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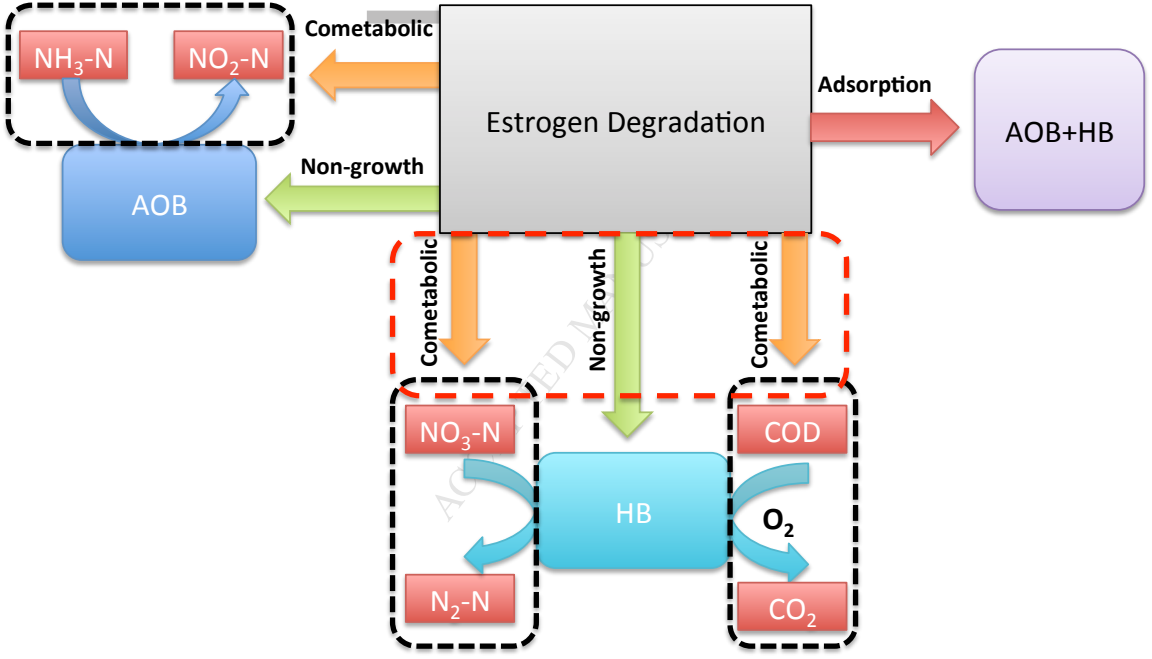
NH3-N
NO2-N
AOB
Cometabolic
Non-growth
Estrogen Degradation
Adsorption
AOB+HB
Cometabolic
Non-growth
Cometabolic
NO3-N
N2-N
HB
COD
O2
CO2

Model-Based Assessment of Estrogen Removal by Nitrifying Activated Sludge

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ABSTRACT

Complete removal of estrogens such as estrone (E1), estradiol (E2), estriol (E3) and ethinylestradiol (EE2) in wastewater treatment is essential since their release and accumulation in natural water bodies are giving rise to environment and health issues. To improve our understanding towards the estrogen bioremediation process, a mathematical model was proposed for describing estrogen removal by nitrifying activated sludge. Four pathways were involved in the developed model: i) biosorption by activated sludge flocs; ii) cometabolic biodegradation linked to ammonia oxidizing bacteria (AOB) growth; iii) non-growth biodegradation by AOB; and iv) biodegradation by heterotrophic bacteria (HB). The degradation kinetics was implemented into activated sludge model (ASM) framework with consideration of interactions between substrate update and microorganism growth as well as endogenous respiration. The model was calibrated and validated by fitting model predictions against two sets of batch experimental data under different conditions. The model could satisfactorily capture all the dynamics of nitrogen, organic matters (COD), and estrogens. Modeling results suggest that for E1, E2 and EE2, AOB-linked biodegradation is dominant over biodegradation by HB at all investigated COD dosing levels. However, for E3, the increase of COD dosage triggers a shift of dominant pathway from AOB biodegradation to HB biodegradation. Adsorption becomes the main contributor to estrogen removal at high biomass concentrations.

Keywords: Ammonia oxidizing bacteria; estrogens; biodegradation; biosorption; heterotrophic bacteria; mathematical modeling.

1. Introduction

Endocrine disrupting chemicals (EDCs) such as synthetic estrogens and natural ones by humans and animals can induce the occurrence of cancer cells in human breast, lead to increasing vitellogenin in fish (Cajthaml et al., 2009), and destroy the sexual equilibrium of wildlife in aquatic environment (Ren et al., 2007a). The natural steroidal estrogens (endogenous EDCs), are always possessing higher estrogenic potency comparing to exogenous or synthetic EDCs such as organochlorine aromatic compounds (Khanal et al., 2006). The natural estrogens from human and livestock include estrone (E1), estradiol (E2), estriol (E3) and ethinylestradiol (EE2). Wastewater treatment plants (WWTPs) are major sources for these contaminants, since the estrogens from urine and feces would be discharged into natural water or land if not completely removed during wastewater treatment (Johnson and Williams, 2004). The estrogen concentrations range from several ng L^{-1} to μg L^{-1} in the effluent of WWTPs. However, the estrogen hormones at nanogram level or even lower can cause adverse impact on aquatic environment.

Removal of estrogen in liquid phase is obtained via volatilization, adsorption and microbial degradation (Khanal et al., 2006). The volatilized natural estrogens are very limited considering the small Henry's law constant. The non-volatile free estrogens, to a large extent, partition readily from the liquid phase by adsorption onto the solid phase, such as the surface of activated sludge in WWTPs. The microorganisms in WWTPs have been demonstrated to be capable of direct degradation of the estrogens as electron donors by heterotrophs or co-metabolic degradation by ammonia oxidizing bacteria (AOB) (Lee and Liu, 2002; Ren et al., 2007a). *Nitrosomonas europaea* is able to degrade E1, E2, E3 and EE2 at initial estrogen concentration of 200 mg L^{-1} with the presence of ammonium

(NH_4^+) (Shi et al., 2004). However, Gaulke et al. (2008) suggested that the removal of EE2 by *Nitrosospira multiformis* in municipal treatment plants is not due to AOB co-metabolic degradation, but most likely due to heterotrophs. A wide variety of microorganisms are able to carry out estrogen biodegradation, such as *Rhodococcus zopfii* and *Rhodococcus equi*, isolates from WWTPs (Yoshimoto et al., 2004), *E. coli*, *Pseudomonas fluorescens* and *Bacillus thuringiensis* strains from activated sludge (Yu and Huang, 2005), and a strain of the while-rot fungus *Trametes versicolor* (Shreve et al., 2016).

Conventional WWTPs for removal of carbon and nutrient are not designed to treat the estrogens. Shieh et al. (2016) identified biological nutrient removal as the optimal option to remove estrogenic contaminants from wastewaters. Tertiary treatment technologies such as membrane filtration, granular activated carbon and advanced oxidation processes, were also used to remove these EDCs, but inevitably resulted in increased energy consumption and carbon footprint and thus increased financial and environmental costs (Rosenfeldt and Linden, 2004; felebuegu et al., 2006). The microorganisms in domestic WWTPs possessed a much higher biodegradation capacity than that in industrial WWTPs (Khanal et al., 2006). Some affecting factors on estrogen removal has been investigated in WWTPs, including seasonal difference (Petrie et al., 2014), microbe diversity (Racz et al., 2012), surface energy (Khan et al., 2013), nitrification activity (Maeng et al., 2013), solid retention time (SRT) (Roh and Chu, 2011; Maeng et al., 2013; Trinh et al., 2016), as well as hydraulic retention time (Estrada-Arriaga and Mijaylova, 2011).

Despite of the experimental demonstration of estrogen removal in WWTPs,

challenges remain in obtaining mechanistic insights and engineered remediation strategies. Mathematical models can be used as a promising tool to help decision-makers to understand the fate and transformation of estrogens in aquatic environment and to optimize the treatment process. Ogunlaja and Parker (2015) implemented a first-order degradation kinetics to simulate E1 and E2 biodegradation in the aerobic, anoxic and anaerobic batch tests, respectively using nitrifying activated sludge. Two estrogen biotransformation models were integrated within the activated sludge model (ASM) to predict estrogen removal in a full-scale plant and a bench sequencing batch reactor (Lust et al., 2012). Andersen et al. (2005) estimated the fraction of the total estrogen concentration that is expected to be sorbed in the activated sludge treatment tanks using distribution coefficients between water and activated sludge particles (Johnson and Williams, 2004). However, these previously-established models based on first-order kinetics of estrogen degradation or biosorption are unlikely to accurately describe the estrogen transformation in WWTPs. A generalized estrogen removal model in activated sludge process, fully considering the complex and combined removal pathways, biologically and physically is urgently needed.

In this work, a comprehensive modeling framework was proposed to evaluate estrogen removal. The developed model explicitly and additively considers different biodegradation pathways by AOB and heterotrophic bacteria (HB) as well as activated sludge adsorption. Literature reported experimental results obtained from batch experiments under varying conditions using nitrifying activated sludge are used to evaluate the proposed model.

2. Materials and methods

2.1. Model development

The proposed mathematical model considers the metabolisms and interactions of AOB, nitrite oxidizing bacteria (NOB) and HB microorganisms in terms of all relevant biological reactions concerning the conversions of nitrogen and organic matters (COD) as well as estrogen removal. In particular, eleven biological or physical processes were included: (1) AOB growth linked to NH_4^+ oxidation and cometabolic biodegradation of trace-level estrogens; (2) non-growth transformation of estrogens by AOB; (3) endogenous decay of AOB; (4) growth of NOB coupled to nitrite oxidation; (5) endogenous decay of NOB; (6) hydrolysis; (7) aerobic growth of HB; (8) anoxic growth of HB coupled to nitrite reduction; (9) estrogen biodegradation by HB; (10) endogenous decay of HB; and (11) adsorption.

Both AOB and HB are able to degrade estrogen via two biochemistry pathways. One is cometabolism, biochemical mechanism of microorganisms to degrade estrogen in the presence of primary substrates for growth. The other is non-growth linked biodegradation, defined as direct estrogen biodegradation without consuming growth substrates. In our model, both biochemistry pathways were considered for AOB. However, due to limited dataset for parameterization, we lumped the cometabolic HB pathways and non-growth linked HB pathway for simplicity. Therefore, the overall estrogen removal is regulated by three biological processes (Process 1, 2 and 9) and one physical process (Process 11). The resulting kinetic rate expressions for AOB cometabolic biodegradation (Equation 1), AOB non-growth linked biodegradation (Equation 2) and HB linked biodegradation (Equation 3) are shown as below (Sathyamoorthy et al., 2013):

$$\frac{dS_E}{dt} = -T_{E,AOB} S_E \mu_{AOB} \frac{S_{NH4}}{S_{NH4}+K_{NH4}^{AOB}} \frac{S_{O2}}{S_{O2}+K_{O2}^{AOB}} X_{AOB} \quad (1)$$

$$\frac{dS_E}{dt} = -k_{E,AOB} X_{AOB} S_E \quad (2)$$

$$\frac{dS_E}{dt} = -\alpha_{E,HB} X_H S_E \quad (3)$$

where $T_{E,AOB}$ is cometabolic estrogen transformation coefficient linked to AOB growth, m^3 g COD^{-1}; μ_{AOB} is growth rate for AOB, h^{-1}; K_{NH4}^{AOB} is S_{NH4} affinity constant for AOB, g N m^{-3}; K_{O2}^{AOB} is S_{O2} affinity constant for AOB, g COD m^{-3}; $k_{E,AOB}$ is non-growth estrogen biodegradation coefficient for AOB, m^3 g COD^{-1} h^{-1}; $\alpha_{E,HB}$ is estrogen biodegradation coefficient for HB, m^3 g COD^{-1} h^{-1}.

The estrogen biodegradation processes are modeled as first-order kinetics with respect to estrogen concentration considering that typical half saturation values for solutes in aquatic environment are always several orders of magnitude greater than the investigated estrogen concentration (trace level in μg L^{-1}) (Alvarez-Cohen and Speitel Jr, 2001; Sathyamoorthy et al., 2013). All of the biodegradation kinetics of estrogens were implemented into ASM framework with biodegradation capacity expressed as Monod equations. In addition, a first-order rate expression of Lagergren is used to describe adsorption of estrogen by activated sludge (Aksu, 2001):

$$\frac{dX_E}{dt} = k_{Ad}(X_{eq} - X_E)X_{tot} \quad (4)$$

where k_{Ad} is the rate constant of first-order biosorption, h^{-1}; X_E is the adsorbed estrogen onto activated sludge, g COD m^{-3}; X_{eq} is the concentration of adsorbed estrogen at equilibrium, g COD m^{-3}; X_{tot} is the total biomass, g COD m^{-3}.

The model framework contains 13 state variables of which 7 are soluble growth substrates (S_{NH4}, S_{NO2}, S_{NO3}, S_{N2}, S_E, S_S and S_{O2}) and 6 are active biomass components

(X_{AOB}, X_{NOB}, X_H, X_S, X_I and X_E) with details referring to Table S1 in the supplementary materials. The process kinetic rate equations for above-listed processes are summarized in Table S2. The overall stoichiometric matrix of the developed model is shown in Tables S3. 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 testing the model

Experimental data previously reported by Ren et al. (2007a) are used to calibrate and validate the proposed estrogen removal model. The activated sludge for experimental use was inoculated from a sequencing batch reactor, fed with swine wastewater. Estrogens are under detection limit in both wastewater and activated sludge. Ammonium, nitrite and nitrate in the sludge were also under detection limit after washing with 0.1M phosphate buffer. Two sets of batch tests were designed and conducted.

In batch test I, the estrogen degradation was evaluated with addition of different concentrations of glucose ranging from ~200 to ~2000 mg COD L^{-1}. Activated sludge were withdrawn to flasks resulting in a biomass concentration of 220 mg VSS L^{-1}. DO in the flasks was above 7.2 mg O_2 L^{-1} and pH was around 7.4. After addition of 12 mg N L^{-1} NH_4^+ and 100 μg L^{-1} of E1, E2, E3, or EE2, the flasks were shaken at 200 rpm in the dark in 20 °C thermostatic room for 48 h. Samples in triplicates were taken over time.

In batch test II, the E1 removal was tested in the nitrifying activated sludge under varying substrate conditions: A) glucose and NH_4^+, B) glucose, C) NH_4^+ and D) no

addition of any substrates. Activated sludge were withdrawn in flasks resulting in a biomass concentration of 1420 mg VSS L^{-1}. All flasks were shaken at 200 rpm in the dark in a 20 °C thermostatic room with DO above 4.0 mg O_2 L^{-1} and pH around 7.7. 655 mg COD L^{-1} of glucose, 55 mg N L^{-1} of NH_4^+ and/or 300 μg L^{-1} of E1 were dosed into the flasks initially and added again on the 4^{th} day during the 8-day experiment. Duplicate samples were taken over time. The analysis of nitrogen, TOC and estrogens was described in details in Ren et al. (2007b).

2.3. Calibration and validation of the proposed model

The proposed model framework contains 27 stoichiometric and kinetic parameters, as summarized in Table S4. To reduce the complexity of model calibration and avoid parameter correlation issues (Peng et al., 2015b), most of model parameter values were adopted from literature directly as they have been well applied in previous studies. Experimental data of nitrogen and COD from batch test I was used to calibrate the key parameters including maximum growth rate of AOB (μ_{AOB}) and maximum growth rate of HB (μ_H). The experimental data of E1, E2, E3 and EE2 were used to calibrate parameters related to estrogen removal including AOB cometabolic estrogen transformation coefficient ($T_{E,AOB}$), AOB non-growth estrogen biodegradation coefficient ($k_{E,AOB}$) and estrogen biodegradation coefficient for HB ($\alpha_{E,HB}$). 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. Parameters in the proposed mathematical model were estimated by minimizing the sum of the square of the weighted deviations between measurements and calculated model results for dynamic simulation.

The objective function to be minimized in the parameter estimation is as follows

$$F^2(p) = \sum_{i=1}^{n} \left(\frac{y_{m,i} - y_i(p)}{\sigma_{m,i}}\right)^2 \quad (5)$$

where $y_{m,i}$ is the measured data at time t_i (i from 1 to n); $y_i(p)$ is the calculated value by the model at time t_i (i from 1 to n); p is the parameters to be estimated; $\sigma_{m,i}$ is the standard deviation of the measurement.

With the built-in simplex and secant algorithms, at each iteration, parameter arrays were replaced by new values until $F^2(p)$ are close enough to fulfill the convergence criterion. The details for the numerical integration procedures refer to Reichert, (1998).

Experimental data of nitrogen, COD and E1 from batch test II was used to validate the parameterized model including both biological degradation kinetics with the same set of parameter values as used in model calibration and activated sludge adsorption kinetics with newly calibrated parameter, the rate constant of first-order biosorption (k_{Ad}).

Furthermore, the established model framework with calibrated and validated parameter values was used to evaluate the shift of estrogen biodegradation pathways by AOB and HB under varying conditions (i.e. at low, medium and high carbon dosing levels).

3. Results and discussion

3.1. Modeling estrogens (E1, E2, E3, and EE2) removal by nitrifying activated sludge with addition of different COD concentrations

In order to accurately estimate the ammonia, COD and estrogen related parameters, a two-phase procedure was applied to calibrate the model. In the first phase, μ_{AOB} and

μ_{HB} were calibrated using the ammonium, nitrate and COD data. Then two key parameters for estrogen biodegradation by AOB ($T_{E,AOB}$ and $k_{E,AOB}$, refer to Table S4) and one key parameters for estrogen biodegradation by HB ($\alpha_{E,HB}$, refer to Table S4) were further calibrated using the estrogen data at different initial COD concentrations in the second phase. As shown in Equation 4, the overall adsorption rate was linearly dependent on the biomass concentration. The process of adsorption was not considered in model calibration since the activated sludge concentration in batch test I was very low. The calibration of the developed model involved optimizing key parameter values for ammonia, nitrate and COD conversions as well as estrogen biodegradation by fitting simulation results to batch experimental data under different conditions.

Figure 1 shows the experimentally observed and model predicted E1, NH_4^+, NO_3^- and COD. The activated sludge was able to remove COD, oxidize NH_4^+ to NO_3^-, and degrade E1 simultaneously (Figure 1A). COD decreased from ~210 mg COD L^{-1} to ~107 mg COD L^{-1}. NH_4^+ decreased from ~12 mg N L^{-1} to ~ 5 mg N L^{-1}, while ~7 mg N L^{-1} of NO_3^- was produced, indicating that the anoxic denitrification was completely suppressed by the high oxygen level (>7.2 mg O_2 L^{-1}) in the flasks. The E1 concentration decreased from 100 μg L^{-1} to 15 μg L^{-1} within the duration of 48 hours (Figure 1A). To elucidate the effect of COD on bacterial activity and estrogen biodegradation in the nitrifying activated sludge, the batch tests were further conducted at higher initial COD concentrations. The trends of nitrogen, COD and E1 in Figure 1B&C were similar with those in Figure 1A. The increase of initial carbon dosage from ~210 mg COD L^{-1} to ~1750 mg COD L^{-1} resulted in an increase of COD removal efficiency from ~50% to ~60%, a decrease of ammonia oxidation rate from ~0.15 mg N L^{-1} h^{-1} to ~0.13 mg N L^{-1} h^{-1} and a drop of E1

removal efficiency from ~85% to ~64% (Figure 1). The removal of Endocrine disruptors was enhanced by implementing nitrifying process in full-scale WWTPs (Kreuzinger et al., 2004) and the biodegradation of micropollutant was promoted at higher specific nitrification rate (Fernandez-Fontaina et al., 2014). Ammonium monooxygenase (AMO) is responsible for degradation of a relatively wide spectrum of substrates (i.e. estrogen; beta blocker, such as atenolol, metoprolol, and sotalol; antibiotics, such as sulfamethoxazole) (Yi and Harper, 2007; Sathyamoorthy et al., 2013; Peng et al., 2017), which supports our observation that E1 degradation is correlated to AOB activity.

The profiles of E2 and EE2 biodegradations along with nitrogen and COD conversions are illustrated in Figure 2&3. A complete removal of E2 was achieved at varying initial glucose concentrations (Figure 2), while 57%-73% of EE2 removal was obtained with higher efficiency at lower COD concentration (Figure 3). The removal efficiency of E2 or EE2 displayed a positive correlation with the nitrification activity. Figure 4 presents the effect of COD supplement on E3 biodegradation. On the contrary, the removal efficiency of E3 were ~77%, ~82% and ~90% at initial glucose concentrations of 200 mg COD L^{-1}, 710 mg COD L^{-1} and 1860 mg COD L^{-1}, respectively. Higher COD supplement caused an increase of heterotroph activity, leading to more E3 degradation. This opposite tendency of E3 against E1, E2 and EE2 revealed a different mechanism involved.

Based on the experimental observation, the calibrated parameter values giving the optimum fit are listed in Table S4. The parameter correlation matrix obtained from model calibration indicates all of the parameter combinations have not shown significant correlation. The obtained parameter value of μ_{AOB} is 0.028 h^{-1} and μ_{HB} is 0.15 h^{-1}, both of

which are relatively lower than literature reported values of 0.085 h^{-1} for μ_{AOB} (Wiesmann, U., 1994) and 0.25 h^{-1} for μ_{HB} (Henze, et al. 2000), respectively, likely due to a lower activated sludge activity after storage in cold room comparing to that in WWTPs. To simplify the calibration process for estrogen related parameters, the basic idea in the model calibration strategy is to change as few constants as possible (Xu and Hultman, 1996), due to the limited variability of experimental data. Hence, we firstly obtained one set of estimated parameter values of $T_{E,AOB}$, $k_{E,AO}$ and $\alpha_{E,HB}$ for E1. Then we only calibrated one parameter for E2, EE2 and E3 degradation with the remaining two kept the same as E1. Table S4 indicates that one set of parameter values ($T_{E,AOB}$ = 0.872 m^3 g COD $^{-1}$, $k_{E,AOB}$ = 0.0061 m^3 g COD $^{-1}$ and $\alpha_{E,HB}$ = 0.00003 m^3 g COD $^{-1}$) is able to describe the biodegradation of E1 and EE2. The $k_{E,AOB}$ for E2 is 0.094 m^3 g COD $^{-1}$, which is higher than that for E1 and EE2, indicating that the observed higher E2 biodegradation capacity of the investigated nitrifying activated sludge (Figure 2) is attributed to AOB non-growth biotransformation. The higher value of $\alpha_{E,HB}$ for E3 (0.0004 m^3 g COD $^{-1}$) comparing to those of other estrogens reveals a larger contribution of HB to E3 biodegradation. The calibrated values for estrogen related parameters are generally higher than literature reported values (Sathyamoorthy et al., 2013; Peng et al., 2015a) with regard to pharmaceutical (PhAC) transformation, possibly owing to the inhibition of PhACs on microorganisms (Sathyamoorthy et al., 2013).

As shown in Figures 1-4, the model predictions with the calibrated parameter values well matched the measured data of NH_4^+, NO_3^-, COD and estrogens (E1, E2, EE2 or E3) concentrations in the batch test I. The good agreement between these simulated and measured data supports that the developed model successfully captures the relationship

between nitrification activity and the degradation of E1, E2 and EE2 as well as the impact of varying COD dosages on E3 biodegradation.

3.2. Modeling E1 removal by nitrifying activated sludge under varying substrate conditions (COD or/and NH_4^+ or none substrate)

To further test the validity and reliability of the estimated parameters obtained from model calibration, the model predictions with these parameters were compared to the experimental data from other batch experiments, which are not used for model calibration. The proposed model framework was assessed using experimental data obtained from batch test II. The experimental conditions for batch II are highly different from batch I. Firstly, the ammonium and E1 concentrations in batch II are higher than those in batch I. Secondly, the biomass concentration in batch II is around 7 times higher than that in batch I, boosting E1 removal via adsorption. Finally, batch II involves different combinations of substrate conditions such as the presence of both glucose and NH_4^+, presence of glucose only, presence of NH_4^+ only and no addition of any substrates.

Figure 5 illustrates the model validation results using experimental data from batch test II. In Figure 5A with the addition of 655 mg COD L^{-1} glucose and 55 mg N L^{-1} NH_4^+, COD decreased to ~100 mg COD L^{-1} at 95th hour after the first pulse addition and down to ~110 at 192nd hours after the second dosage. NH_4^+ was completely oxidized after both additions, resulting a step-wise increase of NO_3^- concentration up to ~ 85 mg N L^{-1} in the end. In Figure 5B with addition of glucose, the profile of COD conversion was similar with Figure 5A, but both NH_4^+ and NO_3^- concentrations were very low. In Figure 5C with addition of NH_4^+, the profiles of NH_4^+ and NO_3^- were in the similar pattern as Figure 5A,

but COD concentration was at a low level. In Figure 5D without addition of any substrates, all substrate concentrations were consequently very low. For these four groups of experiments, E1 displayed a similarly decreasing trend after each addition: the E1 concentration dropped rapidly at the beginning 10 min and further decreased with a much slower decreasing rate. Ren et al. (2007b) revealed that the E1 adsorption from aqueous phase onto activated sludge flocs could be in equilibrium within 10 min, which confirmed the existence of adsorption in this set of batch tests.

As described previously, a first-order rate expression of Lagergren was used to describe adsorption of E1 by activated sludge flocs. The validation results in Figure 5 showed that the model with the same set of calibrated parameters and the newly estimated k_{Ad} can well describe all the dynamics of NH_4^+, NO_3^-, COD and E1 over time, which supports the validity of the developed model. There was no remarkable difference under the four substrate conditions in batch tests II with respect to E1 degradation, suggesting that activated sludge has the same feature for adsorption and E1 biodegradation relied on non-growth biodegradation especially by AOB to a large degree. The proposed model supported that the majority of E1 was removed by adsorption process and AOB non-growth biodegradation was the main contributor of biological E1 transformation (even more contribution when degrading E2, Table S4). The AMO, responsible for ammonia oxidation, is known to be capable of degrading various pollutants even in the absence of ammonia (Kassotaki et al., 2016).

Additional experimental data for removal of E2, EE2 and E3 were not available. Hence, the model validation only involved kinetics concerning E1 removal. Since model-predicted profiles (Figure 1-4) and calibrated parameter values (Table S4) for all E1, E2,

EE2 and E3 biodegradation are very comparable, we believe the robustness and reliability of the proposed model are still valid when extrapolating to removal of E2, EE2 and E3.

In addition, root mean square error (RMSE) analysis was performed for Figure 1-5 to assess the fit of model prediction against experimental data. Each RMSE value and its fraction in maximum measurement were shown in Table S5 in Supplementary Material. Most of the RMSE values are within 10% of the corresponding maximum measurement values, indicating that the model prediction well matches the experimental observation.

3.3. Model-based evaluation of the relative contributions of AOB and HB to the overall estrogen biodegradation

The proposed model framework would be helpful to provide mechanistic insights into the estrogen biodegradation. Model simulations were performed to assess the relative contributions of AOB and HB to estrogen removal. Figure 6A, 6B and 6C illustrate time-course E1 degradation rates by AOB and HB based on model prediction at the initial glucose concentrations of 210 mg COD L^{-1}, 700 mg COD L^{-1} and 1790 mg COD L^{-1}, respectively. A decreasing trend of E1 degradation rates for both AOB and HB over time was observed due to the decrease of E1 concentration (Figure 1). The E1 degradation rate by AOB was substantially higher than that by HB at each COD level. With the increase of COD concentrations, E1 degradation by AOB decreased slightly, whereas the E1 degradation by HB increased. The developed model also predicted that E2 and EE2 biodegradation rates by AOB and HB under varying COD levels displayed a very similar pattern with E1 (data not shown). Figure 6D, 6E and 6F presents the time-course decreasing trends of E3 degradations by AOB and HB at varying COD levels. The

contribution of AOB cometabolic biodegradation and AOB non-growth biodegradation to total E3 transformation was dominant over the contribution of HB linked biodegradation at lower COD concentrations (200 and 710 mg COD L^{-1}) in Figure 6D&E, while E3 degradation by HB become predominant at COD concentration of 1860 mg COD L^{-1} (Figure 6F). The E3 degradation rate by AOB was suppressed by increasing COD dosage, while The E3 degradation rate by HB was largely promoted at higher COD level.

Previous studies used first-order kinetics to simulate the estrogen degradation (Ren et al., 2007a; Lust et al., 2015). These modeling results may not reflect the reality since AOB and HB affect the biotransformation process differently under varying conditions. This study has incorporated different estrogen biodegradation pathways by different microorganisms (AOB and HB). The degrading kinetics were implemented into ASM with consideration of interactions between substrate uptake and microorganism growth as well as endogenous respiration. The proposed model framework differentiated the AOB and HB contributions to estrogen biodegradation under varying conditions (i.e. COD dosage) for the first time. The simulation results on the shift of dominant degradation pathway would give insightful suggestions for on-site remediation. Depending on the composition of estrogen contaminants, different strategies should be applied. When E1, E2 or EE2 is the predominant contaminant, extra carbon addition is not necessary since estrogen biodegradation by AOB serves to be the major pathway, which is suppressed by elevated carbon dosage. When E3 dominates over other estrogens, increase of carbon dosage would boost E3 removal by stimulating HB linked biodegradation pathway. The optimal amount of carbon dosage can be further determined by the proposed model when incorporating more parameters such as the price of carbon source, local discharge limit of

E3, etc. For model implementation at full-scale, future study may focus on testing the proposed model framework for estrogen removal against experimental data from long-term bioreactors with different reactor configuration (suspended growth, attached growth, etc.).

4. Conclusions

In this study, a mathematical model was developed to describe estrogen removal by nitrifying activated sludge, which incorporated the biodegradation processes by AOB and HB as well as the adsorption process by sludge flocs. The validity of the model was demonstrated through testing against experimental data from two sets of batch experiments under varying conditions. The modeling results revealed that for E1, E2 and EE2, AOB-linked biodegradation is dominant over biodegradation by HB at all investigated COD dosing levels. However, for E3, the increase of COD dosage triggers a shift of dominant pathway from AOB biodegradation to HB biodegradation. Adsorption becomes the main contributor to estrogen removal at high biomass concentrations.

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References

Aksu, Z., 2001. Biosorption of reactive dyes by dried activated sludge: equilibrium and kinetic modelling. Biochem. Eng. J. 7, 79-84.

Alvarez-Cohen, L., Speitel Jr, G.E., 2001. Kinetics of aerobic cometabolism of chlorinated solvents. Biodegradation. 12, 105-126.

Andersen, H.R., Hansen, M., Kjølholt, J., Stuer-Lauridsen, F., Ternes, T., Halling-Sørensen, B., 2005. Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. Chemosphere. 61, 139-146.

Cajthaml, T., Kresinová, Z., Svobodová, K., Sigler, K., Rezanka, T., 2009. Microbial transformation of synthetic estrogen 17alpha-ethinylestradiol. Environ. Pollut. 157, 3325-3335.

Estrada-Arriaga, E.B., Mijaylova, P.N., 2011. Influence of operational parameters (sludge retention time and hydraulic residence time) on the removal of estrogens by membrane bioreactor. Environ. Sci. Pollut. Res. Int. 18, 1121-1128.

felebuegu, A.O., Lester, J.N., Churchley, J., Cartmell, E., 2006. Removal of an endocrine disrupting chemical (17α-ethinyloestradiol) from wastewater effluent by activated carbon adsorption: Effects of activated carbon type and competitive adsorption. Environ. Technol. 27, 1343-1349.

Fernandez-Fontaina, E., Carballa, M., Omil, F., Lema, J.M., 2014. Modelling cometabolic biotransformation of organic micropollutants in nitrifying reactors.

Water. Res. 65, 371-383.

Gaulke, L.S., Strand, S.E., Kalhorn, T.F., Stensel, H.D., 2008. 17alpha-ethinylestradiol transformation via abiotic nitration in the presence of ammonia oxidizing bacteria. Environ. Sci. Technol. 42, 7622-7627.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M.C.M., Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M.C.M., 2000. Activated sludge models ASM1, ASM2, ASM2d and ASM3, IWA Publishing.

Johnson, A.C., Williams, R.J., 2004. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environ. Sci. Technol. 38, 3649-3658.

Kassotaki, E., Buttiglieri, G., Ferrando-Climent, L., Rodriguez-Roda, I., Pijuan, M., 2016. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. Water. Res. 94, 111-119.

Khan, M.M., Chapman, T., Cochran, K., Schuler, A.J., 2013. Attachment surface energy effects on nitrification and estrogen removal rates by biofilms for improved wastewater treatment. Water. Res. 47, 2190-2198.

Khanal, S.K., Xie, B., Thompson, M.L., Sung, S., Ong, S.K., Van Leeuwent, J., 2006. Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. Environ. Sci. Technol. 40, 6537-6546.

Kreuzinger, N., Clara, M., Strenn, B., Kroiss, H., 2004. Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. Water Sci. Technol. 50, 149-156.

Lee, H.B., Liu, D., 2002. Degradation of 17β-estradiol and its metabolites by sewage bacteria. Water. Air. Soil. Pollut. 134, 351-366.

Lust, M., Makinia, J., Stensel, H.D., 2012. A mechanistic model for fate and removal of estrogens in biological nutrient removal activated sludge systems. Water Sci. Technol. 65, 1130-1136.

Lust, M.J., Ziels, R.M., Strand, S.E., Gough, H.L., Stensel, H.D., 2015. Biodegradation kinetics of 17α-Ethinylestradiol in activated sludge treatment processes. Environ. Eng. Sci. 32, 637-646.

Maeng, S.K., Choi, B.G., Lee, K.T., Song, K.G., 2013. Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors. Water. Res. 47, 3151-3162.

Ogunlaja, O.O., Parker, W.J., 2015. Assessment of the removal of estrogenicity in biological nutrient removal wastewater treatment processes. Sci. Total. Environ. 514, 202-210.

Peng, L., Chen, X., Xu, Y., Liu, Y., Gao, S., Ni, B.J., 2015a. Biodegradation of pharmaceuticals in membrane aerated biofilm reactor for autotrophic nitrogen removal: A model-based evaluation. J. Memb. Sci. 494, 39-47.

Peng, L., Liu, Y., Gao, S.H., Chen, X., Xin, P., Dai, X., Ni, B.J., 2015b. Evaluation on the nanoscale zero valent iron based microbial denitrification for nitrate removal from groundwater. Sci. Rep. 5, 12331.

Peng, L., Kassotaki, E., Liu, Y., Sun, J., Dai, X., Pijuan, M., Rodriguez-Roda, I., Buttiglieri, G., Ni, B.J., 2017. Modelling cometabolic biotransformation of sulfamethoxazole by an enriched ammonia oxidizing bacteria culture. Chem. Eng.

Sci. 173, 465-473.

Petrie, B., McAdam, E.J., Hassard, F., Stephenson, T., Lester, J.N., Cartmell, E., 2014. Diagnostic investigation of steroid estrogen removal by activated sludge at varying solids retention time. Chemosphere. 113, 101-108.

Racz, L., Muller, J.G., Goel, R.K., 2012. Fate of selected estrogens in two laboratory scale sequencing batch reactors fed with different organic carbon sources under varying solids retention times. Bioresour. Technol. 110, 35-42.

Reichert, P., 1998. AQUASIM 2.0--user manual. Swiss Federal Institute for Environmental Science and Technology. Dubendorf, Switzerland

Ren, Y.X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007a. Effects of bacterial activity on estrogen removal in nitrifying activated sludge. Water. Res. 41, 3089-3096.

Ren, Y.X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007b. A thermodynamic analysis on adsorption of estrogens in activated sludge process. Water. Res. 41, 2341-2348.

Roh, H., Chu, K.H., 2011. Effects of solids retention time on the performance of bioreactors bioaugmented with a 17β-estradiol-utilizing bacterium, Sphingomonas strain KC8. Chemosphere. 84, 227-233.

Rosenfeldt, E.J., Linden, K.G., 2004. Degradation of endocrine disrupting chemicals bisphenol A, ethinyl estradiol, and estradiol during UV photolysis and advanced oxidation processes. Environ. Sci. Technol. 38, 5476-5483.

Sathyamoorthy, S., Chandran, K., Ramsburg, C.A., 2013. Biodegradation and cometabolic modeling of selected beta blockers during ammonia oxidation. Environ.

Sci. Technol. 47, 12835-12843.

Shi, J., Fujisawa, S., Nakai, S., Hosomi, M., 2004. Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium Nitrosomonas europaea. Water. Res. 38, 2322-2329.

Shieh, B.H., Louie, A., Law, F.C., 2016. Factors affecting distribution of estrogenicity in the influents, effluents, and biosolids of Canadian wastewater treatment plants. Arch. Environ. Contam. Toxicol. 70, 682-691.

Shreve, M.J., Brockman, A., Hartleb, M., Prebihalo, S., Dorman, F.L., Brennan, R.A., 2016. The white-rot fungus Trametes versicolor reduces the estrogenic activity of a mixture of emerging contaminants in wastewater treatment plant effluent. Int. Biodeterior. Biodegradation. 109, 132-140.

Trinh, T., van den Akker, B., Coleman, H.M., Stuetz, R.M., Drewes, J.E., Le-Clech, P., Khan, S.J., 2016. Seasonal variations in fate and removal of trace organic chemical contaminants while operating a full-scale membrane bioreactor. Sci. Total Environ. 550, 176-183.

Wiesmann, U., 1994. Biological nitrogen removal from wastewater.InBiotechnics/Wastewater, pp. 113-154, Springer Berlin Heidelberg.

Xu, S., Hultman, B., 1996. Experiences in wastewater characterization and model calibration for the activated sludge process. Water. Sci. Technol. 33, 89-98.

Yi, T., Harper, W.F., 2007. The link between nitrification and biotransformation of 17α-ethinylestradiol. Environ. Sci. Technol. 41, 4311-4316.

Yoshimoto, T., Nagai, F., Fujimoto, J., Watanabe, K., Mizukoshi, H., Makino, T., Kimura, K., Saino, H., Sawada, H., Omura, H., 2004. Degradation of estrogens by

Rhodococcus zopfii and *Rhodococcus equi* isolates from activated sludge in wastewater treatment plants. Appl. Environ. Microbiol. 70, 5283-5289.

Yu, Z., Huang, W., 2005. Competitive sorption between 17α-ethinyl estradiol and naphthalene/phenanthrene by sediments. Environ. Sci. Technol. 39, 4878-4885.

FIGURE CAPTIONS

Figure 1. Model calibration results of E1 degradation along with nitrogen and COD conversions at varying initial glucose concentrations of (A) 210 mg COD L^{-1}; (B) 700 mg COD L^{-1} and (C) 1750 mg COD L^{-1}.

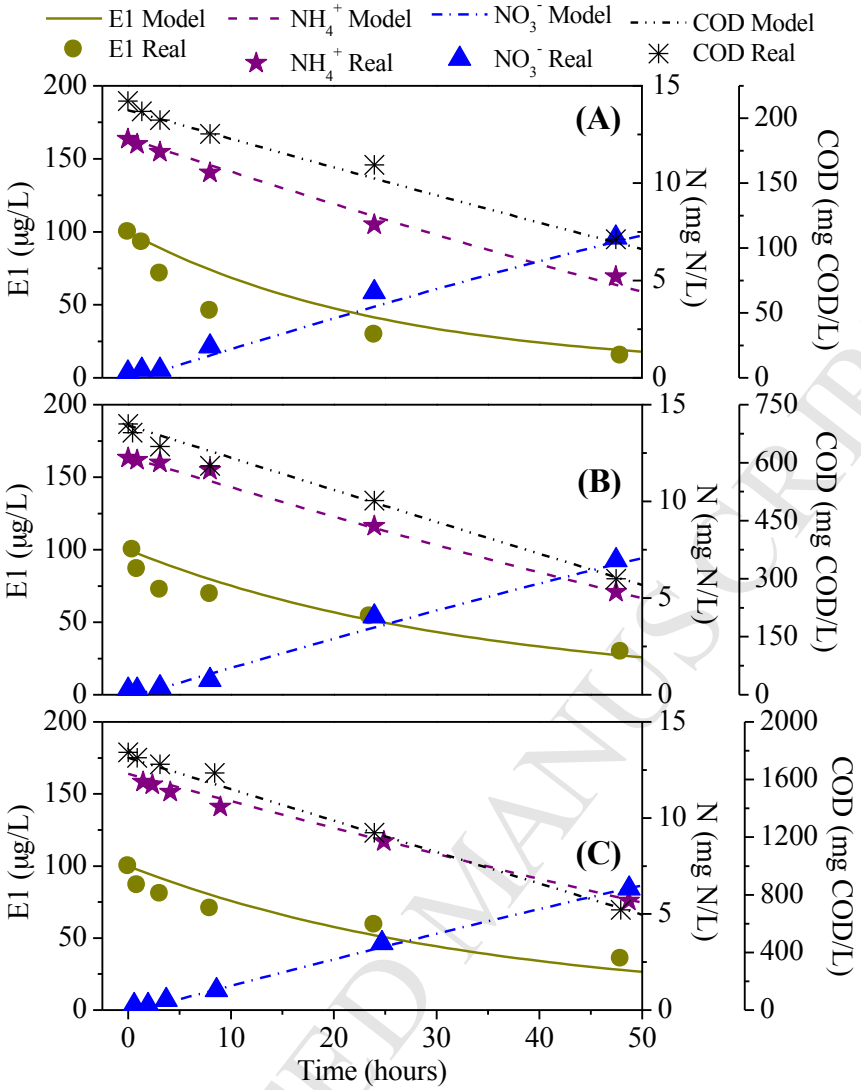
Figure 2. Model evaluation results of E2 degradation along with nitrogen and COD conversions at varying initial glucose concentrations of (A) 210 mg COD L^{-1}; (B) 715 mg COD L^{-1} and (C) 1850 mg COD L^{-1}.

Figure 3. Model evaluation results of EE2 degradation along with nitrogen and COD conversions at varying initial glucose concentrations of (A) 210 mg COD L^{-1}; (B) 700 mg COD L^{-1} and (C) 1790 mg COD L^{-1}.

Figure 4. Model calibration results of E3 degradation along with nitrogen and COD conversions at varying initial glucose concentrations of (A) 200 mg COD L^{-1}; (B) 710 mg COD L^{-1} and (C) 1860 mg COD L^{-1}.

Figure 5. Model validation results of E1 adsorption and biodegradation under different substrate conditions: (A) glucose and NH_4^+, (B) glucose, (C) NH_4^+ and (D) no addition. Substrate was added at 0 and 96 h.

Figure 6. Simulated degradation rates of E1 and E3 by AOB and HB at varying initial glucose concentrations based on the parameterized model framework. E1 degradation: (A) 210 mg COD L^{-1}; (B) 700 mg COD L^{-1} and (C) 1750 mg COD L^{-1}; E3 degradation:(D) 200 mg COD L^{-1}, (E) 710 mg COD L^{-1} and (F) 1860 mg COD L^{-1}.



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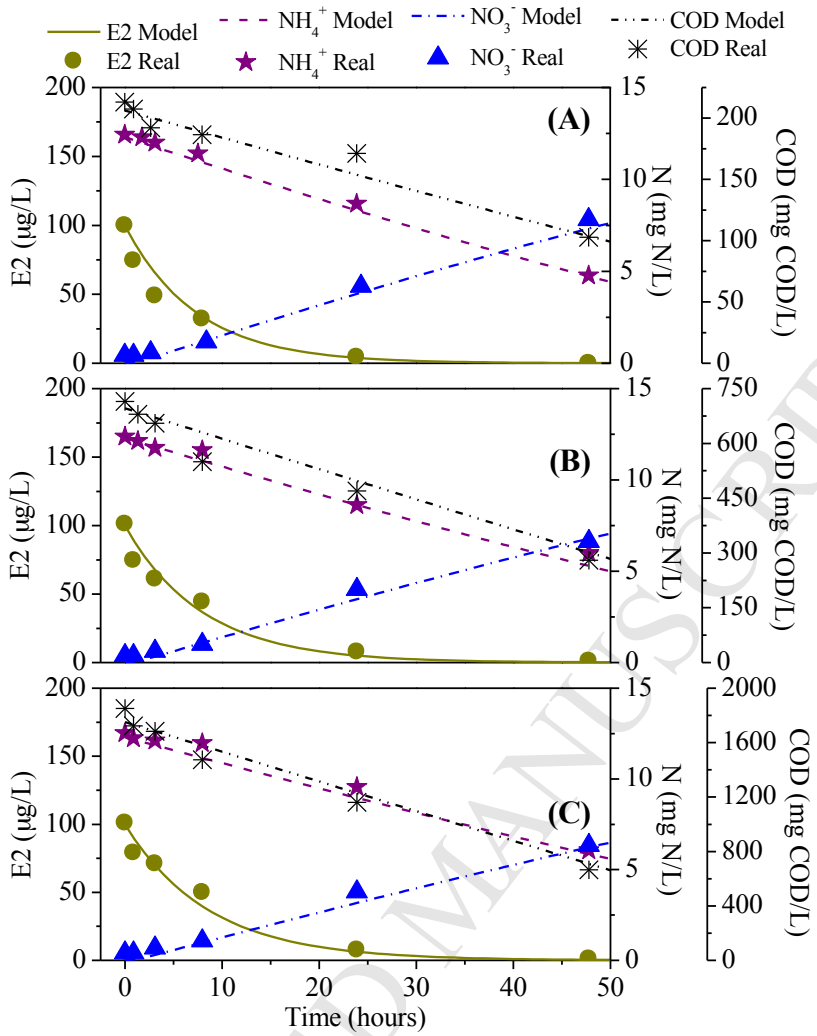

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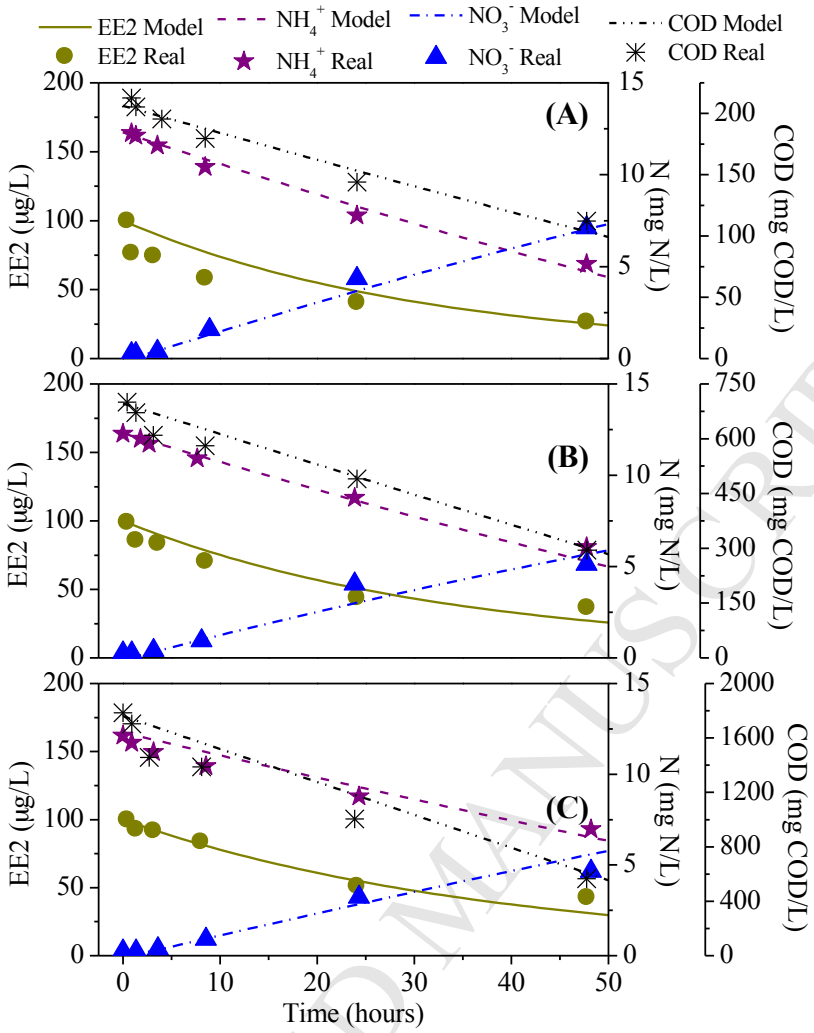

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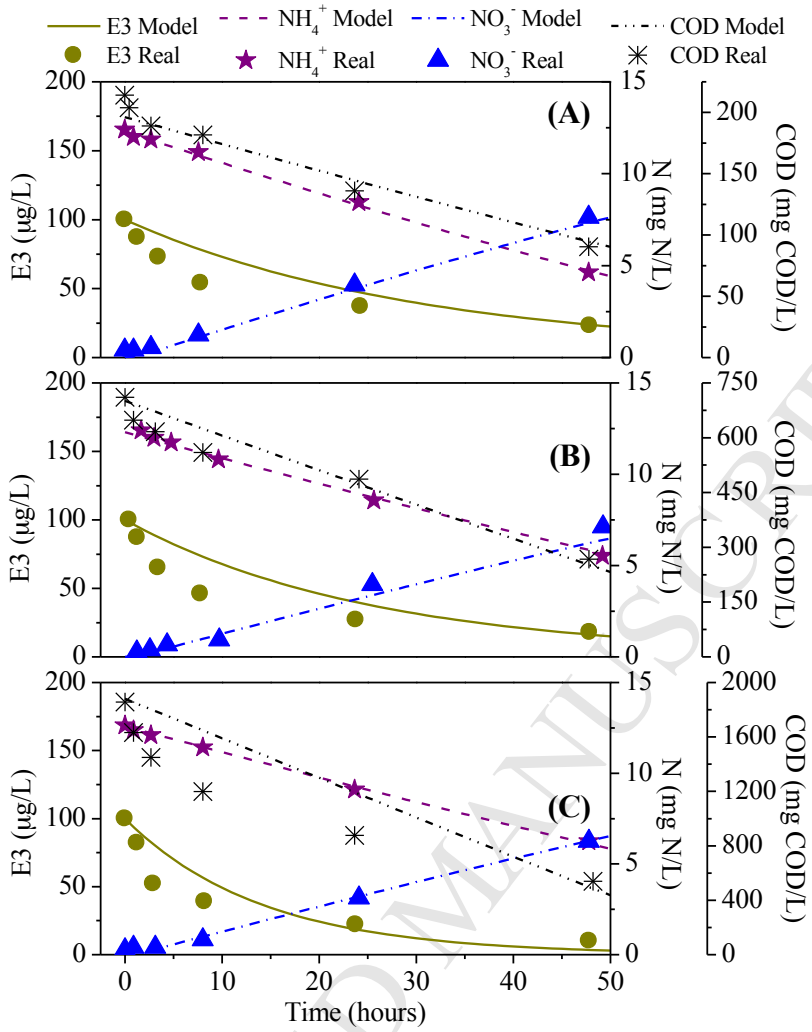

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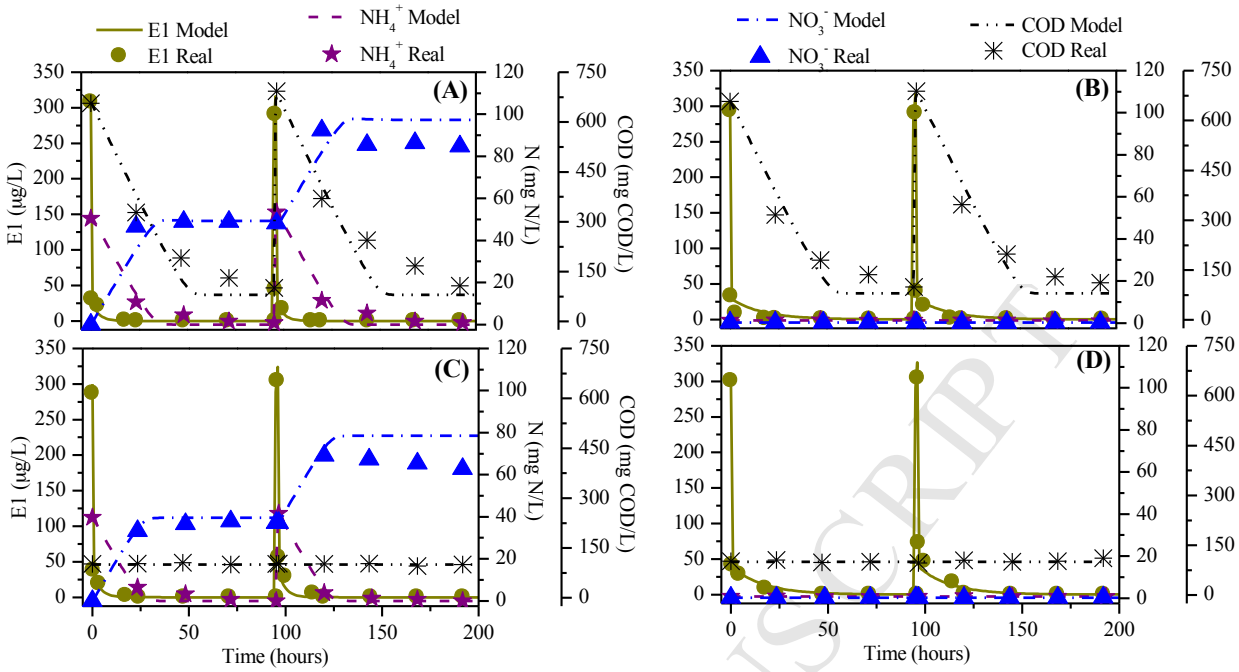

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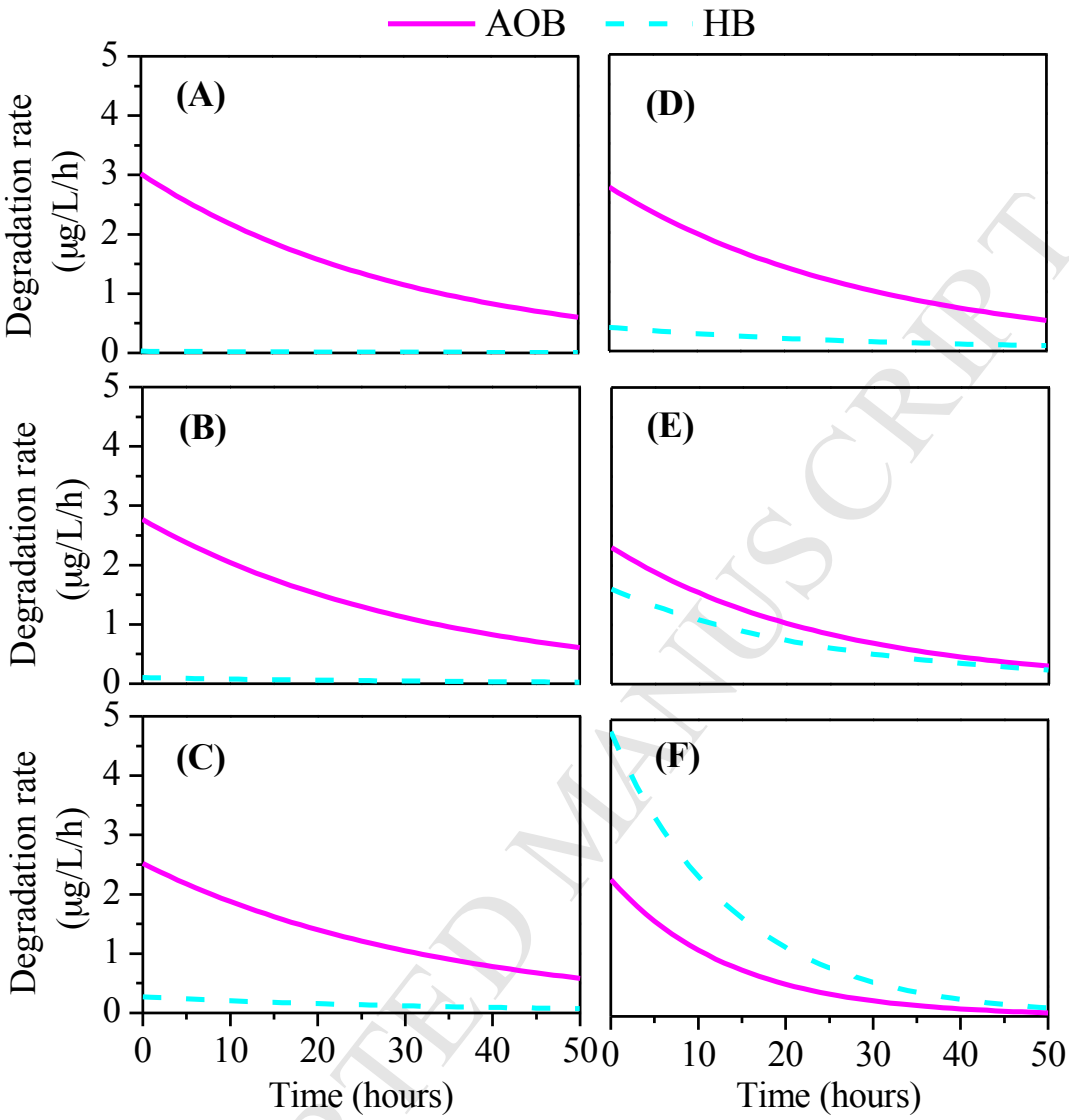

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Highlights

- A model was proposed to describe estrogen removal by nitrifying activated sludge.
- Pathways of biosorption, cometabolic and non-growth degradation were involved.
- The model was assessed by two sets of experimental data under varying conditions.
- The model predicted COD dosage triggered the shift of dominant degradation pathway.