the value estimated in each kinetic experiment (Table 3). The information available in the measured data (soluble and sorbed concentrations of micropollutants) granted the independent determination of the three biokinetic parameters, being the biotransformation rates achieved at different nitrification rates linked to the estimation of T_C, while the variations in the biotransformation rates at different micropollutant concentrations were associated to the reaction order and therefore to the value of K_{SC}. Regarding sorption, the speed on achieving solid–liquid equilibrium conditions in the first samples taken after the addition of micropollutants was related to the estimation of k_{sor}.

The sensitivity analysis was performed employing the estimated parameters T_C and K_{SC} of one of the micropollutants (IBP) as based values and modifying their values in a range of ±50%. The micropollutant transformation capacity (T_C) was the most sensitive parameter and was therefore more accurately estimated (Fig. 2). A decrease in the value of T_C would affect the evolution of C_w very significantly as this means a reduced biodegradability. On the contrary, an increase of T_C would barely decrease C_w as IBP was already highly biodegradable. The micropollutant affinity constant (K_{SC}) was less sensitive, with smaller changes on the output variable (C_w), thus being this parameter estimated with a lower accuracy.

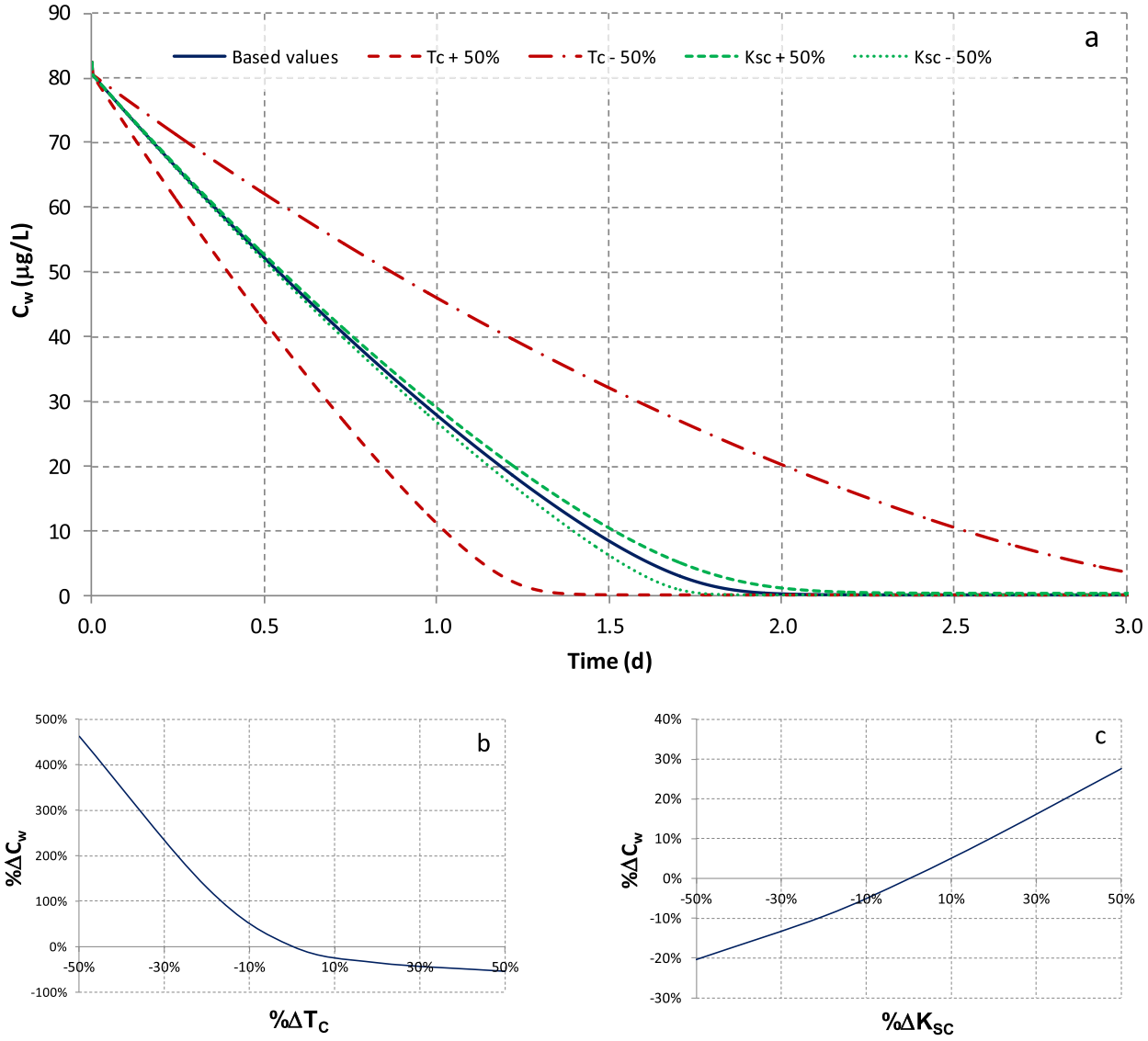

Fig. 2 – Sensitivity analysis of the cometabolic Monod-type kinetic biotransformation model parameters T_C and K_{SC} on the soluble concentration of IBP in the kinetic assay B (based values: $T_C = 480$ µg/g N, $K_{SC} = 3.2$ µg/L) (a). Average variation (%) of the soluble concentration of micropollutant (C_w) depending on parameter change (%): transformation capacity T_C (b) and affinity constant (K_{SC}) (c).

Table 4 – Operational parameters in NAS reactor in sampling periods under steady state conditions used for model validation.

	Sampling period						
	A1	A2	A3	A4	B1	B2	B3
Days of operation	555–565	580–590	600–610	620–630	1090–1100	1100–1110	1135–1145
HRT (d)	3.71	2.91	1.98	1.05	3.75	3.75	3.75
Temperature (°C)	24.9	25.1	25.0	25.0	25.1	25.1	25.3
pH	7.5	7.9	6.9	7.2	7.6	7.2	6.4
X_{VSS} (g/L)	0.60	0.45	0.41	0.28	0.81	0.66	0.64
SRT (d)	45	25	20	10	80	75	75
NLR (g N – NH_4^+/L d)	0.12	0.11	0.10	0.11	0.10	0.10	0.09
SNR (g N – NH_4^+/g VSS d)	0.20	0.26	0.27	0.45	0.12	0.15	0.15
SNA (g N – NH_4^+/g VSS d)	0.95	1.09	1.22	1.46	0.78	0.56	0.43

HRT, Hydraulic Retention Time; SRT, Sludge Retention Time; NLR, Nitrogen Loading Rate; SNR, Specific Nitrification Rate; SNA, Specific Nitrifying Activity.

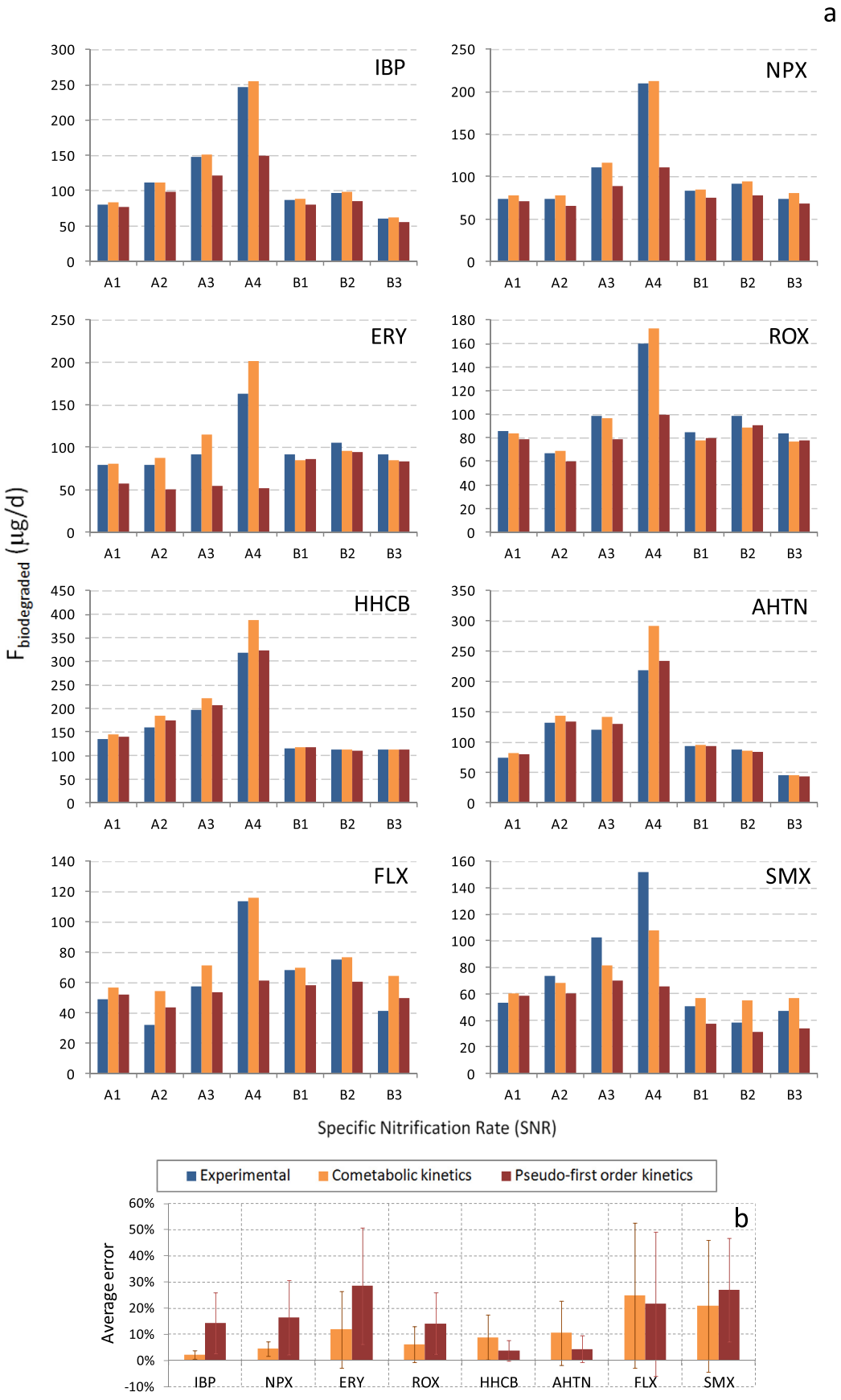

Fig. 3 – Comparison between experimental and predicted biotransformation rates (µg/d) in the different sampling periods (A1-A4 and B1–B3) under steady state conditions (a). The average error is the average value of the absolute deviations of the predicted rates calculated with each model compared to those calculated with the experimental concentrations (b).

3.3. Model validation

The cometabolic Monod-type kinetic model for biotransformation of organic micropollutants should be validated using a different set of experimental results. Consequently, the capability of the proposed model to predict the behaviour of the different organic micropollutants was tested by performing several sampling campaigns in the reactor under steady state conditions (Table 4) right before the kinetic experiments in order to avoid changes in the microbial community present in the reactor, which might affect the biotransformation of micropollutants. Measured and predicted biotransformation rates (Eqs. (15)–(17)) were compared to calculate the errors assumed with each model (Fig. 3; Tables S3 and S4 in Supplementary Data). The fate of IBP, NPX, SMX, ERY and ROX was predicted more accurately employing the cometabolic Monod-type kinetic model, leading to lower errors: IBP (2.1% vs. 14.3%), NPX (4.4% vs. 16.4%), SMX (20.9% vs. 27.0%), ERY (11.8% vs. 28.5%) and ROX (6.2% vs. 14.2%). The higher accuracy of the cometabolic Monod-type kinetics can be further highlighted taking into account that the model was calibrated for all the experimental results, while different k_{biol} were estimated for each kinetic experiment. The cometabolic term was the most relevant, being responsible for a better capability to predict the enhanced biotransformation observed at higher SNR (from A1 to A4) for IBP, NPX, ERY and ROX. As the nitrification rate increased, the cometabolic term played a major role when compared to the pseudo-first order kinetics. Also, regardless of showing ERY and ROX lower k_{biol} values in the kinetic assay A, at the highest nitrification rate, the cometabolic term improved the prediction when increasing SNR in the first experimental period, showing that nitrifiers can biotransform these compounds cometabolically. In the cases of FLX and SMX this trend was not so clear. On the contrary, accounting for nitrification rates with the cometabolic term did not improve the prediction of the effluent concentrations or the biotransformation rates of HHCB (8.8% vs 3.8%) and AHTN (10.5% vs 4.4%). When the SNR was maintained constant in the second experimental period (B1–B3), the accuracy of the predicted concentrations was very similar for both models. The Monod kinetic term could be benefitial in the case of a bigger change in the kinetics order was observed, being simplified to pseudo-first order at low concentrations, which could explain the slight differences observed between Monod and pseudo-first order kinetics.

Cometabolic kinetics could therefore be more suitable to predict the behaviour of micropollutants in real WWTPs where variable loads, not only of organic micropollutants but also organic matter, ammonia and other substrates, are biodegraded by activated sludge, thus leading to different feed/microorganisms (F/M) rates along time. Several studies demonstrated the relationship between biological activities, either anaerobic, heterotrophic or nitrifying, with the biotransformation of different organic micropollutants (Barret et al., 2010; Suárez et al., 2010; Delgadillo-Mirquez et al., 2011; Majewsky et al., 2011; Fernandez-Fontaina et al., 2012; Helbling et al., 2012). Consequently, the parameters obtained with the cometabolic kinetics in nitrifying biomass could be implemented in activated sludge models linking the biotransformation of micropollutants with the autotrophic biomass and the ammonia removal as proposed in the present study. Doing so, not only biomass and micropollutants concentrations are being taken into account, but also nitrogen loading rates, which are highly variable in WWTPs. The parameters obtained in this study at 25 °C are meant to be general for autotrophic biomass due to the high nitrifying activities achieved in the bioreactor; however, the fluctuating nature of activated sludge makes the determination of universal parameters very challenging. On top of the temperature, other limitations to generalize this model are that endogenous respiration has not been taken into account or the influence of other biological processes present in mixed cultures, such as the heterotrophic activity developed from the lysis of the nitrifying biomass, which should not be

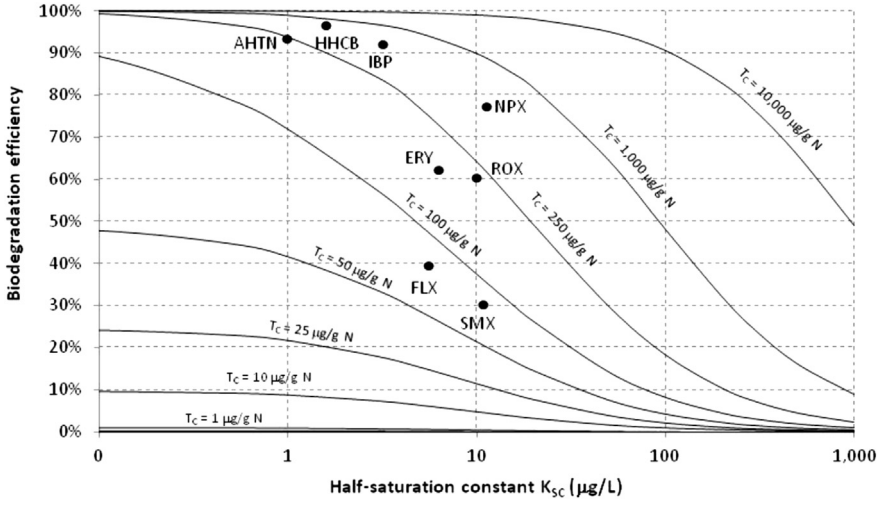

Fig. 4 – Influence of transformation capacity (T_C) and half-saturation constant (K_{SC}) of a micropollutant (K_d: 50 L/kg TSS; inlet concentration: 10 µg/L) on the biotransformation efficiency in a nitrifying activated sludge (NAS) reactor modelled as a continuous stirred tank reactor (HRT: 1 d; SRT: 12 d; T: 25 °C; TSS: 0.85 g/L; VSS: 0.65 g/L; SNR: 0.15 g N/g VSS d). The selected organic micropollutants are also located in the graph according to their experimental kinetic coefficients. The biotransformation efficiency was obtained according to Eq. (18).

discarded. Operational changes in the reactor along time, such as the decrease in SRT during the experiment at a HRT of 1.0 d might have had a significant impact, leading to the washout of different bacterial populations. However, the catabolic capabilities associated to the highly nitrifying biomass developed in the bioreactor, could very likely be extrapolated to any type of NAS, as the non-specific enzymes responsible for the cometabolic biotransformation of organic micropollutants must be intrinsically produced as long as nitrifying activity is present in activated sludge.

3.4. *Assessing/predicting biotransformation efficiency in nitrifying reactors*

The influence of the two biotransformation parameters of the cometabolic model (T_C and K_{SC}) on the performance of a NAS reactor operating at a HRT of 1 d and SRT of 12 d has been theoretically studied. Fig. 4 shows how different combinations of values of these parameters would lead to different biotransformation efficiencies. The selected micropollutants are also plotted according to the experimental values obtained in this study (Table 3). As K_{SC} varies from 11.3 (for NPX) to 1.0 μg/L (for AHTN), the highest sensitivity is observed for intermediate values of T_C (from 50 to 250 μg/g N). In this region, small variations in both parameters due to changes in the biomass composition or its catabolic capabilities could lead to remarkable shifts in the micropollutant concentration leaving the bioreactor. NPX, ERY, ROX, FLX and SMX are classified in this region. In fact, this kinetics could also explain variability of removal efficiencies and biotransformation kinetic constants usually found in the literature for many organic micropollutants. Instead, biotransformation efficiencies higher than 80% are obtained when T_C is higher than 250 μg/g N, with little variations in performance occurring once this

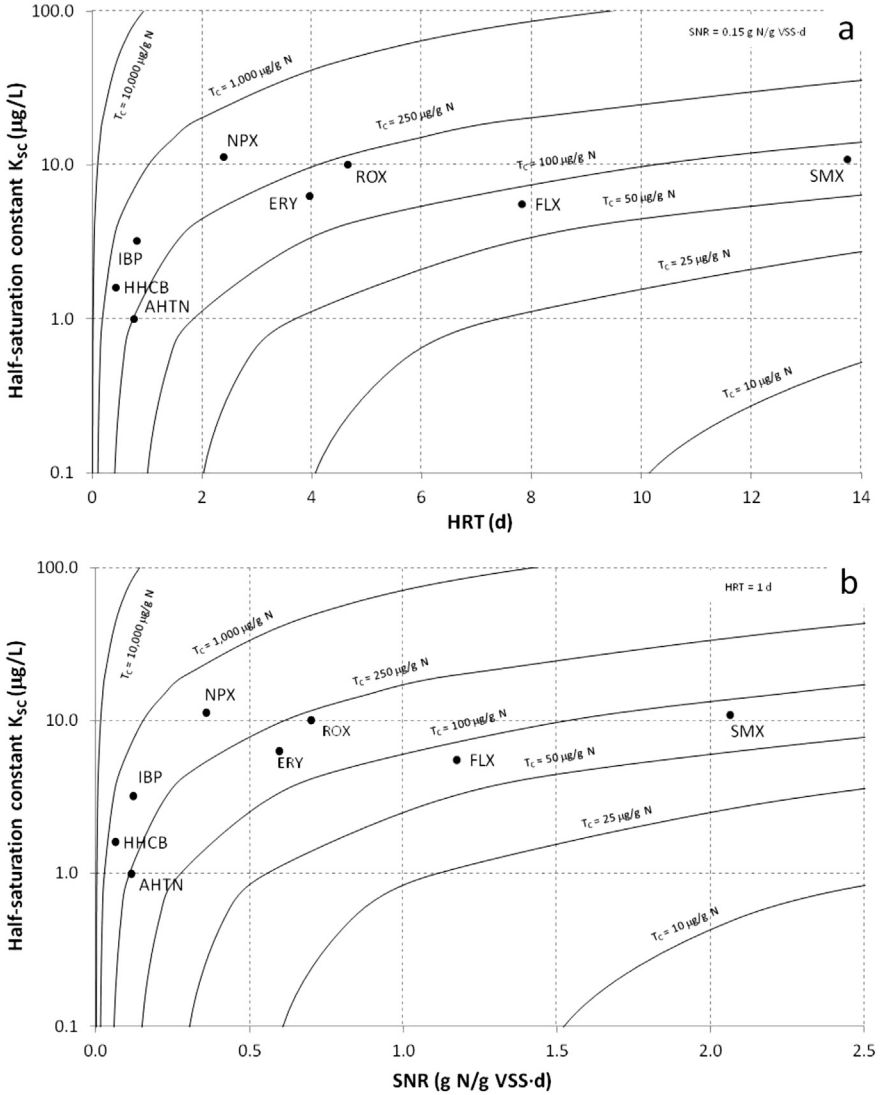

Fig. 5 – Influence of transformation capacity (T_C) and half-saturation constant (K_{SC}) of a micropollutant (K_d: 50 L/kg TSS; inlet concentration: 10 μg/L) on the minimum HRT (a) and on the minimum SNR (b) required to achieve 90% biotransformation efficiency in a NAS reactor modelled as a continuous stirred tank reactor (T: 25 °C; TSS: 0.85 g/L; VSS: 0.65 g/L; SNR: 0.15 g N/g VSS d; HRT: 1 d). The selected organic micropollutants are also located in the graph according to their experimental kinetic coefficients.

value has been surpassed, especially if K_{SC} is lower than 5 μg/L, as it is the case of IBP, HHCB and AHTN. On the contrary, only low biotransformation efficiencies (<25%) could be expected if T_C is too low (<25 μg/g N). It should be noted that the calculations to build these charts have been made employing a continuous stirred tank reactor (CSTR) model for the specific conditions found in the NAS reactor during assay B (T: 25 °C; TSS: 0.85 g/L; VSS: 0.65 g/L).

The effect of T_C and K_{SC} on the minimum HRT and SNR needed to achieve a biotransformation efficiency of at least 90% has been plotted in Fig. 5a and b, respectively. High values of K_{SC} mean longer residence times needed for depleting a micropollutant in a bioreactor, especially if coupled with a low T_C. An HRT of 1 d would only be enough to extensively remove IBP, HHCB and AHTN. Improving the biotransformation efficiency further for other micropollutants would mean an anti-economical increase of the volume of the biological reactor. The SNR exerts a similar effect to that of HRT, with high biotransformation efficiencies achieved (90%) even at low T_C and high K_{SC} values if SNR is high enough. Overall, the cometabolic kinetics could be very useful to assess the removal efficiency of those organic micropollutants which are biotransformed by cometabolic pathways in nitrifying activated sludge.

4. Conclusions

The cometabolic Monod-type kinetics, with two kinetic parameters (T_C and K_{SC}) predicts more accurately the biotransformation of several organic micropollutants (IBP, NPX, ERY and ROX) in a NAS reactor. The cometabolic term (T_C) takes into account the effect of the nitrification rate on the biotransformation of these micropollutants and is the most relevant for improving the prediction accuracy of the biotransformation model. The Monod kinetics (K_{SC}) does not improve accuracy when compared to the pseudo-first order kinetics, but could allow a higher flexibility if the reaction order changes. The parameters (T_C and K_{SC}), estimated at 25 °C, were successfully employed to predict the biotransformation efficiency of these micropollutants in a nitrifying reactor operating under different HRTs and nitrification rates.

The proposed biotransformation kinetics implemented in bioreactor models could be a powerful tool to identify the combination of operational parameters required for obtaining a desired removal efficiency. Also, the estimated parameters for the proposed cometabolic kinetics could also be further implemented in activated sludge models (ASM) for simulation of the cometabolic biotransformation of organic micropollutants performed by autotrophic biomass, adding nitrifying activity as an additional variable to be considered on top of micropollutant and biomass concentrations.

In order to generalize this model for the biotransformation of micropollutants in CAS, a similar procedure should be followed to obtain the parameters for heterotrophic biomass. Other processes such as endogenous respiration must be considered as well. The kinetic parameters should also be determined in a wide range of temperatures to obtain correction factors. This way, a complete and accurate model for the biotransformation of emerging organic micropollutants in activated sludge reactors could be achieved in the near future.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.07.048.

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