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Removal of Estrogens in Municipal Wastewater Treatment under Aerobic and Anaerobic Conditions: Consequences for Plant Optimization

ADRIANO JOSS,*,† HENRIK ANDERSEN,‡ THOMAS TERNES,§ PHILIP R. RICHLE,† AND HANSRUEDI SIEGRIST†

EAWAG, Ueberlandstrasse 133, 8600 Dübendorf, Switzerland, The Danish University of Pharmaceutical Sciences, Institute of Analytical Chemistry, Universitetsparken 2, 2100 Copenhagen, Denmark, and Bundesanstalt für Gewässerkunde, Am Mainzer Tor 1, 56068 Koblenz, Germany

The removal of estrogens (estrone E1, estradiol E2, and ethinylestradiol EE2) was studied in various municipal wastewater treatment processes equipped for nutrient removal. A biological degradation model is formulated, and kinetic parameters are evaluated with batch experiments under various redox conditions. The resulting model calculations are then compared with sampling campaigns performed on different types of full-scale plant: conventional activated-sludge treatment, a membrane bioreactor, and a fixed-bed reactor. The results show a >90% removal of all estrogens in the activated sludge processes. (Due to the analytical quantification limit and low influent concentrations, however, this removal efficiency represents only an observable minimum.) The removal efficiencies of 77% and ≥90% for E1 and E2, respectively, in the fixed-bed reactor represent a good performance in view of the short hydraulic retention time of 35 min. The first-order removal-rate constant in batch experiments observed for E2 varied from 150 to 950 d^{-1} for a 1 gSS L^{-1} sludge suspension. The removal efficiency of E1 and EE2 clearly depends on the redox conditions, the maximum removal rate occurring under aerobic conditions when E1 was reduced to E2. Sampling campaigns on full-scale plants indicate that the kinetic values identified in batch experiments (without substrate addition) for the natural estrogens may overestimate the actual removal rates. Although this paper does not give direct experimental evidence, it seems that the substrate present in the raw influent competitively inhibits the degradation of E1 and E2. These compounds are therefore removed mainly in activated sludge compartments with low substrate loading. Theoretical evaluation leads us to expect that diffusive mass transfer inside the floc (but not across the laminar boundary layer) appreciably influences the observed degradation rates of E1 and E2, but not of EE2.

Introduction

The presence of micropollutants in wastewater treatment plant (WWTP) effluents causing adverse effects in the environment has been extensively documented, mainly with regard to aspects linked to fish health (*1*−*7*). According to several studies, human estrogens (mainly estradiol E2 and estrone E1) and the artificial estrogen ethinylestradiol (EE2) are responsible for a significant part of the endocrine-disrupting effects seen in the aquatic environment (*8*−*10*).

It is known from batch experiments using actived sludge from an WWTP that steroid estrogens degrade slowly in WWTPs, E2 being oxidized to E1 in a few hours. E1 is then degraded over several hours, while EE2 degradation takes as much as a few days (*11*). Steroid estrogens are excreted from humans in the form of conjugates, mainly glucuronides and sulfates. The glucuronides are cleaved to steroid estrogens by actived sludge in a reaction that is complete in less than an hour. The fate of steroid estrogens during sewage treatment in real WWTPs has also been investigated. Matsui et al. (*12*) were probably the first to quantify a complete concentration profile through the treatment process using an immunoassay for measuring E2. An increase in immunoreactivity was observed toward the end of the primary treatment, which may be caused by conjugated estrogens being cleaved to free estrogens. The immunoreactivity was mainly reduced during the denitrification treatment step which was simultaneously the first step of the actived sludge treatment. Andersen et al. (*13*) recently investigated concentrations of steroid estrogens in both water and sludge in a south German WWTP by GC-MS-MS. The measured amount of E1 and E2 approximately doubled in the first step of the actived sludge treatment, which could be explained by cleavage of conjugated steroid estrogens. Analysis of steroid estrogens in both water and actived sludge in the three steps of the actived sludge treatment showed that estrogens were mainly degraded during denitrification.

In this paper, the removal of estrone (E1), estradiol (E2), and ethinylestradiol (EE2) in sludge from a municipal WWTP with nitrogen removal (nitrification/denitrification) is investigated in spiked batch experiments. A biological degradation model is proposed and discussed with sampling campaigns on full-scale WWTPs.

Materials and Methods

Batch experiments were performed in stirred 8-L vessels (Figure 1) equipped with an oxygen sensor and a small bubble aeration unit for automatic O_2 concentration control (LabView application, National Instruments, Austin TX). For the aerobic experiments, air was used to regulate the O_2 concentration in solution between 2 and 3 mgO_2 L^{-1}. During anaerobic experiments, N_2 gas (impurities: $<10^{-5}$, flow rate: 1 L h^{-1}) was used to strip out O_2. Sludge from the conventional activated sludge treatment plant in Kloten/Opfikon (CAS-K, see below) and from a membrane bioreactor pilot plant (MBR, see below) was diluted to approximately 0.3 gSS L^{-1} with treated effluent of the respective WWTP, prior to the experiment and kept in the batch for 2 h before the starting of the experiment. pH was kept in the range of 7.0−8.5 by means of manual adjustment with an addition of 1 N H_2SO_4. The anoxic batch was supplemented with an initial nitrate concentration of 20 mg NO_3−N L^{-1} by stocking up with $NaNO_3$ solution. Depending on the experiment, E1 (aerobic experiments) or E2 (anaerobic experiments) were spiked with an initial concentration of approximately 500 ng L^{-1}, while EE2 was always spiked to 100 ng L^{-1}.

* Corresponding author phone: +41 1 823 54 08; fax: +411 823 53 89; e-mail: adriano.joss@eawag.ch.
† EAWAG.
‡ The Danish University of Pharmaceutical Sciences.
§ Bundesanstalt für Gewässerkunde.

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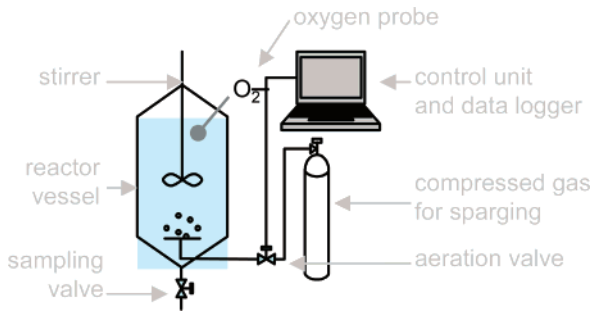

FIGURE 1. Batch reactor setup. Aeration was performed with air or nitrogen for the aerobic and anaerobic experiments, respectively.

Full-scale monitoring was performed at two different locations: at the WWTP of Kloten/Opfikon (Figure 2), a conventional activated sludge (CAS-K) treatment plant is run in parallel with a pilot-scale membrane bioreactor (MBR), and at the WWTP of Altenrhein (Figure 3) a lane of a conventional activated sludge plant (CAS-A) is run in parallel with a fixed-bed reactor (FB).

The CAS-K of Kloten/Opfikon handles 55 000 population equivalents (PE) and is equipped for nutrient removal and is operated with a solid retention time (SRT) of 10−12 days. The plant treats the municipal wastewater from a combined sewer. Primary treatment consists of a screen, an aerated grit-removal tank, and a primary clarifier. The primary effluent is directed to the activated sludge system for denitrification and nitrification. The secondary effluent is discharged to the receiving water after sand filtration. Phosphorus removal is achieved by simultaneous precipitation with Fe^{3+}. The MBR is a 100-PE pilot fed with primary effluent from the CAS-K at a flow rate proportional to the raw wastewater inlet. The pilot is run with a SRT of 30 d and equipped with stirred anaerobic and anoxic compartments followed by aerobic filtration compartments. Three different membrane filtration units of standard design are run in parallel for test purposes, each operated at a maximal flow rate of 1.3 $m^3 h^{-1}$: a microfiltration plate membrane module of type Kubota A50 (40 m^2 membrane surface, 0.4 μm nominal pore size), an ultrafiltration hollow-fiber module of type Mitsubishi Aqua-RM (82 m^2 membrane surface, 0.1 μm nominal pore size), and an ultrafiltration hollow-fiber module of type Zenon ZeeWeed 500-C (46 m^2 membrane surface, 0.04 μm nominal pore size).

The Altenrhein WWTP treats mixed sewage of 120 000 PE: the conventional and fixed-bed lanes each treat half of the incoming wastewater (combined sewer). The primary

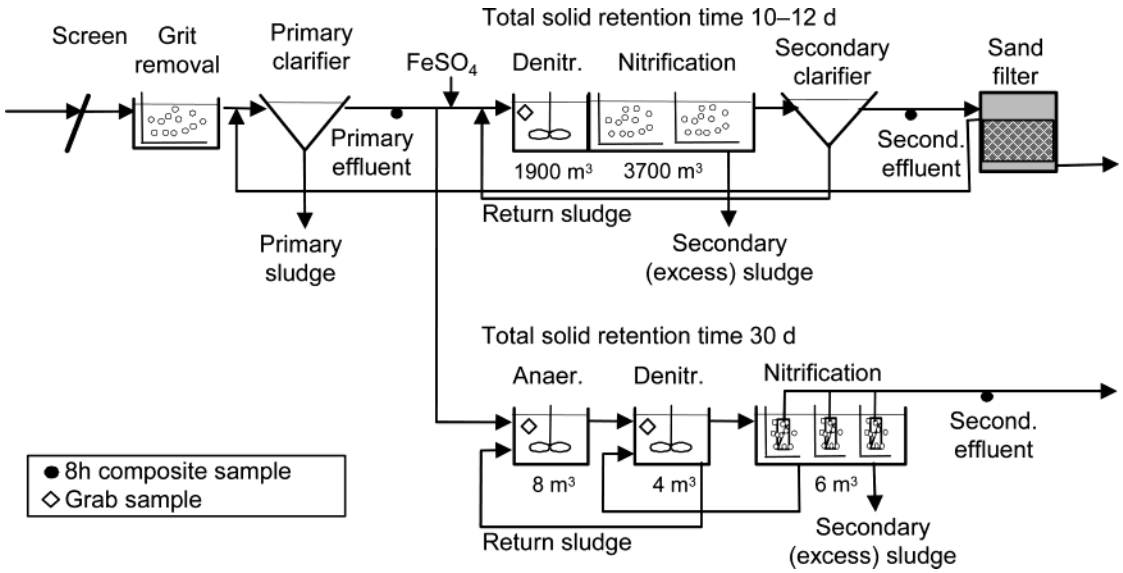

FIGURE 2. Flow scheme of the municipal WWTP of Kloten-Opfikon (CAS-K, 70 000 PE) and of the membrane bioreactor pilot (MBR, 100 PE) with sampling locations.

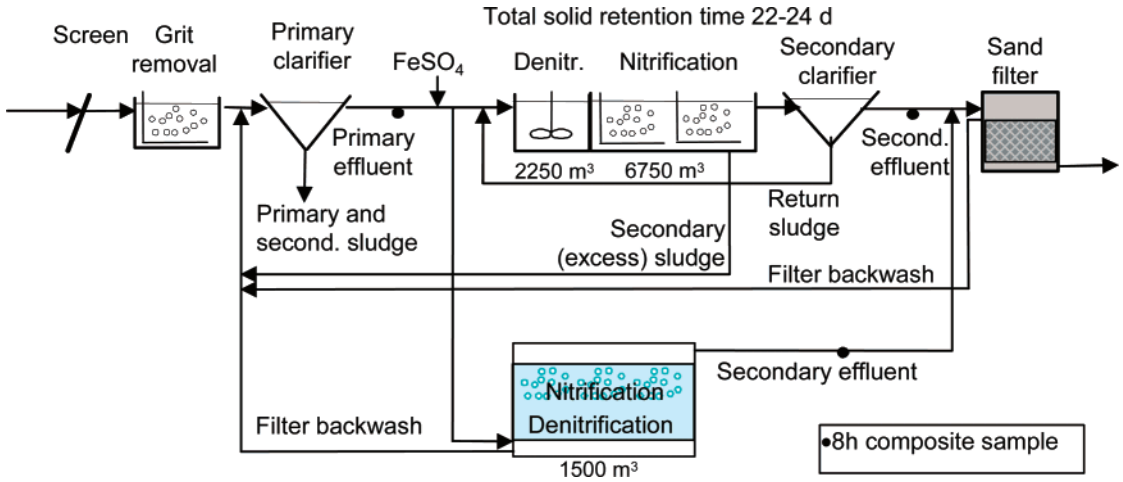

FIGURE 3. Flow scheme of the municipal WWTP of Altenrhein (CAS-A and FB, 60 000 PE each), where a conventional lane and a fixed-bed reactor each treat 50% of the wastewater.

treatment is common to both lanes and consists of a screen, an aerated grit-removal tank, and a primary clarifier. The CAS-A lane is equipped with denitrification and nitrification compartments and is operated at a SRT of 22−24 d. The FB consists of 8 Biostyr (*14*) cells each with a reactor volume of 190 m^3: the wastewater flows upward through a bed composed of 3.6 mm Styrofoam balls with an average hydraulic retention time of 35 min and a biofilm surface of ~600 $m^2\ m^{-3}_{reactor}$. Excess sludge is removed by daily backwash of the fixed bed. The biologically treated wastewater from both the conventional activated-sludge and fixed-bed lanes is mixed and filtered through sand before being discharged to the receiving river.

The full-scale plants were sampled between November 12 and 14, 2002 (CAS-K and MBR) and between November 20 and 22, 2002 (CAS-A and FB). Only minor rain events occurred during both sampling campaigns. Six 8-h samples were taken from each sampling point during a total of 48 h: composite samples were taken at the influent and effluent, while grab samples were taken at 8-h intervals from the anaerobic and anoxic compartments.

Sampling of Batch Experiments. For the batch experiment, 200 mL samples were acidified to pH 3.0 ± 0.1 by the addition of H_2SO_4. Each sample was spiked with 100 ng of E2 acetate as an internal standard prior to SPE extraction on Separtis Isolute C18 cartridges (500 mg/6 mL). The sludge particles in the samples were also transferred to the top of the SPE column so that sorbed estrogens were desorbed during elution of the SPE cartridges. Conditioning and elution of the SPE columns as well as cleanup of the extracts by a silica gel before derivatization and analysis by GC-MS-MS were performed as previously described by Ternes et al. (*6*, *11*). Blank controls and standards for calibrations were made by spiking tap water samples. Recovery experiments were run by spiking the estrogens into acidified samples from the batch reactors taken before addition of the estrogens to the reactors. The limit of quantification (LOQ) of the method was 5 ng L^{-1} for each estrogen. The calibration curve was linear from 2.5 ng L^{-1} to 1000 ng L^{-1}. The absolute recoveries determined with estradiol-acetate as the surrogate standard was 70% ± 10% (n=15).

Sampling of Full-Scale Plants. Twenty-four hour flow proportional composite samples from full-scale plants (1000 mL) were collected online in glass bottles containing enough H_2SO_4 to reach pH 3.0 ± 0.1. Grab samples of 1000 mL were acidified within 15 min of collection to pH 3.0 ± 0.1 by the addition of H_2SO_4. Both types of samples were filtered (Schleicher&Schuell GF6 glass fiber filter) and spiked with 100 ng E2 acetate as an internal standard before extraction and analysis. Extracts of samples taken from the influent and the grab samples were further cleaned by GPC (Gel Permeation Chromatography) as described by Andersen et al. (*13*). Filtration and solid-phase extraction (SPE) were performed after up to 12 h of storage at 4 °C. Conditioning and elution of the SPE columns, as well as cleanup of the extracts by a silica gel before derivatization and analysis by GC-MS-MS, were performed as previously described by Ternes et al. (*6*, *11*). Absolute recoveries varied between 21 and 73% in influent samples and between 52 and 119% in samples from effluent and the CAS effluents. Spike recoveries using the correction for surrogate standard gave recoveries between 93 and 119%, which is consistent with the relative standard deviations of 15% for effluent samples and 20% for influent samples that was found in the original description of the methods (*6*, *13*).

Estrogens in sludge were measured in grab samples taken from the aerobic zone of the plants. After freeze-drying and extraction twice with methanol and subsequently twice with acetone the samples were measured as described in ref *15*.

Blank controls and standards for quantification were made by spiking tap water samples. Recovery experiments were run by spiking the estrogens into acidified and filtered samples. The LOQ of the method was 1 ng/L for each estrogen. The calibration curve was linear from 0.5 ng L^{-1} to 250 ng L^{-1}.

Limit of Quantification for Batch Experiments. For the experimental set up, the limit of quantification for the kinetic parameters was estimated with a blank test performed without sludge and spiked with 100 ng L^{-1} EE2: removal of 25% ± 12% from the solution within 24 h was observed. Assuming a first-order kinetic for the observed abiotic removal (possibly due to chemical instability, sorption to walls or stripping), the limit of quantification for the kinetic parameters measured in batch reactors with 0.3 gSS L^{-1} was estimated to be 1 ± 0.5 L $gSS^{-1}\ d^{-1}$

$$k_{bio,LOQ} = \frac{\ln\left(\frac{C_{w,blank,0}}{C_{w,blank}(t)}\right)}{SS_{batch} \cdot t} \quad (1)$$

where $k_{bio,LOQ}$ is the limit of quantification for kinetic parameter in nonblank batch experiment [L $gSS^{-1}\ d^{-1}$], $C_{w,blank,0}$ is the initial soluble concentration [ng L^{-1}], $C_{w,blank}(t)$ is the soluble concentration at time t [ng L^{-1}], SS_{batch} is the sludge concentration during nonblank experiment [gSS L^{-1}], and t is time [d].

Results and Discussion

Sorption onto Sludge. According to Weber (*16*), there are essentially three consecutive steps in the adsorption of materials from solution by porous adsorbents: diffusion across a surface film (boundary layer), internal diffusion within the pores of the adsorbent, and adsorption to the interior surface. The adsorption process itself is assumed not to be rate-limiting (*16*).

The net mass flux from the soluble phase to the solid sludge particle (sorption) can be expressed as

$$r = -k_{sor} \cdot SS \cdot (C_{w,bulk} - C_{w,floc}) \quad (2)$$

where k_{sor} is the pseudo-first-order sorption rate constant [L $gSS^{-1}\ d^{-1}$], $C_{w,bulk}$ is the soluble estrogen concentration in the bulk liquid phase [g L^{-1}], and $C_{w,floc}$ is the average soluble estrogen concentration inside the floc [g L^{-1}].

Under equilibrium conditions, the distribution coefficient K_D can be measured as follows

$$K_D = \frac{C_{s,floc}}{C_{w,floc}} = \frac{C_{s,reactor}}{SS \cdot C_{w,floc}} \quad (3)$$

where K_D is the distribution coefficient [L gSS^{-1}], $C_{s,floc}$ is the sorbed estrogen amount per amount of solid matter [g gSS^{-1}], $C_{w,floc}$ is the soluble concentration in equilibrium with the sorbed concentration [g L^{-1}], and $C_{s,reactor}$ is the sorbed estrogen amount per reactor volume [g L^{-1}].

Since $C_{w,floc}$ (eq 3) is in equilibrium with the sorbed species, algebraic substitution of eq 3 into eq 2 allows the net mass transfer from the bulk liquid to the suspended solids (and back) to be expressed as

$$r = -k_{sor} \cdot \left(SS \cdot C_{w,bulk} - \frac{C_{s,reactor}}{K_D}\right) \quad (4)$$

Andersen et al. (*13*) have published profiles of steroid estrogen concentrations in a full-scale wastewater treatment plant comparable to those sampled in this study (WWTP Wiesbaden, nutrient-eliminating activated sludge plant run with sludge aged 11−13 d). Since sorbed and soluble estrogen

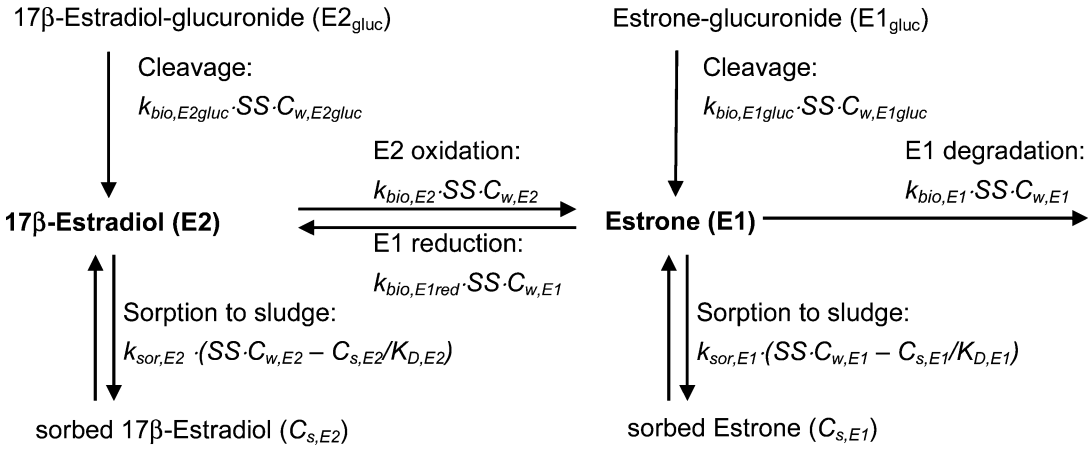

FIGURE 4. Model description of biological degradation and sorption of the natural estrogens E1 and E2. Abbreviations: k_{bio} and k_{sor}, pseudo-first-order reaction rate constant; *SS*, suspended solids; C_w, bulk soluble concentration; C_s sorbed concentration per reactor volume; K_D, sorption coefficient.

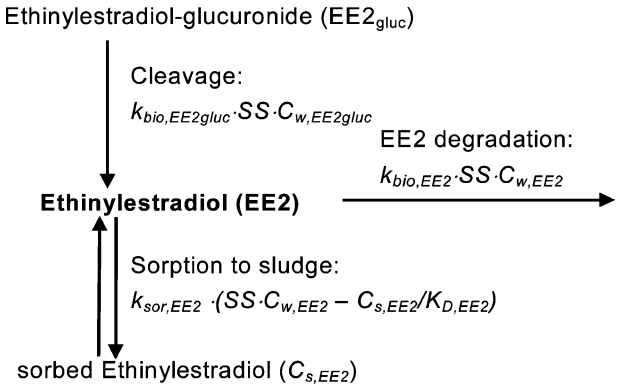

FIGURE 5. Model description of biological degradation and sorption of ethinylestradiol EE2. (Abbreviations see Figure 4).

TABLE 1. Results from the Dynamic Sorption Kinetic Model (Eq 4) with Data from the Wiesbaden WWTP (*13*)

variable	unit	value
$k_{E1,sor}$	L gSS^{-1} d^{-1}	4.1 ± 0.8
$K_{D,E1}$	L gSS^{-1}	0.9 ± 0.1

concentrations were measured in simultaneous grab samples, these data allow the sorption kinetic to be modeled accordingly (see model Figures 4 and 5). The results are shown in Table 1.

No quantitative information on sorption can be gained for E2 and EE2 since the data are too close to their analytical limit of quantification (1 ng L^{-1}). Similar sorption characteristics are nevertheless assumed, since the relationship between K_{OW} and K_D can be regarded as linear (*17*) and K_{OW} is in the same range for E1, E2, and EE2 (log(K_{OW}): 4.0, 4.1, and 4.2 (*18*)).

A K_D value of 0.35 ± 0.04 L gSS^{-1} was measured for radioactive-labeled EE2 at a concentration of 1.7 μg L^{-1} after 0.5, 2, and 14 h of incubation (*19*). Layton et al. (*20*) also observed a similar K_D value (0.26 ± 0.17 L gSS^{-1}) for E2 at a concentration of 58 μg L^{-1} (measured with inactivated sludge and radioactive E2). It is therefore concluded that a K_D value higher by a factor of 2−3 apply at concentrations of 1−50 ng L^{-1} (full-scale plants, Table 1) and ≥1.7 μg L^{-1} (measurements with radioactive E2 in batch experiments (*19*)). This corresponds to the observations of Ying et al. (*21*), who found a logarithmic dependence of the sorption modeled with the Freundlich equation.

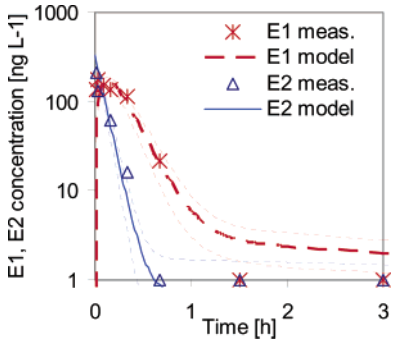

FIGURE 6. Measured and modeled E1 and E2 concentrations during an aerobic batch experiment with MBR sludge (0.3 gSS L^{-1}; temperature 16 ± 1 °C). Light lines indicate 95% confidence interval.

Furthermore, the time indications of the mentioned radioactive-labeled sorption experiment with EE2 allow a minimum value to be calculated for the sorption kinetic ($k_{EE2,sor}$) in batch reactors (according to the first-order kinetic model for sorption and back reaction of Figures 4 and 5; experiments performed with 4.0 gSS L^{-1}; formula derivation, see eq 4 Schwarzenbach et al. (17, pp 473−474) with $k_1 = k_{sor}$·SS and $k_2 = k_{sor}/K_D$)

$$C_s(t) = C_{s,eq} + (C_{s,0} - C_{s,eq})\cdot e^{-k_{sor}\cdot(SS-(1/KD))t} \qquad (5)$$

where $C_s(t)$ is the amount of sorbed compound per reactor volume as a function of time [ng L^{-1}], $C_{s,eq}$ is the amount of sorbed compound per reactor volume in equilibrium with the soluble concentration [ng L^{-1}], $C_{s,0}$ is the initial amount of sorbed compound per reactor volume [ng L^{-1}], k_{sor} is the kinetic constant of the sorption process [L gSS^{-1} d^{-1}], and K_D is the solid water distribution coefficient [L gSS^{-1}].

Since the sorption equilibrium reached at least 90% in the batch experiment after 0.5 h, a value of ≥40 L gSS^{-1} d^{-1} is assumed for k_{sor}.

For the modeling presented here, the K_D and k_{sor} values fitted to the full-scale plant at Wiesbaden were used (Table 1). In any case, sensitivity analysis shows that this modeling is insensitive to the partitioning of estrogens onto sludge by sorption due to the low sludge production and relatively low K_D value (Figure 9). According to the estrogen concentration measured in sludge (Table 2) and the observed sludge production (ca. 0.2 gSS L$^{-1}_{\text{influent wastewater}}$) the total estrogen load associated with the excess sludge is expected in the range of <0.5 ng L$^{-1}_{\text{influent wastewater}}$ (these measured value represents $C_{w,floc} + C_s$).

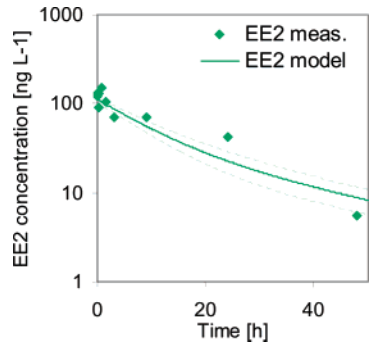

FIGURE 7. Measured and modeled EE2 concentrations during an aerobic batch experiment with MBR sludge (0.3 gSS L^{-1}; temperature 16 ± 1 °C). Light lines indicate 95% confidence interval.

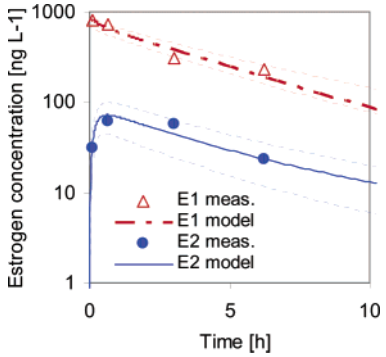

FIGURE 8. Measured and modeled data of the anaerobic batch experiment showing the formation and subsequent removal of E2 in the absence of oxygen and nitrate. Since only E1 was spiked, the initial E2 concentration was zero. Light lines indicate 95% confidence interval.

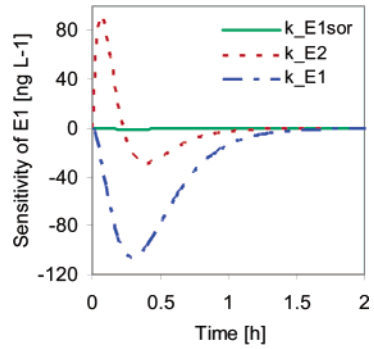

FIGURE 9. The modeled soluble E1 in the batch experiments is much more sensitive to kinetic than to sorption parameters (sensitivity expressed as an absolute signal change for 100% variation of the parameter).

TABLE 2. Estrogen Concentrations Measured in Sludge

	sludge concentration		
plant	E1	E2	EE2
CAS-Kloten	<2 ng gSS^{-1}	2.1 ng gSS^{-1}	2.4 ng gSS^{-1}
CAS-Altenrhein	<2 ng gSS^{-1}	<2 ng gSS^{-1}	<2 ng gSS^{-1}

Internal and External Mass Transfer. Mass transfer by passive diffusion inside the activated sludge floc (internal) as well as across the laminar boundary layer on the floc surface (external) may be relevant to substances that are degraded inside the floc or on its surface.

No information is so far available as to where the various estrogen degradation reactions take place: throughout the floc, on the floc surface (e.g. outer biofilm layer), or in the bulk medium (e.g. catalyzed by extracellular enzymes).

The importance of diffusive mass transfer is estimated in Appendix 1 (see Supporting Information). One important parameter for this is the floc size, which was measured to be 10−100 μm in membrane bioreactors and 100−500 μm for conventional sludge (*22*−*24*). The thickness of the boundary layer is estimated to be 10−20 and 20−30 μm for flocs of membrane and conventional sludge, respectively. Estrogen concentration gradients are estimated by assuming spherical sludge geometry. The estimation allows the conclusion that diffusive mass-transfer resistance across the laminar boundary layer has no major quantitative impact on the observed degradation rate. It therefore follows that the rheological conditions prevailing during the degradation experiments do not affect the observed removal rates. Mass transfer within the floc is relevant for pseudo-first-order rates ≥ 100 L gSS^{-1} d^{-1}. The trend toward higher removal rates in membrane sludge compared to conventional activated sludge (Table 3) may therefore be explained by the smaller floc size (80 vs 400 μm).

Biological Degradation Model. A pseudo-first-order kinetic is assumed for the modeled processes depending on a rate constant k_{deg}, the soluble substance concentration, and the sludge concentration (eq 1, Figures 4 and 5). The latter (or a quantity proportional to it such as the exoenzyme concentration) is assumed to be constant during the experimental observations

$$r = - k_{bio} \cdot SS \cdot C_{w,bulk} \qquad (6)$$

where r is the reaction rate [ng L^{-1} d^{-1}], k_{bio} is the pseudo-first-order constant for biological degradation [L gSS^{-1} d^{-1}], SS is the sludge concentration [gSS L^{-1}], and $C_{w,bulk}$ is the soluble estrogen concentration in the bulk liquid phase [ng L^{-1}].

The relevant reactions are as follows: (1) cleavage of the conjugates (glucuronides and sulfates), (2) oxidations of E2 to E1, (3) reduction of E1 to E2 under anaerobic conditions (see below), (4) degradation of E1 to an unknown product, and (5) degradation of EE2 to an unknown product.

With regards to the amount of conjugated estrogens (mainly as glucuronides but also as sulfonates) in the influent, the assumptions of Adler et al. (*25*) are used in the modeling: a conjugated fraction of 58%, 50%, and 26% is assumed for E1, E2, and EE2, respectively. D'Ascenzo et al. (*26*) found a somewhat smaller amount of conjugates in the influent. In our analytical set up, only the nonconjugated fraction is actually measured.

The rate constant of glucuronide cleavage can be assumed to be 25 L gSS^{-1} d^{-1} (according to the batch measurement (*11*)). The model calculation is insensitive to this parameter.

The biological degradation models (Figures 4 and 5) combined with the different plant setup (Figures 1−3) were implemented in the Aquasim 2.0 software (*27*).

Biological Removal Observed in Batch Experiments. To observe the biological estrogen degradation under different redox conditions, batch experiments were performed with sludge from either CAS-K or MBR. The kinetic pseudo-first-order constants fitted according to the model in Figures 4 and 5 are listed in Table 3. See also Figures 6 and 7 for a typical degradation experiment.

For the natural estrogens E1 and E2, degradation activity is seen to be higher in the MBR sludge than in the CAS-K sludge by a factor of 2−3. The sludge age and the size of the sludge flocs may contribute to this difference in activity. The higher age of the sludge processed in the membrane reactor (30 d as compared to 11 d in the conventional system) allows

TABLE 3. Kinetic Values Fitted According to the Models in Figures 4 and 5 onto the Batch Experiments with Sludge Originating from Conventional Activated Sludge (CAS-K) and from a Membrane Bioreactor (MBR)[a]

experiment	sludge type	sludge age, d	SS, gSS/L	$k_{bio,E1}$, L/(gSS d)	$k_{bio,E1red}$, L/(gSS d	$k_{bio,E2}$, L/(gSS d)	$k_{bio,EE2}$, L/(gSS d)
batch control (LOQ)	no sludge		0.0				1 ± 0.5
batch aerobic	CAS Kloten	12	0.30	162 ± 25		350 ± 42	8 ± 2
batch anoxic	CAS Kloten	12	0.30	30 ± 10		460 ± 60	1.2 ± 0.3
batch anaerobic	CAS Kloten	12	0.30	10 ± 1	52 ± 2	175 ± 10	
batch aerobic	membrane BR	30	0.29	430 ± 55		950 ± 120	6 ± 1
batch anoxic	membrane BR	30	0.27	115 ± 30		280 ± 50	3 ± 2
batch anaerobic	membrane BR	30	0.22	28 ± 3	60 ± 15	500 ± 200	1.5 ± 0.5

[a] Temperature: 16 ± 1 °C.

TABLE 4. Measured and Modeled E1, E2, and EE2 Concentrations[a]

			influent		first compartment		effluent		removal	
plant	temp, °C	sludge age, d	composite sample, ng L^{-1}	load (incl. conjugates), μg m^{-3} d^{-1}	grab sample, ng L^{-1}	model, ng L^{-1}	composite sample, ng L^{-1}	model, ng L^{-1}	observed (incl. conjugates), %	model (incl. conjugates), %
					Estrone (E1)					
CAS Altenrhein	15−16	21	7.3 ± 1.5	37 ± 8	na	na	8.6 ± 1.7	≤0.1 (3.7 ± 0.9)	49 ± 15	≥98.5 (79 ± 6)
CAS Kloten	15−16	12	24 ± 5	310 ± 64	32 ± 6	8.3 ± 3.3 (20 ± 8)	2.4 ± 0.5	0.3 ± 0.1 (2.3 ± 0.6)	96 ± 1	98 ± 1 (94 ± 1)
CAS Wiesbaden[b]	16−17	12	75 ± 15	192 ± 40	37 ± 8	10 ± 3 (32 ± 8)	0.5 ± 0.1	≤0.1 (≤0.1)	≥99	≥99.8 (≥99.8)
membrane BR	15−16	30	25 ± 5	170 ± 35	25 ± 5	3.1 ± 0.7 (24 ± 6)	2.4 ± 0.5	<0.1 (0.12 ± 0.03)	96 ± 1	≥99.5 (≥99.5)
fixed bed	15−16	na	7.3 ± 1.5	202 ± 42	na	na	2.4 ± 0.5	na	90 ± 3	na
					17β-Estradiol (E2)					
CAS Altenrhein	15−16	21	4.9 ± 1.0	19 ± 4	na	na	1.0 ± 0.2	≤0.1 (0.6 ± 0.2)	88 ± 9	≥98 (94 ± 2)
CAS Kloten	15−16	12	7.6 ± 1.5	90 ± 19	2.6 ± 0.5	0.2 ± 0.1 (5.8 ± 2.3)	≤0.5	≤0.1 (0.1)	≥97	≥98.5 (98 ± 1)
CAS Wiesbaden[b]	16−17	12	11 ± 2	23 ± 5	10 ± 2	0.1 (4.0 ± 1)	0.5 ± 0.1[b]	≤0.1 (0.1)	98 ± 1	≥99 (≥99)
membrane BR	15−16	30	6.3 ± 1.3	37 ± 8	4.7 ± 0.9	≤0.1 (5.1 ± 1.2)	≤0.5	≤0.1 (≤0.1)	≥98	≥98 (≥98)
fixed bed	15−16	na	4.9 ± 1.0	95 ± 20	na	na	≤0.5	na	≥95	na
					17α-Ethinylestradiol (EE2)					
CAS Altenrhein	15−16	21	0.7 ± 0.2	1.9 ± 0.4	na	na	≤0.5	≤0.1	71 ± 9	≥95
CAS Kloten	15−16	12	4.3 ± 0.9	26 ± 5	1.7 ± 0.3	2.2 ± 0.9	≤0.5	0.9 ± 0.3	94 ± 2	82 ± 5
CAS Wiesbaden[b]	16−17	12	5.2 ± 1.0	7.6 ± 1.6	1.5 ± 0.3	1.5 ± 0.4	0.5 ± 0.1[b]	0.3 ± 0.1	≥93	96 ± 1
membrane BR	15−16	30	1.6 ± 0.3	6.7 ± 1.4	≤0.5	0.8 ± 0.2	≤0.5	0.13 ± 0.03	≥75	94 ± 1
fixed bed	15−16	na	0.7 ± 0.2	12 ± 2	na	na	0.5 ± 0.1	na	69 ± 9	na

[a] Because the effluent concentrations are at or below the analytical quantification limit for estrogens, most of the observed removal rates (marked with "≥") represent only an observable lower limit of the actual value. The abbreviation "na" means "not available". Figures in brackets indicate the model calculations obtained assuming that the degradation of E1 and E2 occurs only in the last (aerobic) compartment due to substrate inhibition in the preceding compartments (see text below). Volumetric reactor load and removal rates include the conjugates (conjugate amounts in the influent: 58%, 50%, and 26% for E1, E2, and EE2 respectively, according to ref *25*). The removal is calculated according to the load. For the accuracy estimation the following assumptions were made: analytical accuracy ±20%; flow measurements ±5%; accuracy of the kinetic constants as reported in Table 3. [b] An average of three samples were taken in the Wiesbaden CAS (*13*). The composite sample was taken over 24 h, while the grab samples were taken between 9 and 11 a.m. (i.e. at a time when the daily estrogen peak load is seen in the influent, although it will take another 6 h to reach the secondary settling tank).

the growth and higher accumulation of specialist-degrading estrogens. The difference in microbial populations between membrane-bioreactor and conventional activated-sludge systems is well documented (*28*) and supports this hypothesis. Floc size may be another relevant factor causing the difference between the two processes (*22*−*24*): the flocs of a membrane bioreactor have a characteristic size of 10−100 μm as compared to 100−500 μm for conventional sludge. The smaller average distance between microorganisms and the floc surface imply shorter distances to be overcome within the floc by diffusion. Furthermore, according to the estimation in Appendix 1 (see Supporting Information), the floc surface per unit reactor volume is higher in MBR than in CAS systems by a factor of 10 (8 gSS L^{-1} and 3.5 gSS L^{-1}, respectively).

The degradation of estrone ($k_{bio,E1}$) takes place under all redox conditions but at significantly different rates: an increase by a factor of between 3 and 5 is observed in the transition from anaerobic to anoxic (nitrate available but no molecular oxygen) as well as between anoxic and aerobic (O_2 available in solution).

The oxidation of estradiol ($150 < k_{bio,E2} < 1000$) was observed at a high rate under all redox conditions tested: the rate difference observed between anaerobic and aerobic systems was below a factor of 3. The electron acceptor for E2 oxidation under anaerobic conditions is still unknown: before the start of the batch experiments, fresh activated sludge was incubated long enough to remove O_2 and nitrate. Nevertheless, it is expected that several mg Fe^{3+} and various organic oxidative compounds were still present in the sludge and

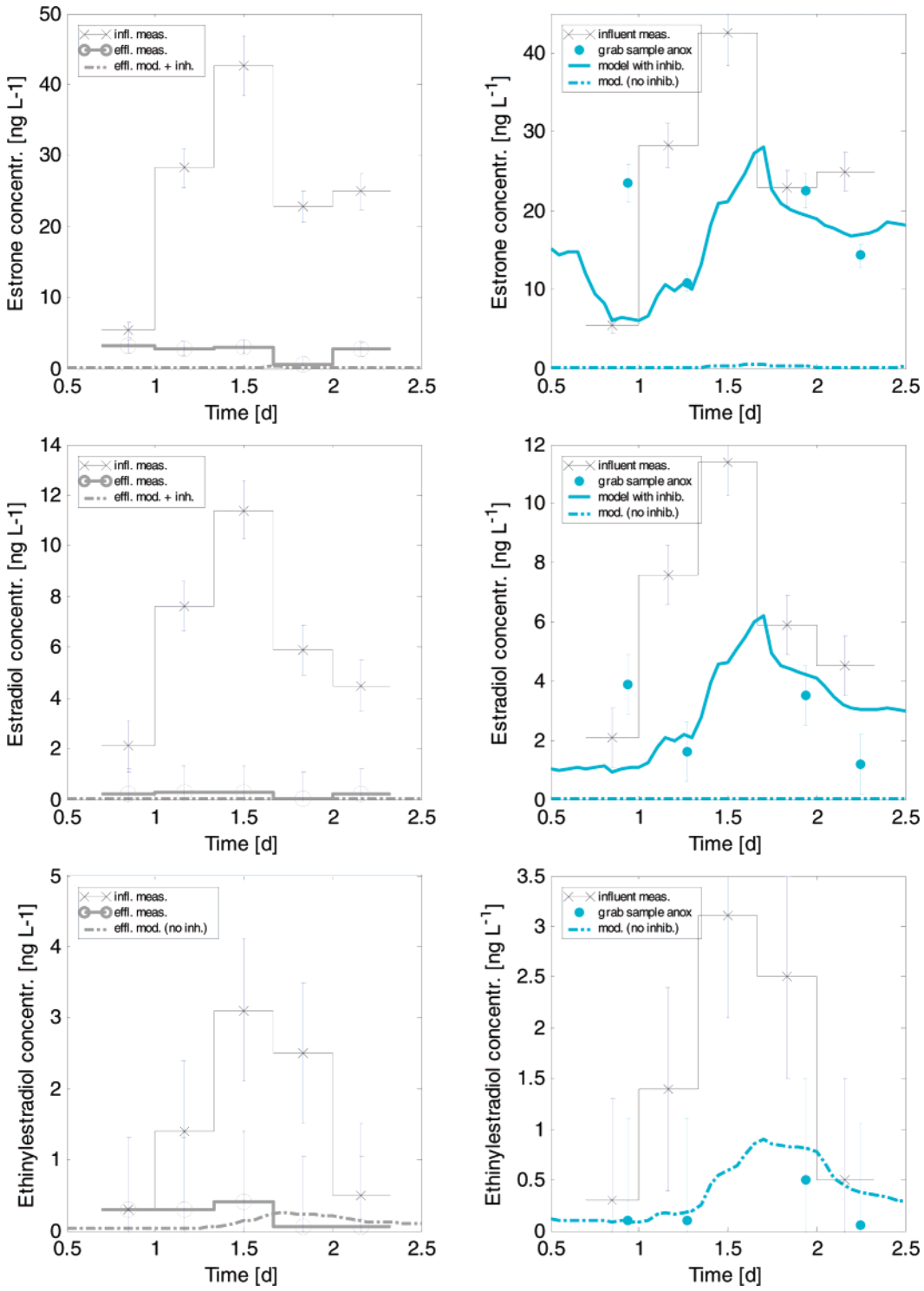

FIGURE 10. Measured and modeled estrogen concentrations of the membrane bioreactor pilot plant (MBR). The left-hand graphs show the 8 h composite samples measured in the influent and effluent: i.e., the typical daily influent variation but no significant effluent variation. The right-hand side shows the measured grab samples and model calculations for the anoxic compartment. Two model calculations are illustrated: according to the kinetic parameters found in the batch experiments (Table 3, no inhibition) and assuming that no degradation of E1 and E2 took place in the anaerobic and anoxic compartments due to competitive substrate inhibition (see text for further discussion).

may have acted as electron acceptors. This will also be the case in full-scale plants, where the retention time in the anaerobic compartment is about 1 h.

The reduction of estrone to estradiol could be shown to take place under anaerobic conditions without nitrate (Figure 8 shows the data for the MBR sludge; the experiment with CAS-K sludge is not shown): after spiking E1, a constant ratio of the concentrations of E1 and E2 was reached after 1−2 h, yielding a similar value for $k_{bio,E1,red}$ of ∼55 ± 10 L gSS^{-1} d^{-1} for both sludges (Table 3).

Ethinylestradiol was removed at a significant rate only under aerobic conditions, while in the absence of molecular O_2 the fitted values were in the range of the quantification limit (1 ± 0.5 L gSS^{-1} d^{-1}; eq 9). The degradation rates observed

in our experiment are in the same range as reported by Vader et al. (*29*) for experiments performed with EE2 concentrations of 1000 to 50 000 ng L^{-1}.

Sampling of Full-Scale Municipal Plants. Table 4 shows the elimination rates observed in our full-scale experiments as well as the elimination expected from the model using kinetic data obtained from the batch experiments (plant CAS-W represents data published by Andersen et al. (*13*)).

A relatively high variation of estrogen concentration in the influent (up to a factor of 10) was seen between different plants over the sampling period (average of six 8 h composite samples taken within 2 days). The low E1 and EE2 concentrations observed in the untreated wastewater from Altenrhein (CAS-A and FB) were particularly surprising, since 70% of the load comes from human sources and the influent volume of 0.38 m^3 PE^{-1} d^{-1} is characteristic of average dry wastewater flow in Switzerland. This may be explained by the relatively large drainage area of ~1000 km^2 (52 inhabitants km^{-2}) with correspondingly high retention times within the sewer.

A clear daily fluctuation of the influent load and concentration is seen in Figure 10 (similar daily variations were seen also in the influents of CAS-K and CAS-A). Nevertheless, no significant peak load is seen in the effluent, the measured values being close to or below the quantification limit. The observed removal rates in most cases thus represent only a lower limit (indicated by "≥" in Table 4). The effluent concentrations (often below the analytical limit of quantification of 0.5 ng L^{-1}) and the rather low influent concentration are responsible for this accuracy limit of the observable relative removal rate. A removal rate ≥90% is found for all estrogens in conventional and activated-sludge treatments. Considering the low hydraulic retention time in the fixed bed of 35 min (dry weather average), the removal rates of 75% and ≥90% for E1 and E2 represent a good performance.

As illustrated in Figure 10 (left-hand graphs E1 and E2), model calculations (Figures 4 and 5) and the kinetic constants obtained in the batch experiments (Table 3) led to significantly lower concentrations for the natural estrogens than for the measured values. A significantly better match between the model and the measurement is obtained if we assume that the degradation of E1 and E2 does not take place in the first reactors (Figure 10, right-hand graphs). The contradiction between the substantial removal rates observed in the anaerobic and anoxic batch experiments and the absence of removal in the corresponding compartment of the full-scale plants is interpreted in terms of competitive inhibition of estrogen degradation by the influent substrate. (The batch experiments were run without addition of primary effluent, while the full-scale plants were monitored during regular operation.) Although this observation was confirmed in all the plants sampled, further experimental evidence is required to confirm this interpretation (i.e. competitive substrate inhibition). (See Table 4, observed and modeled estrogen concentration in the first compartment; the model values in brackets are calculated assuming competitive inhibition.) The model calculation is insensitive to the cleavage rate of the glucuronides, the sorption coefficient K_D, and the kinetic sorption constant k_{sor}; so these parameters may not be responsible for the difference between the measurements and the values calculated without the assumption of competitive inhibition by the substrate.

The reason for the low removal rate found for E1 and EE2 in the conventional plant in Altenrhein (CAS-A) is not clear. A greater amount of conjugated compound in the influent than expected (not measured directly in our study; see below) could explain this finding.

The degradation of artificial estrogen EE2 was observed in the expected range or slightly below it (Figure 10, below).

Consequences for Plant Optimization. A biological degradation >90% of the E1, E2, and EE2 load can be expected from conventional activated sludge plants and membrane bioreactors with nitrification and denitrification (SRT of 12−15 d). According to the model calculation, higher removal rates can be expected (mainly for E2), but this could not be confirmed in this study by monitoring full-scale plants due to the analytical limit of quantification of 0.5 ng L^{-1}. Several authors quote similar removal rates (*3*, *9*, *13*, *30*), while others report lower ones (*7*, *11*, *12*, *26*, *31*). The data from the old Wiesbaden WWTP with only BOD removal (SRT < 4 d (*11*)) and that obtained after its upgrade to a nutrient removal plant (i.e. to an SRT of 12 d (*13*)) as well as this work suggest that the different sludge ages explain at least part of the difference. Vader et al. (*29*) showed that the EE2 degradation capability of sludge correlated with the nitrifying activity. According to the hypothesis presented in this paper that substrate contained in the primary effluent competitively inhibits the degradation of the natural estrogens E1 and E2, it is expected that sludge loading is a key parameter influencing the removal of estrogens from a wastewater treatment plant. This is confirmed by the low degradation rate observed in the first compartments of the monitored reactors. Due to the first-order kinetics, a much better E1 and E2 removal rate can be expected from a reactor cascade than from a completely stirred tank.

Sludge originating from a membrane bioreactor (sludge age 30 d) showed significantly higher activity in batch experiments than in conventional activated sludge with a sludge retention time of 11 d (increase by a factor of 2−3). Again, this increased removal activity could not be confirmed by sampling full-scale plants due to the analytical quantification limit.

As for the fixed-bed reactor, the removal rates of 90% and ≥95% for E1 and E2 represent a good performance considering the low hydraulic retention time of 35 min. This parameter thus seems to have little impact on estrogen removal capability, and the rather high age of the biofilm sludge is expected to be one of the main reasons for the observed removal.

Due to the relatively low K_D value (0.9 L gSS^{-1}) and the biological degradation, it is expected that <10% of the influent partitions to sludge by sorption (sludge production ~200 $gSS\ m^{-3}_{influent}$; see also ref *9*).

Nevertheless, high estrogenic activity is expected in the sludge digestion supernatant. First, conjugated compounds originating from primary sludge are expected to be cleaved in the digester; second, the dissolution of particles due to the digestion process may release estrogens by desorption; and third, the E1 to E2 reverse reaction could be shown to take place in an anaerobic environment (E2 has a 5−10-fold higher estrogenic activity than E1 (*3*, *12*, *32*)).

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Supporting Information Available

Appendix of diffusive mass transfer across the boundary layer and inside the floc. This material is available free of charge via the Internet at http://pubs.acs.org.

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