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Biodegradation and Attenuation of Steroidal Hormones and Alkylphenols by Stream Biofilms and Sediments

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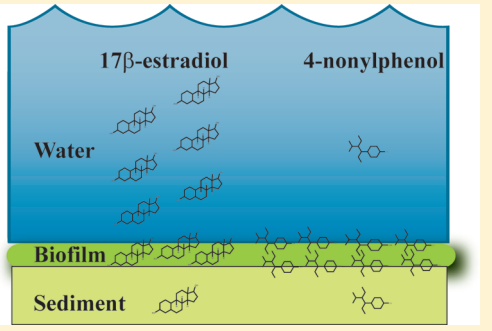
Supporting Information

ABSTRACT: Biodegradation of select endocrine-disrupting compounds (17β-estradiol, estrone, 17α-ethynylestradiol, 4-nonylphenol, 4-nonylphenol-monoexthoylate, and 4-nonylphenoldiethoxylate) was evaluated in stream biofilm, sediment, and water matrices collected from locations upstream and downstream from a wastewater treatment plant effluent discharge. Both biologically mediated transformation to intermediate metabolites and biologically mediated mineralization were evaluated in separate time interval experiments. Initial time intervals (0−7 d) evaluated biodegradation by the microbial community dominant at the time of sampling. Later time intervals (70 and 185 d) evaluated the biodegradation potential as the microbial community adapted to the absence of outside energy sources. The sediment matrix was more effective than the biofilm and water matrices at biodegrading 4-nonylphenol and 17β-estradiol. Biodegradation by the sediment matrix of 17α-ethynylestradiol occurred at later time intervals (70 and 185 d) and was not observed in the biofilm or water matrices. Stream biofilms play an important role in the attenuation of endocrine-disrupting compounds in surface waters due to both biodegradation and sorption processes. Because sorption to stream biofilms and bed sediments occurs on a faster temporal scale (<1 h) than the potential to biodegrade the target compounds (50% mineralization at >185 d), these compounds can accumulate in stream biofilms and sediments.



INTRODUCTION

Compounds that have the potential to disrupt the endocrine system of aquatic organisms have been identified in surface waters worldwide.[1−4] Endocrine-disrupting compounds (EDCs) include steroidal hormones, alkylphenol nonionic surfactant degradation products, plasticizers, inorganic compounds, and pharmaceuticals.[5−8] The fate, transport, and biological impact of EDCs in surface waters is controlled by several attenuation processes, of which photolysis, sorption, and biodegradation are believed to be the most significant.[9−13]

Biofilms ubiquitously coat streambed sediments and play an important role in biogeochemical cycling as well as being the base of the aquatic ecosystem.[14] Stream biofilms are dynamic matrices that undergo changes in the composition of photosynthetic periphyton, heterotrophic microbes, and extra-cellular polymers in response to environmental conditions.[15,16] Under laboratory conditions, steroidal hormones and alkylphenols rapidly partition to the organic matter of stream biofilms,[17] but the extent to which these compounds undergo biodegradation by stream biofilms is not known. The purpose of this work was to evaluate the biodegradation and attenuation of model EDCs by stream biofilm, sediment, and water matrices to better understand EDC fate in surface waters. Model EDCs included the steroidal hormones, 17β-estradiol, estrone, 17α-ethynylestradiol, and the alkylphenol and alkylphenolpolyethoxylate compounds, 4-nonylphenol, 4-*n*-nonylphenol, 4-nonylphenolmonoethoxylate (NP1EO), and 4-nonylphenoldiethoxylate (NP2EO). The chemical structures of these compounds are provided in the Supporting Information (SI) Figure S1.

METHODS

Sample Collection. Experiments were conducted with environmental samples and in situ colonized artificial substrata collected from Boulder Creek upstream (260 m) and downstream (100 m) from the City of Boulder's municipal wastewater-treatment plant (WWTP) outfall. Both sites are influenced by urban stormwater discharge and the downstream site is also influenced by effluent discharge from the City of Boulder WWTP.[18] Previous studies on the distribution, fate, and biological impact of EDCs have been performed on Boulder Creek

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and show that WWTP effluent discharge influences stream chemistry and corresponding biological function.[4,12,13,19]

The stream biofilm (epilithon) was sampled by collecting 10−15 cobbles and boulders (size range 10−30 cm in diameter) per site from Boulder Creek, using a polytetrafluoroethylene template to isolate a circular area (70 cm^2), and scraping and brushing the attached biofilm into a wide-mouth amber glass jar. At each site, sediment grab samples were collected from 3 to 5 locations from the top 5 cm of the bed surface and composited. The sediments consisted of sand and gravel (size range 1−10 mm) and associated detritus. Boulder Creek water upstream and downstream from the WWTP, and wastewater-effluent samples were collected in 1 L amber glass jars. In addition to collecting epilithon samples, stainless steel substrata were placed in Boulder Creek and colonized by a biofilm for a period of three weeks at locations upstream and downstream from the WWTP.[17]

Analytical. Analytical procedures and experiments are described elsewhere,[17] and in more detail in the SI. Briefly, organic matter mass was determined by the difference between the total mass dried at 105 °C for 2 h and the total mass after combustion at 500 °C for 4 h. Chlorophyll *a* was determined by spectrophotometry after pheophytin correction.[20] Chlorophyll *a* was converted to algal carbon [21] and a ratio was determined between algal carbon and total organic matter to evaluate the relative proportion of the phototrophic community in the stream biofilm. Concentrations of steroidal hormones were determined by gas chromatography-tandem mass spectrometry (GC−MSMS) using isotope dilution techniques (deuterated d_4-17β-estradiol, d_4-estrone, d_4-17α-ethynylestradiol). Concentrations of 4-nonylphenol, NP1EO, and NP2EO (collectively referred to as alkylphenols in following discussion) were determined by gas chromatography−mass spectrometry (GC−MS) using surrogate standard techniques (4-*n*-nonylphenol, 4-*n*-nonylphenolmonoethoxylate, and 4-*n*-nonylphenoldiethoxylate).

Sorption and Transformation Studies. Sorption isotherm experiments were performed using biofilm-colonized stainless steel screen substrata and bed sediments collected from the top 5 cm of Boulder Creek.[17] Colonized screens (biofilm mass 80−700 mg) and sediment (about 5−10 g) were placed in preweighed 250-mL glass jars with 30 mL of ionic strength and pH adjusted water (10^{-3} M NaCl, 10^{-3} M $NaHCO_3$), and the target compounds were added to the respective jars at four different concentrations (ranges of 1−100 $\mu g\ L^{-1}$ for 17β-estradiol and 17α-ethynylestradiol and 10−1000 $\mu g\ L^{-1}$ for 4-nonylphenol, NP1EO, and NP2EO). Each target concentration was run in triplicate. Estrone was not added in the sorption experiments, to evaluate potential transformation from 17β-estradiol. The experimental concentrations were higher than environmental concentrations, but the objective was to evaluate the sorptive capacity of the stream biofilms and sediments and to ensure aqueous concentrations after equilibration were above the detection limits of the analytical method. To evaluate biological activity, a control set of Boulder Creek sediments samples were base-rinsed (pH = 11.6 for 1 h) to inactivate the biological community, and thoroughly rinsed with deionized water.

The batch experiments were placed on a shaking table for 1 h (90 rpm), at which point the aqueous solution was decanted into the centrifuge tube and centrifuged. The target compounds were isolated by C_{18} solid phase extraction (SPE), derivatized to form their trimethylsilyl esters, and analyzed by GC−MS. Concentrations in the aqueous phase (C_{aq}) were determined using isotope (or surrogate standard) dilution techniques. Isotopes and surrogate standards were added onto the SPE cartridges prior to sample application. Analytically, it was not possible to simultaneously evaluate organic matter content (a critical measurement to evaluate sorption) and concentration of the target compounds in the sorbent phase; therefore, sorbent phase concentrations (C_s) were determined by the difference between the mass added and the mass measured in the aqueous phase normalized to the mass of the sorbent phase (biofilms or sediments). Linear sorption isotherms were used to determine partition coefficients.[17]

Transformation of 17β-estradiol to estrone and NP2EO degradation to 4-nonylphenol was evaluated following a similar procedure, with the exception that target compound concentrations were directly measured in both the aqueous phase (as previously described) and sorbent phase over time. To evaluate the relative degree of sorption and chemical transformation, 17β-estradiol (spiked at 5 $\mu g\ L^{-1}$), 4-nonylphenol, NP1EO, and NP2EO (all spiked at 25 $\mu g\ L^{-1}$) concentrations in the aqueous and sorbent phases were determined for batch experiments lasting 0.5, 1, 2, and 4 h. After removal of the aqueous phase, the sorbent matrices were spiked with isotope or surrogate standards (listed previously), dried at 105 °C for 2 h, and a total mass of the sorbent material determined. Methanol (25 mL) was used to extract the sorbed target compounds while sonicating (5 min). The methanol extract was taken to dryness and the target compounds derivatized for GC−MS analysis.

Mineralization Experiments. ^{14}C-radiolabeled substrates were used to assess the potential for microbial mineralization of 4-*n*-nonylphenol, 17β-estradiol, and 17α-ethynylestradiol in aerobic microcosms containing stream biofilm (epilithon), sediment, or water-only, as described previously.[12,13] The production of $^{14}CO_2$ and $^{14}CH_4$ over time was monitored by gas chromatographic separation with radiometric detection at time intervals of approximately 0, 7, 70, and 185 d (actual time intervals varied slightly by treatment). The time intervals were selected to provide an initial evaluation (7 d) of metabolism by the dominant microbial community at the time of sampling, and the biodegradation potential of the community, which will change at the later time intervals (>7 d).

Mineralization experiments were set up by matrix within 48 h of sample collection by dispensing approximately 5 mL of the biofilm matrix solution (about 2.5 mg dry mass mL^{-1}), approximately 5 g dry streambed sediment and 3 mL water, or approximately 8 mL of Boulder Creek water into microcosms (11 mL volume). Triplicate experimental controls, duplicate autoclaved controls, and a single sediment-free control were prepared for each matrix and location. Treatments were incubated concurrently in the dark at 23 ± 2°C.

RESULTS

Evaluation of Organic Matter Mass and Chlorophyll *a*. The fraction of organic matter (f_{om}) of the biofilm colonized stainless steel substrata ($f_{om} \approx 0.1$) was substantially lower than the epilithon, or mature stream biofilm collected on natural substrate ($f_{om} \approx 0.5$), but much higher than the f_{om} of the surficial (0−5 cm) streambed sediments ($f_{om} < 0.005$, SI Table S1). The sediments had a lower ratio of algal carbon to organic matter than the biofilm (for samples collected within 7 d of each other), which indicates that the ratio of phototrophic organisms to heterotrophic organisms is lower in the sediments. This finding is consistent with limitations on light penetration in the stream

Table 1. Organic Matter Partition Coefficients (K_{om}) for Target Compounds

compound	range of literature values	stream biofilm	stream sediment
	log K_{om}[a] (L kg^{-1})	log K_{om}[g] (L kg^{-1})	log K_{om}[g] (L kg^{-1})
17β-estradiol	2.5−3.3 [b,c,d]	**2.8 ± 0.07** (2.3−2.8)[h]	**2.1 ± 0.1**[h]
estrone	2.8−3.2[b]	ND[i]	ND[i]
17α-ethynylestradiol	2.6−3.7 [b,c,d]	**2.9 ± 0.05** (2.5−2.9)	**2.5 ± 0.04**
4-nonylphenol	3.7−5.1 [e,f]	**4.6 ± 0.1** (3.4−4.6)	**3.3 ± 0.04**
4-nonylphenolmonoethoxylate	5.3[f]	**4.0 ± 0.06** (3.5−4.0)	**3.1 ± 0.08**
4-nonylphenoldiethoxylate	4.9[f]	3.9 ± 0.06 (3.9−4.3)	4.1 ± 0.03

[a] Reported log K_{oc} converted to log K_{om} assuming organic matter is 50% organic carbon.[22] [b] Ref 23. [c] Ref 24. [d] Ref 25. [e] Ref 26. [f] Ref 27. [g] Because of the influence of environmental conditions, results are presented as the mean ±95% confidence interval for stream biofilm and sediment samples collected at the same time (5/14/09) upstream of the WWTP, bold text indicates a significant ($p < 0.05$) difference between stream biofilm and stream sediment; the range from multiple batch sorption experiments from 5/14−10/21/09 using Boulder Creek stream biofilm is listed in parentheses.[17] [h] Sum of 17β-estradiol and estrone. [i] Parameter not determined.

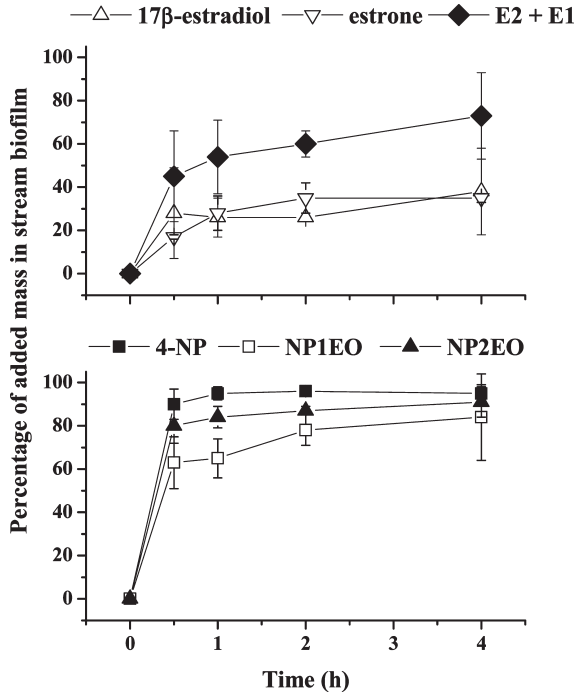

Figure 1. Percentage of total recovered mass in the stream biofilm in sorption experiments conducted at different exposure times; estrone (E1) was not added to the experiments, but was observed in both matrices due to oxidation of the added 17β-estradiol (E2); biofilm colonized (4/07/09−4/28/09) on stainless steel screens placed in Boulder Creek upstream from the City of Boulder wastewater treatment plant; 4-nonylphenol (4-NP); 4-nonylphenolmonoethoxylate (NP1EO); 4-nonylphenoldiethoxylate (NP2EO).

bed sediments (SI Table S1). The ratio of algal carbon to total organic matter for the biofilm colonized on the screens and the epilithon was consistently higher in samples collected downstream from the City of Boulder's WWTP. The increased algal carbon to total organic matter ratio was attributed to wastewater-induced nutrient enrichment.

Concentrations of Target Compounds in the Epilithon and Wastewater Effluent. Concentrations of target compounds measured in the wastewater effluent were less than the detection limit (<1 ng L^{-1}) for 17β-estradiol, estrone, and 17α-ethynylestradiol, 210 ng L^{-1} for 4-nonylphenol, 1400 ng L^{-1} for NP1EO, and 740 ng L^{-1} for NP2EO. Concentrations of target compounds in the epilithon collected upstream from the WWTP discharge were below the detection limit (<0.1 ng g^{-1}) for 17β-estradiol and 17α-ethynylestradiol, 0.18 ng g^{-1} for estrone, and 1700 ng g^{-1} for 4-nonylphenol. Concentrations of target compounds measured in the epilithon downstream from the WWTP discharge were 0.9 ng g^{-1} for 17β-estradiol, 0.6 ng g^{-1} for estrone, below the detection limit (<0.1 ng g^{-1}) for 17α-ethynylestradiol, and 1600 ng g^{-1} for 4-nonylphenol. Matrix interferences prevented quantification of NP1EO and NP2EO in the epilithon samples.

Sorption/Transformation Experiments. Because of the influence of environmental conditions on the stream biofilm and sediment samples, comparisons are made only between samples collected during the same time period (SI Table S1). Organic matter partition coefficients (K_{om}) for the biofilm were significantly higher (*t*-test used on subsequent statistical analyses unless otherwise noted, $p < 0.05$) than the K_{om} values for the bed sediments for the sum of estrone and 17β-estradiol (E1+E2), 17α-ethynylestradiol, 4-nonylphenol, and NP1EO (Table 1), indicating the stream biofilm was more effective than the sediments in sorbing the target compounds from the aqueous phase. The K_{om} values for the biofilm were not significantly different ($p > 0.05$) from samples collected upstream and downstream from the WWTP for E1+E2 and 17α-ethynylestradiol. The K_{om} values for the biofilm were significantly ($p < 0.05$) greater for the sample upstream from the WWTP than the sample downstream from the WWTP for both 4-nonylphenol ($10^{3.9\pm0.2}$, $10^{3.4\pm0.2}$, respectively) and NP2EO ($10^{4.3\pm0.05}$, $10^{4.2\pm0.03}$, respectively). Measured K_{om} values for the stream biofilm were comparable to literature values for a variety of environmental matrices,[22−27] whereas K_{om} values for the stream bed sediment were lower (Table 1).

Sorption approached equilibrium for 4-nonylphenol, NP1EO, and NP2EO after a period of about 1 h (Figure 1). 17β-Estradiol partitioned relatively slower than 4-nonylphenol, NP1EO, and NP2EO. Oxidation of 17β-estradiol to estrone was observed in less than 1 h, after which estrone concentrations remained relatively constant (Figure 1). The percentage of 17β-estradiol that was oxidized to estrone was defined as follows:

$$E_{ox} = \frac{\text{estrone}}{\text{estrone} + 17\beta\text{-estradiol}} \times 100 \quad (1)$$

In 1 h batch sorption experiments, oxidation to estrone was greater in the sediments than in the stream biofilm, and the

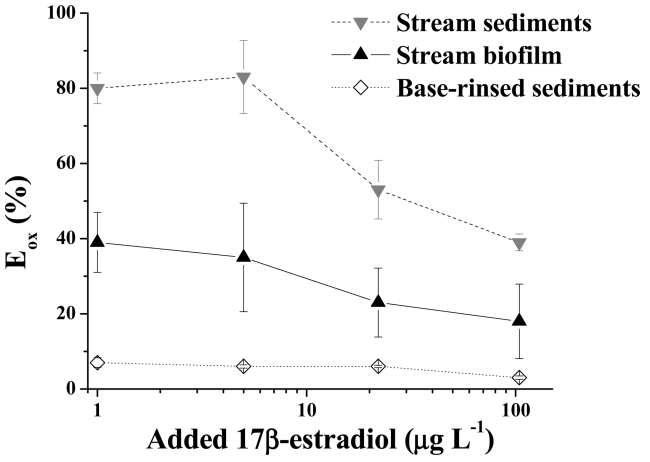

Figure 2. Percentage of estrogen in the oxidized state (E_{ox}), defined as estrone divided by the sum of estrone and 17β-estradiol after 1 h sorption experiments; results expressed as the mean ± standard deviation for triplicate aqueous/sorbed phase solutions; stream biofilm results from five different experiments; sediments were collected above the City of Boulder wastewater treatment plant.

percentage of 17β-estradiol that was oxidized to estrone in both the sediments and the biofilm decreased with increasing initial estradiol concentrations (Figure 2). Minimal oxidation of 17β-estradiol to estrone was observed in base-rinsed sediments.

Mineralization Experiments. No significant mineralization ($p > 0.05$) of 4-*n*-nonylphenol and 17β-estradiol was observed in the biofilm or water matrices during the initial time step (7 d), whereas significant mineralization of 4-*n*-nonylphenol and 17β-estradiol was observed in the sediment matrices (Figure 3a,b). Mineralization of 17α-ethynylestradiol was not observed in the initial time step (7 d) for any of the environmental matrices (Figure 3c).

After 70 d, mineralization of 4-*n*-nonylphenol and 17β-estradiol was observed in the biofilm and sediment matrices, and after 185 d biodegradation of these compounds was observed in all matrices. After 185 d, the relative amount of mineralization of 4-*n*-nonylphenol by matrix followed the order,

$$\begin{matrix}\text{sediments}\\\text{upstream}\end{matrix} > \begin{matrix}\text{sediments}\\\text{downstream}\end{matrix} \approx \begin{matrix}\text{biofilm}\\\text{upstream}\end{matrix} > \begin{matrix}\text{biofilm}\\\text{downstream}\end{matrix}$$

$$\approx \begin{matrix}\text{water}\\\text{downstream}\end{matrix} > \begin{matrix}\text{water}\\\text{upstream}\end{matrix}$$

After 185 d, the relative amount of mineralization of 17β-estradiol by matrix followed the order,

$$\begin{matrix}\text{sediments}\\\text{upstream}\end{matrix} \approx \begin{matrix}\text{biofilm}\\\text{upstream}\end{matrix} > \begin{matrix}\text{sediments}\\\text{downstream}\end{matrix}$$

$$\approx \begin{matrix}\text{biofilm}\\\text{downstream}\end{matrix} > \begin{matrix}\text{water}\\\text{downstream}\end{matrix} > \begin{matrix}\text{water}\\\text{upstream}\end{matrix}$$

The order of mineralization in the matrices was determined by statistically significant differences assessed using a one-way analysis of variance and the Holm-Sidak multiple comparison test ($p < 0.05$). After 185 d, significant ($p < 0.05$) mineralization of 17α-ethynylestradol was observed only in the sediments.

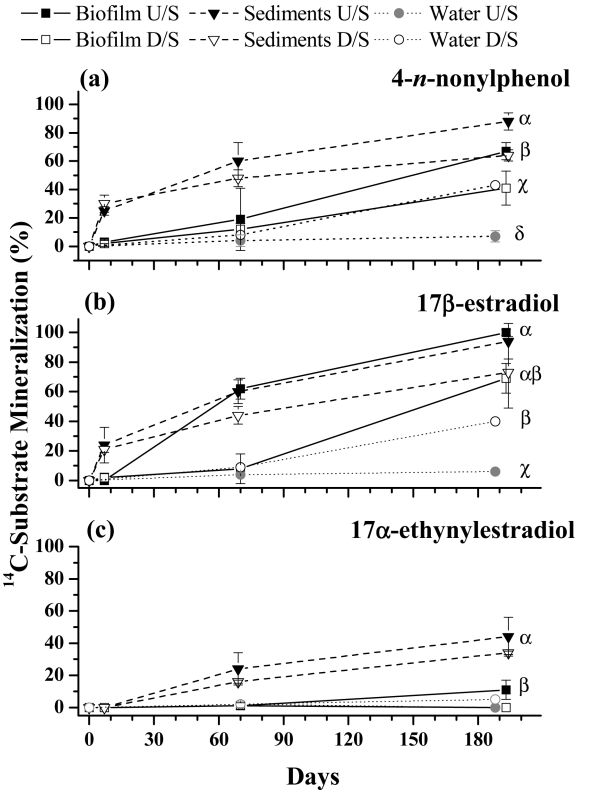

Figure 3. Percentage mineralization of ^{14}C-labeled target compounds to $^{14}CO_2$ in oxic microcosms containing matrices collected from Boulder Creek, CO, at locations upstream (U/S) and downstream (D/S) from the City of Boulder municipal wastewater treatment plant on 7/23/09; data are shown as mean ± standard deviation for triplicate experimental microcosms; different symbols represent microcosms having statistically significant differences in the final recovery of $^{14}CO_2$ (one-way analysis of variance and Holm-Sidak multiple comparison test, $p < 0.05$); no significant mineralization recovery was observed in autoclaved control microcosms.

Autoclaved control samples for each matrix showed no production of $^{14}CO_2$ or $^{14}CH_4$.

DISCUSSION

Attenuation of steroidal hormones and alkylphenols in surface waters by the stream biofilm and sediment matrices appears to be a combination of both sorption and biodegradation processes. Limited sampling of the stream biofilm (epilithon) from Boulder Creek upstream and downstream from the WWTP discharge indicated that steroidal hormones and 4-nonylphenol compounds accumulate in this matrix (even though the steroidal hormones were below detection in the water samples), primarily due to hydrophobic interactions with organic matter.[17] The bulk of biofilm organic matter is hydrophobic extracellular polymeric substrate, with autotrophic organic matter comprising approximately 10%, and heterotrophic organic matter comprising <1% of the total organic matter.[28] Sorption of hydrophobic contaminants to extracellular material has been shown to limit microbial degradation.[29]

Oxidation of 17β-estradiol to estrone was observed in both the biofilms and sediments, although greater relative oxidation (E_{ox}) was observed in the sediments (Figure 2). Less oxidation was observed in control sediments in which the biological

community was inactivated by a base-rinse, which suggests that the oxidation of 17β-estradiol was biologically mediated. Production of estrone from 17β-estradiol plateaued after a period of 1 h (Figure 1), suggesting that the majority of the hormone was sorbed to the extra-cellular polymeric substrate and less available for oxidation by extra-cellular enzymes or the microbial community. Sorption experiments with higher concentrations of 17β-estradiol (>10 μg L^{-1}) had lower E_{ox} values, suggesting that enzyme saturation (Monod) kinetics limited oxidation at the higher concentrations used in these experiments.[30]

Although additional factors influence biodegradation of the target compounds in the stream (e.g., temperature and light variability, availability of other carbon substrates, oxygen concentrations), the mineralization experiments provided information on the biodegradation potential of the different microbial communities over time. Mineralization of 4-*n*-nonylphenol or 17β-estradiol was not observed in the biofilm treatments during the initial time step (0−7 d), whereas mineralization of these compounds was observed in the sediment treatments with no lag time, both upstream and downstream from the WWTP. Mineralization was not observed in autoclaved matrices; therefore, 4-*n*-nonylphenol and 17β-estradiol mineralization in the sediments was attributed to biodegradation. Because the amount of mineralization in the biofilm treatments was similar at later time intervals to what was observed in sediment treatments, the biological communities of both matrices have the ability to mineralize 4-*n*-nonylphenol and 17β-estradiol. Mineralization of 4-*n*-nonylphenol and 17β-estradiol was limited in the water matrices until the 185 d time step. Also, mineralization of both compounds was significantly higher ($p < 0.05$) in water collected downstream from the WWTP than from upstream, likely due to microbial enrichment from the wastewater effluent.

To evaluate the fraction of the ^{14}C-labeled compounds in the aqueous phase (f_{aq}) in the mineralization experiments the following relation was used:

$$f_{aq} = \frac{1}{1 + K_{om} f_{om} \frac{M_s}{V_w}} \quad (2)$$

where K_{om} (L kg^{-1}) is the organic matter partition coefficient, f_{om} is the fraction of organic matter in the sorbent matrix, M_s (kg) is the mass of the sorbent matrix, and V_w (L) is the volume of water added to each microcosm. Although K_{om} values for the target compounds were higher for the stream biofilm than the sediments (Table 1), the greater mass of sediment organic matter relative to the mass of stream biofilm organic matter (about 25 mg vs 7.5 mg) results in a smaller percentage of 17β-estradiol and 17α-ethynylestradiol being sorbed in the stream biofilm experiments (about 30%) as compared to the sediment experiments (about 60%). Using eq 2, greater than 90% of 4-*n*-nonylphenol was sorbed in both the biofilm and sediment experiments. Because sorption generally limits biodegradation,[29] the faster mineralization of 17β-estradiol and 4-*n*-nonylphenol in the sediments relative to the stream biofilm during the initial time period is not consistent with greater relative sorption of target compounds in the sediments. Therefore, sorption does not appear to explain the observed differences in mineralization.

The differences in mineralization between the sediment and stream biofilm treatments may be due to microbial community structure and carbon metabolism. The structure of the stream biofilm can be characterized as an extracellular polysaccharide matrix containing channels that can deliver water and solutes to dispersed patches of microbial colonies.[16] Photosynthesis is a fundamental component of energy conversion in stream-bed biofilm communities with significant exposure to solar radiation. The availability of labile carbon exudates from the photosynthetic community influences the associated metabolic pathways of the biofilm heterotrophic community.[31,32] Due to light limitations on photosynthetic carbon fixation below the sediment surface, the subsurface sediment heterotrophic community primarily metabolizes exogenous and generally more recalcitrant carbon substrates. Consequently, the delayed onset of 4-*n*-nonylphenol and 17β-estradiol mineralization in biofilm treatments may reflect the greater availability of labile carbon substrates produced by the photosynthetic community of the biofilms prior to sample retrieval. The eventual onset of mineralization activity in the biofilm treatments is consistent with depletion of these labile photosynthetic products and a shift toward more recalcitrant substrates, including the ^{14}C-substrates. This conclusion is consistent with research that showed the extracellular polymeric substrate incorporates nutrients and organic carbon that can later act as reserve during times of starvation.[33]

Biodegradation of 17α-ethynylestradiol typically is assumed to be slow in aquatic sediments, and limited direct assessments have been conducted.[9] Results from this work provide evidence that 17α-ethynylestradiol mineralization can occur in surface water sediments; however, the delayed onset and slower rate of mineralization indicates that 17α-ethynylestradiol is more recalcitrant than 17β-estradiol or 4-*n*-nonylphenol. The K_{om} values for 17β-estradiol and 17α-ethynylestradiol are similar and nearly an order of magnitude lower than that of 4-nonylphenol (Table 1), which suggests that the relative recalcitrance of 17α-ethynylestradiol as compared to 17β-estradiol is not due to sorption differences.

Mineralization of 17β-estradiol was not significantly different ($p > 0.05$) in the biofilm and sediment matrices collected upstream and downstream from the WWTP. On the basis of final recovery of the ^{14}C substrate ($t > 185$ d), mineralization of 4-*n*-nonylphenol was lower in the sediments collected downstream from the WWTP than the sediments collected upstream from the WWTP. Organic matter concentrations were not significantly different between sediment matrices collected upstream and downstream from the WWTP. Previous studies have shown reduced mineralization downstream from WWTPs for several other compounds (caffeine, cotinine, estrone, and testosterone), and this inhibitory effect was attributed to biological oxygen demand (BOD) and oxygen limitations.[12,13,34] Antimicrobial compounds such as triclosan are frequently detected in wastewater effluent,[19] and have been shown to be toxic to stream biofilms.[35] However, limitations of the microbial community to degrade these compounds is not supported by results from this work, which showed that mineralization in the water microcosms downstream from the WWTP was significantly greater ($p < 0.05$) than the water microcosms upstream from the WWTP. Further research is required to define the effect of wastewater effluent on the ability of the microbial community to degrade steroidal hormones, alkylphenols, and other wastewater compounds.

Results of these experiments suggest that biodegradation of target compounds by stream biofilms is controlled by (1) partitioning onto organic matter, which effectively limits compound

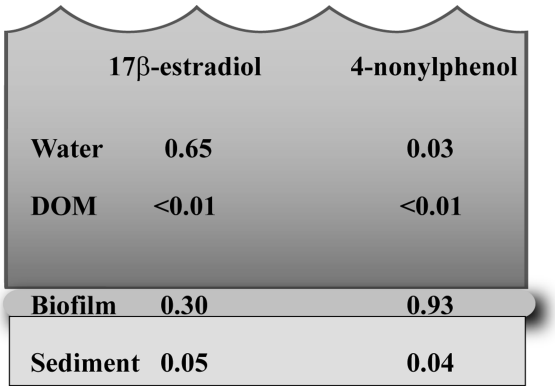

Figure 4. Mass fraction of 17β -estradiol and 4-nonylphenol in relevant environmental matrices of a stream, assuming degradation not substantial relative to organic carbon partitioning, K_{om} values from Table 1, and equilibrium conditions; listed parameters consistent with measured values in Boulder Creek, Colorado; water depth = 0.7 m, dissolved organic matter concentration = 11 mg L^{-1}; biofilm thickness = 0.001 m, fraction of organic matter biofilm $f_{om,\,bf}$ = 0.5; sediment depth = 0.05 m, f_{om} = 0.005; DOM, dissolved organic matter.

bioavailability, and (2) metabolic differences in the heterotrophic community in response to differences in available organic energy sources. Biodegradation of the target compounds in stream biofilms appears to be limited due to the availability of labile authochtonous organic carbon produced by an active photosynthetic community. This work showed that sorption to both the stream biofilm and to the sediments occurred on a temporal scale much faster (<1 h) than the potential of these matrices to completely mineralize the target compounds (20−30% mineralization of 4-*n*-nonylphenol and 17β-estradiol occurred in the sediments at 7 d, and less than 10% mineralization of 4-*n*-nonylphenol and 17β-estradiol occurred in the stream biofilms at 7 d). Consequently, steroidal hormones and alkylphenols can accumulate in stream biofilms. Biological transformation to intermediate metabolites (e.g., 17β-estradiol oxidation to estrone) occurred relatively faster (less than 1 h), but slowed markedly as sorption limited the bioavailability of the compounds. Transformation processes mediated by the microbial community in the sediments and epilithon can generate intermediate metabolites (e.g., estrone) which may still have estrogenic properties.

The applicability of the laboratory experiments described in this work relative to an actual stream setting can be illustrated by assuming equilibrium organic matter partitioning in a unit stream typical of environmental conditions found in Boulder Creek. The fraction of the target compounds in each of the principal matrices (f_{aq}) of water, dissolved organic matter (DOM), biofilm, and sediment can be estimated according to the following relationship:

$$f_{aq} = \frac{1}{1 + K_{om}[\mathrm{DOM}] + K_{om}f_{om,bf}\dfrac{M_{bf}}{V_w} + K_{om}f_{om,sed}\dfrac{M_{sed}}{V_w}} \quad (3)$$

where [DOM] is the dissolved organic matter concentration (11 mg L^{-1}), $f_{om,bf}$ is the fraction of organic matter in the biofilm, M_{bf} (kg) is the mass of biofilm, $f_{om,sed}$ is the fraction of organic matter in the sediment, M_{sed} (kg) is the mass of sediment. Assuming degradation will not be substantial relative to sorption (discussed previously in this section) and K_{om} values from Table 1, the relative mass fraction of 17β-estradiol and 4-nonylphenol in the stream biofilm is greater than the mass fraction in the sediments (Figure 4). The actual distribution and concentrations in the principal environmental matrices will depend on in-stream vertical mixing and contact with the streambed surface. The stream biofilm plays an important role in surface water attenuation, and further research is warranted to quantify the role of this matrix in the fate of EDCs and other trace organic contaminants. As EDCs accumulate in the biofilm,[36,37] direct or indirect consumption of this base of the aquatic ecosystem represents a potential exposure mechanism to higher trophic level organisms.[38,39] Additionally, biofilm senescence due to seasonal conditions and higher stream velocities [15] may result in pulses of accumulated biofilm EDCs being released and transported downstream.

■ ASSOCIATED CONTENT

Supporting Information. One figure showing the chemical structure of the model compounds, one table describing characteristics of the sampled matrices, and detailed descriptions of the analytical procedures and experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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