

A mechanistic model for fate and removal of estrogens in biological nutrient removal activated sludge systems

M. Lust, J. Makinia and H. D. Stensel

ABSTRACT

Two estrogen fate and transformation models were integrated with a comprehensive activated sludge model (ASM) to predict estrogen removal based on biomass and solids production. Model predictions were evaluated against published full-scale plant data as well as results from a laboratory-scale sequencing batch reactor (SBR) fed synthetic wastewater. The estrogen fate model relating the rate of total estrogen degradation to soluble estrogen concentrations successfully predicted estrogen removals when compared with measured concentrations. Model fit 17α -ethinylestradiol (EE2) biodegradation rate constant was 19 to 43% of the estrone (E1) value and 31 to 72% of the 17β -estradiol (E2) value.

Key words | activated sludge, endocrine disrupting compound, estrogen, mathematical model

M. Lust

H. D. Stensel

Department of Civil and Environmental Engineering,
University of Washington,
201 More Hall, Box 352700,
Seattle, WA 98195,
USA

J. Makinia (corresponding author)

Faculty of Civil and Environmental Engineering,
Gdansk University of Technology,
ul. Narutowicza 11/12, 80-233 Gdansk,
Poland
E-mail: jmakinia@pg.gda.pl

INTRODUCTION

Wastewater treatment plant (WWTP) effluents are a primary source of endocrine-disrupting compounds in the environment. The majority of their endocrine-disrupting activity is from anthropogenic estrogen compounds, including synthetic estrogen, 17α -ethinylestradiol (EE2), and the natural estrogens, estrone (E1) and 17β -estradiol (E2). Influent wastewater concentrations may be in the range of 50–200 $\mu\text{g}/\text{m}^3$, and there is a need to understand design and operating conditions that can lead to minimal effluent concentrations as significant endocrine disruptor effects on fish have been found at EE2 concentrations below 1 $\mu\text{g}/\text{m}^3$ (Purdom *et al.* 1994). Numerous literature sources show that estrogen biodegradation occurs in full-scale biological nutrient removal (BNR) systems, but the removal efficiencies vary widely (10–98%), with EE2 being the most resistant to biodegradation and having the greatest endocrine disruptor activity (Combalbert & Hernandez-Raquet 2010; Muller *et al.* 2010).

Two conceptual models for the fate and transformation of estrogens in activated sludge treatment have been proposed by Joss *et al.* (2004) and Urase & Kikuta (2005). Both conceptual models based estrogen removals on biodegradation and sorption to solids; however, Joss *et al.* proposed sorption and biodegradation may occur in parallel while Urase and Kikuta proposed these processes occur sequentially with sorption followed by biodegradation. Joss

et al. also included cleavage of conjugated estrogens in their model resulting in production of free estrogens. Both Urase & Kikuta (2005) and Joss *et al.* (2004) applied the conceptual models to predict the fate of estrogens during batch experiments. In addition, Joss *et al.* modeled estrogen removals across full-scale WWTPs using Aquasim software. Integration of an estrogen fate model with a comprehensive activated sludge model (ASM) was performed by Monteith *et al.* (2008) using GPS-X software. Simulations predicted the fate of estrogens across a WWTP consisting of primary sedimentation and secondary activated sludge treatment (anaerobic, anoxic and aerobic bioreactors in series) and were based on estrogen biodegradation and sorption occurring in parallel. Biodegradation was modeled based on volatile suspended solids (VSS) concentrations.

In this paper, these modeling efforts have been extended by integrating estrogen removal mechanisms into a comprehensive ASM, which is important for two reasons. First, estrogen degraders are grown primarily on influent wastewater substrates as the amount of influent estrogen is too low to support a sufficient biomass to account for observed estrogen removal rates. Second, the amount of estrogen removed by solids partitioning is a function of the solids produced from site specific influent wastewater characteristics and operating conditions. Therefore, the aim of this work was (1) a comparison of

the reported conceptual models for estrogen removal based on the reported results of experimental studies, (2) review of kinetic expressions and parameter values for estrogen removal, and (3) development of a mechanistically-based mathematical model coupled with the IWA Activated Sludge Model No. 1 (ASM1) and its evaluation based on available data. An additional important advancement with this model effort is that the model accounts for the fact some of the estrogens in the influent wastewater can be in a conjugated form and deconjugation with the production of free estrogen can occur in the activated sludge process.

METHODS

Model development

Removal of micropollutant compounds from the liquid phase can be achieved through four possible pathways: biotic and abiotic degradation, adsorption onto solids and volatilization to the gas phase. Due to physico-chemical properties of the estrogens, their removal mainly occurs by adsorption/biosorption onto activated sludge flocs and biodegradation. Biodegradation of E1, E2 and EE2 by activated sludge comprise degradation of E1 and EE2 to unknown products and oxidation of E2 to E1 (fully or partially, with the remaining to an unknown product). Two conceptual models based on sorption and biodegradation occurring either in parallel (Figure 1(a)) or sequentially (Figure 1(b)) were considered. Biodegradation based on the parallel model related total estrogen degradation rates to soluble estrogen concentrations while the sequential model related total estrogen degradation rates to sorbed estrogen concentrations. Cleavage of conjugated estrogens

to free estrogens was also incorporated in the estrogen fate model (but is not shown in Figure 1).

The two conceptual models of the estrogen fate were written as a set of mathematical expressions and implemented in the GPS-X simulation software as an expansion of the ASM1. General kinetic expressions for the processes incorporated in the new models are presented in Table 1.

Definitions for terms shown in Table 1 are as follows: k_{cle} is the cleavage rate for conjugated estrogens (m^3/g CODd), S_{CE} is the conjugated estrogen concentration ($\mu g/m^3$), X_H is the active heterotrophic biomass concentration (g COD/ m^3), X_{VSS} is the VSS concentration (g/m^3), S_S is the readily biodegradable substrate concentration (g COD/ m^3), S_E is the soluble estrogen concentration ($\mu g/m^3$), X_E is the sorbed estrogen concentration ($\mu g/m^3$), S_O is the dissolved oxygen concentration (g O₂/ m^3), and S_{NO} is the sum of nitrite and nitrate concentrations (g N/ m^3). All other terms are described in Table 3.

Model calibration and validation

Model predictions were evaluated based on published data of a full-scale plant (Andersen et al. 2003) as well as our results from a laboratory-scale sequencing batch reactor (SBR). Descriptions of the WWTP and SBR operation and model calibration are provided below.

Full-scale WWTP in Wiesbaden (Germany)

The total volume of the activated sludge bioreactor was 63,000 m³ (1/3 and 2/3 occupied by the anoxic and aerobic compartments, respectively) and the process temperature

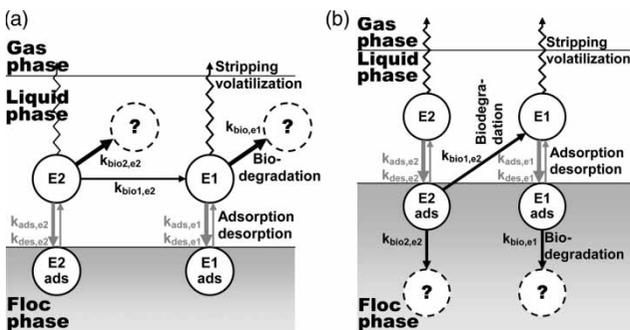


Figure 1 | Conceptual models of free estrogen (E1 and E2) removals in activated sludge systems based on (a) sorption and biodegradation occurring in parallel or (b) sorption and biodegradation occurring sequentially (EE2 model is similar to E1 except for not having any generation source).

Table 1 | General kinetic expressions for the processes incorporated in the new models

Process	Parallel model
Cleavage	$k_{cle}S_{CE}X_H$
Adsorption	$k_{ads} \left(\frac{K_{S,a}}{K_{S,a} + S_S} \right) S_E X_{VSS}$
Desorption	$k_{des} \left(\frac{S_S}{K_{S,d} + S_S} \right) X_E X_{VSS}$
Aerobic biodegradation	$k_{bio} \left(\frac{K_{S,b}}{K_{S,b} + S_S} \right) \left(\frac{S_O}{K_O + S_O} \right) S_E X_H$
Anoxic biodegradation	$\eta_{bio} k_{bio} \left(\frac{K_{S,b}}{K_{S,b} + S_S} \right) \left(\frac{K_O}{K_O + S_O} \right) \times \left(\frac{S_{NO}}{K_{NO} + S_{NO}} \right) S_E X_H$

The equations for the sequential model are the same except for biodegradation where S_E is replaced by X_E .

was 16–17 °C. The influent flow rate to the WWTP was 66,000 m³/d, whereas the return activated sludge (RAS) and mixed liquor recirculation (MLR) were about 50 and 200% of the inlet flow rate, respectively. During a 2-day measurement campaign, total estrogen concentrations were measured in samples of wastewater withdrawn as 24 h flow-proportional composite samples from the primary and secondary effluents. Sorbed and soluble estrogen concentrations were also measured in three grab samples withdrawn in the sampling points located inside the bio-reactor including the anoxic zones (ANOX1 and ANOX2) and aerobic zone (AER2). The studied models were calibrated based on these measurements. The operating parameters (SRT, MLSS and WAS load) are presented in Figure 2(a). More information about the studied WWTP can be found in Andersen *et al.* (2003) and Joss *et al.* (2004). Conjugated estrogens were not measured during the sampling campaign and were therefore not modeled for the full-scale WWTP.

Laboratory-scale SBR at the University of Washington, Seattle (USA)

A laboratory-scale SBR was operated at 20 °C at a 10-day aerobic SRT (13-day total SRT) with four cycles per day.

Each 6 h cycle consisted of a 1 h anoxic period followed by a 4.5 h aeration period. Feeding occurred during the first 5 min of the anoxic period. Acetate, propionic acid, peptone and casein were the main components of the synthetic wastewater feed to which 330 µg/m³ of E1 and E2 and 280 µg/m³ of EE2 were added. No conjugated estrogens were added to the synthetic feed. Estrogen concentrations in the feed and effluent were measured in duplicate as described by Gaulke *et al.* (2008) using LC-MS-MS analysis. Following operation to steady state, an *in situ* estrogen degradation test was conducted where the SBR was spiked to an initial concentration of 200 µg/m³ E1, E2 and EE2 and the total estrogen concentrations measured throughout the cycle. The data collected during this test were used for calibration of the studied models.

Simulation environment

GPS-X ver. 5.0.2 (Hydromantis, Canada) was used as a simulator environment for implementing the developed models and running simulations. For model calibration, a special utility called ‘Optimizer’ was used (parameters were estimated based on the Nelder-Mead simplex method with the maximum likelihood as an objective function).

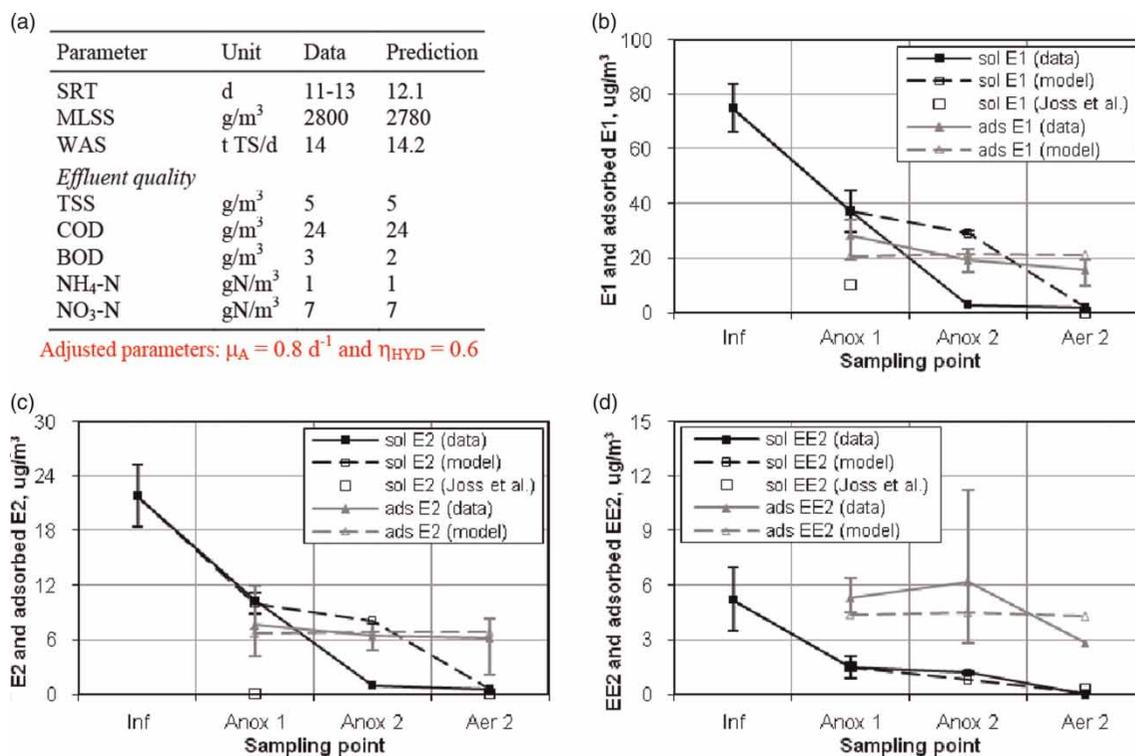


Figure 2 | Measured values versus steady-state model predictions for the published data from the Wiesbaden WWTP: (a) operating parameters and effluent concentrations and (b) E1, (c) E2 and (d) EE2 concentrations across the system. Soluble and sorbed estrogen concentrations are shown in black and gray, respectively. Measured values by Andersen *et al.* (2003) indicated by solid lines; predicted values based on parallel model indicated by dashed lines. Predictions of Joss *et al.* (2004) also shown for E1, E2 and EE2.

RESULTS AND DISCUSSION

Full-scale WWTP

Figure 2(a) shows the operating and effluent quality parameters and their predictions obtained with the calibrated ASM1 for the Wiesbaden WWTP (Andersen *et al.* 2003). Figure 2(b)–(d) illustrates the parallel model results predicting E1, E2 and EE2 concentration profiles in both liquid and floc phases for the same full-scale plant data of Andersen *et al.* (2003). Predictions by Joss *et al.* (2004) based on the same sampling campaign have also been included. Predicted sorbed estrogen concentrations based on the parallel model were similar to measured values; however, predicted soluble estrogen concentrations in the second anoxic zone (ANOX2) were relatively high for both E1 and E2 (the model could not explain these discrepancies). Model results showed most of the estrogen removed was via biodegradation (>90%).

Figure 3 compares the results from the calibrated estrogen fate models (parallel and sequential) for the Wiesbaden WWTP (Andersen *et al.* 2003). The two estrogen fate models produced similar predictions of soluble and sorbed E1, E2 and EE2 in the first anoxic zone (ANOX1) and aeration zone (AER2). However, the detailed analysis of mass balances revealed a different level of importance of the sorption process. For the sequential model, for which the mass balance is presented in Figure 4(b), the sorption rate is an order of magnitude higher compared with the parallel model (Figure 4(a)).

Laboratory-scale SBR

Batch simulations of the *in situ* SBR estrogen degradation test using the same modelling parameters used for the full-scale WWTP with the exception of modified η_{bio} values are shown in Figure 5 for the two estrogen models (parallel and sequential). The two models produced significantly

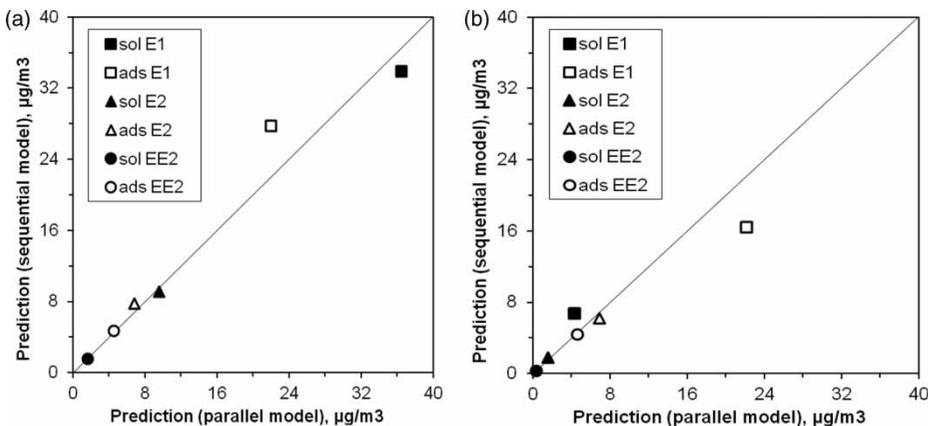


Figure 3 | Comparison of predicted estrogen concentrations in (a) the first anoxic zone (ANOX1) and (b) the aeration zone (AER2) of the Wiesbaden WWTP according to the sequential model versus the parallel model.

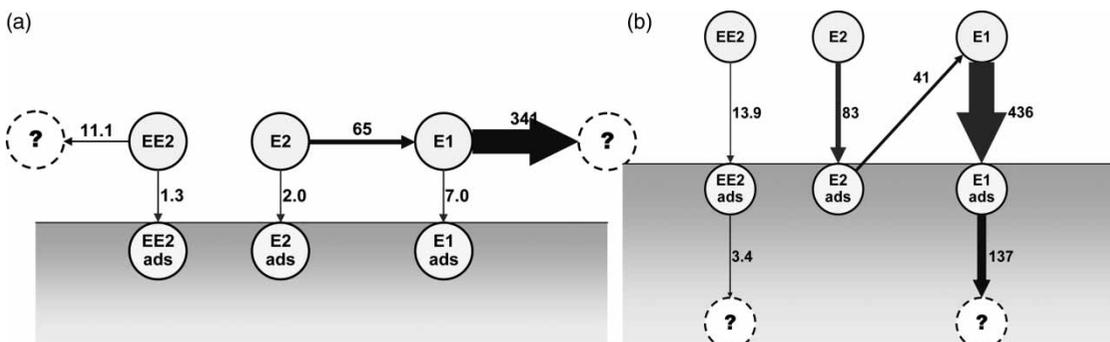


Figure 4 | Predicted estrogen mass flows ($\mu\text{g}/(\text{m}^3 \text{d})$) in the first anoxic zone (ANOX1) of the Wiesbaden WWTP according to (a) the parallel model and (b) the sequential model.

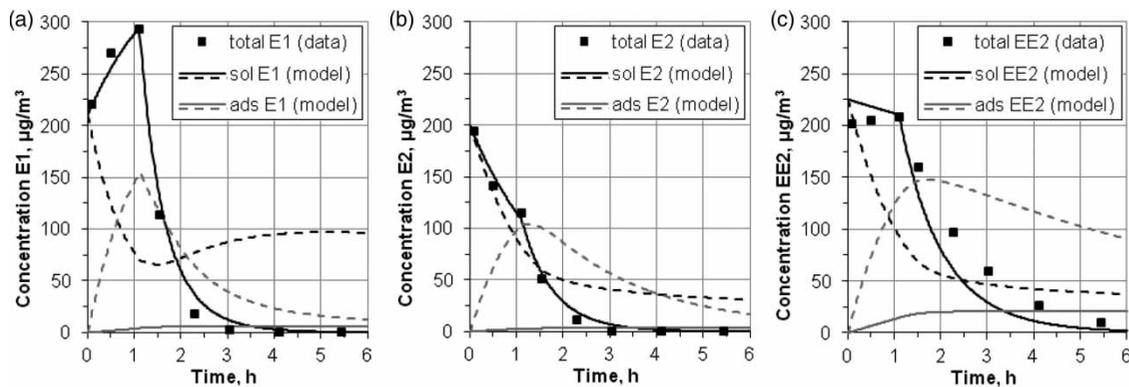


Figure 5 | Measured data versus model predictions for the anoxic/aerobic *in situ* SBR tests with 1 h anoxic followed by 4.5 h aeration: (a) E1, (b) E2, (c) EE2 (solid line – predictions of the parallel model, dashed line – predictions of the sequential model).

different predictions. The parallel model provided the best fit to actual measurements and was therefore selected as the model to use for future simulations.

A dynamic SBR simulation was performed with the parallel model over 10 days under steady-state operating conditions after which the estrogen feed concentrations were increased to model spiking of the *in situ* estrogen degradation test. Different estrogen modelling parameters were used for the dynamic SBR simulation than for the full-scale WWTP. The k_{ads} , k_{des} and $K_{S,a}$ values used for the full-scale WWTP resulted in predicted adsorbed estrogen concentrations being greater than the total measured estrogen concentrations when applied to the laboratory-scale SBR. The operating and effluent quality parameters and their predictions based on the dynamic SBR simulation are shown in Table 2. Figure 6 shows the modeled estrogen performance of the SBR along with the data from the *in situ* estrogen degradation test. A mass balance on model predictions showed biodegradation accounted for over 97% of the fate of the incoming estrogens.

Table 2 | Comparison of measured versus predicted operating parameters for laboratory-scale anoxic/aerobic SBR. Measured data (\pm one standard deviation) taken from period of one SRT prior to *in situ* estrogen degradation test

Parameter	Unit	Data	Prediction
Aerobic SRT	d	10	10
MLSS	g/m ³	1,185 \pm 61	1,193
MLVSS	g/m ³	1,056 \pm 54	1,062
<i>Effluent quality</i>			
NH ₄ -N	g N/m ³	<1	<1
NO ₃ -N	g N/m ³	10.8 \pm 3.4	10.7

Adjusted parameter: $b_H = 0.28 \text{ d}^{-1}$.

Modeling parameters

Table 3 lists the values used in the simulations conducted on both the Wiesbaden WWTP and laboratory-scale SBR. The only temperature dependent parameter was the biodegradation rate constant, k_{bio} , and the temperature correction factor, θ , was set to 1.03 for k_{bio} .

Fitting of the model to estrogen measurements from the laboratory-scale SBR *in situ* degradation test resulted in k_{bio} coefficients (normalized to biomass concentration) of 0.126, 0.078 and 0.024 m³/(g COD d) for E1, E2 and EE2, respectively. A lower but similar EE2 k_{bio} value of 0.020 m³/(g COD d) was determined based on fitting the model to measured EE2 effluent concentrations prior to spiking the SBR for the *in situ* test. All k_{bio} values were based on a 1:1 conversion of E2 to E1. Partial conversion of E2 to E1 would reduce the production of E1 and result in a lower estimated E1 k_{bio} value.

Adsorption and desorption rate constants of 0.021 and 0.044 m³/g d were applied to all estrogens for the laboratory-scale SBR model, achieving sorption near equilibrium within 0.5 h and reaching an equilibrium solid/liquid partitioning (K_D) coefficient of 0.45 m³/kg VSS within 5 h. This is in agreement with Andersen *et al.* (2005) who showed E1, E2 and EE2 sorption to activated sludge reached near equilibrium within 0.5 h with K_D coefficients of 0.40, 0.48 and 0.58 m³/kg solids, respectively.

Selection of $K_{S,a}$ of 10 g COD/m³ for the Wiesbaden WWTP simulations effectively reduced the adsorption rates by greater than 70%. Predicted solid/liquid partitioning (m³/kg solids) of estrogens in the first anoxic zone (ANOX1) were 0.2, 0.3 and 1.0 for E1, E2 and EE2, respectively. These respective values increased to 1.9, 1.6

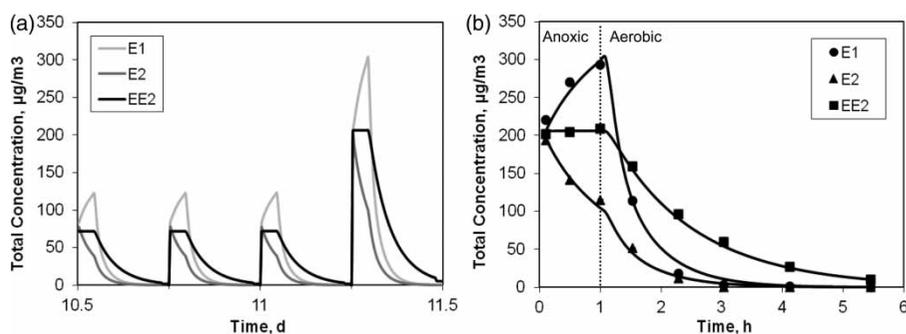


Figure 6 | Modeled estrogen performance of SBR (a) after 10 days of dynamic simulation followed by an increase in estrogen feed concentration to simulate estrogen spiking of the *in situ* estrogen degradation test (b) fitted to measured data. Markers correspond to estrogen measurements taken after spiking SBR to initial concentration of 200 µg/m³. Solid lines indicate predicted estrogen concentrations based on selection of k_{bio} values to fit *in situ* estrogen degradation test measurements.

Table 3 | List of kinetic parameters and their values at $T = 20^\circ\text{C}$ in the ASM1 extension for modeling estrogen removal (parallel model)

Symbol	Definition	Unit	Wiesbaden WWTP ^a			Laboratory-scale SBR		
			E1	E2	EE2	E1	E2	EE2
k_{ads}	Adsorption rate constant	m ³ /(g d)	0.0048	0.0053	0.002	0.021	0.021	0.021
k_{des}	Desorption rate constant	m ³ /(g d)	0.0002	0.0002	0.0002	0.044	0.044	0.044
$K_{\text{S,a}}$	Substrate half-saturation constant for adsorption	g COD/m ³	10	10	10	1,000	1,000	1,000
$K_{\text{S,d}}$	Substrate half-saturation constant for desorption	g COD/m ³	0	0	0	0	0	0
$K_{\text{S,b}}$	Substrate half-saturation constant for biodegradation	g COD/m ³	1,000	1,000	1,000	1,000	1,000	1,000
k_{bio}	Biodegradation rate constant	m ³ /(g COD d)	0.077	0.046	0.033	0.126	0.078	0.024
η_{bio}	Anoxic factor for biodegradation	–	0.19	0.29	0.5	0	0.24	0
K_{O}	Oxygen half-saturation constant for biodegradation	g O ₂ /m ³	0.2	0.2	0.2	0.2	0.2	0.2
K_{NO}	Nitrate half-saturation constant for biodegradation	g N/m ³	0.1	0.1	0.1	0.1	0.1	0.1

^aThe simulations were run at $T = 16.5^\circ\text{C}$.

and 4.8 in the aeration zone (AER2). For the laboratory-scale SBR simulations, predicted solid/liquid partitioning (m³/kg solids) of estrogens during the anoxic cycle ranged from 0.03 to 0.3 for E1, 0.03–0.5 for E2 and 0.04–0.4 for EE2. During the aerobic cycle, these respective values increased to 1.7, 1.5 and 0.6 m³/kg solids. Reported equilibrium solid/liquid partitioning coefficients (m³/kg solids), given as 95% confidence intervals, are 0.40 ± 0.13 for E1, 0.48 ± 0.19 for E2 and 0.58 ± 0.14 for EE2 (Andersen *et al.* 2005). Greatest deviation from equilibrium was predicted during aerobic treatment when estrogen concentrations were low and biodegradation rates were high indicating adsorption and desorption rates are important in predicting estrogen partitioning under these conditions.

Conjugated estrogens were not modeled for the full-scale WWTP as these were not measured during the WWTP sampling campaign by Andersen *et al.* (2003). Sampling of WWTPs in Germany showed conjugated estrogens comprised up to 50% of the total influent steroids

(Adler *et al.* 2001). Therefore, larger k_{bio} values would likely have been used in the model had production of free estrogens from deconjugation been included for the full-scale WWTP.

Model limitations and future research needs

Simulations conducted with the estrogen fate and transformation model highlighted many areas needing additional research. Model limitations and future research needs are summarized below.

- Estrogen degrader kinetics – An understanding of variations in estrogen degrading populations and kinetics due to different activated sludge process designs is needed to model biodegradation.
- Sorption and desorption rates – Sorption and desorption rates are needed to accurately model estrogen removals due to solids sorption.
- Substrate inhibition – Presence of readily biodegradable substrates may reduce sorption sites and/or binding sites

of enzymes available to the estrogens. The model currently has the ability to account for substrate inhibition of both sorption and biodegradation, however, substrate half-saturation constants are unknown.

- Deconjugation rates – Continuation of research addressing cleavage rates of conjugated estrogens under different operating conditions will lead to improvements in modeling production of free estrogens from deconjugation.
- Conversion fraction of E2 to E1 – Knowledge of the fraction of E2 removed via oxidation to E1 is needed to appropriately model the production and fate of E1.
- Temperature effects – A better understanding of the effects of temperature on sorption/desorption, biodegradation and deconjugation rates is needed to appropriately use the temperature correction factors included in the model.
- Calibration and WWTP sampling – Calibration of the model to a WWTP would require measurements of both free and conjugated estrogens at multiple points along the treatment train, preferably between staged reactors. Dynamic simulations would also require multiple samples to be taken over the course of a day. The feasibility associated with this sampling demand requires development of a careful sampling protocol.

CONCLUSIONS

Two estrogen fate and transformation models based on sorption and biodegradation occurring either in parallel or sequentially were integrated into the IWA ASM1. Both models were applied to published full-scale plant data as well as results from a laboratory-scale SBR, and the parallel model was selected as providing the best fit to measured estrogen concentrations. This model may also be applied for predicting the fate and removal of other micropollutants. Although this modeling effort lays a foundation for a prediction tool that may be used to evaluate different scenarios to optimize estrogen removals, many limitations of the model need to be addressed with further research.

ACKNOWLEDGEMENTS

This research was supported by graduate student fellowships awarded by the King County Department of

Natural Resources and Parks, Wastewater Treatment Division. This paper has not been formally reviewed by King County. The views expressed in this document are solely those of the authors. King County does not endorse any products or commercial services mentioned in this publication. During the time of the study, J. Makinia was a visiting professor at the Department of Civil and Environmental Engineering, University of Washington.

REFERENCES

- Adler, P., Steger-Hartmann, T. & Kalbfus, W. 2001 [Distribution of natural and synthetic estrogenic steroid hormones in water samples from southern and middle Germany](#). *Acta Hydrochim. Hydrobiol.* **29**, 227–241.
- Andersen, H., Siegrist, H., Halling-Sorensen, B. & Ternes, T. 2003 [Fate of estrogens in a municipal sewage treatment plant](#). *Environ. Sci. Technol.* **37**, 4021–4026.
- Andersen, H. R., Hansen, M., Kjølholt, J., Stuer-Lauridsen, F., Ternes, T. & Halling-Sorensen, B. 2005 [Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment](#). *Chemosphere* **61**, 139–146.
- Combalbert, S. & Hernandez-Raquet, G. 2010 [Occurrence, fate, and biodegradation of estrogens in sewage and manure](#). *Appl. Microbiol. Biotechnol.* **86**, 1671–1692.
- Gaulke, L. S., Strand, S. E., Kalthorn, T. F. & Stensel, H. D. 2008 [17 \$\alpha\$ -ethinylestradiol transformation via abiotic nitration in the presence of ammonia oxidizing bacteria](#). *Environ. Sci. Technol.* **42**, 7622–7627.
- Joss, A., Andersen, H., Ternes, T., Richle, P. R. & Siegrist, H. 2004 [Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization](#). *Environ. Sci. Technol.* **38**, 3047–3055.
- Monteith, H., Andres, H., Snowling, S. & Schraa, O. 2008 [Modeling the fate of estrogenic hormones in municipal wastewater treatment](#). In: *Proceedings of the 81st Annual WEF Technical Exhibition & Conference*, 18–22 October 2008, Chicago, USA, pp. 3477–3495.
- Muller, M., Patureau, D., Godon, J. J., Delgenes, J. P. & Hernandez-Raquet, G. 2010 [Molecular and kinetic characterization of mixed cultures degrading natural and synthetic estrogens](#). *Appl. Microbiol. Biotechnol.* **85**, 691–701.
- Purdom, C. E., Hardiman, P. A., Bye, V. J., Eno, N. C., Tyler, C. R. & Sumpter, J. P. 1994 [Estrogenic effects of effluents from sewage treatment works](#). *Chem. Ecol.* **8**, 275–285.
- Urase, T. & Kikuta, T. 2005 [Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process](#). *Water Res.* **39**, 1289–1300.