Modeling of Pharmaceutical Biotransformation by Enriched Nitrifying Culture under Different Metabolic Conditions

Xu, Yifeng; Chen, Xueming; Yuan, Zhiguo; Ni, Bing-Jie

Published in:
Environmental Science & Technology

Link to article, DOI:
[10.1021/acs.est.8b00705](https://doi.org/10.1021/acs.est.8b00705)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Xu, Y., Chen, X., Yuan, Z., & Ni, B-J. (2018). Modeling of Pharmaceutical Biotransformation by Enriched Nitrifying Culture under Different Metabolic Conditions. *Environmental Science & Technology*, 52(5), 2835-2843. <https://doi.org/10.1021/acs.est.8b00705>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Article

Modeling of Pharmaceutical Biotransformation by Enriched Nitrifying Culture under Different Metabolic Conditions

Yifeng Xu, Xueming Chen, Zhiguo Yuan, and Bing-Jie Ni

Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.8b00705 • Publication Date (Web): 15 Feb 2018

Downloaded from <http://pubs.acs.org> on February 20, 2018

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

**Modeling of Pharmaceutical Biotransformation by Enriched Nitrifying Culture under
Different Metabolic Conditions**

Yifeng Xu¹, Xueming Chen^{1,2}, Zhiguo Yuan¹, Bing-Jie Ni^{1,*}

¹Advanced Water Management Centre, The University of Queensland, St. Lucia, Brisbane,
QLD 4072, Australia

²Process and Systems Engineering Center (PROSYS), Department of Chemical and
Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

***Corresponding author:**

Phone: + 61 7 3346 3219; Fax: +61 7 3365 4726; E-mail: bingjieni@gmail.com

Table of Contents (TOC) Art

Abstract

Pharmaceutical removal could be significantly enhanced through cometabolism during nitrification processes. So far pharmaceutical biotransformation models have not considered the formation of transformation products associated with the metabolic type of microorganisms. Here we reported a comprehensive model to describe and evaluate the biodegradation of pharmaceuticals and the formation of their biotransformation products by enriched nitrifying cultures. The biotransformation of parent compounds was linked to the microbial processes via cometabolism induced by ammonium oxidizing bacteria (AOB) growth, metabolism by AOB, cometabolism by heterotrophs (HET) growth and metabolism by HET in the model framework. The model was calibrated and validated using experimental data from pharmaceuticals biodegradation experiments at realistic levels, taking two pharmaceuticals as examples, i.e., atenolol and acyclovir. Results demonstrated the good prediction performance of the established biotransformation model under different metabolic conditions, as well as the reliability of the established model in predicting different pharmaceuticals biotransformations. The linear positive correlation between ammonia oxidation rate and pharmaceutical degradation rate confirmed the major role of cometabolism induced by AOB in the pharmaceutical removal. Dissolved oxygen was also revealed to be capable of regulating the pharmaceutical biotransformation cometabolically and the substrate competition between ammonium and pharmaceuticals existed especially at high ammonium concentrations.

38

Keywords: Cometabolism, pharmaceutical, model, ammonia oxidizing bacteria, biotransformation product, substrate competition

41

42 **Introduction**

43 The ubiquitous occurrence and fate of pharmaceuticals in the environment and
44 engineering systems have attracted the concerns of the scientists and the public for decades
45 due to their potential ecotoxic impact on aquatic ecosystems.^{1,2} These organic compounds
46 were present in the wastewater at concentrations ranging from pg L^{-1} to $\mu\text{g L}^{-1}$.^{3,4} As the
47 wastewater treatment plants (WWTPs) were originally designed for chemical oxygen demand
48 and other nutrients removal, the incomplete removal was found for pharmaceuticals in the
49 treatment processes, being a major pathway for pharmaceuticals to enter the environment.⁵

50 Autotrophic biomass (e.g., enriched nitrifying sludge) was capable of transforming the
51 pharmaceuticals cometabolically during the wastewater treatment process and thus the
52 pharmaceutical removal was reported to be positively correlated to nitrification rate.^{6,7}
53 Ammonia oxidizing bacteria (AOB) in the nitrifying biomass could degrade a broad range of
54 substrates including aromatic and aliphatic compounds due to the non-specific enzyme
55 ammonia monooxygenase (AMO).⁸⁻¹⁰ The presence of the growth substrate (i.e. ammonium)
56 was required for cometabolism which should be taken into account when predicting the fate
57 of pharmaceuticals.¹¹ In addition to cometabolism, pharmaceuticals could also be degraded as
58 the energy and carbon source for microorganisms through metabolic biotransformation.¹¹
59 Furthermore, the formed biotransformation products might be more toxic and persistent.¹²
60 Hence the biotransformation products should be considered for a more comprehensive
61 understanding of the fate of pharmaceuticals in the nitrifying activated sludge.

62 Mathematical modeling offers a useful tool and is adopted widely to analyze complicated
63 metabolic pathways. Cometabolic biotransformations were previously modeled through first-
64 order kinetics and mixed order kinetics like Monod expression¹³⁻¹⁵ and have evolved from
65 only considering the cometabolic substrates to incorporating the relationships between
66 cometabolic substrates and growth substrates, such as competitive interaction and toxicity

inhibition.¹⁵ However, the previous literature has rarely considered the formation of biotransformation products in the cometabolic biotransformation models for pharmaceuticals.

The aim of this work is to develop and test a comprehensive modeling framework to describe the pharmaceuticals biotransformation at realistic levels as well as the formation of their biotransformation products by the enriched nitrifying sludge under different metabolic conditions. Microbial processes contributing to the pharmaceutical biotransformation were considered as follows: growth-linked cometabolism by AOB, metabolic transformation by AOB, growth-linked cometabolism by heterotrophs (HET) and metabolic transformation by HET. To this end, atenolol and acyclovir were selected as the model compounds in this study as they were frequently found in the wastewater with the highest concentrations of 25 and 1.8 $\mu\text{g L}^{-1}$, respectively, which have been reported to be increasingly removed under nitrifying conditions.¹⁶⁻¹⁸ It has been reported that they can be biotransformed into atenolol acid and carboxy-acyclovir, respectively.^{18,19} Model calibration and validation were carried out with experimental data using atenolol as parent compounds under different metabolic conditions. Model evaluation was also conducted using the experimental data from acyclovir biotransformation. The effects of dissolved oxygen (DO) and ammonium concentrations on pharmaceutical biotransformation were investigated using the validated model to provide insights into the process dynamics. The reported model in this work is expected to be used as a tool to fully understand the fate of pharmaceuticals associated with different metabolisms by responsible microorganisms in the complicated activated sludge system.

Materials and Methods

Model development

91 A multi-species and multi-substrate model was developed to describe the pharmaceutical
92 biotransformation processes by the enriched nitrifying sludge. This biotransformation model
93 comprehensively considered the consumption of the pharmaceuticals and the formation of
94 transformation products accompanied with the simultaneous nitrification in the enriched
95 nitrifying sludge. It describes the relationships among eight soluble substrates as defined in
96 Table S1 in Supporting Information (SI), i.e., ammonium (S_{NH_4}), nitrite (S_{NO_2}), nitrate (S_{NO_3}),
97 readily biodegradable substrates (S_S), oxygen (S_{O_2}), pharmaceutical (parent compound, PC,
98 S_{PC}), primary biotransformation product (BP, S_{BP}) and other biotransformation products (OP,
99 S_{OP}), and five particulate species, i.e., AOB (X_{AOB}), HET (X_{HET}), NOB (nitrite oxidizing
100 bacteria, X_{NOB}), slowly biodegradable substrates (X_S) and inert biomass (X_I). Nine processes
101 are considered: (1) metabolic transformation of PC by AOB; (2) cometabolic transformation
102 of PC coupled to growth of AOB; (3) endogenous decay of AOB; (4) hydrolysis; (5)
103 metabolic transformation of PC by HET; (6) cometabolic transformation of PC coupled to
104 growth of HET; (7) endogenous decay of HET; (8) growth of NOB; and (9) endogenous
105 decay of NOB. The kinetic expressions and the stoichiometric matrix of the proposed
106 biotransformation model are summarized in Tables S2 and S3 in SI, respectively. The
107 definitions, values, units and sources of all parameters used in the biotransformation model
108 are listed in Table S4 in SI.

109 Pharmaceutical biodegradation was reported to be linked to AOB due to the non-specific
110 enzyme AMO as well as HET, which was not related to the activity of NOB.²⁰ In this model,
111 the microbial growth-linked kinetic expressions (processes 2 and 6 in Table S2 in SI) are
112 described using the Monod equations, which are associated with cometabolic
113 biotransformation of pharmaceuticals.²⁰ The concentration of growth substrates S_{NH_4} and S_S
114 is also involved in the Monod equations. The basis of the cometabolic biotransformation
115 expressions is the concept of transformation coefficient parameters such as AOB growth-

linked T_{PC-AOB}^c and HET growth-linked T_{PC-HET}^c . The pharmaceutical biotransformation reactions directly conducted via metabolism by AOB and HET are described by pseudo-first order kinetic expressions (processes 1 and 5 in Table S2 in SI). For each reaction, the rate is expressed by an explicit function of the concentrations of relevant pharmaceuticals in the process. For microbial metabolic biodegradation of PC, the key parameters are biomass normalized PC degradation rate coefficients in the absence of AOB and HET growth, i.e. k_{PC-AOB} and k_{PC-HET} . Processes 1, 2, 5 and 6 together contribute to pharmaceutical biotransformation in the enriched nitrifying sludge.

The formation of biotransformation products is modeled using the specific stoichiometry coefficients in processes 1, 2, 5 and 6. The coefficients α_{BP}^m and α_{BP}^c indicate the transformation of PC to BP under metabolism and cometabolism conditions by AOB, respectively. Similarly, the coefficients β_{BP}^m and β_{BP}^c present the transformation of PC to BP under metabolism and cometabolism conditions by HET, respectively.

Atenolol and acyclovir biotransformation experiments

Experimental data from our previous biodegradation experiments of atenolol (Case *I*) and acyclovir (Case *II*) under different conditions by an enriched nitrifying sludge were used for model evaluation in this work.^{21,22} The chemicals used in the batch experiments and the enrichment of nitrifying cultures in the sequencing batch reactor (SBR) are described in Text S1 and S2 in SI. Details of the experimental conditions applied in different scenarios are provided in Table S5 in SI. Briefly, 4-L beaker was used as the batch reactor with enriched nitrifying cultures inoculated to degrade parent compounds at an initial $15 \mu\text{g L}^{-1}$. The mixed liquid suspended solid (MLVSS) concentration was kept at approximately 1 g L^{-1} . All the batch experiments were conducted in duplicates. The designs for Experiments 1-3 were same for atenolol (Case *I*) and acyclovir (Case *II*). In Experiment 1, 30 mg L^{-1} allylthiourea (ATU)

141 was added to inhibit nitrifying activities,^{20,23,24} leading to the dominant contribution from
142 HET to pharmaceutical biotransformation.¹¹ Initial ammonium concentration was provided at
143 50 mg-N L⁻¹. No external ammonium was supplied during the entire experimental period
144 (240 h). In Experiment 2, no initial and external ammonium was provided during 240 h. In
145 Experiment 3, constant ammonium concentration was maintained at 50 mg-N L⁻¹ by dosing a
146 mixture of ammonium bicarbonate and potassium bicarbonate as ammonium feeding solution
147 and pH buffer at the same time, which could ensure the cometabolic biotransformation by
148 AOB. The Experiment 4 was exclusively designed for atenolol biotransformation, where
149 constant ammonium concentrations of 25 mg-N L⁻¹ were provided using the dosing method
150 in Experiment 3 during the experimental period. Samples were collected periodically to
151 analyse mixed liquid suspended solid (MLSS) concentration and its volatile fraction (i.e.,
152 MLVSS), NH₄⁺, NO₂⁻, NO₃⁻, atenolol, acyclovir and their biotransformation products
153 atenolol acid and carboxy-acyclovir. The detailed chemical analysis procedures could be
154 found in the previous work.^{21,22,25}

155 The contribution of sorption to removal of atenolol and acyclovir was insignificant based
156 on our previous studies.^{22,25} This is in consistent with low sorption coefficient K_D (0.04) of
157 atenolol and low octanol-water partition coefficient Log K_{OW} (0.16) of atenolol as well as Log
158 K_{OW} (-1.59) of acyclovir.²⁶⁻²⁸ Volatilization was considered negligible given the low values of
159 Henry's law constants for atenolol (1.37×10^{-18} atm m³ mol⁻¹) and acyclovir (3.2×10^{-22} atm m³
160 mol⁻¹).²⁹ Hydrolysis would not contribute to the degradation of atenolol and acyclovir, which
161 was confirmed previously and was in consistent with the absence of their transformation
162 products.^{22,25} Photodegradation was also insignificant considering the turbidity of the sludge
163 and the aluminum foil covering the reactor. Therefore, microbially induced biodegradation
164 should be the main mechanism for pharmaceutical removal in both atenolol and acyclovir
165 biotransformation experiments.

Model calibration and validation

The biotransformation model used in this work consists of 9 biochemical processes and 27 stoichiometric and kinetic parameters (as shown in Tables S2 and S4 in SI). Most of these parameters were well established in previous literature, therefore the reported values were directly used in this developed model. However, the information on biomass growth-linked PC transformation coefficients T_{PC-AOB}^c and T_{PC-HET}^c and microbial endogenous transformation coefficients k_{PC-AOB} and k_{PC-HET} was limited.²⁰ Considering the key role of cometabolism induced by AOB growth in biotransformation, the maximum specific growth rate of AOB $\mu_{max, AOB}$ was of significance to the developed model. Furthermore, the sensitivity analysis suggested the four key parameters k_{PC-AOB} , k_{PC-HET} , T_{PC-AOB}^c and $\mu_{max, AOB}$ are highly sensitive to the biotransformation processes in terms of the experimental measurements (examples shown in Figure S1 in SI). Model calibration was therefore conducted to estimate the values of k_{PC-AOB} , k_{PC-HET} , T_{PC-AOB}^c and $\mu_{max, AOB}$ based on experimental measurements through minimizing the sum of squares of the deviations between the measured and modeled values for the concentrations of parent compounds and biotransformation products under different conditions. In addition, the four stoichiometric coefficients, i.e., α_{BP}^m , α_{BP}^c , β_{BP}^m and β_{BP}^c , for the transformation of PC to BP under metabolism and cometabolism conditions could be determined based on the respective molecular mass and concentrations of BP and PC measured in the experiments.

Experimental data from atenolol biotransformation (Case I) of Experiments 1-3 were firstly used for model calibration. Concentrations of ammonium, nitrite, DO, atenolol and atenolol acid from Experiment 1 and Experiment 2 were fitted by model simulations to estimate k_{PC-HET} and k_{PC-AOB} , respectively, whereas concentrations of ammonium, nitrite, DO,

190 atenolol and atenolol acid from Experiment 3 were fitted to estimate $\mu_{max, AOB}$ and T_{PC-AOB}^c ,
191 using the k_{PC-HET} and k_{PC-AOB} values obtained in previous experiments (Experiment 1 and
192 Experiment 2). Model validation was then carried out with the calibrated parameters using
193 the independent experimental data sets from atenolol biotransformation of Experiment 4.²¹
194 Specifically, in Experiment 4, batch experiments with atenolol as the parent compound at an
195 initial concentration of 15 $\mu\text{g L}^{-1}$ were conducted using the same enriched nitrifying sludge
196 (i.e., same microbial composition) in the constant presence of ammonium of 25 mg-N L^{-1} and
197 at DO of around 2.5 mg L^{-1} . There were no significant gaps between batch experiments,
198 leading to insignificant biomass changes. The ammonium and DO concentrations applied
199 were different from of Experiment 3 at ammonium of 50 mg-N L^{-1} and DO of 3.0 mg L^{-1}
200 (Table S5 in SI). To further verify the validity and applicability of the model, the model was
201 also applied to evaluating the acyclovir biotransformation data from Case II of Experiments
202 1-3. The key model parameters were recalibrated for Case II using the three sets of batch
203 experimental data (Table S5 in SI).

204 The sensitivity analysis, parameter estimation, parameter uncertainty evaluation and
205 model simulations were done through employing a modified version of software AQUASIM
206 2.1d according to Batstone et al.³⁰, with a 95% confidence level for significance testing and
207 parameter uncertainty analysis. The standard errors and 95% confidence intervals of
208 individual parameter estimates were calculated from the mean square fitting errors and the
209 sensitivity of the model to the parameters. Residual sum of squares (RSS) between the
210 objective data and model was used as the objective function.

211

212 **Results**

213

214 **Model calibration with experimental data from atenolol biotransformation**

As atenolol acid was the sole biotransformation products with no other products identified in all batch experiments, the dynamics of the substrate S_{OP} was not modeled herein. The model was first calibrated to illustrate the biotransformation of atenolol catalysed solely by HET in Experiment 1 (i.e. with addition of ATU to inhibit the nitrifying activity). Given that no exogenous organic carbon was supplied during culture enrichment and the only organic carbon in the batch experiments was pharmaceuticals, the growth of HET was considered extremely low and the cometabolic transformation rate of pharmaceuticals linked to growth of HET was not modeled with T_{PC-HET}^c omitted for estimation.²⁰ With AOB related parameters k_{PC-AOB} and T_{PC-AOB}^c set to zero, only the parameter k_{PC-HET} was estimated with its best-fit value shown in Table 1 for Experiment 1. The predicted atenolol and atenolol acid concentration profiles with the established model were demonstrated in Figure 1A, along with the measured experimental values. Atenolol experienced a continuous decrease by 94.3% from the beginning to the end of experiments accompanied with a gradual increase of atenolol acid until 168 h and a stable stage until 240 h at a conversion efficiency of 62.6% (Figure 1A), which was well captured by the model predictions.

The experimental data obtained from Experiment 2 (i.e., in the absence of ammonium) were used to further calibrate the developed model in terms of atenolol and atenolol acid dynamics. Without the presence of the growth substrate, the ammonium released from cell lysis process during bacterial decay was minor and AOB growth-linked cometabolism would be considered to have negligible contribution to atenolol biotransformation. Therefore, only the metabolic biotransformation by AOB and HET were involved in the biotransformation of atenolol for Experiment 2. The parameter value of k_{PC-HET} obtained in Experiment 1 was used directly without any modification. Another key model parameter k_{PC-AOB} related to AOB metabolism was thus reliably estimated during atenolol biotransformation (value as shown in Table 1). As shown in Figure 1B, although atenolol demonstrated a sharp decrease by 97.4%

over the whole experimental period, the production of atenolol acid indicated a lower transformation efficiency in the absence of ammonium (29.1%) compared with the experiments with addition of ATU (see Figure 1A), again well matching the model predictions.

In Experiment 3, the presence of ammonium at 50 mg-N L^{-1} was provided constantly to ensure the cometabolic biodegradation of atenolol by both AOB and HET at DO of 3.0 mg L^{-1} . Together with the rest of the parameters involved, the parameter values of k_{PC-HET} and k_{PC-AOB} estimated in the previous two experiments were applied in the biotransformation model. The key parameters related to AOB induced cometabolism, i.e., T_{PC-AOB}^c and $\mu_{max, AOB}$, were then estimated with the optimum values listed in Table 1. Figure S2A in SI showed the well agreement between predicted and measured concentrations of ammonium, nitrite and DO based on the proposed model, supporting the capability of the model to describe the two-step nitrification processes in terms of nitrite accumulation, as well as the suitability of the selected parameters related to DO dynamics for the cometabolic biodegradation processes by the enriched nitrifying culture (i.e., the $K_{O_2, AOB}$ and $K_{O_2, HET}$ values for AOB and HET). It should be noted that the nitrate concentrations were not specifically modeled, which were slightly higher than that in the SBR in all experiments since the biomass in batch experiments was taken directly from SBR with a background nitrate concentration up to 1000 mg L^{-1} . As shown in Figure 1C, concomitant with the gradual decrease of atenolol at a removal efficiency of 88.0%, atenolol acid was formed at an increasing trend with 86.9% conversion efficiency. This was obviously higher than the experiments in the absence of ammonium and with the addition of ATU, indicating a positive role of AOB induced cometabolism in atenolol transformation. The model described these observations reasonably well.

Overall, the developed model could satisfactorily capture all dynamics associated with atenolol and atenolol acid in all batch biodegradation experiments under different metabolic

conditions. The good agreement between model simulations and measured data in Figure 1 supports the capability of the developed model in describing the microbial growth related biotransformation of atenolol in enriched nitrifying cultures. The obtained parameter linked to AOB growth during ammonia oxidation, i.e., AOB-induced cometabolic atenolol transformation coefficient T_{PC-AOB}^c , was estimated at $0.012 \pm 0.000036 \text{ m}^3 \text{ g COD}^{-1}$. It was lower than the reported value of $0.0715 \pm 0.0227 \text{ m}^3 \text{ g COD}^{-1}$ for atenolol biodegradation by an enriched nitrifying sludge.²⁰ The non-growth metabolism by HET and the non-growth metabolism by AOB on atenolol biodegradation also described the experimental data with the addition of ATU and in the absence of ammonium well. The estimated parameters of k_{PC-HET} and k_{PC-AOB} were 0.000180 ± 0.000017 and $0.000140 \pm 0.000012 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$, which were lower than but in the same order of magnitude as the literature reported values (0.00093 ± 0.00018 and $0.00067 \pm 0.00023 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$, respectively).²⁰ The discrepancy in these parameters values could be probably ascribed to the difference in the community structure in the adopted nitrifying cultures or different operating conditions. The model could be potentially applied to a widespread extent despite that the parameter values would vary according to the experimental conditions. As suggested, it was difficult to compare these coefficients (k_{PC-HET} , k_{PC-AOB} and T_{PC-AOB}^c) with other pharmaceuticals as most existing models did not consider the specific biochemical processes.²⁰

Model validation with atenolol biotransformation under different conditions

In order to further confirm the validity and reliability of the developed model, model validation was carried out to compare the model simulations to the independent experimental data, which were not used for model calibration. Based on the measured concentrations of atenolol and atenolol acid, the stoichiometric coefficients α_{BP}^c and α_{BP}^m were calculated as 0.58 and 0.58, respectively. Applied with previously calibrated parameters in Table 1, the

proposed biotransformation model was used to predict dynamics of ammonium, nitrite, DO, atenolol and atenolol acid in the presence of ammonium at a constant concentration of 25 mg-N L⁻¹ and at DO of around 2.5 mg L⁻¹ (significantly different from the ammonium of 50 mg-N L⁻¹ and DO of 3.0 mg L⁻¹ used for model calibration). The model captured the dynamics of ammonium, nitrite and DO, again suggesting the validity of the two-step nitrification model and the suitability of the selected parameters related to DO (see Figure S2B). As shown in Figure 2, atenolol continuously dropped from initial 15 µg L⁻¹ with a final degradation efficiency of 92.9%. The conversion rate of atenolol acid transformed from atenolol was calculated as 57.9%. The model predictions could capture these trends of atenolol degradation and atenolol acid formation very well, which again supports the validity of the developed model for atenolol biotransformation.

Model evaluation with experimental data from acyclovir biotransformation

The experimental results obtained with Case II for biotransformation of acyclovir were used to further evaluate the developed model. The developed biotransformation model was recalibrated for acyclovir biodegradation and carboxy-acyclovir formation dynamics under different conditions. Most of the literature reported model parameters were employed at same values as the case of atenolol except the stoichiometry coefficients (α_{BP}^m , α_{BP}^c , β_{BP}^m , β_{BP}^c) for formation of carboxy-acyclovir associated with specific biochemical processes (as shown in Table S4 in SI), which were calculated based on the experimental data. The values for the three key parameters k_{PC-HET} , k_{PC-AOB} and T_{PC-AOB}^c were recalibrated, which were associated with the investigated parent compound. As the enriched nitrifying biomass utilized in the batch biodegradation experiments of acyclovir were same as those in case of atenolol, the maximum growth rate of AOB $\mu_{max, AOB}$ was set to be the same as in case of atenolol during model calibration for acyclovir biotransformation in the presence of ammonium. The

obtained parameter values for acyclovir biotransformation were $0.00035 \pm 0.00002 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$ (k_{PC-HET}), $0.00005 \pm 0.00003 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$ (k_{PC-AOB}) and $0.00093 \pm 0.00049 \text{ m}^3 \text{ g COD}^{-1}$ (T_{PC-AOB}^c).

The model predictions of acyclovir biotransformation matched the experimental results well under different conditions (Figure 3), further demonstrating the validity of the established model. Parameters values giving the optimum fits with the experimental data were difficult to compare reliably with literature values as this study firstly reported the AOB cometabolic acyclovir transform coefficient T_{PC-AOB}^c . However, compared to other reported compounds, e.g. atenolol,²⁰ it was obvious that parameters k_{PC-AOB} and T_{PC-AOB}^c for acyclovir were lower than those values for atenolol (Table 1), indicating a stronger degradation ability of the AOB culture studied on atenolol than acyclovir. Considering the molecular differences between these two pharmaceuticals, this may imply an affinity property of AOB for different compounds probably due to a preferential substrate selection to AMO active sites.³¹ The parameter k_{PC-HET} for acyclovir was $0.00035 \pm 0.00002 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$, which was in the same order of magnitude of the value estimated in this study ($0.000180 \pm 0.000017 \text{ m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$) for atenolol. The conversion efficiencies from acyclovir to carboxy-acyclovir were 83.9%, 43.0% and 29.9% in Experiments 1, 2 and 3, respectively (see Figure 3). These results indicated the importance of metabolism of acyclovir by HET. Oxidation of acyclovir to carboxy-acyclovir might be dominated by unspecific monooxygenase from HET,³² which needs to be confirmed in the further work.

Discussion

In this work, a comprehensive mathematical model is developed to describe the biotransformation of pharmaceuticals and the formation of their products by enriched nitrifying cultures. In the proposed model, processes 1 and 2 (Table S2 in SI) depict the

AOB-induced cometabolic and metabolic biotransformation of pharmaceuticals, while processes 5 and 6 (Table S2 in SI) describe the HET-induced cometabolic and metabolic biotransformation of pharmaceuticals, respectively. Sensitivity analysis indicated that four key parameters k_{PC-HET} , k_{PC-AOB} , T_{PC-AOB}^c and $\mu_{max, AOB}$ were critical to the model output and therefore estimated through model calibration. The validity of this biotransformation model is confirmed by independent atenolol biodegradation data and further evaluated by acyclovir biotransformation experiments. Compared to the previous studies where atenolol biodegradation was investigated through experiments and modeling approaches,^{20,21} the proposed model in this work considers the formation of biotransformation products and describes biotransformation of different pharmaceuticals under different metabolic conditions. This microbial processes-linked biotransformation model could enhance our ability to predict the fate of pharmaceuticals and their transformation products during wastewater treatment processes.

Since we estimated four model parameters for fitting the experimental data, parameter uniqueness is important, since it is possible that different parameter combinations can give similar simulation accuracy. In our work, we applied a least-squared analysis and evaluated standard errors and 95% confidence intervals of individual parameter estimates. The parameter confidence intervals showed a well-defined range in which the optimum values of parameters reside (Table 1), which indicates good uniqueness of these parameters. In addition to the analysis of the confidence intervals, two other aspects of our experimental design support the uniqueness of the parameter values. First, we used five different experimental parameters (ammonium, nitrite, DO, parent compound, and biotransformation product), which reflect different aspects of the kinetics of the two-step nitrification and pharmaceutical biotransformation by enriched nitrifying culture. Second, we carried out independent experiments to validate the estimated parameters. In particular, the good correspondence for

independent experimental data supports the validity of the new model and the uniqueness of the parameters for pharmaceutical biotransformation.

The modeling results in this work suggested the cometabolism induced by AOB could play an important role in the pharmaceutical removal in the studied ratio ranges of pharmaceuticals to ammonia for cometabolism. Indeed a positive linear relationship was observed between ammonia oxidation rate and pharmaceutical degradation rates in terms of atenolol and acyclovir based on the validated model (Figure 4A). The atenolol degradation rate increased from 0.012 to 0.16 $\mu\text{g g VSS}^{-1} \text{ h}^{-1}$ while the nitrification rate increased from 2.84 to 59.15 $\text{mg NH}_4^+\text{-N g VSS}^{-1} \text{ h}^{-1}$. With respect to acyclovir, the degradation rate changed from 0.014 to 0.10 $\mu\text{g g VSS}^{-1} \text{ h}^{-1}$ whereas the ammonia oxidation rate showed an increase from 2.37 to 36.63 $\text{mg NH}_4^+\text{-N g VSS}^{-1} \text{ h}^{-1}$. Such a positive correlation was also reported in previous literature under certain conditions,^{7,22,25} supporting the notion that majority of atenolol and acyclovir could be cometabolically degraded in the enriched nitrifying cultures. A further assessment on the wide application of the relationship was carried out by simulating the concentration profiles of pharmaceuticals after 240 h. The molar ratios of atenolol to ammonia from 8.42×10^{-7} to 1.91×10^{-5} calculated based on their concentrations was observed to be still within the range for a linearly positive relationship regarding the cometabolic biodegradation of atenolol by the enriched nitrifying cultures used in this work, and the relationship maintained at a same slope (Black solid squares in Figure 4A demonstrated the predicted atenolol degradation rate after 240 h). However, a different slope was found for the relationship between ammonia oxidation rate and the acyclovir degradation rate after 240 h predicted using the developed model (Figure 4B). If the ammonia oxidation rate was higher than the critical value (2.3 $\text{mg NH}_4^+\text{-N g VSS}^{-1} \text{ h}^{-1}$ in this study), the lower slope might indicate a slower increasing trend in acyclovir degradation rate with an increasing ammonia oxidation rate (Figure 4A). Compared with the situation at the lower

ammonia oxidation rate, a higher increasing trend in acyclovir degradation rate would arise at higher slope (Figure 4B). The observation that pharmaceutical would not be degraded until the ammonia was depleted³³ revealed a higher pharmaceutical degradation rate at lower ammonia oxidation rate, which supported the findings in this study. Regardless of the different slopes for the relationship, the molar ratios of acyclovir to ammonia ranging from 1.62×10^{-11} to 2.26×10^{-5} was obtained to be a valid application range for the cometabolic biodegradation of acyclovir by the enriched nitrifying cultures used in this work.

The proposed model framework was expected to be a useful tool to predict the biotransformation of pharmaceuticals and the formation of transformation products under varying conditions, therefore providing the guidance in designing, upgrading and optimizing of the relevant biological wastewater treatment processes. The influence of DO on pharmaceutical biotransformation was investigated by performing model simulations in the enriched nitrifying systems. The pharmaceutical removal efficiencies at 240 h at different DO concentrations ranging from 0 to 4 mg L⁻¹ with ammonium concentration of 50 mg-N L⁻¹ are shown in Figure 5. Overall DO concentration had a positive effect on pharmaceutical removal efficiencies. The concentrations of atenolol and acyclovir decreased rapidly with a prompt increase of atenolol acid and carboxy-acyclovir as DO increased to 1 mg L⁻¹. With DO further increased to 4 mg L⁻¹, a gradual decrease of pharmaceutical concentrations was observed accompanied with a slight increase of their biotransformation products. The degradation efficiencies for atenolol at DO concentrations of 0, 1 and 4 mg L⁻¹ were 44.3%, 83.2% and 94.0%, respectively. With regard to acyclovir, its degradation efficiencies were observed to be 36.2%, 81.2% and 87.3%, respectively at DO of 0, 1 and 4 mg L⁻¹. The simulation results revealed that the DO concentration would play an important role in pharmaceutical biotransformation. This was contrary to the previous report that DO in the WWTP had no influence on oxidative biotransformation of selected micropollutants.³⁴ The

possible reason could be that the experiments conducted in this study were nitrifying culture based instead of the regular activated sludge in WWTP, suggesting that DO might regulate the pharmaceutical biotransformation cometabolically. It should be noted that the simulation results are to provide insight into the potential impact of DO on pharmaceutical biotransformation by enriched nitrifying culture rather than to accurately predict the reality, which remain to be verified in future work.

The growth substrate might also have an impact on the pharmaceutical biotransformation. Different ammonium concentrations ranging from 0 to 100 mg L⁻¹ were applied in the model simulations at different DO concentrations as shown in Figure 6. It was obvious that the degradation efficiencies of studied pharmaceuticals and the formation rates of their transformation products would increase dramatically when ammonium concentrations increase from 0 to 20 mg-N L⁻¹, especially in case of atenolol suggesting the importance of cometabolism on its biotransformation. However, there was no significant enhancement with the increase of ammonium concentrations from 20 to 250 mg-N L⁻¹ (data of 100-250 mg-N L⁻¹ were not shown). This was contrary to the previous report where the removal efficiencies of the selected pharmaceuticals were enhanced at higher initial ammonium concentrations.³⁵ This could be probably due to the substrate competition between growth substrate (ammonium) and cometabolic substrates (e.g. atenolol or acyclovir). Pharmaceutical levels applied in this study were several orders of magnitude lower than the investigated ammonium concentrations, leading to a competition for AMO active sites and therefore potential decreasing degradation rates at higher ammonium concentrations.^{31,33}

In summary, a comprehensive model that considers all microbial processes contributing to pharmaceutical biotransformation as well as the formation of biotransformation products by the enriched nitrifying cultures is developed in this work. The proposed model was successfully calibrated and validated using the biotransformation experiments of atenolol and

acyclovir under different metabolic conditions. The linear positive correlation between ammonia oxidation rate and pharmaceutical degradation rate confirmed the major role of cometabolism induced by AOB in the pharmaceutical removal. DO was revealed to be capable of regulating the pharmaceutical biotransformation cometabolically and the substrate competition between ammonium and pharmaceuticals existed at high ammonium concentrations. More verification should be conducted using other pharmaceuticals' biotransformation data for this developed model to facilitate its application as a useful tool in prediction of pharmaceutical fate, especially in the real municipal wastewater systems, where other processes (e.g., the competition between different parent compounds on the enzyme active sites) need to be considered in future work.

Acknowledgement

This study was supported by the Australian Research Council (ARC) through Future Fellowship FT160100195. Dr. Bing-Jie Ni acknowledges the support of ARC Discovery Project DP130103147.

Supporting Information

Additional texts, tables and figures are shown in Supporting Information.

Reference

- (1) Ternes, T. A., Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* **1998**, *32* (11), 3245-3260.
- (2) Benner, J.; Helbling, D. E.; Kohler, H. P. E.; Wittebol, J.; Kaiser, E.; Prasse, C.; Ternes, T. A.; Albers, C. N.; Aamand, J.; Horemans, B.; Springael, D.; Walravens, E.; Boon, N., Is biological treatment a viable alternative for micropollutant removal in drinking water treatment processes? *Water Res.* **2013**, *47* (16), 5955-5976.

- 466 (3) Petrie, B.; Barden, R.; Kasprzyk-Hordern, B., A review on emerging contaminants in
467 wastewaters and the environment: Current knowledge, understudied areas and
468 recommendations for future monitoring. *Water Res.* **2015**, *72*, 3-27.
- 469 (4) Evgenidou, E. N.; Konstantinou, I. K.; Lambropoulou, D. A., Occurrence and removal of
470 transformation products of PPCPs and illicit drugs in wastewaters: A review. *Sci. Total*
471 *Environ.* **2015**, *505*, 905-926.
- 472 (5) Carballa, M.; Omil, F.; Lema, J. M.; Llompарт, M. a.; García-Jares, C.; Rodríguez, I.;
473 Gómez, M.; Ternes, T., Behavior of pharmaceuticals, cosmetics and hormones in a
474 sewage treatment plant. *Water Res.* **2004**, *38*, (12), 2918-2926.
- 475 (6) Batt, A. L.; Kim, S.; Aga, D. S., Enhanced biodegradation of iopromide and trimethoprim
476 in nitrifying activated sludge. *Environ. Sci. Technol.* **2006**, *40* (23), 7367-7373.
- 477 (7) Yi, T.; Harper Jr, W. F., The link between nitrification and biotransformation of 17 α -
478 ethinylestradiol. *Environ. Sci. Technol.* **2007**, *41* (12), 4311-4316.
- 479 (8) Keener, W. K.; Arp, D. J., Kinetic studies of ammonia monooxygenase inhibition in
480 *Nitrosomonas europaea* by hydrocarbons and halogenated hydrocarbons in an optimized
481 whole- cell assay. *Appl. Environ. Microbiol.* **1993**, *59* (8), 2501-2510.
- 482 (9) Keener, W. K.; Arp, D. J., Transformations of aromatic compounds by *Nitrosomonas*
483 *europaea*. *Appl. Environ. Microbiol.* **1994**, *60* (6), 1914-1920.
- 484 (10) Xu, Y.; Yuan, Z.; Ni, B.-J., Biotransformation of pharmaceuticals by ammonia
485 oxidizing bacteria in wastewater treatment processes. *Sci. Total Environ.* **2016**, *566*–567,
486 796-805.
- 487 (11) Tran, N. H.; Urase, T.; Ngo, H. H.; Hu, J.; Ong, S. L., Insight into metabolic and
488 cometabolic activities of autotrophic and heterotrophic microorganisms in the
489 biodegradation of emerging trace organic contaminants. *Bioresour. Technol.* **2013**, *146*,
490 (0), 721-731.
- 491 (12) Quintana, J. B.; Weiss, S.; Reemtsma, T., Pathways and metabolites of microbial
492 degradation of selected acidic pharmaceutical and their occurrence in municipal
493 wastewater treated by a membrane bioreactor. *Water Res.* **2005**, *39* (12), 2654-2664.
- 494 (13) Fernandez-Fontaina, E.; Carballa, M.; Omil, F.; Lema, J. M., Modelling cometabolic
495 biotransformation of organic micropollutants in nitrifying reactors. *Water Res.* **2014**, *65*,
496 371-383.
- 497 (14) Oldenhuis, R.; Vink, R. L. J. M.; Janssen, D. B.; Witholt, B., Degradation of
498 chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing
499 soluble methane monooxygenase. *Appl. Environ. Microbiol.* **1989**, *55* (11), 2819-2826.
- 500 (15) Liu, L.; Binning, P. J.; Smets, B. F., Evaluating alternate biokinetic models for trace
501 pollutant cometabolism. *Environ. Sci. Technol.* **2015**, *49* (4), 2230-2236.
- 502 (16) Verlicchi, P.; Al Aukidy, M.; Zambello, E., Occurrence of pharmaceutical compounds
503 in urban wastewater: Removal, mass load and environmental risk after a secondary
504 treatment—A review. *Sci. Total Environ.* **2012**, *429*, 123-155.

- (17) Prasse, C.; Schlüsener, M. P.; Schulz, R.; Ternes, T. A., Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance? *Environ. Sci. Technol.* **2010**, *44* (5), 1728-1735.
- (18) Prasse, C.; Wagner, M.; Schulz, R.; Ternes, T. A., Biotransformation of the antiviral drugs acyclovir and penciclovir in activated sludge treatment. *Environ. Sci. Technol.* **2011**, *45* (7), 2761-2769.
- (19) Radjenović, J.; Pérez, S.; Petrović, M.; Barceló, D., Identification and structural characterization of biodegradation products of atenolol and glibenclamide by liquid chromatography coupled to hybrid quadrupole time-of-flight and quadrupole ion trap mass spectrometry. *J. Chromatogr. A* **2008**, *1210* (2), 142-153.
- (20) Sathyamoorthy, S.; Chandran, K.; Ramsburg, C. A., Biodegradation and cometabolic modeling of selected beta blockers during ammonia oxidation. *Environ. Sci. Technol.* **2013**, *47* (22), 12835-12843.
- (21) Xu, Y.; Yuan, Z.; Ni, B.-J., Impact of Ammonium Availability on Atenolol Biotransformation during Nitrification. *ACS Sustainable Chem. Eng.* **2017**, *5* (8), 7137-7144.
- (22) Xu, Y.; Yuan, Z.; Ni, B.-J., Biotransformation of acyclovir by an enriched nitrifying culture. *Chemosphere* **2017**, *170*, 25-32.
- (23) Ginestet, P.; Audic, J. M.; Urbain, V.; Block, J. C., Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. *Appl. Environ. Microbiol.* **1998**, *64* (6), 2266-2268.
- (24) Ali, T. U.; Kim, M.; Kim, D. J., Selective inhibition of ammonia oxidation and nitrite oxidation linked to n₂o emission with activated sludge and enriched nitrifiers. *J. Microbiol. Biotechnol.* **2013**, *23* (5), 719-723.
- (25) Xu, Y.; Radjenovic, J.; Yuan, Z.; Ni, B. J., Biodegradation of atenolol by an enriched nitrifying sludge: Products and pathways. *Chem. Eng. J.* **2017**, *312*, 351-359.
- (26) Kasim, N. A.; Whitehouse, M.; Ramachandran, C.; Bermejo, M.; Lennernäs, H.; Hussain, A. S.; Junginger, H. E.; Stavchansky, S. A.; Midha, K. K.; Shah, V. P.; Amidon, G. L., Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol. Pharmaceutics* **2004**, *1* (1), 85-96.
- (27) Maurer, M.; Escher, B. I.; Richle, P.; Schaffner, C.; Alder, A. C., Elimination of β -blockers in sewage treatment plants. *Water Res.* **2007**, *41* (7), 1614-1622.
- (28) Mohsen-Nia, M.; Ebrahimabadi, A. H.; Niknahad, B., Partition coefficient n-octanol/water of propranolol and atenolol at different temperatures: Experimental and theoretical studies. *J. Chem. Thermodyn.* **2012**, *54*, 393-397.
- (29) Küster, A.; Alder, A. C.; Escher, B. I.; Duis, K.; Fenner, K.; Garric, J.; Hutchinson, T. H.; Lapen, D. R.; Péry, A.; Römbke, J.; Snape, J.; Ternes, T.; Topp, E.; Wehrhan, A.; Knackerk, T., Environmental risk assessment of human pharmaceuticals in the European union: A case study with the β -blocker atenolol. *Integr. Environ. Assess. Manage.* **2010**, *6* (SUPPL. 1), 514-523.

- 545 (30) Batstone, D. J.; Pind, P. F.; Angelidaki, I., Kinetics of thermophilic anaerobic
546 oxidation of straight and branched chain butyrate and valerate. *Biotechnol. Bioeng.* **2003**,
547 *84* (2), 195-204.
- 548 (31) Fernandez-Fontaina, E.; Omil, F.; Lema, J. M.; Carballa, M., Influence of nitrifying
549 conditions on the biodegradation and sorption of emerging micropollutants. *Water Res.*
550 **2012**, *46* (16), 5434-5444.
- 551 (32) Men, Y.; Han, P.; Helbling, D. E.; Jehmlich, N.; Herbold, C.; Gulde, R.; Onnis-
552 Hayden, A.; Gu, A. Z.; Johnson, D. R.; Wagner, M.; Fenner, K., Biotransformation of
553 Two Pharmaceuticals by the Ammonia-Oxidizing Archaeon *Nitrososphaera gargensis*.
554 *Environ. Sci. Technol.* **2016**, *50* (9), 4682-4692.
- 555 (33) Dawas-Massalha, A.; Gur-Reznik, S.; Lerman, S.; Sabbah, I.; Dosoretz, C. G., Co-
556 metabolic oxidation of pharmaceutical compounds by a nitrifying bacterial enrichment.
557 *Bioresour. Technol.* **2014**, *167*, 336-342.
- 558 (34) Helbling, D. E.; Johnson, D. R.; Honti, M.; Fenner, K., Micropollutant
559 biotransformation kinetics associate with WWTP process parameters and microbial
560 community characteristics. *Environ. Sci. Technol.* **2012**, *46* (19), 10579-10588.
- 561 (35) Tran, N. H.; Urase, T.; Kusakabe, O., The characteristics of enriched nitrifier culture
562 in the degradation of selected pharmaceutically active compounds. *J. Hazard. Mater.*
563 **2009**, *171* (1-3), 1051-1057.
- 564
- 565

Table and Figure Legends

Table 1. Estimated parameter values for the biotransformation model in this study

Figure 1. Model calibration with experimental data from atenolol biodegradation: (A) Experiment 1, with addition of allylthiourea (ATU); (B) Experiment 2, in the absence of ammonium; and (C) Experiment 3, in the presence of ammonium (50 mg NH_4^+ -N L^{-1}).

Figure 2. Model validation results of atenolol biotransformation by the enriched nitrifying culture in the presence of ammonium of 25 mg-N L^{-1} (Experiment 4).

Figure 3. Model evaluation with experimental data from acyclovir biodegradation: (A) Experiment 1, with addition of allylthiourea (ATU), (B) Experiment 2, in the absence of ammonium and (C) Experiment 3, in the presence of ammonium (50 mg NH_4^+ -N L^{-1}).

Figure 4. (A) The relationship between ammonia oxidizing rate and the pharmaceutical degradation rates in terms of atenolol and acyclovir (black solid squares indicate the atenolol degradation rates after 240 h); and (B) The relationship between ammonia oxidizing rate and the acyclovir degradation rate after 240 h at a different linear fit slope.

Figure 5. Predicted final concentrations of (A) atenolol and atenolol acid and (B) acyclovir and carboxy-acyclovir at time of 240 h at different concentrations of dissolved oxygen (DO) in the enriched nitrifying culture system.

Figure 6. Predicted concentrations of pharmaceuticals and their transformation products at time of 240 h at initial concentrations of 15 $\mu\text{g L}^{-1}$ with different ammonium concentrations ranging from 0 to 100 mg-N L^{-1} at different DO levels.

593 **Table 1.** Estimated parameter values for the biotransformation model in this study

Parameters	Definition	Unit	Estimated	
			atenolol	acyclovir
k_{PC-HET}	Heterotrophs (HET) transformation coefficient	$\text{m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$	0.000180	0.00035 $\pm$
			$\pm$	0.00002
			0.000017	
k_{PC-AOB}	Ammonia oxidizing bacteria (AOB) transformation coefficient	$\text{m}^3 \text{ g COD}^{-1} \text{ h}^{-1}$	0.000140	0.00005 $\pm$
			$\pm$	0.00003
			0.000012	
T_{PC-AOB}^c	Parent compound biotransformation coefficient rate linked to AOB growth (cometabolism)	$\text{m}^3 \text{ g COD}^{-1}$	0.012 $\pm$	0.00093 $\pm$
			0.000036	0.00049
$\mu_{max, AOB}$	Maximum specific growth rate of AOB	h^{-1}	0.012 $\pm$ 0.0023	

594

Figure 1. Model calibration with experimental data from atenolol biodegradation: (A) Experiment 1, with addition of allylthiourea (ATU); (B) Experiment 2, in the absence of ammonium; and (C) Experiment 3, in the presence of ammonium (50 mg $\text{NH}_4^+\text{-N L}^{-1}$).

Figure 2. Model validation results of atenolol biotransformation by the enriched nitrifying culture in the presence of ammonium of 25 mg-N L^{-1} (Experiment 4).

Figure 3. Model evaluation with experimental data from acyclovir biodegradation: (A) Experiment 1, with addition of allylthiourea (ATU), (B) Experiment 2, in the absence of ammonium and (C) Experiment 3, in the presence of ammonium (50 mg NH₄⁺-N L⁻¹).

Figure 4. (A) The relationship between ammonia oxidizing rate and the pharmaceutical degradation rates in terms of atenolol and acyclovir (black solid squares indicate the atenolol degradation rates after 240 h); and (B) The relationship between ammonia oxidizing rate and the acyclovir degradation rate after 240 h at a different linear fit slope.

Figure 5. Predicted final concentrations of (A) atenolol and atenolol acid and (B) acyclovir and carboxy-acyclovir at time of 240 h at different concentrations of dissolved oxygen (DO) in the enriched nitrifying culture system.

Figure 6. Predicted concentrations of pharmaceuticals and their transformation products at time of 240 h at initial concentrations of 15 $\mu\text{g L}^{-1}$ with different ammonium concentrations ranging from 0 to 100 mg-N L^{-1} at different DO levels.