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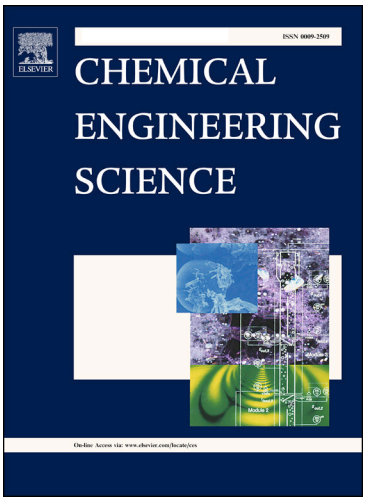


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# Modelling Cometabolic Biotransformation of Sulfamethoxazole by an Enriched Ammonia Oxidizing Bacteria Culture

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**ABSTRACT**

Antibiotics such as sulfamethoxazole (SFX) are environmentally hazardous after being released into the aquatic environment and challenges remain in the development of engineered prevention strategies. In this work, a mathematical model was developed to describe and evaluate cometabolic biotransformation of SFX and its transformation products (TPs) in an enriched ammonia oxidizing bacteria (AOB) culture. The growth-linked cometabolic biodegradation by AOB, non-growth transformation by AOB and non-growth transformation by heterotrophs were considered in the model framework. The production of major TPs comprising 4-Nitro-SFX, Desamino-SFX and $N^4$-Acetyl-SFX was also specifically modelled. The validity of the model was demonstrated through testing against literature reported data from extensive batch tests, as well as from long-term experiments in a partial nitritation sequencing batch reactor (SBR) and in a combined SBR+membrane aerated biofilm reactor performing nitrification/denitrification. Modelling results revealed that the removal efficiency of SFX increased with the increase of influent ammonium concentration, whereas the influent organic matter, hydraulic retention time and solid retention time exerted a limited effect on SFX biodegradation with the removal efficiencies varying in a narrow range. The variation of influent SFX concentration had no impact on SFX removal efficiency. The established model framework enables interpretation of a range of experimental observations on SFX biodegradation and helps to identify the optimal conditions for efficient removal.

**Keywords:** ammonia oxidizing bacteria; cometabolic biotransformation; mathematical model; sulfamethoxazole; transformation products; removal efficiency

## 1. Introduction

The release of pharmaceutically active compounds (PhACs) and of their transformation products (TPs) into the aquatic environment has led to increasing concerns with regard to the resulting exposure of wildlife and human beings. The adverse effects of PhACs are associated with their toxicity, endocrine disruption and antimicrobial resistance (Luo et al., 2014). Antibiotics, as a major category of PhACs, are of special interest due to their widespread use and increasing consumption (Göbel et al., 2007; Larcher and Yargeau, 2012). Of special interest are the sulfonamide antibiotics which were the first antimicrobial drugs utilized worldwide (Kassotaki et al., 2016). Sulfamethoxazole (SFX) is the most prominent short-acting representative of sulfonamide antibiotics used in high amounts in human and veterinary applications to treat and prevent bacterial infections (Majewsky et al., 2014), hence studying elimination strategies is of great importance. Although the removal of SFX largely relies on microbial activity (Müller et al., 2013), its reported removal efficiencies vary between different wastewater treatment plants (WWTPs) mainly because they are not designed to biologically remove PhACs (Bolong et al., 2009). Moreover, for most PhACs including SFX, there is still a lack of discharge standards and regulations (Luo et al., 2014).

Research on mechanisms of microbial elimination of SFX is of great significance since the findings are able to provide suggestions and insights for future WWTPs design and operation aiming at PhACs treatment. Up-to-date,a lot of studies have characterized the biodegradation of PhACs, including SFX, under varying operational conditions (Bendz et al., 2005; Levine et al., 2006; Müller et al., 2013; Collado et al., 2013; Alvarino et al., 2015). In some cases it was found that PhACs removal was favored in the presence of external organic carbon sources or nutrient sources (nitrogen, phosphorus etc.) (Boiesen et al., 1993; Fava et al., 1995), whereas other

reports revealed higher removal efficiency at longer solid retention time (SRT), indicating that the slow growing nitrifying bacteria may play an important role (Clara et al., 2005a; Joss et al., 2006). Moreover,the biodegradation of PhACs in wastewater was reported to be linked to the cometabolic activity (Tran et al., 2009). Batt et al. (2006) and Yi and Harper (2007) found that ammonia oxidizing bacteria (AOB) were responsible for PhACs removal in nitrifying activated sludge considering that ammonium monooxygenase (AMO) has a relatively wide spectrum for the degradation of substrates. Fernandez-Fontaina et al. (2014) reported that SFX was slowly biodegradable at mg/L concentration by autotrophic nitrifying bacteria, whilst higher specific nitrification rate led to higher biodegradation rate.

Mathematical models could be used as a promising tool to help decision-makers to understand the fate and transformation of PhACs in the aquatic environment and to optimize the treatment process. Among the cometabolic transformation processes of PhACs, the biodegradation kinetics can be of major practical importance for application of in situ remediation (Liu et al., 2015). First-order (Oldenhuis et al., 1991), Michaelis-Menten kinetics (Gälli and McCARTY, 1989; Chang and Criddle, 1997; Boonchayaanant et al., 2008) and models considering both cometabolic and primary substrate (Verce et al., 2002; Sathyamoorthy et al., 2013; Fernandez-Fontaina et al., 2014) were used to predict biotransformation of PhACs in activated sludge systems. Interactions between growth substrates and PhACs could occur in different manners including competitive inhibition on primary substrate (Rittmann, 1992; Sathyamoorthy et al., 2013) and vice versa (Chang and Alvarez-Cohen, 1995; Plósz et al., 2010). Fernandez-Fontaina et al. (2012) demonstrated the correlation between the biotransformation rate of PhACs and the nitrification activity. Cometabolic biodegradation could be responsible for the removal of some compounds due to the

action of the AMO enzyme of AOB. Hence, the cometabolic model is more suitable to describe biotransformation of PhACs in nitrifying activated sludge reactors. However, a comprehensive model for describing SFX removal by enriched AOB sludge with the consideration of its multiple transformation products (TPs) is currently not available.

This work aims to develop a new modelling framework to evaluate the cometabolic biotransformation of SFX and its TPs, which explicitly and additively considers: i) the correlation between SFX removal and the specific ammonium oxidation rate ($AOR_{sp}$: the amount of ammonium oxidized per sludge mass and time); ii) the effect of external carbon dosage on SFX degradation; iii) the role of AMO enzyme during this process; and iv) the generation of two TPs of SFX: 4-Nitro-SFX (4-$NO_2$-SFX) and Desamino-SFX as well as of a human metabolite: $N^4$-Acetyl-SFX and their correlation with the parent compound. Experimental results obtained from the literature are used to calibrate (Kassotaki et al., 2016) and validate (Kassotaki et al., 2016; Zhu et al., 2017) the model. The verified model is then used to systematically explore the effects of key operational conditions on SFX removal and TPs formation, which would be useful for the development of engineered prevention strategies.

## 2. Materials and methods

### 2.1. Model development

A model framework is developed to describe the biotransformation of SFX and its TPs formation in an enriched AOB culture. The model considers the metabolism and interactions of AOB and heterotrophic bacteria (HB) in terms of all relevant biological reactions concerning the conversions of nitrogen and organic matter (COD)

as well as SFX degradation and TPs formation. The framework contains 15 state variables of which 10 are soluble growth substrates ($S_{NH4}$, $S_{NO2}$, $S_{NO3}$, $S_{O2}$, $S_S$, $S_{SFX}$, $S_{4\text{-}NO2\text{-}SFX}$, $S_{desamino\text{-}SFX}$, $S_{N4\text{-}Acetyl\text{-}SFX}$, $S_{others}$) and 5 are active biomass components ($X_{AOB}$, $X_{NOB}$, $X_H$, $X_S$, $X_I$).

The kinetics related to growth and metabolism of AOB, NOB and HB are adapted from widely-accepted activated sludge models (ASMs) by the international water association. The microbial processes related to SFX transformation are determined based on our experimental observations: i) the correlation between ammonia oxidation rate and SFX degradation rate illustrates the cometabolic growth-linked process by AOB; ii) the SFX degradation in the absence of ammonium confirms the non-growth linked processes by AOB and HB; iii) the improved SFX degradation in the presence of COD reveals the contribution of non-growth linked process by HB. As for the formation of the TPs, we consider that the formation of the three main TPs (4-$NO_2$-SFX, Desamino-SFX and $N^4$-Acetyl-SFX) are solely linked to AOB cometabolism, while other minor TPs (unidentified in this study) can be generated by either AOB or HB. Specifically, eleven microbial processes are considered in the model: i) Growth of AOB, coupled to ammonia oxidation and cometabolic biodegradation of the trace-level pollutant SFX; ii) non-growth transformation of SFX by AOB; iii) endogenous decay of AOB; iv) growth of NOB; v) endogenous decay of AOB; vi) hydrolysis; vii) aerobic growth of HB; viii) anoxic growth of HB, coupled to nitrite reduction; ix) anoxic growth of HB, coupled to nitrate reduction; x) non-growth biodegradation of SFX by HB; and xi) endogenous decay of HB. From all the processes previously described, three (process i, ii, and x) contribute to the biodegradation of SFX. The transformation capacity of SFX is modelled by Monod type expressions (Sathyamoorthy et al., 2013), which integrate

SFX biodegradation kinetics into the activated sludge model (ASM) framework. The related SFX biodegradation processes are modeled as first-order kinetics with respect to SFX concentration due to the fact that typical half saturation values for solutes in aquatic environment are always several orders of magnitude greater than the investigated SFX concentration (trace level in μg/L) (Alvarez-Cohen and Speitel Jr, 2001; Sathyamoorthy et al., 2013). The resulting kinetic rate expressions for cometabolic biodegradation of SFX by AOB (Equation 1), SFX biodegradation by AOB (Equation 2) and SFX biodegradation by HB (Equation 3) are shown as below:

$$\frac{dS_{SFX}}{dt} = -\varphi_{SFX,AOB} X_{AOB} S_{SFX} \mu_{AOB} \frac{S_{NH4}}{S_{NH4}+K_{NH4}^{AOB}} \frac{S_{O2}}{S_{O2}+K_{O2}^{AOB}} \quad (1)$$

$$\frac{dS_{SFX}}{dt} = -r_{SFX,AOB} X_{AOB} S_{SFX} \quad (2)$$

$$\frac{dS_{SFX}}{dt} = -\theta_{SFX,H} X_H S_{SFX} \quad (3)$$

Where $\varphi_{SFX,AOB}$ is cometabolic SFX transformation coefficient linked to AOB growth; $\mu_{AOB}$ is growth rate for AOB; $K_{NH4}^{AOB}$ is $S_{NH4}$ affinity constant for AOB ; $K_{O2}^{AOB}$ is $S_{O2}$ affinity constant for AOB; $r_{SFX,AOB}$ is non-growth SFX biodegradation coefficient for AOB; $\theta_{SFX,H}$ is non-growth SFX biodegradation coefficient for HB; $S_{SFX}$, $S_{NH4}$ and $S_{O2}$ are soluble SFX, $NH_4^+$ and $O_2$ concentrations, respectively; $X_{AOB}$ and $X_H$ are active AOB and HB biomass concentrations, respectively.

In the model framework, for the first time, the formation of TPs is modeled using distribution coefficients in four individual process: i) biodegradation of SFX to 4-SFX-$NO_2$; ii) biodegradation of SFX to Desamino-SFX; iii) biodegradation of SFX to $N^4$-Acetyl-SFX; and iv) biodegradation of SFX to other TPs. The rate expressions of TPs generation are listed as below:

$$\frac{dS_{TPs}}{dt} = \alpha_i \varphi_{SFX,AOB} X_{AOB} S_{SFX} \mu_{AOB} \frac{S_{NH4}}{S_{NH4}+K_{NH4}^{AOB}} \frac{S_{O2}}{S_{O2}+K_{O2}^{AOB}} \quad (4)$$

$$\frac{dS_{others}}{dt} = (1-\sum_{i=1}^{3}\alpha_i) \varphi_{SFX,AOB} X_{AOB} S_{SFX} \mu_{AOB} \frac{S_{NH4}}{S_{NH4}+K_{NH4}^{AOB}} \frac{S_{O2}}{S_{O2}+K_{O2}^{AOB}} \quad (5)$$

$$\frac{dS_{others}}{dt} = r_{SFX,AOB} X_{AOB} S_{SFX} \quad (6)$$

$$\frac{dS_{others}}{dt} = \theta_{SFX,H} X_H S_{SFX} \quad (7)$$

Where $\alpha_i$ (i=1, 2 or 3) is the distribution coefficient for AOB cometabolic TPs: 4-SFX-$NO_2$, Desamino-SFX or $N^4$-Acetyl-SFX, respectively; Equation 4 is the production rate of 4-SFX-$NO_2$, Desamino-SFX or $N^4$-Acetyl-SFX; Equation 5-7 is the production rate of other TPs during AOB cometabolic degradation, biotransformation of SFX in the absence of AOB growth and biotransformation of SFX due to the presence of HB, respectively.

We make several assumptions in the model framework of this study, which include: i) 4-SFX-$NO_2$, Desamino-SFX and $N^4$-Acetyl-SFX can not be removed by the investigated systems, as described in Kassotaki et al. (2016) (refer to Figure S1); ii) the generation of the three known TPs is solely linked to AOB cometabolism (Equation 4) in line with the experimental observation by Kassotaki et al. (2016); iii) Other transformation products (not detected specifically) were lumped into one soluble parameter ($S_{others}$) for simplicity, which can be produced by AOB cometabolic degradation (Equation 5) as well as by non-growth biodegradation by AOB and HB (Equation 6 and 7); iv) the biodegradation process of other possible TPs is not involved due to the lack of a comprehensive dataset for kinetic evaluation.

The overall kinetics and stoichiometry of the developed model are summarized in Tables S1 and S2 in the Supplementary Material. Both growth and decay processes are considered for each microbial species. Kinetic control of all the enzymatic reaction rates is described by the Michaelis-Menten equation. The rate of each reaction is modeled by an explicit function of the concentrations of all substrates involved in the biological reaction.

2.2. Experimental data for model testing

The model was first tested using the experimental data obtained by Kassotaki et al. (2016), under both aerobic short term experiments, as well as long term experiments in a partial nitrification SBR. The experimental approaches are being briefly summarized below.

An 8L SBR was enriched with ammonia oxidizing bacteria (AOB) in order to treat synthetic reject wastewater with a concentration of 1g $NH_4^+$-N/L. The short term experiments were conducted after more than a year of operation, with a stable AOB population (more than 80% of the total microbial community), and a stable nitritation performance (95% of $NH_4^+$ converted to $NO_2^-$ and no $NO_3^-$ detected in the effluent), with the aim of exploring the biodegradation capacity of the culture towards SFX. Different experiments were conducted with sludge withdrawn from the SBR (under aerobic conditions and with a contact time of 6h) in order to evaluate: i) the effect of different specific ammonium oxidation rates ($AOR_{sp}$), ii) the role of AMO enzyme (addition of the inhibitor allylthiourea; ATU) and iii) the role of the heterotrophic fraction of the biomass (addition of acetate), on SFX degradation and TPs production (4-$NO_2$-SFX, Desamino-SFX and the human metabolite: $N^4$-Acetyl-SFX). Moreover, separate experiments were conducted adding only TPs to assess possible degradation or re-transformation to SFX. Finally, long term experiments (up to 10 weeks) were conducted directly adding SFX in the influent medium of the SBR to investigate a longer hydraulic retention time (HRT) (contact time of 24h) and possible acclimation factors. The long term experiments were executed after all the batch experiments were completed to avoid any inconsistency attributable to the potential biomass adaptation to SFX.

Moreover, the proposed model was further evaluated based on experimental data

from an attach-growth system under nitrification conditions. The investigated system involved a 5-L anoxic SBR and a 10-L membrane aerated biofilm reactor (MABR). The HRT was 6 hours. The combined system was operated for 275 days since inoculation. The removal of nitrogen and COD and biotransformation of SFX were monitored under varying SFX influent concentrations. For more details refer to Zhu et al. (2017).

2.3. Model calibration and validation

The developed model contains 29 stoichiometric and kinetic parameters, as summarized in Table S3. Most of model parameter values were adopted from literature directly as they have been well applied in previous studies. This strategy reduces the complexity of model calibration and avoids parameter correlation issues (Ni and Yu, 2008). Experimental data from the batch experiments i - iii were used to calibrate the key parameters related to SFX biodegradation and TPs formation including the: cometabolic SFX transformation coefficient ($\varphi_{SFX,AOB}$), non-growth SFX biodegradation coefficient for AOB ($r_{SFX,AOB}$), non-growth SFX biodegradation coefficient for HB ($\theta_{SFX,H}$) and the distribution coefficients of AOB cometabolic TPs ($\alpha_i$ (i=1,2 or 3)). AQUASIM 2.1 is used to perform the estimation of parameters (Reichert, 1998), using the mixed reactor compartment module to present fully mixed reactor conditions and biofilm compartment module to present the MABR reactor conditions (Sun et al., 2017). Parameters were estimated by minimizing the sum of the squared residuals.

The SFX removal efficiencies at varying $AOR_{sp}$ were then used to validate the developed model and the obtained parameter values. To verify the validity of the established model, model evaluation was also carried out using experimental data in

terms of SFX and its TPs from long-term partial nitritation SBR operation with different influent concentrations of SFX. Further model evaluation was performed using the experimental data from the combined SBR+MABR system under nitrification conditions. In this scenario, the most sensitive parameter ($\theta_{SFX,HB}$) according to sensitivity analysis was recalibrated.

## 3. Results

### 3.1. Model calibration

The calibration of the developed model involved optimizing key parameter values for SFX biodegradation and TPs formation by fitting simulation results to batch experimental data under different conditions. The calibrated parameter values giving the optimum fit are listed in Table S3.

The experimentally observed and model predicted SFX, TPs, $NH_4^+$ and $NO_2^-$ are presented in Figure 1. As it can be observed, the calibrated model was able to simulate accurately the influence of the specific ammonium oxidation rate in the removal of SFX and production of TPs. It managed to adapt to the decreasing nitrification rate (Figure 1B, 1D, and 1F) and to capture the decreasing levels of SFX removal, and consequently of the 4-$NO_2$-SFX production (Figure 1A, 1C, and 1E). The formation of Desamino-SFX and $N^4$- Acetyl-SFX was negligible in most of the cases.

Figure 2 illustrates the effect of the addition of the inhibitor ATU and of an external carbon source (acetate; ACE) on SFX biodegradation. SFX concentrations are reasonably well reproduced, demonstrating the effect of ATU (null removal – Figure 2A) and of acetate in the presence and absence of $NH_4^+$ (moderate removal – Figures 2C&D, respectively). Moreover, the model fitted well to the results of the

experiment where both ATU and acetate were added and it simulated with null removal of SFX removal (Figure 2B). It is known that ATU inhibits AMO activity and thus cease the ammonia oxidation and the resulting AOB-linked SFX degradation. The discrepancy between the ATU inhibition and the enhanced removal of SFX with ACE in Figure 2 is possibly attributed to two reasons or their combination: 1) there exists a intrinsic regulation mechanism of AMO on SFX degradation by HB (Kassotaki et al., 2016); 2) ATU can inhibit some enzymes in HB and thus inhibit the HB-linked SFX degradation, since ATU is not a specific inhibitor for AMO (Men et al., 2017).

The good agreement between model prediction and experimental data indicates that the proposed model framework is able to describe the effect of influent $NH_4^+$ ($AOR_{sp}$) on SFX biodegradation and TPs formation. The obtained parameter value of cometabolic SFX transformation coefficient ($\varphi_{SFX,AOB}$ = 0.04 $m^3$ g $COD^{-1}$) is comparable with literature reported value of 0.0715 $m^3$ g $COD^{-1}$ for PhAC degradation in both suspended growth system and membrane aerated biofilm reactor (Sathyamoorthy et al., 2013; Peng et al., 2015). The distribution coefficients of AOB cometabolic products ($\alpha_i$ (i=1,2 or 3)) are newly obtained parameters in the developed model. The values for stoichiometric fraction of 4-$NO_2$-SFX ($\alpha_1$), Desamino-SFX ($\alpha_2$) and $N^4$-Acetyl-SFX ($\alpha_3$) from SFX cometabolic transformation are 0.3, 0.03 and 0.01, respectively, supporting the observation that 4-SFX-$NO_2$ is the dominated SFX degradation product among the three detected TPs. Furthermore, the proposed model framework could well describe the connection between AMO and microorganisms including AOB and HB (Figure 2A&B). The non-growth SFX biodegradation by AOB (Equation 2) and non-growth SFX biodegradation by HB (Equation 3) were well characterized by the experimental data in the absence of $NH_4^+$

(Figure 1E) and in the presence of ACE (Figure 2D). The calibrated parameter value of biomass normalized SFX biodegradation rate coefficient for AOB ($r_{SFX,AOB}$ = 0.00001 $m^3$ g $COD^{-1}$ $h^{-1}$) was much lower than the $r_{SFX,AOB}$ value of 0.00067 $m^3$ g $COD^{-1}$ $h^{-1}$ in the cometabolic kinetics developed by Sathyamoorthy et al. (2013), while the obtained values of biomass normalized SFX removal coefficients for HB ($\theta_{SFX,HB}$) in these two studies were relatively comparable (0.00063 $m^3$ g $COD^{-1}$ $h^{-1}$ in this study; 0.00093 $m^3$ g $COD^{-1}$ $h^{-1}$ in Sathyamoorthy et al. (2013)). The parameter correlation matrix obtained from model calibration indicated that none of the parameter combinations showed significant correlations.

3.2. Model validation

Model validation aims to further test the validity and reliability of the model framework and of the estimated parameters (Table S3). The model predictions with these parameters were compared to the experimental data from other batch experiments, those are not used for model calibration. The proposed model framework was assessed using SFX degradation data obtained from varying batch tests with different $NH_4^+$ loading rates. The calculated SFX removal efficiencies were plotted against the corresponding $AOR_{sp}$ in Figure 3 and a positive correlation was found experimentally. In the absence of $NH_4^+$ where there is no ammonia oxidation, ~25% degradation of SFX was observed. The model with the same set of calibrated parameters can well describe the relationship between SFX removal efficiencies and $AOR_{sp}$.

The experimental results obtained from long-term experiments that were conducted in the partial nitritation SBR by adding SFX in the influent medium, were used to further validate the established model. As shown in Figure 4, the influent SFX

was ~ 11 μg/L from day 0 to day 35, while ~102 μg/L of SFX was provided from day 36 to day 70. The simulation results fit well with the experimental data under both operational conditions (Figure. 4). The developed model with the same set of parameter values used for the batch experiment (Table S3) could generate accurate predictions in terms of effluent SFX concentrations (below 1 μg/L) as well as SFX removal efficiencies (over 97%) in the partial nitritation SBR. Moreover, in agreement with experimental measurement, the model simulation indicates 4-$NO_2$-SFX was the main TP, whereas the production of Desamino-SFX and $N^4$-Acetyl-SFX was minimum. The developed model is also able to describe SFX degradation and TPs formation in the long-term partial nitritation SBR with different influent SFX, further confirming the validity of the model framework.

Figure 5 presents the model evaluation results based on the combined SBR+MABR system performing simultaneous nitrification/denitrification and SFX degradation. The proposed model for partial nitritation SBR, when adapting to the attached-growth system and nitrification conditions, possesses a good predictive ability and captures all the dynamics of nitrogen, COD and SFX. And the calibrated parameter value of $\theta_{SFX,HB}$ in this scenario is lower than that in the partial nitritation SBR due to fact that the supplement of COD in MABR+SBR may alter the microbial community, especially the HB in the system.

**4. Discussion**

In this study, a comprehensive model framework for describing SFX biodegradation and its TPs formation in an enriched nitrifying culture dominated by AOB was proposed. The biodegradation of SFX was modeled via three biological processes: cometabolic biodegradation of SFX by AOB, linked to AOB growth; non-

growth SFX biodegradation by AOB and non-growth SFX biodegradation by HB. Distribution coefficients were used to model the production of TPs of 4-SFX-$NO_2$, Desamino-SFX and $N^4$-Acetyl-SFX from AOB cometabolic biodegradation of SFX. The proposed model framework was assessed against experimental data from extensive batch tests, as well as from long-term experiments in a partial nitritation SBR and in a combined SBR+MABR performing nitrification/denitrification.

The good agreement between the model predictions and experimental data demonstrates the validity of the developed model in predicting SFX degradation and TPs formation in the enriched AOB culture with varying operational conditions applied. The developed model of this study would be useful to enhance our ability to understand and predict cometabolic SFX transformation in wastewater treatment, especially during biological nitrogen removal.

A pseudo-first order kinetic has been widely used for biotransformation of micropolluants (Fernandez-Fontaina et al., 2014). Criddle (1993) established a cometabolic model in association with biomass growth and the uptake of primary substrate. In the present study, the proposed model framework directly adopted the first-order kinetics for non-growth SFX transformation by AOB and HB, while SFX cometabolic biodegradation was linked to enzymatic reactions during AOB growth. A high variation among the values of biotransformation kinetic constant have been reported in the literature, which could be attributed to varying nitrification rates, different types of micropollutants with low or high biodegradability, and/or various culture community in activated sludge (Pomiès et al., 2013). It is difficult to directly compare the values of biotransformation kinetic constants identified here to those from literature based on pseudo-first order kinetics, since this study has incorporated different SFX biodegradation pathways by different microorganisms (AOB and HB)

and implemented SFX degrading kinetics into ASM with consideration of interactions between substrate update and microorganism growth as well as endogenous respiration. However, the estimated parameter value of cometabolic biodegradation coefficient ($\varphi_{SFX,AOB}$ = 0.04 $m^3$ g $COD^{-1}$) is comparable to those in the study of Sathyamoorthy et al. (2013) evaluating the fate of atenolol, a selected beta blocker by biodegradation and cometabolic model (0.0715 $m^3$ g $COD^{-1}$).

The developed model successfully described the effect of the specific ammonia oxidation rate on the removal of SFX (Figure 3). The kinetics and model framework could be extrapolated to describe the biodegradation of other PhACs since positive relationship was also found between the nitrifying activity and the removal of erythromycin, ibuprofen and roxithromycin in an aerobic conventional activated sludge system (Alvarino et al., 2014). According to the estimated values of distribution coefficients of TPs ($\alpha_1$, $\alpha_2$ and $\alpha_3$), over 30% of TPs would be generated during cometabolic biotransformation of SFX with 4-$NO_2$-SFX being the main contributor. As it has been previously reported, in aquifer material and under denitrifying conditions, 4-$NO_2$-SFX was cleaved back to SFX when the concentration of nitrite decreased (Barbieri et al., 2012). On the contrary, in this study it was shown that 4-SFX-$NO_2$, Desamino-SFX and $N^4$-Acetyl-SFX were neither degraded nor cleaved back to SFX during both batch and long-term experiments (the experimental data along with model prediction results are presented in Figure S1). The results were in accordance with those of Kassotaki et al. (2016), which further demonstrated the good predictive ability of the developed model framework. In this study, the aim is not to provide a full screening of all the TPs by the three pathways, but rather to focus on the three major cometabolic transformation products by AOB. Hence, for simplicity we have lumped these undetected TPs into one model component ($S_{others}$).

Future work will be needed to identify/quantify the potential other TPs in the system. Once such information will be available, the model would be easily modified to accurately predict all the other TPs. SFX adsorption onto the biomass was not considered since the sorption control experiment revealed that SFX did not show any remarkable removal during a period of four days (Kassotaki et al., 2016). Therefore, it was assumed that biodegradation became the predominant removal mechanism once adsorption equilibrium had been established, as it was previously demonstrated by Yang et al. (2011).

As for the enriched AOB culture with minor presence of HB growing on cell lysate, the biotransformation via AOB processes was the major contributor in the system. Indeed, in the presence of ATU in batch experiment, nitrification was completely inhibited and SFX was experimentally demonstrated to be not degraded. However, the dosage of COD promoted SFX degradation rate, suggesting the existence of transformation pathway via HB. The model did consider the possible transformation of SFX by HB (Process 10, Tables S1 and S2), which enabled the model to be capable of describing the non-growth linked pathway by HB contributing to SFX degradation. Furthermore, in a long-term SBR+MABR with COD present in the influent, the HB biotransformation pathway plays an even more important role. The parameter $\theta_{SFX,HB}$ was identified as the most sensitive parameter in terms of SFX biodegradation.

We use almost the same parameter values (small modification of $\theta_{SFX,HB}$ due to the COD supplement) to describe substrates dynamics and SFX conversion in both long-term partial nitritation SBR and SBR+MABR, revealing that different reactor configurations would not affect the model prediction. The proposed model framework is able to possess good predictive ability upon varying operational conditions, i.e.

sidestream partial nitritation conditions with high $NH_4^+$ concentration (~1000 mg N/L) vs. mainstream complete nitrification conditions with low $NH_4^+$ concentration (~40 mg N/L). The successful model validation using experiment datasets from different research groups, systems and conditions demonstrated the reliability and wide applicability of the proposed model.

The proposed model framework could be a useful tool to predict SFX degradation under varying conditions and thus provides support for reactor design, operation and optimization in terms of SFX removal. In this work, model simulations were performed to assess the influence of key parameters on SFX removal in a partial nitritation SBR. Figure 6 illustrates the system performance in terms of SFX degradation and TPs formation in response to influent wastewater with varying characteristics, while HRT and SRT of the SBR were kept at 24 hours and 15 days, respectively. The effluent concentrations of SFX, 4-$NO_2$-SFX**,** Desamino-SFX and $N^4$-Acetyl-SFX increased linearly with the increase of influent SFX from 0 to 1000 μg/L under the conditions of 1000 mg N/L influent $NH_4^+$ (Figure 6A). Hence, the change of influent SFX exerts no obvious effect on the removal efficiency of SFX, which was maintained at 89.4% for all cases. However, as $NH_4^+$ concentration in the influent increased from 50 to 500 mg N/L during treatment of wastewater containing 100 μg/L of SFX, there was a rapid drop of SFX concentration in the effluent, accompanied by a rise of effluent 4-$NO_2$-SFX**,** Desamino-SFX and $N^4$-Acetyl-SFX (Figure 6B). When influent $NH_4^+$ further increased to 5000 mg N/L, the decreasing rate for SFX or increasing rate of the TPs became much lower. The removal efficiencies for SFX in the presence of 50, 500 and 5000 mg N/L of influent $NH_4^+$ were 26%, 80.6% and 97.7%, respectively. The simulation results reveal that the influent $NH_4^+$ plays an important role in SFX biodegradation and thus side-stream

wastewater offers much more favorable conditions for concomitant SFX removal comparing to mainstream wastewater. Since organic matter can also be present in large amounts in wastewater, its effect on SFX conversions was also assessed in the model simulation. During treatment of wastewater containing 100 μg/L of SFX and 1000 mg N/L of $NH_4^+$, effluent SFX and TPs displayed a decreasing trend with the increase of influent COD from 100 to 7500 mg COD/L, while the decreasing rates decreased (Figure 6C). The removal efficiencies of SFX didn't vary a lot in this scenario (between 91.5% and 99.5%), indicating that the organic matter may have a limited impact on SFX biodegradation. For all simulation cases in Figure 6, 4-$NO_2$-SFX was always the major TP with Desamino-SFX and $N^4$-Acetyl-SFX as minor contributor. High TPs accumulations were observed under the conditions of high SFX and $NH_4^+$ in the influent, which may raise special attention since they may be of further environmental concern (Kassotaki et al., 2016). Although the increase of COD could decrease the build-up of 4-$NO_2$-SFX, Desamino-SFX and $N^4$-Acetyl-SFX from cometabolic SFX biodegradation by AOB, the additional transformed products other than the three major TPs mentioned above may be still environmentally hazardous, which need to be further evaluated.

The effect of HRT and SRT on SFX transformation processes in partial nitritation SBR treating 100 μg/L SFX and 1000 mg N/L $NH_4^+$ was also investigated (Figure 7). A slight increase of the effluent SFX and a decrease of the effluent 4-$NO_2$-SFX**,** Desamino-SFX and $N^4$-Acetyl-SFX were observed as HRT increased from 8 to 96 hours (Figure 7A). The removal efficiencies of SFX varied between 90% and 92.9%. Since influent $NH_4^+$ was a constant parameter in this simulation scenario, the increase of HRT not only increased contact time between the microbial community and SFX, but also decreased the $NH_4^+$ loading rate. It has been reported that more

antibiotics were biodegraded at longer HRTs (Fernandez-Fontaina et al., 2012) and high removal of micropollutants occurred in wastewater treatment plants with operational HRT longer than the half-life time of these compounds (García-Galán et al., 2011). On the other hand, as demonstrated previously (Figure 6B), the SFX removal is highly dependent on $NH_4^+$ loading. Hence, less SFX would be removed at low $NH_4^+$ rate (long HRT). The simulated trend of SFX removal against varying HRT is attributed to the combined effect of reaction time and loading. Upon increasing SRT from 5 to 25 days, the effluent SFX as well as the three TPs decreased slowly (Figure 7B). 87.5% - 90% of SFX were removed with higher efficiency obtained at longer SRT. Fernandez-Fontaina et al. (2012) found no obvious correlation between SRT and the biodegradation efficiency of micropollutants. On the contrary, higher removal efficiencies of micropollutants were obtained in biological nitrogen removal process, operating at long SRT (Clara et al., 2005b; Göbel et al., 2007; Clara et al., 2005a; Joss et al., 2006), which is consistent with our model simulation results. A long SRT condition favors slow growing autotrophs (i.e. AOB) and leads to a more diverse microbial community. The proposed cometabolic biotransformation kinetics implemented in biological reactor models could be a powerful tool to identify the combination of operational parameters required for obtaining a desired removal efficiency (Rubirola et al., 2014; Collado et al., 2014).

## 5. Conclusions

The developed model framework incorporating both AOB cometabolic biodegradation and non-growth transformation was successfully calibrated and validated using experimental data from short-term batch tests, long-term SBR and SBR+MABR reactors experiments. The model was able to describe the SFX

degradation and the production of its TPs at varying conditions. Simulation results indicate that influent ammonium concentration plays an important role in affecting transformation of SFX and production of TPs, with removal efficiency being enhanced at high influent ammonium level. Influent organic matters, HRT and SRT also affect SFX biodegradation, but the removal efficiencies varied in a narrow range. In contrast, influent SFX concentration exerts no significant effect on SFX removal efficiency. Finally, the proposed model framework could be a useful tool to predict SFX degradation under varying conditions and thus provides support for reactor design, operation and optimization in terms of SFX removal.

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## FIGURE CAPTIONS

**Figure 1.** Model calibration results of SFX degradation, TPs generation and nitrogen conversion in the presence of $NH_4^+$concentration of 435 mg N/L (A & B); 185 mg N/L (C & D) and 0 mg N/L (E & F).

**Figure 2.** Model calibration results of SFX degradation, TPs generation and nitrogen conversion in the presence of A) ATU + $NH_4^+$; B) ATU + ACE + $NH_4^+$; C) ACE + $NH_4^+$ and D) ACE.

**Figure 3.** Model validation results of SFX removal efficiency under varying $AOR_{sp}$.

**Figure 4.** Model validation results of SFX and TPs transformation in a long-term partial nitritation SBR experiments with the presence of 10 µg/L (A) and 100 µg/L (B) of SFX in the influent.

**Figure 5.** Model validation based on a combined SBR + MABR system performing nitrification and denitrification. (A) influent and effluent $NH_4^+$; (B) effluent $NO_3^-$; (C) influent and effluent COD; (D) influent and effluent SFX.

**Figure 6.** The effects of influent SFX (A), influent $NH_4^+$ (B) and influent COD (C) on the transformation of SFX and TPs formation in the partial nitritation SBR based on model simulations.

**Figure 7.** The effects of HRT (A) and SRT (B) on the transformation of SFX and TPs

formation in the partial nitritation SBR based on model simulations.

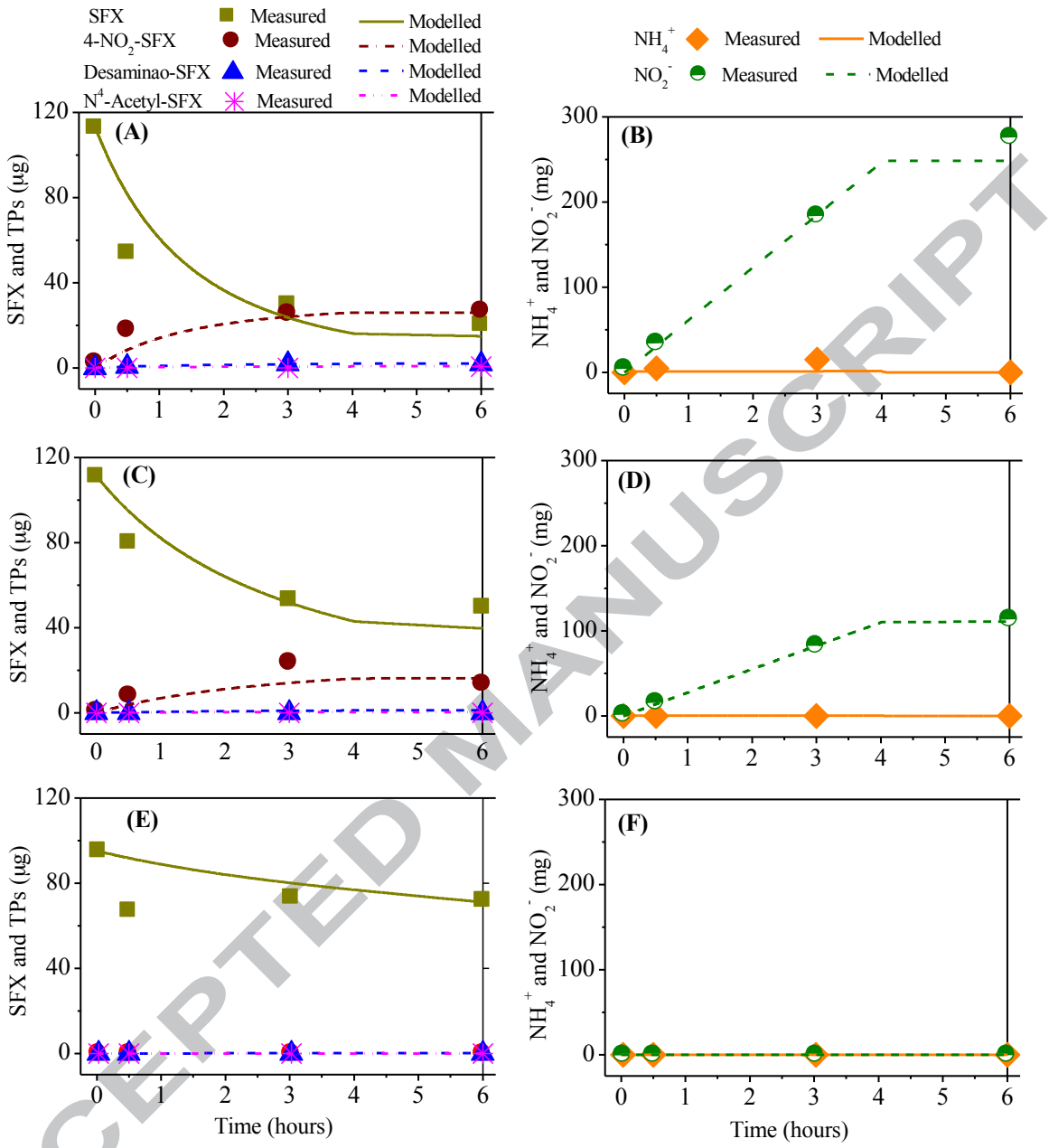


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Figure 2
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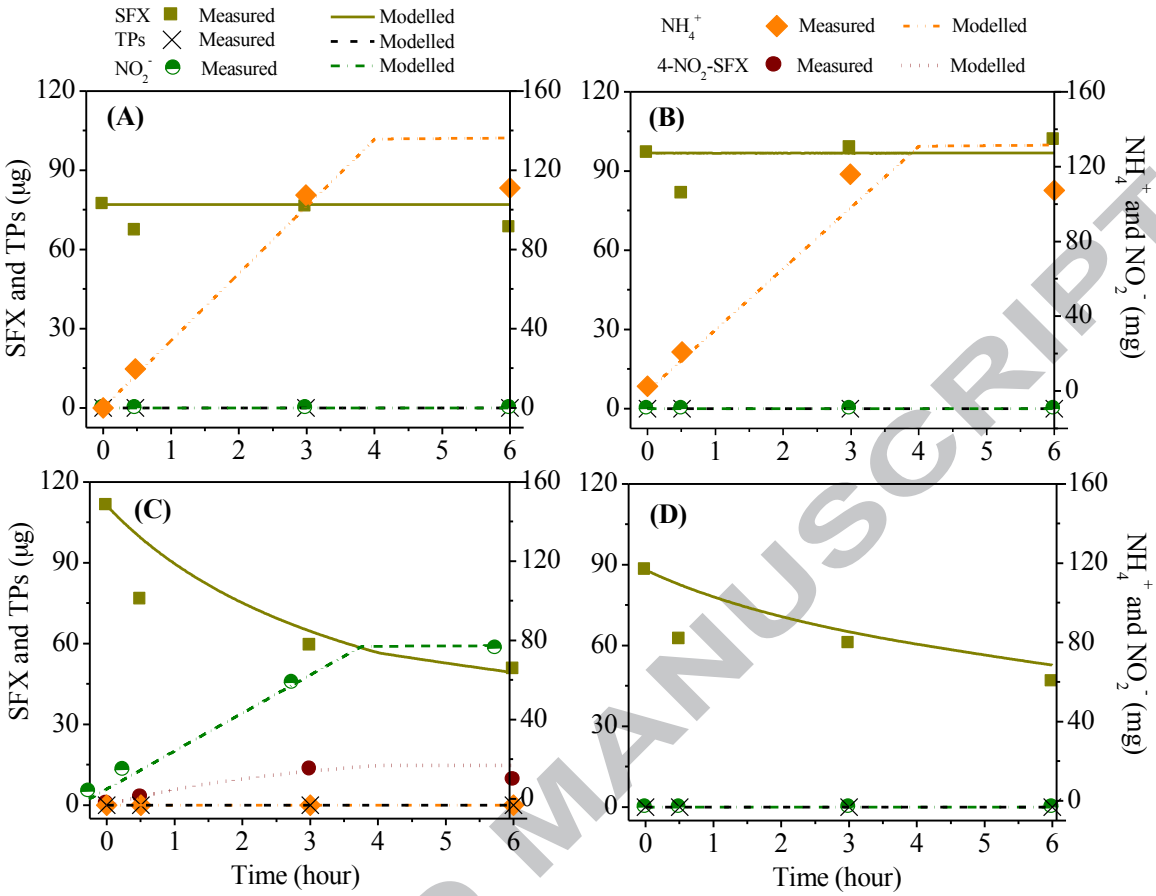


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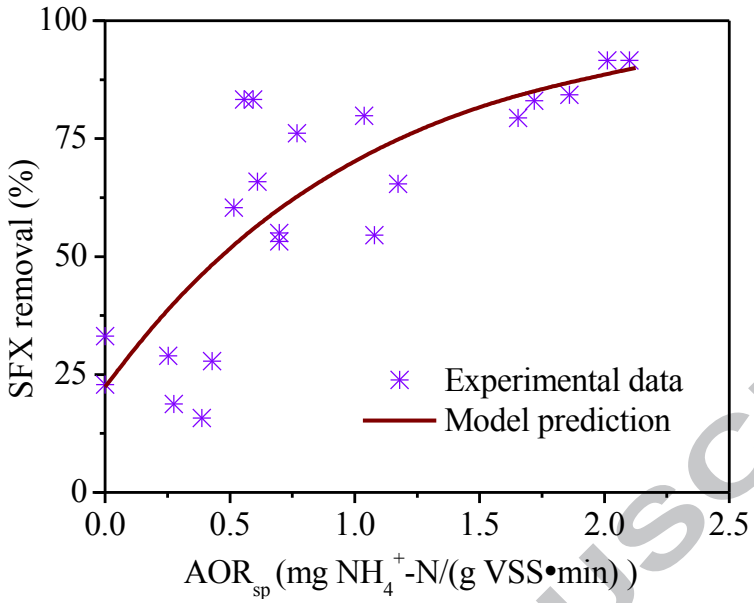


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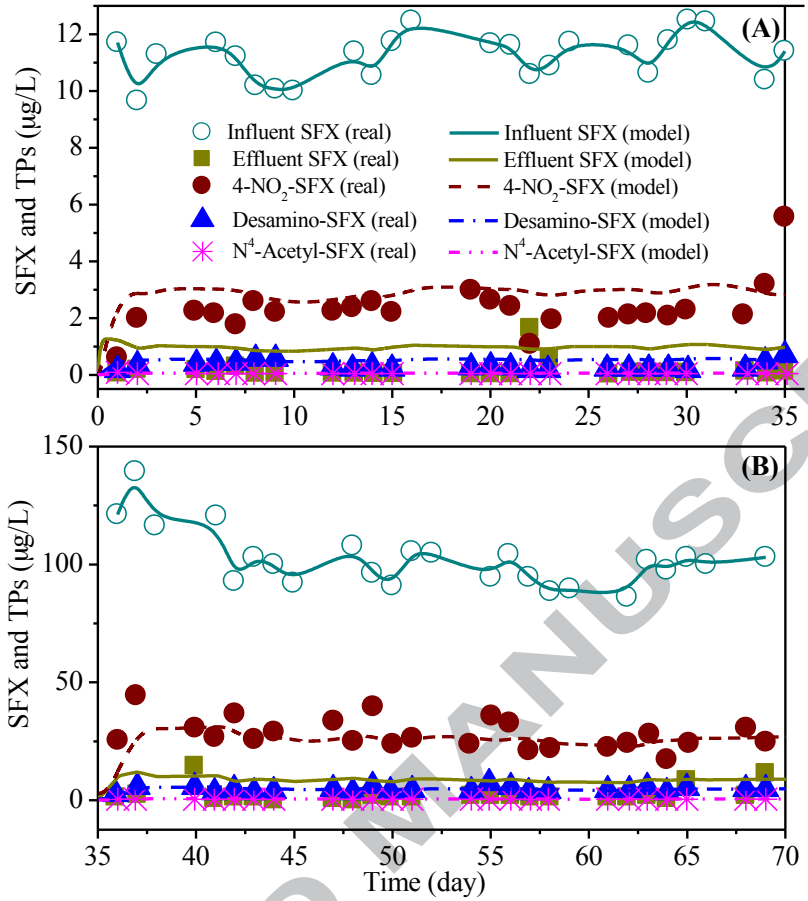


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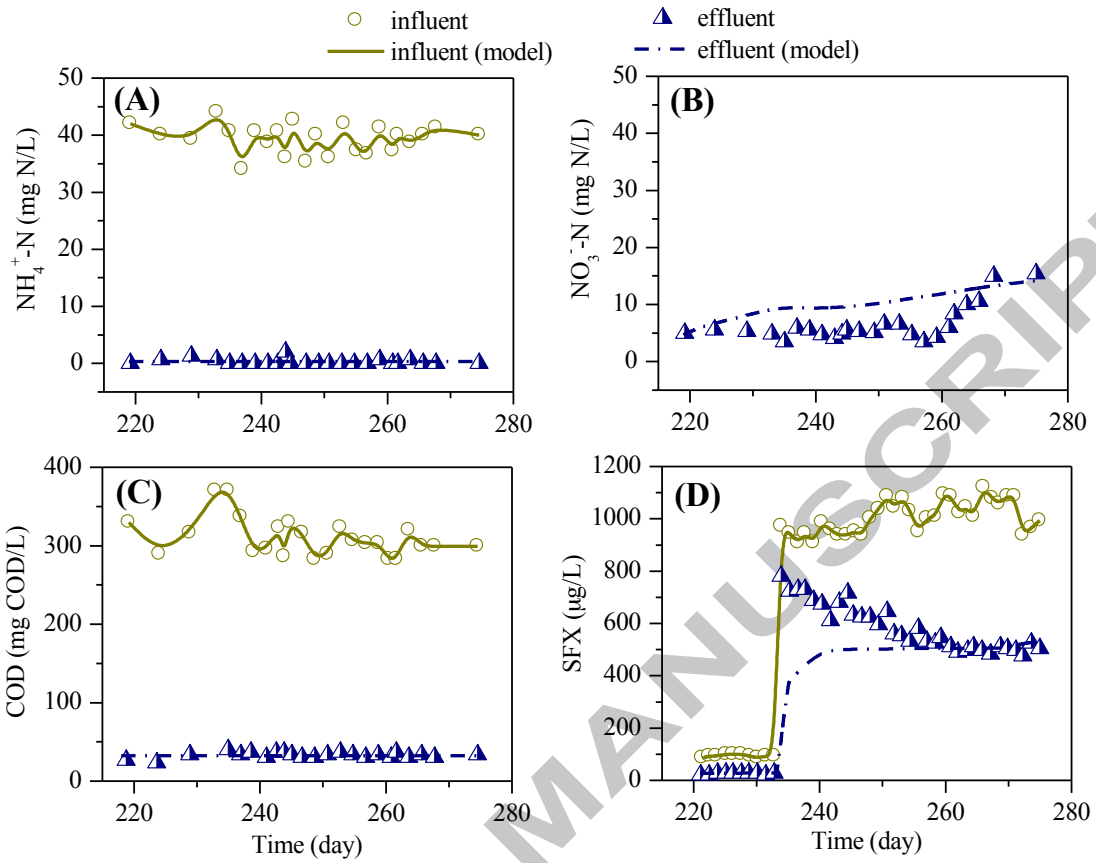


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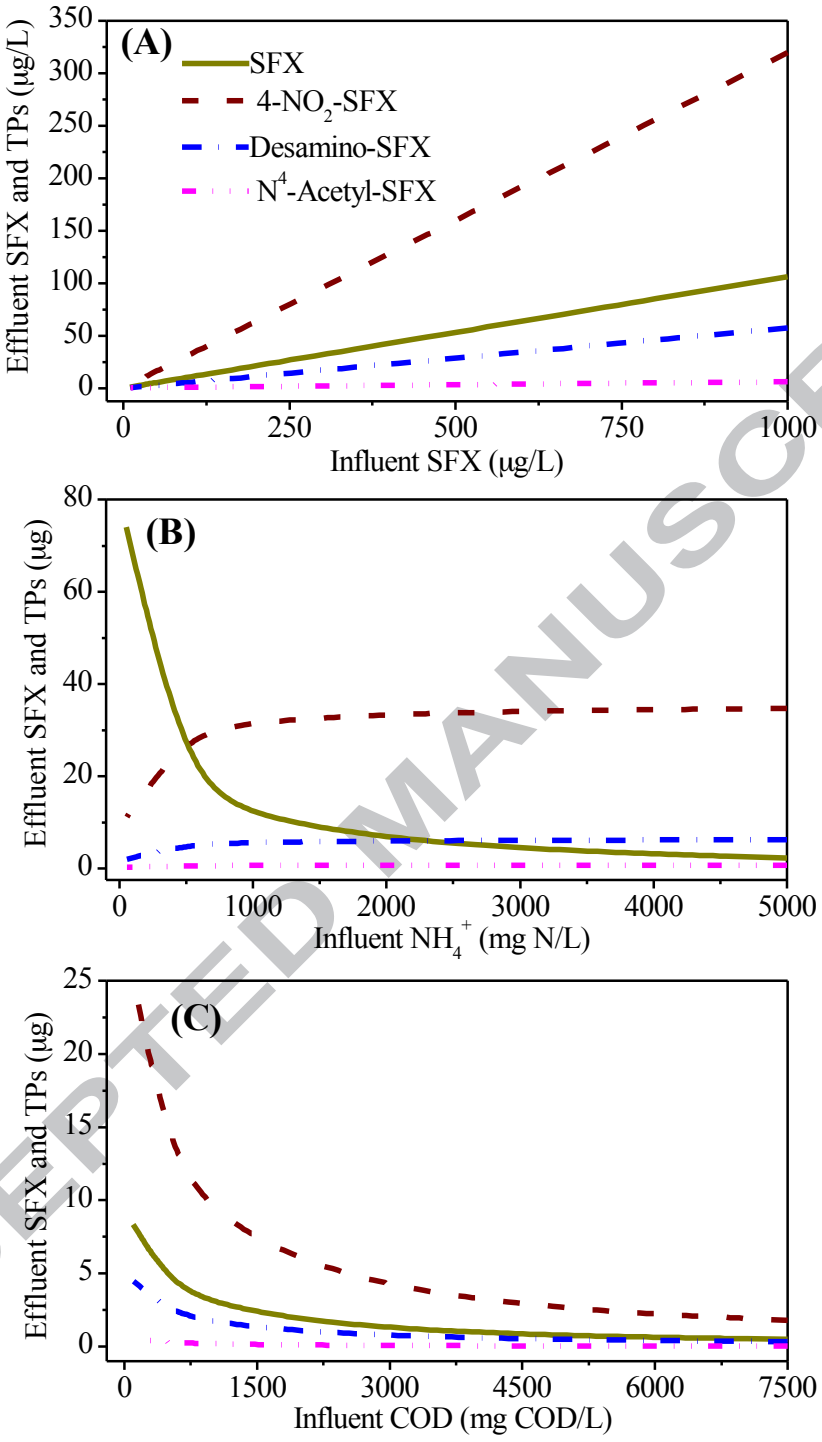


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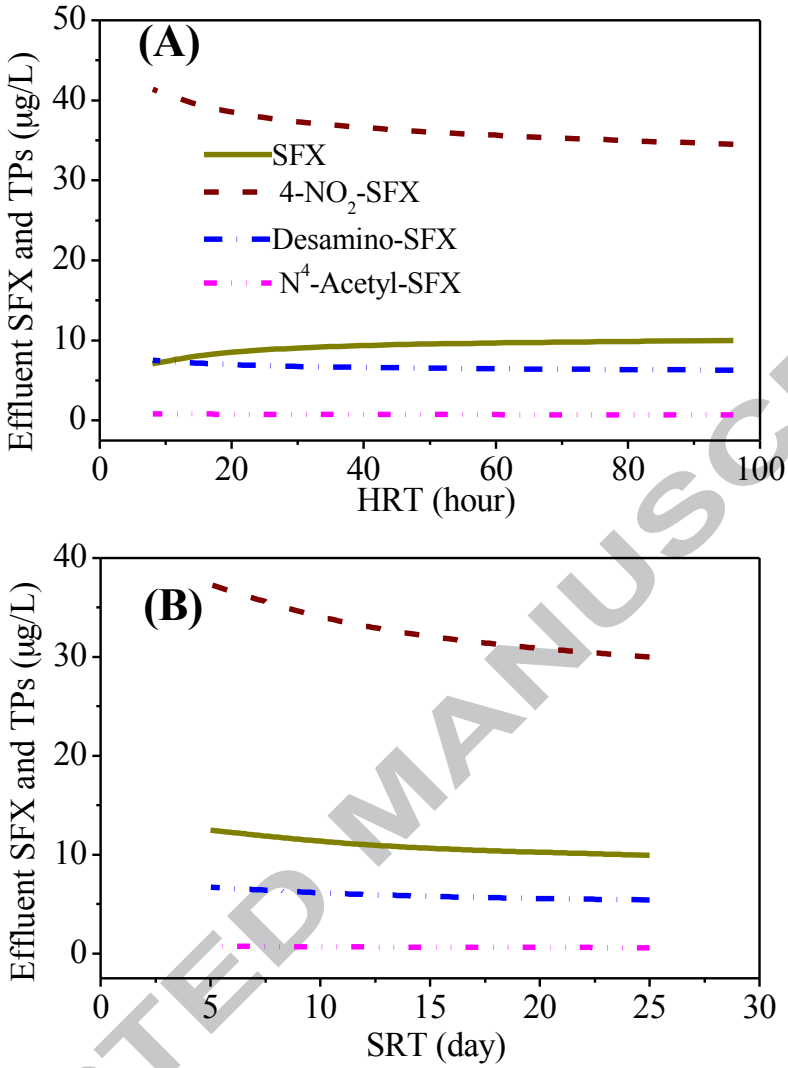


**Figure 7.** The effects of HRT (A) and SRT (B) on the transformation of SFX and TPs formation in the partial nitritation SBR based on model simulations.

## Highlights

- The model involved one cometabolic and two non-growth degradation pathways of SFX
- The production of three SFX transformation products was specifically modelled
- The model was assessed by both short-term and long-term experimental data.
- The model predicted the effect of several operational parameters on SFX removal

Graphical abstract