Transformation and sorption of illicit drug biomarkers in sewer systems: understanding the role of suspended solids in raw wastewater

Ramin, P.; Libonati Brock, A.; Polesel, F.; Causanilles, A.; Emke, E.; de Voogt, P.; Plósz, B.G.

DOI
10.1021/acs.est.6b03049

Publication date
2016

Document Version
Final published version

Published in
Environmental Science and Technology

Environmental Science & Technology

Article

pubs.acs.org/est

Transformation and Sorption of Illicit Drug Biomarkers in Sewer Systems: Understanding the Role of Suspended Solids in Raw Wastewater

Pedram Ramin,[*,†] Andreas Libonati Brock,[†] Fabio Polesel,[†] Ana Causanilles,[‡] Erik Emke,[‡] Pim de Voogt,[‡,§] and Benedek Gy. Plósz[*,†]

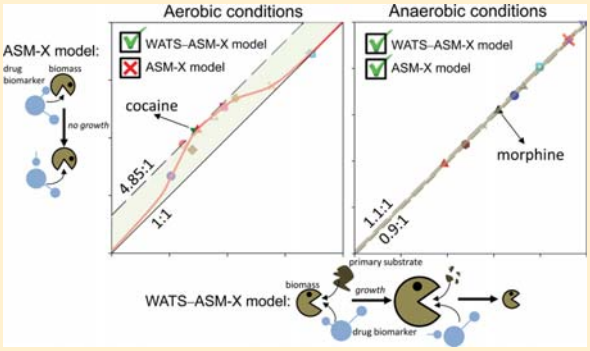
[†]Technical University of Denmark (DTU), Department of Environmental Engineering, Miljøvej 113, 2800 Kgs. Lyngby, Denmark

[‡]KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB Nieuwegein, The Netherlands

[§]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands

Supporting Information

ABSTRACT: Sewer pipelines, although primarily designed for sewage transport, can also be considered as bioreactors. In-sewer processes may lead to significant variations of chemical loadings from source release points to the treatment plant influent. In this study, we assessed in-sewer utilization of growth substrates (primary metabolic processes) and transformation of illicit drug biomarkers (secondary metabolic processes) by suspended biomass. Sixteen drug biomarkers were targeted, including mephedrone, methadone, cocaine, heroin, codeine, and tetrahydrocannabinol (THC) and their major human metabolites. Batch experiments were performed under aerobic and anaerobic conditions using raw wastewater. Abiotic biomarker transformation and partitioning to suspended solids and reactor wall were separately investigated under both redox conditions. A process model was identified by combining and extending the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) model and Activated Sludge Model for Xenobiotics (ASM-X). Kinetic and stoichiometric model parameters were estimated using experimental data via the Bayesian optimization method DREAM$_{(ZS)}$. Results suggest that biomarker transformation significantly differs from aerobic to anaerobic conditions, and abiotic conversion is the dominant mechanism for many of the selected substances. Notably, an explicit description of biomass growth during batch experiments was crucial to avoid significant overestimation (up to 385%) of aerobic biotransformation rate constants. Predictions of in-sewer transformation provided here can reduce the uncertainty in the estimation of drug consumption as part of wastewater-based epidemiological studies.



INTRODUCTION

Over the past decade, wastewater-based epidemiology (WBE) has emerged as a promising approach to provide policy makers with improved knowledge of consumption and abuse of illicit drugs, based on the analysis of excreted parent drugs and/or their human metabolites in untreated sewage.[1,2] In this emerging field, temporal and spatial patterns of drug use have been identified and characterized in selected urban sewer catchments,[3−6] allowing, more recently, for the undertaking of international comparative studies.[7,8] Therefore, WBE has the potential to complement the conventional surveillance data on drug abuse.[9] In order to ensure reliable and robust epidemiological engineering tools (mathematical and experimental methods that can be used to predict the substance usage rate in an urban catchment), ongoing research is currently addressing various sources of uncertainties and deficiencies,[4,10] the most common being associated with the performance of the analytical methods used (e.g., matrix effect, analytical variability, and validation).[4,11] The notion of in-sewer *stability* has also been introduced to describe the transformation of drug biomarkers between a theoretical discharge point and the sampling point at the influent of a wastewater treatment plant (WWTP).[12−16] However, very few attempts have been made to refine calculations of drug consumption by accounting for in-sewer transformation of drug biomarkers.[3]

Accounting for in-sewer fate of drug biomarkers in back-calculation schemes requires a mathematical description of physico-chemical and biological processes. Considering drug biomarkers as organic micropollutants (such as pharmaceuticals, personal care products, and their metabolites), models developed for these chemicals could be relevant, such as multimedia

Received: June 17, 2016
Revised: October 6, 2016
Accepted: October 14, 2016
Published: October 14, 2016

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

fugacity and activity-based models[17−19] or concentration-based models.[13,20] More specifically, the Activated Sludge Model for Xenobiotic trace chemicals (ASM-X)[13] was proposed to describe transformation and sorption processes for pharmaceuticals in wastewater treatment systems and has been further applied for predicting the fate of cocaine biomarkers in wastewater.[3]

The application of water quality models to sewer systems is based on the concept that the sewer network is a bioreactor where biochemical transformations occur.[21] Transformation kinetics, and thus the wastewater composition in sewers, can be impacted by the design features and the operation regimes (e.g., gravity-driven or pressurized pipe) implemented in sewer systems.[22] The microbial community and the underlying biochemical processes in sewers require a different characterization than for WWTPs in terms of availability of growth substrates, terminal electron acceptors, and fraction of active biomass. For instance, high-substrate-to-microorganism ratios are often expected for raw wastewater in sewers, while lower ratios occur in activated sludge reactors of full-scale WWTPs.[23] On the basis of these concepts, the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) modeling framework was introduced to describe microbially mediated aerobic transformation of organic carbon[24,25] and biochemical processes related to the nitrogen and sulfur cycle.[23,26,27] Furthermore, high substrate-to-microorganism ratios in untreated sewage require accounting for significant microbial growth when describing biotransformation of drug biomarkers during stability tests, thus possibly influencing the estimation of transformation kinetics.

To date, comprehensive studies assessing the influence of different factors (e.g., redox conditions, abiotic processes) on the in-sewer transformation of drug biomarkers are still limited.[3,28] Moreover, while the majority of studies have focused on the stability of individual biomarkers, drug metabolites present in spiking solutions during targeted experiments can potentially transform to each other (an observations that can be made only with adequate chemical labeling). These transformation pathways should be included in fate models, and the common term *stability* appears to simplify this challenge.

The main objectives of this study were (i) to characterize abiotic and microbially mediated transformation and sorption of illicit drugs in raw wastewater under aerobic and anaerobic conditions, by means of targeted batch experiments, (ii) to identify and calibrate a mathematical model for a combined description of in-sewer microbial growth kinetics (based on WATS) and drug biomarker sorption and transformation (based on ASM-X), (iii) to identify the simplest transformation pathways and structures for ASM-X process model extensions for selected illicit drug biomarkers, and (iv) to evaluate the optimal model complexity for the reliable prediction of biomarker fate in bulk raw wastewater.

MODELING FRAMEWORK

In-sewer processes for the utilization of a primary organic substrate (measured as chemical oxygen demand—COD), electron acceptors (oxygen, sulfate), and the fate of drug biomarkers are described separately. The structure of process models, rate equations, stoichiometric coefficients, and definitions of model state variables and model parameters are presented in Table 1. Since experiments in this study were carried out strictly under either aerobic or anaerobic conditions, the processes relevant for each distinct redox condition are formulated separately for the WATS model. In Table 1, drug biomarker transformation under aerobic and anaerobic conditions is defined based on previously suggested mathematical expressions.[3]

Primary Metabolic Processes (WATS). In-sewer transformation of organic matter and growth of heterotrophic (X_{Hw}) and sulfate reducing bacteria (SRB, X_{SRB}) were described according to literature.[23,24,29−32] Oxygen (S_O) and sulfate (S_{SO4}) were considered as terminal electron acceptors under aerobic and anaerobic conditions, thus neglecting processes under denitrifying conditions. Process rates only describe transformation and partitioning of chemicals, and the simulation model does not account for in-sewer transport processes. Evaporation of methanol (S_{Me}) was additionally considered and described using a first-order equation (Supporting Information, section S1.3). All process rates include an Arrhenius-based correction to account for the effect of temperature. Further details of the WATS model can be found in SI, section S1.

Secondary Metabolic Processes (ASM-X). A model for the fate of drug biomarkers in wastewater was developed based on the ASM-X modeling framework.[3] Biotransformation of drug biomarkers as nongrowth substrates was expressed as a second-order rate equation proportional to (i) the aqueous concentration of the drug biomarker, C_{LI}, and (ii) the concentration of active biomass, X_{Hw} and/or X_{SRB}. Due to their high diversity and their ability to oxidize a variety of organic compounds,[33−35] SRB species were also considered capable of degrading drug biomarkers under anaerobic conditions. Hence, the impact of the utilization of organic matter fractions and the associated significant microbial growth on biomarker biotransformation was considered by combining WATS and ASM-X (WATS−ASM-X). The extent of biotransformation kinetics is described by the biotransformation rate constant k_{bio} (L gCOD^{-1} d^{-1}).

ASM-X was further extended to account for additional fate processes, namely, (i) first-order abiotic transformation, described by the abiotic transformation rate constant k_{abio} (d^{-1}), and (ii) sorption and desorption of drug biomarkers onto the reactor wall, with definition of the partition coefficient (K_{dw}) between the reactor wall and liquid. The latter processes were considered to reflect observed drug biomarker concentrations in blank experiments (typically a decreasing trend, with a pronounced initial drop indicative of partitioning to the reactor wall; Figure 1 and SI, Figure S11). Sorption to and desorption from particulate matter were regarded as two opposite equilibrium processes.[20] Drug biomarkers in the aqueous phase were considered capable of partitioning onto total suspended solids, TSS (X_{SS}, gTSS L^{-1}) including hydrolyzable organic matter (X_{S1} + X_{S2} as TSS) and active biomass (X_{Hw} + X_{SRB} as TSS). The solid−liquid partition coefficient, K_d (L g^{-1}), was normalized to the total suspended solids (TSS) concentration, and a fixed conversion factor (f_{SS}, gTSS gCOD^{-1}) was used to convert COD-based state-variables to TSS using experimental data (SI, Table S1). C_{SL} and C_{SW} denote the concentration of drug biomarkers in the solid phase and on the reactor wall, respectively. Due to varying areas of the reactor wall in contact with the liquid phase during batch experiments (caused by sample withdrawal), a variable wet-surface-area-to-volume ratio (σ_w) was defined (SI, section S5.4).

Transformation pathways of drug biomarkers were assessed individually considering their possible transformation and simultaneous formation from other biomarkers present in the spiking mixture. An additional state variable (C_{CJ}) was thus

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

Table 1. Primary and Secondary Metabolic Processes under Aerobic and Anaerobic Conditions Considered in the WATS−ASM-X Framework[a]

	State variables →	X_{Hw}	X_{SRB}	S_F	S_A	S_S	S_{Me}	X_{S1}	X_{S2}	S_O	S_{SO4}	C_{LI}	C_{SL}	C_{CJ}	C_{SW}	
	Definition →	Heterotrophic biomass	Sulfate reducing bacteria	Fermentable substrate	Fermentation product	Readily degradable COD	Methanol	Rapid hydrolysable substrate	Slow hydrolysable substrate	Dissolved oxygen	Sulfate	Biomarker in aqueous phase	Biomarker in suspended solids	Biomarker transforming to C_{LI}	Biomarker onto reactor wall	Process rates ↓
	Processes ↓ Unit →	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gO$_2$ m^{-3}	gS m^{-3}	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	
WATS - aerobic	Growth of X_{Hw}	1				$-\frac{1}{Y_{Hw}}$				$-\frac{(1-Y_{Hw})}{Y_{Hw}}$						$\mu_H S_S/(S_S+K_{Sw})X_{Hw}\alpha_w^{(T-20)}$
	Maintenance	-1				-1				-1						$q_m X_{Hw}\alpha_w^{(T-20)}$
	Hydrolysis, rapid					1		-1								$k_{h1}(X_{S1}/X_{Hw})/(X_{S1}/X_{Hw}+K_{X1})X_{Hw}\alpha_w^{(T-20)}$
	Hydrolysis, slow					1			-1							$k_{h2}(X_{S2}/X_{Hw})/(X_{S2}/X_{Hw}+K_{X2})X_{Hw}\alpha_w^{(T-20)}$
	Methanol evaporation						-1									$k_{eva,ae}S_{Me}$
WATS - anaerobic	Decay of X_{Hw}	-1							1							$d_H X_{Hw}\alpha_S^{(T-20)}$
	Growth of X_{SRB} for S_F		1	$-\frac{1}{Y_{SRB}}$							$-0.5\frac{1-Y_{SRB}}{Y_{SRB}}$					$\mu_{SRB}\frac{S_F}{S_F+K_{SRB,S}}\frac{S_F}{S_F+S_A}\frac{S_{SO4}}{(S_{SO4}+K_{SRB,SO4})}X_{SRB}\alpha_S^{(T-20)}$
	Growth of X_{SRB} for S_A		1		$-\frac{1}{Y_{SRB}}$						$-0.5\frac{1-Y_{SRB}}{Y_{SRB}}$					$\mu_{SRB}\frac{S_A}{S_A+K_{SRB,S}}\frac{S_A}{S_F+S_A}\frac{S_{SO4}}{(S_{SO4}+K_{SRB,SO4})}X_{SRB}\alpha_S^{(T-20)}$
	Hydrolysis, rapid			1				-1								$\eta_h k_{h1}(X_{S1}/X_{Hw})/(X_{S1}/X_{Hw}+K_{X1})X_{Hw}\alpha_S^{(T-20)}$
	Hydrolysis, slow			1					-1							$\eta_h k_{h2}(X_{S2}/X_{Hw})/(X_{S2}/X_{Hw}+K_{X2})X_{Hw}\alpha_S^{(T-20)}$
	Fermentation			-1	1											$q_{fe}\frac{S_F}{S_F+K_{fe}}X_{Hw}\alpha_S^{(T-20)}$
	Methanol evaporation						-1									$k_{eva,an}S_{Me}$
ASM-X (aerobic / anaerobic)	Desorption from wall											1		1	-1	$k_{des,W}C_{SW}$
	Sorption to wall											-1		-1	1	$\sigma_W k_{des,W}K_{d,W}C_{LI}$ (or C_{CJ})
	Desorption from suspended solids											1	-1	1		$k_{des}C_{SL}$
	Sorption to suspended solids											-1	1	-1		$k_{des}K_d(X_{Hw}+X_{SRB}+X_{S1}+X_{S2})f_{SS}10^{-3}C_{LI}$ (or C_{CJ})
	Abiotic transformation											-1				$k_{abio,LI}C_{LI}$
	Abiotic formation											$\frac{M_{LI}}{M_{CJ}}$		-1		$k_{abio,CJ}C_{CJ}$
	Biotransformation											-1				$k_{bio,LI}C_{LI}(X_{Hw}+X_{SRB})10^{-3}$
	Biotic formation											$\frac{M_{LI}}{M_{CJ}}$		-1		$k_{bio,CJ}C_{CJ}(X_{Hw}+X_{SRB})10^{-3}$

[a]WATS aerobic: μ_H, maximum specific growth rate of X_{Hw}; Y_{Hw}, heterotrophic growth yield; K_{Sw}, affinity constant of X_{Hw} for S_S; q_m: maintenance rate; k_{h1}, rapid hydrolysis rate; k_{h2}, slow hydrolysis rate; K_{X1}, affinity constant for rapid hydrolysis; K_{X2}, affinity constant for slow hydrolysis; α_w, aerobic Arrhenius temperature coefficient; $k_{eva,ae}$, aerobic methanol evaporation rate; T, temperature. WATS anaerobic: μ_{SRB}, maximum specific growth rate of X_{SRB}; Y_{SRB}, growth yield of X_{SRB}; $K_{SRB,S}$, affinity constant of X_{SRB} for S_S; $K_{SRB,SO4}$, affinity constant of X_{SRB} for S_{SO4}; d_H, decay rate of X_{Hw}; η_h, anaerobic reduction factor for hydrolysis; q_{fe}, maximum fermentation rate; K_{fe}, affinity constant for S_F; α_S, anaerobic Arrhenius temperature coefficient; $k_{eva,an}$, anaerobic methanol evaporation rate. ASM-X: $k_{des,w}$, desorption rate from reactor wall; K_{dw}, wall-liquid partition coefficient; k_{des}, desorption rate from suspended solids; K_d, solid−liquid partition coefficient; σ_W, wet-surface-to-volume ratio; $k_{abio,LI}$, abiotic transformation rate; $k_{abio,CJ}$, abiotic formation rate; $k_{bio,LI}$, biotransformation rate constant; $k_{bio,CJ}$, biotic formation rate constant; f_{SS}, TSS-to-particulate-CO D ratio; M, biomarker molecular weight.

considered, denoting the concentration of other biomarkers transforming to C_{LI}. Identified transformation pathways and complete Gujer matrices defined for each group of drug biomarkers are presented in SI section S6. Abiotic transformation and biotransformation pathways for each chemical in water and wastewater, respectively, were primarily identified based on relevant literature[3,36−38] and confirmed by statistical analysis via postprocessing after model calibration. Feasibility of biotransformation pathways was also attested using the EAWAG-BBD Pathway Prediction System[39] (SI, Figure S27).

MATERIALS AND METHODS

Selection of Trace Organic Biomarkers. We selected 16 illicit drug biomarkers based on their relevance and frequency of occurrence as demonstrated through a recent wastewater monitoring campaign in European cities[8] and reports by European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[40] Biomarkers were subdivided into five groups: (i) mephedrone (MEPH); (ii) methadone (METD) and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP); (iii) cocaine (COC) and its metabolites benzoylecgonine (BE), ecgonine methyl ester (EME), and cocaethylene (CE); (iv) heroin (HER) and its metabolites 6-monoacetylmorphine (6MAM), morphine (MOR), and morphine-3-β-D-glucuronide (MORG); codeine (COE) and its metabolite norcodeine (NCOE); and (v) tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ9-THC (THCOH) and 11-nor-9-carboxy-Δ9-THC (THCCOOH). Analytical standards and their isotopically labeled internal standard (ILIS) analogues were purchased from Sigma-Aldrich (Brøndby, Denmark) at concentrations of 0.1 mg mL^{-1} and 1 mg mL^{-1}, respectively. Corresponding stock solutions were prepared by dilution in methanol (MeOH) at final concentrations of 10 and 42 μg mL^{-1}. Physicochemical properties of the compounds are presented in SI Table S3.

Laboratory-Scale Batch Experiments. Sorption and transformation of selected drug biomarkers were assessed using batch experiments in jacketed reactors. An external recirculating bath was used to control wastewater temperature at 14−15 °C throughout the experiments. A diffuser was placed at the bottom of each reactor, and sparging of dry compressed atmospheric air or pure nitrogen was used to create aerobic or

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

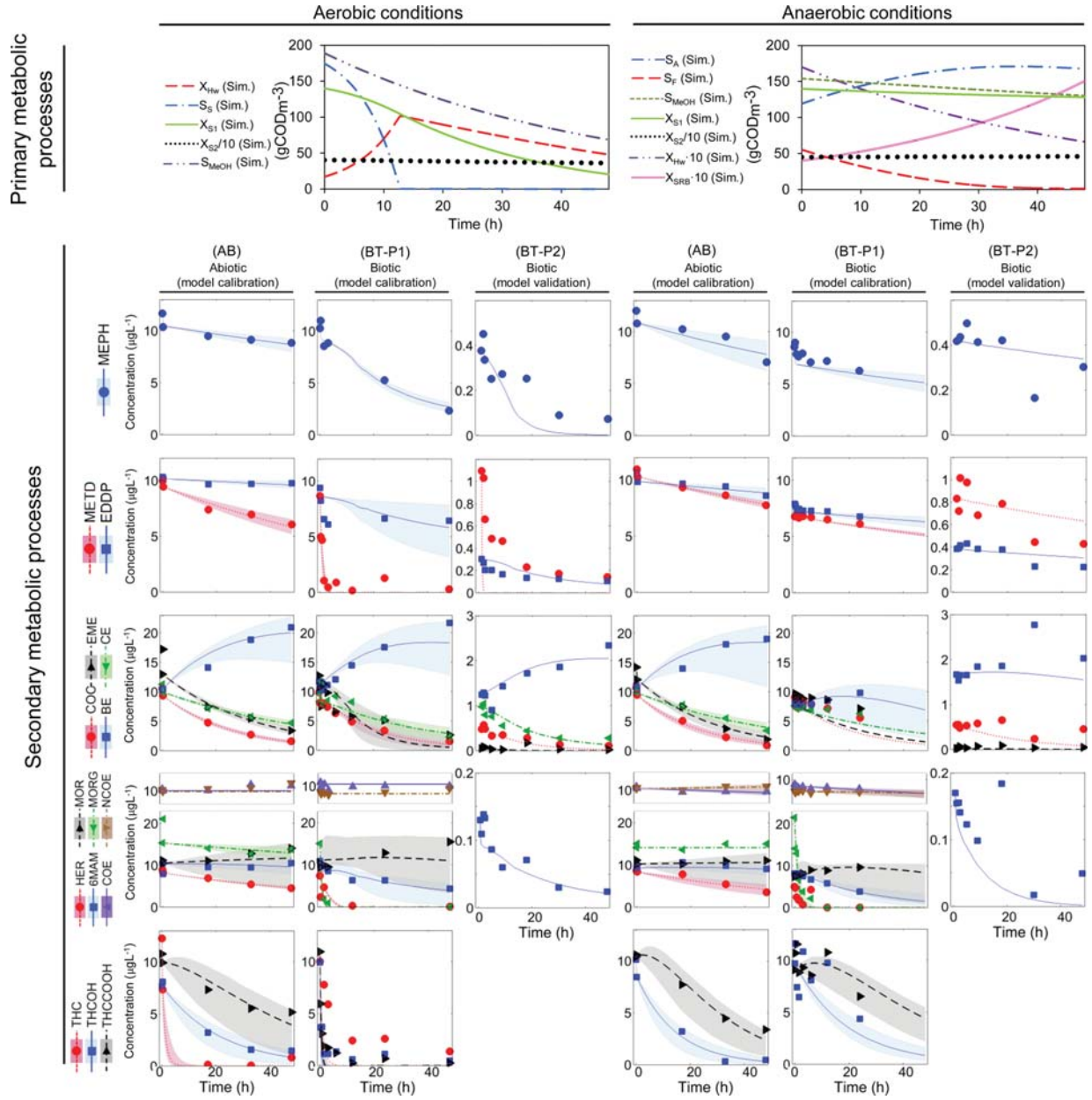


Figure 1. Experimental data and simulation results for primary and secondary metabolic processes under aerobic and anaerobic conditions. *Primary metabolic processes:* aerobic WATS model outputs including heterotrophic biomass X_{Hw}, soluble substrate S_S, and rapid, X_{S1}, and slow, X_{S2}, hydrolyzable fractions. Anaerobic WATS model outputs, including X_{Hw}, sulfate reducing bacteria, X_{SRB}, fermentation product (VFA), S_A, fermentable substrate, S_F, X_{S1}, and X_{S2}. Evaporation of methanol, S_{Me}, is also simulated for both redox conditions. *Secondary metabolic processes:* data related to AB-BT aerobic, AB-BT anaerobic, BT-P1 aerobic, BT-P1 anaerobic, BT-P2 aerobic, and BT-P2 anaerobic experiments. While WATS−ASM-X is calibrated with AB and BT-P1 data, BT-P2 data are used to validate the WATS−ASM-X model. Markers are measured data, and lines are simulation results. The shaded area reflects a 95% credibility interval of model prediction.

anaerobic conditions, respectively. Reactors were further equipped with a mixing impeller from the top.

Three different sets of batch experiments were conducted: (i) biotransformation experiments with raw wastewater (BT), (ii) sorption experiments with diluted primary sludge with the addition of sodium azide (SO), and (iii) abiotic experiments with mineral water (AB). Two procedures of BT experiments were carried out. In the first procedure (BT-P1), analytical standards were spiked with an initial concentration at 10 μg L^{-1} (higher than background concentrations) in the reactors and considered as the main target chemicals. Isotopically labeled internal standards (ILIS) were used to evaluate the analytical procedure and spiked into collected samples prior to sample treatment. In the second procedure (BT-P2), ILIS were spiked at 2 μg L^{-1} in the reactors and targeted, thus allowing for the determination of direct transformation of illicit drugs without any interference from background concentrations.[15] For other experiments, (i.e., SO and AB) only the first procedure (P1) was employed. For all experiments, grab samples of raw wastewater and primary sludge were collected from Mølleåværket

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

WWTP (Lundtofte, Denmark; SI section S2). The solution containing the drug biomarkers was added in the batch reactors, and the first-sample (t = 0) was collected after 2 min to allow for mixing of biomarkers in the medium (each sample volume = 260 mL). TSS, volatile suspended solids (VSS), temperature, and pH were monitored during experiments and are reported as average value ± standard deviation. An overview of all experiments is presented in SI Table S4.

Biotransformation Experiments (BT). For BT-P1 experiments (aerobic: 0.32 ± 0.04 gTSS L^{-1}, pH = 8.8 ± 0.1, T = 14.3 ± 0.1 °C; anaerobic: 0.32 ± 0.05 gTSS L^{-1}, pH = 8.3 ± 0.2, T = 15.3 ± 0.2 °C), raw wastewater was collected in June 2015, 3 h prior to start-up of batch experiments. For BT-P2 experiments (aerobic: final 1.28 ± 0.14 gTSS L^{-1}, pH = 8.7 ± 0.1, T = 15 ± 0.4 °C; anaerobic: 1.29 ± 0.07 gTSS L^{-1}, pH = 8.1 ± 0.3, T = 15.5 ± 0.1 °C) raw wastewater was collected in October 2014 and kept overnight at 4 °C for decantation. Settled wastewater solids were subsequently diluted (1:2) with newly sampled raw sewage and used for experiments. Spiking solution for BT-P2 experiments contained ILIS only for MEPH, METD, EDDP, COC, BE, EME, CE (not for anaerobic experiment), and 6MAM. Aerobic and anaerobic experiments were conducted in parallel over 48 h, with an initial wastewater volume of 7 L. Over the course of experiments, nine and 12 samples were collected for BT-P1 and BT-P2, respectively. Due to deficiency in the nitrogen sparging system, oxygen was transferred to an anaerobic BT-P1 reactor (2−3.8 mgO_2 L^{-1}) over the last 4 h of the experiment. Hence, all data at t = 48 h for anaerobic BT-P1 were neglected for model calibration purposes.

Respirometry tests were used to monitor microbial respiration and characterize different COD fractions in the wastewater inoculum according to their biodegradability.[41−45] Briefly, aliquots of the wastewater used as inoculum for BT-P1 (t = 0) were collected and used for biological oxygen demand (BOD) monitoring (Oxitop, WTW, Germany) over 48 h (T = 20 °C), based on which oxygen uptake rates (OUR) were calculated. We assumed that the biomass activity in the BOD bottles would be approximately identical with the biomass activity in the BT-P1 aerobic experiment as both systems were operated with the same wastewater medium without limitation of oxygen. To account for temperature differences, Arrhenius-based correction factors of bacterial growth were used. Moreover, the COD fractionation was assumed to be applicable for the BT-P1 anaerobic experiment at t = 0. A detailed description of the respirometry method is presented in SI section S1.2.

Sorption Experiments (SO). Primary sludge samples were first mixed with tap water to remove already sorbed chemicals for a period of 12 h (wash-off step). The amount of sorbed chemicals that remained in the solid phase was assumed to be negligible compared to the spiked amount (initial concentration of 10 μg L^{-1}). Following centrifugation (20 min, 4700 rpm) and dilution of the extract with wastewater effluent, sodium azide (0.05% v/v) was added to the mixture to inhibit microbial degradation. SO1 experiments with an initial volume of 7 L (0.32 ± 0.02 gTSS L^{-1}, pH = 8.4 ± 0.1, T = 15.2 ± 0.1 °C) and SO2 with initial volume of 4 L (0.41 ± 0.03 gTSS L^{-1}, pH = 7.8 ± 0.1, T = 15 ± 0.1 °C) were performed at different pH levels representative of conditions in corresponding aerobic and anaerobic BT experiments.

Abiotic Experiments (AB). AB experiments were performed: (i) to assess abiotic process kinetics independent of microbial transformation and estimate abiotic degradation rate constants k_{abio}; (ii) to quantify partitioning of drug biomarkers to the reactor wall; and (iii) to correct the estimation of K_d by accounting for mass loss e.g. by hydrolysis and sorption to reactor wall. Therefore, in parallel with BT-P1 experiments, two abiotic control experiments were conducted under aerobic (AB-BT aerobic, pH = 8.8 ± 0.02, T = 14.9 ± 0.4 °C) and anaerobic (AB-BT anaerobic, pH = 8.7 ± 0.6, T = 15.2 ± 0.1 °C) conditions. An initial 7 L working volume of mineral water spiked with biomarkers was used. Two additional control experiments, AB-SO1 at pH = 8.7 ± 0.04 and T = 14.2 ± 0.1 °C and AB-SO2 at pH = 7.9 ± 0.1 and T = 14.8 ± 0.2 °C, were also carried out with mineral water to mimic the conditions of SO1 and SO2 experiments, respectively, in terms of pH, redox conditions, presence of sodium azide, and reactor volume.

Sample Preparation and Analysis. Chemical analysis was carried out using colorimetric methods for total COD and soluble COD (Hach Lange, Germany) and sulfate (Merck, Germany) according to international standards.[46] Samples for dissolved chemical analyses were filtered (0.45 μm cellulose acetate filters, Sartorius, Germany) and stored at −20 °C until analysis. Concentrations of selected volatile fatty acids (formate, acetate, and propionate) and lactate were also quantified in filtered samples. After thawing, samples were injected through an HPLC Fast Acid Analysis Column (100 mm × 7.8 mm, BIO-RAD, Denmark). For quantification, a calibration curve with six points was prepared ranging from 0.5 to 100 mg L^{-1}. TSS was measured using gravimetric analysis following filtration (0.6 μm glass fiber filter, GA-55, Advantec, USA).

For drug biomarkers determination (BT-P1 experiments), samples were spiked with ILIS at 360 ng L^{-1} immediately after sampling and stored at −20 °C until analysis. Following thawing at room temperature, samples were filtered using a 0.6 μm glass fiber filter (GA-55, Advantec, Germany) before further treatment. In SO experiments, samples were filtered immediately after collection to avoid additional contact time between the aqueous phase and suspended solids during storage and thawing. The difference between the nominal spiked concentration and the measured initial (t = 0) concentration may be attributed to the chemical loss through sample filtration. However, for samples with internal standards and ILIS, the loss of internal standards was corrected by a loss of ILIS. All samples were extracted by solid phase extraction (150 mg, 6 cc, Oasis HLB, Waters, Denmark) and analyzed with liquid chromatography coupled to high resolution mass spectrometry (HPLC-LTQ-Orbitrap).[47] Further details on the analytical method for drug biomarkers determination can be found in SI section S3. Experimental parameters used for drug biomarker determination are presented in SI Table S5.

Model Parameter Estimation. A number of WATS and ASM-X model parameters (underlined parameters in Table 1) were estimated via direct calculation from experimental results or parameter estimation using a global optimization algorithm (for details see SI section S7).

Direct Estimation of Parameters. OUR results derived from respirometry tests with the wastewater inoculum were used for (i) estimation of the initial concentrations of different COD fractions in BT-P1 experiments and (ii) calculation of maximum specific growth rate (μ_H), maintenance rate (q_m), and heterotrophic yield (Y_{Hw}), the latter by analyzing the OUR response to propionate spiking. A six-step methodology for COD fractionation and parameter calculation is presented in detail in SI section S1.2. Partition coefficients K_{dw} and K_d were

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

estimated using AB-BT and SO experimental data, respectively, and by assuming that sorption onto wall and suspended solids reached equilibrium within 15 min and 4 h, respectively. These assumptions were based on previous considerations[3] and observation of measured data. K_{dw} was calculated as

$$K_{dw} = \frac{C_{SW,eq}}{C_{LI,eq}\sigma_w} \quad (1)$$

in which C_{LI} is the aqueous concentration at equilibrium (t = 15 min) and C_{SW} (g L^{-1}) is equal to the difference $C_{LI,t=15\ min} - C_{LI,t=0}$ in AB-SO experiments. A similar equation was derived for K_d at equilibrium:

$$K_d = \frac{C_{SL,eq} - C_{loss}}{(C_{LI,eq} + C_{loss})X_{SS}} \quad (2)$$

C_{loss} (equal to the difference $C_{LI,t=4h} - C_{LI,t=0}$ in AB-SO1 and AB-SO2 experiments) was deducted from the sorbed concentration ($C_{SL,eq}$) and added to the aqueous concentration ($C_{LI,eq}$) at equilibrium to account for any mass loss not attributable to sorption onto suspended solids (i.e., by hydrolysis or sorption to reactor wall). Additional information on the calculation of partition coefficients is presented in SI section S5.3.

Parameter Estimation via Optimization. The rapid hydrolysis rate (k_{h1}) in aerobic WATS was estimated by comparing simulation results with corresponding OUR data, obtained from respirometry experiments. Transformation rate constants (k_{abio} and k_{bio}) in ASM-X and the WATS−ASM-X combined model were estimated using AB-BT and BT-P1 experimental data. Parameter estimation was carried out using the Bayesian optimization method Differential Evolution Adaptive Metropolis (DREAM$_{(ZS)}$).[48] The objective function was defined as the normalized sum of squared error (SSE):

$$SSE = \sum_{i=1}^{n}\sum_{j=1}^{m}\left(\frac{O_{i,j} - P_{i,j}}{O_{i,j,max} - O_{i,j,min}}\right)^2 \quad (3)$$

where n is the number of measurements series, m is the number of the data points in each series, O denotes measured data and P the model predictions, and $O_{i,jmax}$ and $O_{i,jmin}$ the maximum and minimum of measurements, respectively. Details on the calibration methodology and identifiability of model parameters are presented in SI section S.7.

Model Simulation and Evaluation. Model simulation and calibration were performed using Matlab R2014a (MathWorks, US). WATS was initialized using the measured and estimated concentrations of different COD fractions and sulfate. ASM-X was initialized using measurements for C_{LI}, estimations of C_{SL} from eq 2 based on measured C_{LI} data prior to spiking (SI Figure S8), and assuming negligible initial C_{sw}.

In order to assess the importance of accounting for microbial growth, the estimation of k_{bio} was carried out using two model complexity levels: (i) the full WATS−ASM-X framework (Table 1), thus accounting for the dynamics of active biomass concentration (unit of k_{bio}: L $gCOD^{-1}$ d^{-1}) and (ii) simplified ASM-X framework with fixed biomass (unit of k_{bio}: L $gCOD^{-1}$ d^{-1}), i.e., no microbial growth. An additional modeling scenario was considered for the estimation of TSS-normalized k^*_{bio} values (unit of k^*_{bio}: L $gTSS^{-1}$ d^{-1}) that could be compared with findings from previous studies[3,14,49] and used to assess the relative contribution of abiotic and biotic processes to the overall transformation of a drug biomarker.

The accuracy of predictions by the WATS−ASM-X model was further assessed by comparing the simulation outputs with the BT-P2 data set. Since no additional internal standards (rather than the ILIS listed in Table S3) were spiked to correct for any mass loss during sample treatment or the effect of sample matrix,[47] the data set from BT-P2 was only used for model evaluation (as an independent data set) and not for parameter estimation. BT-P2 experiments differed from BT-P1 in terms of raw sewage composition and TSS concentration (3-fold difference) and of the use of nondeuterated or deuterated internal standards (ILIS).

RESULTS AND DISCUSSION

Wastewater Characterization. On the basis of the analysis of respirometric data, the total COD (977 gCOD m^{-3}) in raw wastewater used for the BT-P1 experiment was characterized as 1.8% X_{Hw}, 12.9% S_A, 6% S_F, 15.2% X_{S1}, 43.6% X_{S2}, and 20.5% S_{Me} (SI Table S2). X_{SRB} was assumed to be 4 gCOD m^{-3} and, only for the aerobic experiment, considered as a fraction of X_{S2}.[32]

The comparison with reference respirometric results revealed that the presence of MeOH (0.024% v/v) in the biomarker spiking solution did not significantly affect the respiration process, thus indicating limited utilization of MeOH as a growth substrate over the 2-d experiment (SI section S1.3). Methanol utilization by SRB species under anaerobic conditions was considered negligible as only a few SRB strains can utilize MeOH.[34] The wastewater sample used for BT-P2 experiments was assumed to have the same relative composition as the BT-P1 sample by adjusting COD fractions to measured total COD (5440 gCOD m^{-3}) and methanol (1800 gCOD m^{-3}).

Primary Substrates. Using the WATS model, concentration dynamics of different COD fractions (substrate and biomass) during BT-P1 batch experiments were predicted (Figure 1). Simulation results for the aerobic batch experiment, following WATS calibration with respirometric data, revealed a significant variation of X_{Hw} (5-fold increase followed by a 53% decrease) over the course of the batch experiment, as expected by the initial substrate-to-microorganism ratio. This likely influenced the kinetics of drug biomarker biotransformation and shows the limited validity of the nongrowth assumption typically considered in stability studies. X_{Hw} was predicted to reach a maximum concentration of 100 gCOD m^{-3} after 13 h, when S_S became growth limiting. While X_{S1} was reduced via hydrolysis, X_{S2} remained almost constant during the experiment (due to extremely low hydrolysis rate k_{h2}). Significant evaporation of MeOH (66% during BT-P1) was predicted, based on the results obtained in an additional set of evaporation experiments (SI section S1.3). Under aerobic conditions, the calibrated WATS model predicted OUR measurements from the respirometry tests as well as measured total and soluble COD during BT-P1 aerobic experiments (SI Figures S4 and S7).

WATS model predictions under anaerobic conditions (Figure 1) showed a 61% decrease of X_{Hw}, with simultaneous growth of X_{SRB} (2.8-fold increase) over the 2-d experiment. Concentration profiles for X_{S1} and X_{S2} indicated comparably slow hydrolysis, with limited formation of S_F. Almost complete fermentation of S_F to S_A within 30 h was predicted (Figure 1), with initial net formation and subsequent decrease of S_A. The predicted nonlimiting S_F (during the first 30 h) and S_A

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

(over the entire experiment) were expected to support growth of X_{SRB}. Notably, the MeOH evaporation rate in anaerobic experiments was 2-fold lower than in the aerobic experiment (see also SI Figure S6), partly justifying the lower removal of total and soluble COD in the anaerobic experiment. Even though calibration of the anaerobic WATS model was not performed and previously suggested parameter values were used (SI Table S1), it was possible to predict S_{SO4} variations under anaerobic conditions with reasonable approximation (SI Figure S7). Discrepancies between WATS simulations and total and soluble COD measured values could have resulted from, among others, underestimation of maximum specific growth rate for X_{SRB} (μ_{SRB} = 0.8 d^{-1}, originally estimated for anaerobic biofilm[50]). Nevertheless, it should be noted that available methods to determine WATS anaerobic model parameters are less structured and less conclusive[23,31] than for the aerobic model.[30]

Sorption and Transformation of Drug Biomarkers. *Solid–Liquid Partitioning.* Two wall-liquid partition coefficients, $K_{dw,1}$ (from AB-BT aerobic) and $K_{dw,2}$ (from AB-BT anaerobic), and two solid–liquid partition coefficients, $K_{d,1}$ (from SO1 and AB-SO1) and $K_{d,2}$ (from SO2 and AB-SO2), were estimated from respective experimental data (Figure 1 and SI Figure S9) using eqs 1 and 2. Obtained K_{dw} and K_d values are presented in SI Figure S13 and Table S12. Based on the similarity of pH conditions (SI Figure S10), $K_{dw,1}$ and $K_{d,1}$ determinations were considered relevant to BT-P1 and BT-P2 aerobic experiments and $K_{dw,2}$ and $K_{d,2}$ to BT-P1 and BT-P2 anaerobic experiments. Partitioning to the reactor wall was found to be relevant (K_{dw} up to 0.16 L dm^{-2} for THC) for all drug biomarkers except for MORG and 6MAM. Partitioning to suspended solids was found to be relevant for MEPH, METD, EDDP, BE, 6MAM, THCOH, and THCCOOH, with K_d values ranging from 0.11 L $gTSS^{-1}$ (METD) to 0.80 L $gTSS^{-1}$ (THCCOOH). Although THC is highly hydrophobic (log K_{ow} = 7.61), for THC, sorption onto the wall was the dominant partitioning process (poly(methyl methacrylate), Plexiglas). Notably, recorded pH data showed a pH increase during experiments, crossing the pK_a of some of the drug biomarkers. Variations of pH can potentially alter the speciation of the drug biomarker and possibly affect their sorption potential (see SI section S5.2).

Transformation of Drug Biomarkers: Pathways and Kinetics. Measured and simulated (using combined WATS–ASM-X model) drug biomarker concentrations in batch experiments AB-BT, BT-P1 and BT-P2 are presented in Figure 1. All posterior distributions (densities) of estimated parameters are reported in SI Figures S22 and S23.

The calibrated WATS–ASM-X model was then evaluated via forward simulations using the BT-P2 data set. The effect of different redox conditions on transformation kinetics and the relative contribution of abiotic and biotic processes to the overall transformation of each drug biomarker (quantified by comparing the transformation rates k_{abio}, d^{-1}, and $k^*_{bio}{\cdot}X_{SS}$, d^{-1}) are summarized in Figure 2 (a–b and c–d, respectively). The results obtained are presented separately for each group of drug biomarkers in the following paragraphs. In this study, biotransformation rate constants (k_{bio}, L $gCOD^{-1}$ d^{-1}) for illicit drugs were estimated for the first time by accounting for microbial growth using the WATS–ASM-X framework. Thus, our results were compared with published literature in terms of TSS-normalized biotransformation rate constants ($k^*_{bio}{\cdot}X_{SS}$, d^{-1}) or relative conversion (%) during batch experiments.

Mephedrone. Under aerobic conditions, biotransformation ($k^*_{bio,ae,MEPH}{\cdot}X_{SS}$ = 0.58 d^{-1}) was found to dominate MEPH conversion over abiotic mechanisms ($k_{abio,ae,MEPH}$ = 0.1 d^{-1}), which is not the case under anaerobic conditions ($k^*_{bio,an,MEPH}{\cdot}X_{SS}$ = 0 d^{-1}; $k_{abio,an,MEPH}$ = 0.18 d^{-1}). Model predictions were in good agreement with measurements from the BT-P2 data set (Figure 1). A few studies assessed the transformation of MEPH in wastewater. Ostman et al.[6] reported 5% and 6% removal of MEPH in Milli-Q water and sewage, respectively, at room temperature over 24 h, being significantly less than what observed in the present study. MEPH is a relatively new psychoactive substance, and its consumption has been estimated by measuring MEPH itself as biomarker in wastewater influent.[51]

Methadone. Net formation of EDDP (Figure 1) as a result of significant METD transformation (especially under aerobic conditions) was not observed. Thus, our data do not suggest EDDP as the major METD transformation product, as shown for human metabolism.[12,37] Moreover, *N*-demethylation of METD to EDDP was predicted to be unfeasible in wastewater.[39] Hence, the transformations of EDDP and METD were considered as independent processes (further discussion in SI section S8). The abiotic METD transformation rate was higher under aerobic conditions ($k_{abio,ae,METD}$ = 0.25 d^{-1}; $k_{abio,an,METD}$ = 0.15 d^{-1}). Furthermore, aerobic biotransformation of METD was found to be significantly higher than under anaerobic conditions ($k_{bio,ae,METD}$ = 1495 L $gCOD^{-1}$ d^{-1}; $k_{bio,an,METD}$ = 0 L $gCOD^{-1}$ d^{-1}). Similarly, for EDDP, aerobic biotransformation was significantly higher than that obtained under anaerobic conditions ($k_{bio,ae,EDDP}$ = 2.90 L $gCOD^{-1}$ d^{-1}, $k_{bio,an,EDDP}$ = 0.81 L $gCOD^{-1}$ d^{-1}). The WATS–ASM-X model did not adequately predict BT-P2 experimental data for METD under aerobic conditions, whereas the model could be validated for other BT-P2 data sets. Former studies were inconclusive as to the removal of METD in wastewater, ranging from almost complete (wastewater in closed container at 4 °C after 3 d)[52] to low (<5%, in unfiltered wastewater at 19 °C, pH = 7.4 after 1 d)[53] or even negative removal (−8%, in wastewater at 20 °C and pH ∼ 7.5 after 12 h).[12] Our results suggest that no formation of EDDP should be considered from METD, if EDDP is to be used as a METD biomarker in WBE studies.

Cocaine. The transformation pathway for COC drug biomarkers was defined according to Bisceglia et al.,[14] with negligible transformation of COC to EME as reported previously (see SI Figure S16).[3] For all the experiments, the measured data (Figure 1) indicated net removal of COC, EME, and CE and net formation of BE. For all COC biomarkers, abiotic processes dominated the overall transformation under aerobic conditions and especially under anaerobic conditions, at which (except for BE) no contribution of biotic processes was found. Slightly higher anaerobic k_{abio} were found compared to aerobic rates (Figure 2a).

For COC, EME, and CE, simulation results obtained with the calibrated model agreed well with the measured independent data set (BT-P2 aerobic and anaerobic), thereby validating the identified model structure. We note that the model for BE could be validated if only abiotic transformation was considered. The estimated transformation rates for COC, EME, and BE were in the range reported by Bisceglia et al.[36] (untreated sewage at T = 9 °C and T = 23 °C and pH = 7). In agreement with the latter study,[36] our results indicate that hydrolysis is the governing transformation mechanism for COC and transformation products except for BE under anaerobic conditions (Figure 2d). Furthermore, since blank experiments

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397–13408

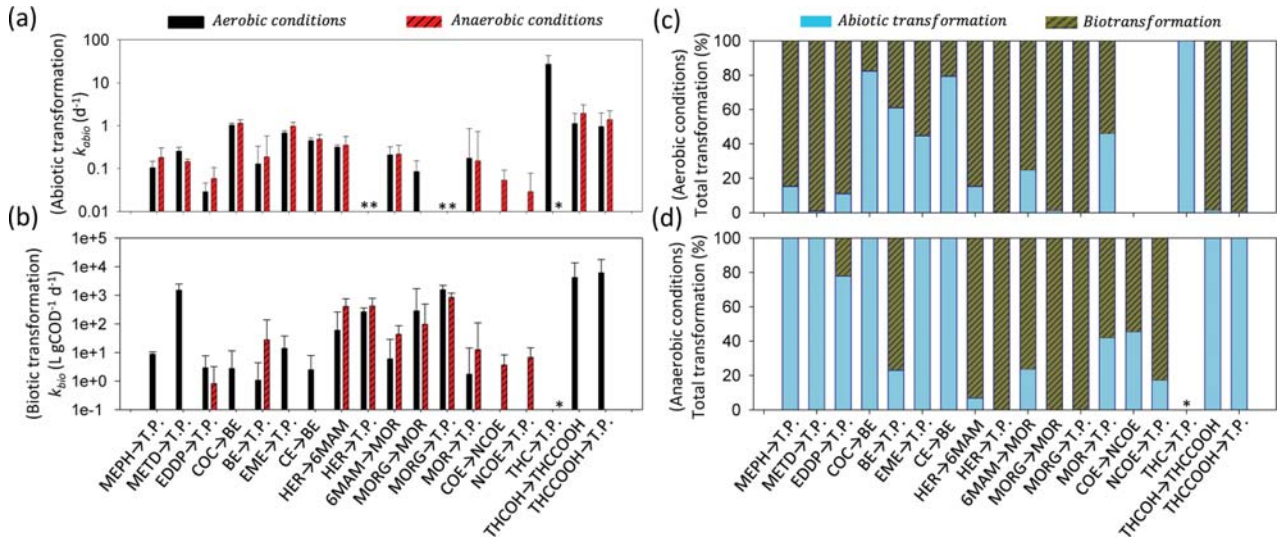


Figure 2. Comparing the effect of aerobic and anaerobic conditions on abiotic transformation (a) and biotransformation rates (b) as well as comparing abiotic transformation with biotransformation under aerobic (c) and anaerobic (d) conditions. Undetermined transformations are indicated with an asterisk (*). Abbreviations: T.P. = transformation product(s). (a and b) k_{bio} (L $gCOD^{-1}$ d^{-1}) is the biotransformation rate constant using WATS−ASM-X. Error bar is the upper bound of the 95% credibility interval of estimated parameters. (c and d) Estimated transformation rates (k_{abio} and $k^{*}_{bio}{\cdot}X_{SS}$) are used as indicators of the contribution of transformation processes—abiotic (filled blue) against biotic (shaded brown)—to the overall transformations (k_{abio} + $k^{*}_{bio}{\cdot}X_{SS}$). k^{*}_{bio} (L $gTSS^{-1}$ d^{-1}) is the TSS-normalized biotransformation rate estimated using the ASM-X model without WATS.

were performed in mineral water, it may be concluded that hydrolysis is not solely bacterially mediated, as reported previously.[36] The estimated aerobic biotransformation rate for COC ($k^{*}_{bio,ae,COC}{\cdot}X_{SS}$ = 0.22 d^{-1}) data was also comparable to estimated rates in unfiltered wastewater at 10 °C (0.1 d^{-1}) and 20 °C (0.48 d^{-1}) with pH = 7.5.[49] However, transformation rates obtained in the present study for COC were lower than those reported by Plósz et al. (8.8 d^{-1})[3] for activated sludge (T = 21 °C and pH = 7.4), likely due to the presence of a biocenosis different from that prevailing in sewer systems. In WBE, BE is normally used as a suitable biomarker for back-calculation of COC consumption. This study demonstrates that formation of BE from both COC and CE (when ethanol and cocaine coexist in blood) is significant (especially under aerobic conditions) and should be considered in back-calculation schemes.

Heroin. HER transformation to 6MAM and then to MOR via two-step deacetylation has been reported.[54] However, rapid HER conversion (overall $k_{bio,ae,HER}$ = 321.4 L $gCOD^{-1}$ d^{-1}, $k_{bio,an,HER}$ = 824.1 L $gCOD^{-1}$ d^{-1}) did not result in a significant 6MAM formation in BT-P1 experiments. Thus, an additional biotransformation product for HER was considered in the pathway. Furthermore, a mass balance analysis over MOR revealed that the fast decrease of MORG concentration (overall $k_{bio,ae,MORG}$ = 1842.8 L $gCOD^{-1}$ d^{-1}, $k_{bio,an,MORG}$ = 942.6 L $gCOD^{-1}$ d^{-1}) could be described if MORG was transformed not only to MOR[55] but also to another (unknown) transformation product. This assumption was supported by the EAWAG-BBD pathway prediction system (SI Figure S27)[39] and by experimental data reported by Senta et al.,[49] who also found an imbalance between formed MOR and removed MORG and 6MAM amounts. These two additional pathways were not considered for abiotic transformation of HER and MORG. As presented in Figure 1, MORG remained nearly unchanged in mineral water ($k_{abio,ae,MORG}$ = 0.08 d^{-1}, $k_{abio,an,MORG}$ = 0 d^{-1}) but was rapidly transformed in wastewater, possibly via extracellular β-glucuronidase enzymes (abundant, e.g., in fecal bacteria).[56,57] Further details on the transformation pathways for HER and MORG are presented in SI section S8. Although COE can potentially be metabolized to MOR in the human body,[37] we considered MOR as a minor transformation product of COE in wastewater as previously reported.[38]

Significant abiotic conversion was observed for 6MAM and MOR under both redox conditions, while abiotic transformation of COE was observed only under anaerobic conditions. Other identified transformations are dominantly microbially mediated transformations (Figure 2c–d). HER removal of 40% and 80% (T = 4 °C) after 1 d and 3 d in wastewater, respectively, has been previously reported.[52] However, our results for HER are in closer agreement with data presented by Baker et al.,[53] i.e., 80% removal after 12 h (raw wastewater; T = 19 °C, pH = 7.4). In the same study, comparably high removal (85%) for MORG in both filtered and unfiltered wastewater and relatively low removal of 6MAM (12%) were also observed. Biotransformation rates for 6MAM and MORG under aerobic conditions (overall $k^{*}_{bio,ae,MORG}{\cdot}X_{SS}$ = 32.2 d^{-1}, $k^{*}_{bio,ae,6MAM}{\cdot}X_{SS}$ = 0.63 d^{-1}) were found to be significantly higher than the values reported in wastewater at pH = 7.5 (0.94 d^{-1} for MORG, 0.12 d^{-1} for 6MAM at 10 °C; 2.4 d^{-1} for MORG and 0.19 d^{-1} for 6MAM at 20 °C).[49] Previous studies on COE are inconclusive and estimated removal rates (1-d batch experiments) exhibit significant variation from no removal (sewage, room temperature)[6] to comparably high (~50% removal in 1:20-diluted activated sludge).[58] Since 6MAM often occurs at nondetectable levels in samples taken from sewer systems, MOR has been proposed as the best biomarker to estimate heroin abuse levels.[59] This approach necessitates the quantification of the therapeutic consumption of MOR that must be subtracted from the total MOR load measured in wastewater.[38,60] In addition, evidence from this study shows the necessity of accounting for MOR formation from 6MAM and MORG. To our knowledge, this is the first

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

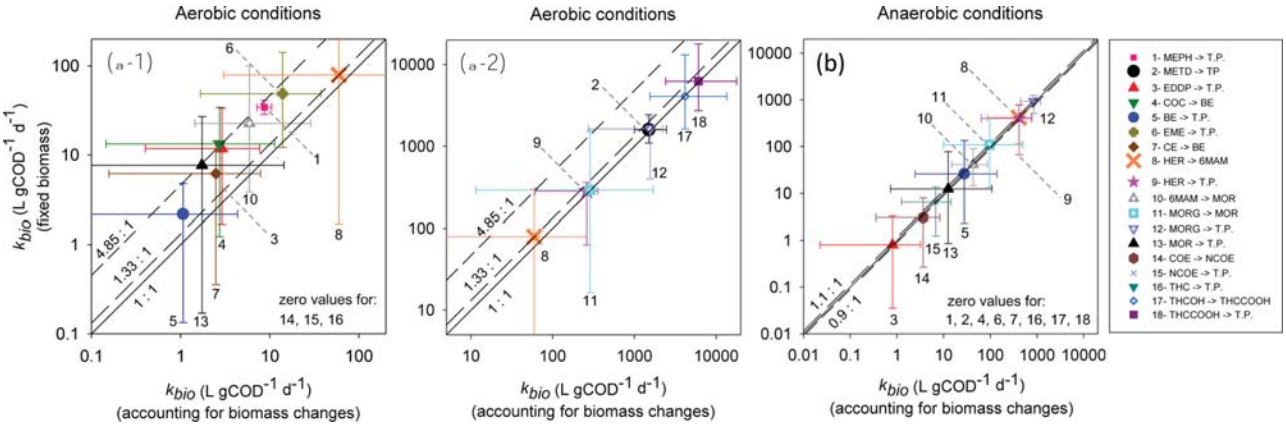


Figure 3. Comparing estimated k_{bio} using WATS−ASM-X considering biomass changes (X axis) with k_{bio} estimated using ASM-X with a fixed biomass fraction (Y axis) under aerobic conditions (a-1 and a-2) and under anaerobic conditions (b). Dashed lines indicate the ratio of estimated parameters.

study to evaluate transformation kinetics of six heroin biomarkers simultaneously.

THC. With respect to the pathway identification (SI Figure S18), we initially hypothesized that THC transformation would be different from THC metabolic pathways in humans as transformation of THC to THCOH appears unfeasible in wastewater[39] (SI Figure S27) while transformation of THCOH to THCCOOH may occur.[37,39] This hypothesis was confirmed by our experimental results (Figure 1), which indicated no clear formation of THCOH, and independent in-sewer transformation for THC was thus considered. THC under aerobic conditions and THCOH and THCCOOH under anaerobic conditions (Figure 2c−d) underwent significant abiotic transformation ($k_{abio,ae,THC}$ = 27.2 d^{-1}, $k_{abio,an,THCOH}$ = 1.9 d^{-1}, $k_{abio,an,THCCOOH}$ = 1.4 d^{-1}). We note that the THC concentration could not be quantified during AB-BT and BT-P1 anaerobic experiments due to ILIS signal suppression.

Removal rates reported in the literature for THC biomarkers show significant variations. THCOH removal up to 20% (unfiltered wastewater; T = 20 °C, pH = 7.5; duration: 3 d) has been reported.[49] Another investigation in wastewater showed 40% THCOH removal (4 °C) after 3 d[52] and 40% THC removal and negligible THCCOOH removal (−20 °C) after 3 d.[61] Castiglioni et al.[55] have reported 8% removal of THCCOOH in wastewater (4 °C) after 3 d. These results do not agree with our findings, which show significantly higher conversion rates for THC, THCOH, and THCCOOH (Figure 1). Furthermore, it is unclear to what extent the reported elimination was due to sorption—which in our study was found to be significant for THCOH and THCCOOH ($K_{d,THCOH}$ ∼ 0.7, $K_{d,THCCOOH}$ ∼ 0.8 L gTSS^{-1})—or to transformation.

Factors Influencing Biomarker Transformation. *Redox conditions.* Aerobic and anaerobic conditions were found to have no major impact on abiotic transformation rates for most of the investigated substances, except for MORG, COE, and NCOE (Figure 2a). Conversely, differences between k_{bio} values estimated under the two redox conditions were found to be significant for nearly all drug biomarkers (Figure 2b). Thus, redox conditions prevailing in sewers may significantly influence the microbially mediated transformation of drug biomarkers.

Transformation Mechanisms. Abiotic transformation processes were found to be the dominating mechanism to the overall biomarker transformation (Figure 2c−d) for THC (aerobic conditions) MEPH, METD, COC, EME, CE, THCOH, and THCCOOH (anaerobic conditions). Conversely, insignificant abiotic contribution was observed for MEPH, METD, EDDP, HER, MORG, THCOH, and THCCOOH (aerobic conditions) and HER, MORG, and NCOE (anaerobic conditions). Overall, these results highlight the necessity of distinguishing between abiotic and microbially mediated transformation (e.g., through control experiments in the absence of active biomass) when assessing the fate of illicit drugs in sewer systems.

Model Complexity. The uncertainty imposed by neglecting biomass growth processes and propagating to the estimated parameter values was additionally assessed (Figure 3). Values of k_{bio} (L gCOD^{-1} d^{-1}) were estimated with the BT-P1 data set using two model complexity levels, i.e., ASM-X with no biomass growth and the combined WATS−ASM-X implementation. The comparison revealed that neglecting active biomass concentration dynamics during a batch experiment can result in up to 385% (4.85:1) overestimation of k_{bio} under aerobic conditions, whereas no major difference was observed under anaerobic conditions. For drug biomarkers with comparably high k_{bio} (e.g., METD, MORG, THCCOOH), estimated parameter values were less sensitive to the dynamics of active biomass concentrations than for those chemicals with $k_{bio} \leq$ 20 L gCOD^{-1} d^{-1} (Figure 3a-1 and a-2). This can be explained by the fact that at high biotransformation rate constants, complete removal of the drug biomarker would be achieved before biomass undergoes significant growth. Our results suggest that the increased model complexity of the combined WATS−ASM-X model can be justified by the avoided parameter uncertainties introduced by the prediction of the microbial growth processes under aerobic conditions. This was not the case under anaerobic conditions (Figure 3b), and reliable parameter estimation was possible by calibrating a simplified modeling framework with ASM-X only. These conclusions were drawn on the optimal kinetic model complexity and can also be considered true for sewer catchment simulation models used to back-calculate drug abuse rates in urban areas.

In this study, we have presented an assessment of the removal of illicit drug biomarkers in wastewater, comprising the partitioning onto a solid medium (i.e., suspended solids and reactor wall), abiotic transformation, and microbiologically mediated transformations. Results obtained demonstrate that redox conditions can have a significant impact on transformation

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

kinetics. Modeling the transformation of drug biomarkers in raw wastewater required consideration of the significant growth of biomass under aerobic conditions and thus describing the dynamics of different COD fractions. Our results suggest that the estimation of transformation rates and rate constants are significantly influenced by transformation pathways, as drug biomarkers present in the medium can often be formed from other biomarkers. These findings underscore the importance of accounting for in-sewer transformation of drug biomarkers and may lead to more accurate estimations of drug consumption. While this study focused on the fate of selected drug biomarkers in the presence of suspended biomass, current research is also focusing on transformation of drug biomarkers in sewer biofilms.[62] Along with in-sewer transformation, a more comprehensive assessment of all sources of uncertainty is required for the selection of a suitable biomarker candidate for back-calculation purposes. Further research activities are also required to consider in-sewer transport processes and thus calculate residence time distribution, at a catchment or subcatchment level. Wastewater-based epidemiological engineering is an emerging field, in which mathematical models, such as the WATS−ASM-X developed in this study, can play a key role as decision support tools for epidemiological studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03049.

Additional information about details of WATS−ASM-X model and modeling transformation pathways (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Address: Bygningstorvet 115, 2800 Kongens, Lyngby, Denmark. Telephone: +45 4525 1608. E-mail: pear@env.dtu.dk.

*Address: Bygningstorvet 115, 2800 Kongens, Lyngby, Denmark. Telephone: +45 4525 1694. E-mail: beep@env.dtu.dk.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the European Union's Seventh Framework Programme for research, technological development, and demonstration [grant agreement 317205, the SEWPROF MC ITN project]. We also thank Borja Valverde Pérez, PhD for helpful discussions and inputs to develop a new methodology for model calibration.

■ REFERENCES

(1) Daughton, C. G. Illicit drugs in municipal sewage: proposed new non-intrusive tool to heighten public awareness of societal use of illicit/abused drugs and their potential for ecological consequences. In *American Chemical Society, Symposium Series*; American Chemical Society: Washington, DC, 2001; pp 348−364.

(2) Zuccato, E.; Chiabrando, C.; Castiglioni, S.; Calamari, D.; Bagnati, R.; Schiarea, S.; Fanelli, R. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environ. Health* **2005**, *4* (10), 1−7.

(3) Plósz, B. G.; Reid, M. J.; Borup, M.; Langford, K. H.; Thomas, K. V. Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. *Water Res.* **2013**, *47* (7), 2129−2140.

(4) Castiglioni, S.; Bijlsma, L.; Covaci, A.; Emke, E.; Hernández, F.; Reid, M.; Ort, C.; Thomas, K. V.; Van Nuijs, A. L. N.; De Voogt, P.; Zuccato, E. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. *Environ. Sci. Technol.* **2013**, *47* (3), 1452−1460.

(5) Zuccato, E.; Castiglioni, S.; Tettamanti, M.; Olandese, R.; Bagnati, R.; Melis, M.; Fanelli, R. Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. *Drug Alcohol Depend.* **2011**, *118*, 464−469.

(6) Ostman, M.; Fick, J.; Näsström, E.; Lindberg, R. H. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. *Sci. Total Environ.* **2014**, *472*, 862−871.

(7) Thomas, K. V.; Bijlsma, L.; Castiglioni, S.; Covaci, A.; Emke, E.; Grabic, R.; Hernández, F.; Karolak, S.; Kasprzyk-Hordern, B.; Lindberg, R. H.; Lopez de Alda, M. J.; Meierjohann, A.; Ort, C.; Pico, Y.; Quintana, J. B.; Reid, M.; Rieckermann, J.; Terzic, S.; van Nuijs, A. L. N.; de Voogt, P. Comparing illicit drug use in 19 European cities through sewage analysis. *Sci. Total Environ.* **2012**, *432*, 432−439.

(8) Ort, C.; van Nuijs, A. L. N.; Berset, J. D.; Bijlsma, L.; Castiglioni, S.; Covaci, A.; de Voogt, P.; Emke, E.; Fatta-Kassinos, D.; Griffiths, P.; Hernandez, F.; Gonzalez-Marino, I.; Grabic, R.; Kasprzyk-Hordern, B.; Mastroianni, N.; Meierjohann, A.; Nefau, T.; Ostman, M.; Pico, Y.; Racamonde, I.; Reid, M.; Slobodnik, J.; Terzic, S.; Thomaidis, N.; Thomas, K. V. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. *Addiction* **2014**, *109* (8), 1338−1352.

(9) Reid, M. J.; Langford, K. H.; Grung, M.; Gjerde, H.; Amundsen, E. J.; Morland, J.; Thomas, K. V. Estimation of cocaine consumption in the community: a critical comparison of the results from three complimentary techniques. *BMJ. Open* **2012**, *2* (6), e001637.

(10) Burgard, D. a; Banta-Green, C.; Field, J. a. Working Upstream: How Far Can You Go with Sewage-Based Drug Epidemiology? *Environ. Sci. Technol.* **2014**, *48* (3), 1362−1368.

(11) Kinyua, J.; Covaci, A.; Maho, W.; McCall, A.-K.; Neels, H.; van Nuijs, A. L. N. Sewage-based epidemiology in monitoring the use of new psychoactive substances: Validation and application of an analytical method using LC-MS/MS. *Drug Test. Anal.* **2015**, *7* (9), 812−818.

(12) van Nuijs, A. L. N.; Abdellati, K.; Bervoets, L.; Blust, R.; Jorens, P. G.; Neels, H.; Covaci, A. The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? *J. Hazard. Mater.* **2012**, *239*−*240*, 19−23.

(13) Plósz, B. G.; Langford, K. H.; Thomas, K. V. An activated sludge modeling framework for xenobiotic trace chemicals (ASM-X): Assessment of diclofenac and carbamazepine. *Biotechnol. Bioeng.* **2012**, *109* (11), 2757−2769.

(14) Bisceglia, K. J. K.; Lippa, K. a. Stability of cocaine and its metabolites in municipal wastewater - the case for using metabolite consolidation to monitor cocaine utilization. *Environ. Sci. Pollut. Res.* **2014**, *21* (6), 4453−4460.

(15) Thai, P. K.; Jiang, G.; Gernjak, W.; Yuan, Z.; Lai, F. Y.; Mueller, J. F. Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. *Water Res.* **2014**, *48*, 538−547.

(16) Jelic, A.; Rodriguez-Mozaz, S.; Barceló, D.; Gutierrez, O. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Res.* **2014**, *68*, 98−108.

(17) Struijs, J. *SimpleTreat 4.0: A Model to Predict Fate and Emission of Chemicals in Wastewater Treatment Plants Background Report Describing the Equations*; Bilthoven: The Netherlands, 2014.

(18) Trapp, S.; Franco, A.; Mackay, D. Activity-Based Concept for Transport and Partitioning of Ionizing Organics. *Environ. Sci. Technol.* **2010**, *44* (16), 6123−6129.

(19) Polesel, F.; Plósz, B. G.; Trapp, S. From consumption to harvest: environmental fate prediction of excreted ionizable trace organic chemicals. *Water Res.* **2015**, *84*, 85−98.

(20) Joss, A.; Zabczynski, S.; Göbel, A.; Hoffmann, B.; Löffler, D.; McArdell, C. S.; Ternes, T. a.; Thomsen, A.; Siegrist, H. Biological degradation of pharmaceuticals in municipal wastewater treatment:

Proposing a classification scheme. *Water Res.* **2006**, *40* (8), 1686−1696.
(21) Hvitved-Jacobsen, T.; Vollertsen, J.; Matos, J. S. The sewer as a bioreactor - A dry weather approach. *Water Sci. Technol.* **2002**, *45* (3), 11−24.
(22) Nielsen, P. H.; Raunkjrer, K.; Norsker, N. H.; Jensen, N. A.; Hvitved-Jacobsen, T. Transformation of wastewater in sewer systems - a review. *Wat. Sci. Technol.* **1992**, *25* (6), 17−31.
(23) Hvitved-Jacobsen, T.; Vollertsen, J.; Nielsen, A. H. *Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks*, 2nd edition; CRC Press: Boca Raton, FL, 2013.
(24) Hvitved-Jacobsen, T.; Vollertsen, J.; Tanaka, N. Wastewater quality changes during transport in sewers - an integrated aerobic and anaerobic model concept for carbon and sulfur microbial transformations. *Water Sci. Technol.* **1998**, *38* (10), 257−264.
(25) Hvitved-Jacobsen, T.; Vollertsen, J.; Nielsen, P. H. A process and model concept for microbial wastewater tranformations in gravity sewers. *Water Sci. Technol.* **1998**, *37* (1), 233−241.
(26) Hvitved-Jacobsen, T.; Vollertsen, J.; Tanaka, N. An integrated aerobic/anaerobic approach for prediction of sulfide formation in sewers. *Water Sci. Technol.* **2000**, *41* (6), 107−115.
(27) Tanaka, N.; Hvitved-Jacobsen, T.; Horie, T. Transformations of Carbon and Sulfur Wastewater Components under Aerobic-Anaerobic Transient Conditions in Sewer Systems. *Water Environ. Res.* **2000**, *72* (6), 651−664.
(28) McCall, A.-K.; Bade, R.; Kinyua, J.; Lai, F. Y.; Thai, P. K.; Covaci, A.; Bijlsma, L.; van Nuijs, A. L. N.; Ort, C. Critical review on the stability of illicit drugs in sewers and wastewater samples. *Water Res.* **2016**, *88*, 933−947.
(29) Tanaka, N.; Hvitved-Jacobsen, T. Transformations of wastewater organic matter in sewers under changing aerobic/anaerobic conditions. *Water Sci. Technol.* **1998**, *37* (1), 105−113.
(30) Vollertsen, J.; Hvitved-Jacobsen, T. Stoichiometric and kinetic model parameters for microbial transformation of suspended solid in combined sewer systems. *Water Res.* **1999**, *33* (14), 3127−3141.
(31) Rudelle, E.; Vollertsen, J.; Hvitved-Jacobsen, T.; Nielsen, A. H. Anaerobic transformations of organic matter in collection systems. *Water Environ. Res.* **2011**, *83* (6), 532−540.
(32) Rudelle, E.; Vollertsen, J.; Hvitved-Jacobsen, T.; Nielsen, A. H. Modeling anaerobic organic matter transformations in the wastewater phase of sewer networks. *Water Sci. Technol.* **2012**, *66* (8), 1728−1734.
(33) Muyzer, G.; Stams, A. J. M. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **2008**, *6*, 441−454.
(34) Hao, T.; Xiang, P.; Mackey, H. R.; Chi, K.; Lu, H.; Chui, H.; van Loosdrecht, M. C. M.; Chen, G.-H. A review of biological sulfate conversions in wastewater treatment. *Water Res.* **2014**, *65*, 1−21.
(35) Gibson, G. R. Physiology and ecology of the sulphate-reducing bacteria. *J. Appl. Bacteriol.* **1990**, *69* (6), 769−797.
(36) Bisceglia, K. J.; Lippa, K. A. Stability of cocaine and its metabolites in municipal wastewater - the case for using metabolite consolidation to monitor cocaine utilization. *Environ. Sci. Pollut. Res.* **2014**, *21* (516), 4453−4460.
(37) Castiglioni, S.; Zuccato, E.; Fanelli, R. *Illicit Drugs in the Environment: Occurrence, Analysis, and Fate Using Mass Spectrometry*; John Wiley & Sons, Inc: New York, 2011.
(38) Zuccato, E.; Chiabrando, C.; Castiglioni, S.; Bagnati, R.; Fanelli, R. Estimating Community Drug Abuse by Wastewater Analysis. *Environ. Health Perspect.* **2008**, *116* (8), 1027−1032.
(39) EAWAG-BBD. Pathway Prediction System. http://eawag-bbd.ethz.ch/predict/index.html (accessed Mar 15, 2016).
(40) EMCDDA. European Drug Report 2015. http://www.emcdda.europa.eu/publications/edr/trends-developments/2015 (accessed Apr 1, 2016).
(41) Spanjers, H.; Takács, I.; Brouwer, H. Direct parameter extraction from respirograms for wastewater and biomass characterization. *Water Sci. Technol.* **1999**, *39* (4), 137−145.
(42) Wentzel, M. C.; Mbewe, A.; Ekama, G. A. Batch test for measurement of readily biodegradable COD and active organism concentrations in municipal waste waters. *Water SA* **1995**, *21* (2), 117−124.
(43) Smets, B. F.; Jobbágy, A.; Cowan, R. M.; Grady, C. P. L., Jr. Evaluation of respirometric data: identification of features that preclude data fitting with existing kinetic expressions. *Ecotoxicol. Environ. Saf.* **1996**, *33* (1), 88−99.
(44) Choubert, J. M.; Rieger, L.; Shaw, A.; Copp, J.; Spérandio, M.; Sørensen, K.; Rönner-Holm, S.; Morgenroth, E.; Melcer, H.; Gillot, S. Rethinking wastewater characterisation methods for activated sludge systems - a position paper. *Water Sci. Technol.* **2013**, *67* (11), 2363−2373.
(45) Orhon, D.; Çokgör, E. U. COD Fractionation in Wastewater Characterization - The State of the Art. *J. Chem. Technol. Biotechnol.* **1997**, *68* (3), 283−293.
(46) *Standard Methods for the Examination of Water and Wastewater. American Public Health Association*, 19th ed.; American Public Health Association: Washington, DC, 1995.
(47) Bijlsma, L.; Emke, E.; Hernández, F.; De Voogt, P. Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water. *Anal. Chim. Acta* **2013**, *768* (1), 102−110.
(48) Laloy, E.; Vrugt, J. a. High-dimensional posterior exploration of hydrologic models using multiple-try DREAM(ZS) and high-performance computing. *Water Resour. Res.* **2012**, *48* (1), 1−18.
(49) Senta, I.; Krizman, I.; Ahel, M.; Terzic, S. Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology. *Sci. Total Environ.* **2014**, *487*, 659−665.
(50) Jiang, F.; Leung, D. H.-W. W.; Li, S.; Chen, G.-H. H.; Okabe, S.; van Loosdrecht, M. C. M. A biofilm model for prediction of pollutant transformation in sewers. *Water Res.* **2009**, *43* (13), 3187−3198.
(51) Castiglioni, S.; Borsotti, A.; Senta, I.; Zuccato, E. Wastewater Analysis to Monitor Spatial and Temporal Patterns of Use of Two Synthetic Recreational Drugs, Ketamine and Mephedrone, in Italy. *Environ. Sci. Technol.* **2015**, *49* (9), 5563−5570.
(52) González-Mariño, I.; Quintana, J. B.; Rodríguez, I.; Cela, R. Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 1748−1760.
(53) Baker, D. R.; Kasprzyk-Hordern, B. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2011**, *1218* (44), 8036−8059.
(54) Poochikian, G.; Cradock, J. Simple high-performance liquid chromatographic method for the separation of 3,6- diacetylmorphine hydrochloride (heroin) and hydrolysis products. *J. Chromatogr.* **1979**, *171*, 371−376.
(55) Castiglioni, S.; Zuccato, E.; Crisci, E.; Chiabrando, C.; Fanelli, R.; Bagnati, R. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **2006**, *78* (24), 8421−8429.
(56) Zuccato, E.; Castiglioni, S. Illicit drugs in the environment. *Philos. Trans. R. Soc., A* **2009**, *367*, 3965−3978.
(57) D'Ascenzo, G.; Di Corcia, A.; Gentili, a.; Mancini, R.; Mastropasqua, R.; Nazzari, M.; Samperi, R. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. *Sci. Total Environ.* **2003**, *302* (1−3), 199−209.
(58) Wick, A.; Wagner, M.; Ternes, T. a. Elucidation of the transformation pathway of the opium alkaloid codeine in biological wastewater treatment. *Environ. Sci. Technol.* **2011**, *45* (8), 3374−3385.
(59) Khan, U.; Nicell, J. A. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. *Environ. Int.* **2011**, *37* (7), 1236−1252.
(60) van Nuijs, A. L. N.; Castiglioni, S.; Tarcomnicu, I.; Postigo, C.; de Alda, M. L.; Neels, H.; Zuccato, E.; Barcelo, D.; Covaci, A. Illicit drug consumption estimations derived from wastewater analysis: A critical review. *Sci. Total Environ.* **2011**, *409* (19), 3564−3577.

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408

(61) Heuett, N. V.; Ramirez, C. E.; Fernandez, A.; Gardinali, P. R. Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: Communal assessment of consumption. *Sci. Total Environ.* **2015**, *511*, 319−330.
(62) McCall, A.-K.; Scheidegger, A.; Madry, M. M.; Steuer, A. E.; Weissbrodt, D. G.; Vanrolleghem, P. A.; Kraemer, T.; Morgenroth, E.; Ort, C. Influence of different sewer biofilms on transformation rates of drugs. *Environ. Sci. Technol.* **2016**, DOI: 10.1021/acs.est.6b04200.

NOTE ADDED AFTER ASAP PUBLICATION

This article published November 21, 2016 with an incorrect version of Figures 2 and 3. The legend and axis of Figure 2 and 3 are updated and the article reposted on November 29, 2016.

DOI: 10.1021/acs.est.6b03049
Environ. Sci. Technol. 2016, 50, 13397−13408