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

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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, and 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 Wastewater Aerobic/anaerobic Transformations in Sewers model (WATS) 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, 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 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 physical and biochemical 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 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 considered as 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 sewer, while lower ratios occur in activated sludge reactors of full-scale WWTPs.[23] Based on 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 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 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 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 conditions are formulated separately for WATS model. In Table 1, ASM-X process rates under aerobic and anaerobic conditions are considered identical as previously suggested.[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 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 non-growth substrates was expressed as a second-order rate equation proportional to (i) the aqueous concentration of the drug biomarker, C_{Ll}; 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 biomarker 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 reactor wall, with definition of the partition coefficient (K_{dw}) between reactor wall and liquid. The latter processes were considered to reflect observed drug biomarker concentrations in blank experiments (typically a decreasing trend, with pronounced initial drop indicative of partitioning to 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 aqueous phase were considered capable of partitioning onto suspended solids (X_{SS}, gTSS L^{-1}) including hydrolysable 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 (not shown). C_{SL} and C_{SW} denote the concentration of drug biomarkers in the solid phase and on reactor wall, respectively. Due to varying area 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 biomarker were assessed individually considering the possible transformation of biomarkers and simultaneous formation from other biomarkers present in the spiking mixture. An additional

state-variable (C_{CJ}) was thus 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 post-processing after model calibration. Feasibility of biodegradation pathways were also attested using 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 EMCDDA reports.[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); (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 concentration 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

149 experiments. A diffuser was placed at the bottom of each reactor and sparging of dry compressed atmospheric
150 air or pure nitrogen was used to create aerobic or anaerobic conditions, respectively. Reactors were further
151 equipped with a mixing impeller from the top.
152 Three different sets of batch experiments were conducted: (i) biotransformation experiments with raw
153 wastewater (BT); (ii) sorption experiments with diluted primary sludge with addition of sodium azide (SO); and
154 (iii) abiotic experiments with mineral water (AB). Two procedures of BT experiments were carried out. In the
155 first procedure (BT-P1), analytical standards were spiked with an initial concentration at 10 μg L^{-1} (higher than
156 background concentrations) in the reactors and considered as the main target chemicals. Isotopically labeled
157 internal standards (ILIS) were used to evaluate the analytical procedure and spiked into collected samples prior
158 to sample treatment. In the second procedure (BT-P2), ILIS were spiked at 2 μg L^{-1} in the reactors and targeted,
159 and thus allowing for the determination of direct transformation of illicit drugs without any interference from
160 background concentrations.[15] For other experiments, (i.e. SO and AB) only the first procedure (P1) was
161 employed. For all experiments, grab samples of raw wastewater and primary sludge were collected from
162 Mølleåværket WWTP (Lundtofte, Denmark; SI Section S2). The solution containing the drug biomarkers was
163 added in the batch reactors and the first sample (t = 0) was collected after two minutes to allow for mixing of
164 biomarkers in the medium (each sample volume=260 mL). TSS, volatile suspended solids (VSS), temperature,
165 and pH were monitored during experiments and are reported as average value ± standard deviation. An overview
166 of all experiments is presented in SI Table S4.
167
168 **Biotransformation Experiments (BT)**
169 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
170 L^{-1}, pH=8.3±0.2, T=15.3±0.2 °C), raw wastewater was collected in June 2015, three hours prior to start-up of
171 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;
172 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
173 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 twelve samples were collected for BT-P1 and BT-P2, respectively. Due to deficiency in nitrogen sparging system, oxygen was transferred to 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 was 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 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 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 at 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 experiment with 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.

199

200 **Abiotic Experiments (AB)**

201 AB experiments were performed: (i) to assess abiotic process kinetics independent of microbial transformation
202 and estimate abiotic degradation rate constants k_{abio}; (ii) to quantify partitioning of drug biomarkers to reactor
203 wall; and (iii) correcting the estimation of K_d by accounting for mass loss e.g. by hydrolysis and sorption to
204 reactor wall. Therefore, in parallel with BT-P1 experiments, two abiotic control experiments were conducted
205 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,
206 T=15.2±0.1 °C) conditions. An initial 7-L working volume of mineral water spiked with biomarkers was used.
207 Two additional control experiments, AB-SO1 at pH=8.7±0.04, T=14.2±0.1 °C, and AB-SO2 at pH=7.9±0.1,
208 T=14.8±0.2 °C, were also carried out with mineral water to mimic the conditions of SO1and SO2 experiments
209 respectively in terms of pH, redox conditions, presence of sodium azide and reactor volume.

210

211 **Sample preparation and analysis**

212 Chemical analysis was carried out using colorimetric methods for total COD and soluble COD (HACH Lange,
213 Germany) and sulfate (Merck, Germany) according to international standards.[46] Samples for dissolved chemical
214 analyses were filtered (0.45 μm cellulose acetate filters, Sartorius, Germany) and stored at -20 °C until analysis.
215 Concentrations of selected volatile fatty acids (formate, acetate and propionate) and lactate were also quantified
216 in filtered samples. After thawing, samples were injected through HPLC Fast Acid Analysis Column (100 mm x
217 7.8 mm, BIO-RAD, Denmark). For quantification, a calibration curve with six points was prepared ranging from
218 0.5 to 100 mg L^{-1}. TSS was measured using gravimetric analysis following filtration (0.6 μm glass fiber filter,
219 Advantec, USA).
220 For drug biomarkers determination (BT-P1 experiments), samples were spiked with ILIS at 360 ng L^{-1}
221 immediately after sampling and stored at -20 °C until analysis. Following thawing at room temperature, samples
222 were filtered using a 0.6 μm glass fiber filter (GA-55, Advantec, Germany) before further treatment. In SO
223 experiments, samples were filtered immediately after collection to avoid additional contact time between

aqueous phase and suspended solids during storage and thawing. The difference between the nominal spiked concentration and the measured initial (t=0) concentration can be due to the chemical loss through sample filtration. However, for samples with internal standards and ILIS, the loss of internal standards can be corrected by a loss of ILIS. All samples were extracted by solid phase extraction (150 mg, 6 cc, Oasis HLB, Waters, Denmark) and analysed 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 biomarkers 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 initial concentrations of different COD fractions in BT-P1 experiments; (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 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 $C_{LI,t=15min} - C_{LI,t=0}$ 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 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 the number of the data points in each series, O denotes measured data and P the model predictions, $O_{i,j\,max}$ and $O_{i,j\,min}$ 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 was performed using Matlab R2014a (MathWorks, US). WATS was initialized using the measured and estimated concentrations of different COD fractions and SO_4. 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}); (ii) simplified modeling framework of ASM-X with fixed initial 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 dataset. We note that, for BT-P2 experiments, 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 dataset from BT-P2 was only used for model evaluation (as an independent dataset) 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 non-deuterated or deuterated internal standards (ILIS).

RESULTS AND DISCUSSION

Wastewater characterization

Based on 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 growth substrate over the 2-d experiment (SI Section S1.3). Methanol utilization by SRB species

295 under anaerobic conditions was considered negligible as only few SRB strains can utilize MeOH.[34] Wastewater
296 sample used for BT-P2 experiments assumed to have the same characterization as BT-P1 sample by adjusting
297 COD fractions to measured total COD (5440 gCOD m^{-3}) and methanol (1800 gCOD m^{-3}).
298
299 **Primary substrates**
300 Using the WATS model, concentration dynamics of different COD fractions (substrate and biomass) during BT-
301 P1 batch experiments were predicted (Figure 1). Simulation results for the aerobic batch experiment, following
302 WATS calibration with respirometric data, revealed a significant variation of X_{Hw} (5-fold increase followed by a
303 53% decrease) over the course of the batch experiment, as expected by the initial substrate-to-microorganism
304 ratio. This likely influences the kinetics of drug biomarker biotransformation, and shows the limited validity of
305 the non-growth assumption typically considered in stability studies. X_{Hw} was predicted to reach a maximum
306 concentration of 100 gCOD m^{-3} after 13 h, when S_S became growth limiting. While X_{S1} was reduced via
307 hydrolysis, X_{S2} remained almost constant during the experiment (due to extremely low hydrolysis rate k_{h2}).
308 Significant evaporation of MeOH (66% during BT-P1) was predicted, based on the results obtained in an
309 additional set of evaporation experiments (SI Section S1.3). Under aerobic conditions, the calibrated WATS
310 model well predicted OUR measurements from the respirometry tests as well as measured total and soluble COD
311 during BT-P1 aerobic experiment (SI Figure S4 and Figure S7).
312 WATS model predictions under anaerobic conditions (Figure 1) showed 61% decrease of X_{Hw}, with
313 simultaneous growth of X_{SRB} (2.8-fold increase) over the 2-d experiment. Concentration profiles for X_{S1} and X_{S2}
314 indicated comparably slow hydrolysis, with limited formation of S_F. Almost complete fermentation of S_F to S_A
315 within 30 h was predicted (Figure 1), with initial net formation and subsequent decrease of S_A. The predicted
316 non-limiting S_F (during the first 30 h) and S_A (over the entire experiment) were expected to support growth of
317 X_{SRB}. Notably, MeOH evaporation rate in anaerobic experiments was 2-fold lower than in the aerobic experiment
318 (see also SI Figure S6), partly justifying the lower removal of total and soluble COD in the anaerobic
319 experiment. Even though calibration of anaerobic WATS model was not performed and previously suggested

parameter values were used (SI Table S1), it was possible to predict SO_4 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 Eq. 1 and Eq. 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 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 MPEH, METD, EDDP, BE, 6MAM, THCOH, and THCCOOH, with K_d values ranging from 0.11 L $gTSS^{-1}$ (METD) to 0.80 L $gTSS^{-1}$ (THCOH). Although THC is highly hydrophobic ($\log K_{ow}$=7.61), we observed that all sorption of THC was related to partitioning to the reactor wall (poly(methyl methacrylate), Plexiglas). Notably, recorded pH data show 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 Figure S22–23.

The calibrated WATS–ASM-X model was then evaluated via forward simulations using the BT-P2 dataset. 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 (Figure 2c–d).

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 dataset (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 and thus our data do not suggest EDDP as the major METD transformation product, as suggested for human metabolism.[12,37] Moreover, *N*-demethylation of METD to EDDP was predicted to be unfeasible in wastewater.[39] Hence, the transformation 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 for 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 datasets. 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 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 dataset (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 other 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 were performed in mineral water, it may be concluded that hydrolysis is not solely bacterially-mediated, as reported previously.[36] 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 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 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 transformation pathway model (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 day and 3 days 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 non-detectable 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 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 literature for THC biomarkers show significant variations. THCOH removal up to 20% (unfiltered wastewater; T=20 °C, pH=7.5; duration: 3 days) has been reported[49]. Another investigation in

wastewater showed 40% THCOH removal (4 °C) after 3 days[52], 40% THC removal and negligible THCCOOH removal (-20 °C) after 3 days.[61] Castiglioni et al.[55] have reported 8% removal of THCCOOH in wastewater (4 °C) after 3 days. These results do not agree with our findings, which show significantly higher conversion rates for THC, THCOH and THCCOH (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 THCOOH ($K_{d,THCOH}$~0.7, $K_{d,THCOOH}$~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 sewer 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 dataset 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 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 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 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. in-sewer transformation of drug biomarkers, and may lead to more accurate estimations of drug consumption. While this study focused on fate of selected drug biomarkers in presence of suspended biomass, ongoing research activity focuses on transformation and sorption of drug biomarkers in sewer biofilms. Along with in-sewer transformation, a more comprehensive assessment of all sources of uncertainty is required for the

494 selection of suitable biomarker candidate for back-calculation purposes. Further research activity is also required
495 to consider in-sewer transport processes, and thus calculate residence time distribution, at a catchment or sub-
496 catchment level. Wastewater-based epidemiological engineering is an emerging field, in which mathematical
497 models, such as the WATS–ASM-X developed in this study, can play a key role as decision support tools for
498 epidemiological studies.
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507 **ASSOCIATED CONTENT**

508 **Supporting information**

509 Additional information about details of WATS–ASM-X model and modeling transformation pathways. This
510 material is available free of charge via the Internet at http://pubs.acs.org.
511

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Table 1. Primary and secondary metabolic processes under aerobic and anaerobic conditions considered in the WATS–ASM-X framework.

	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 products	Readily degradable COD	Methanol	Rapid hydrolizable substrate	Slow hydrolizable substrate	Dissolved oxygen	Sulfate	Biomarker in aqueous phase	Biomarker in suspended solids	Biomarker transforming to C_{LI}	Biomarker onto reactor wall	Process rates ↓
	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}	
	Processes ↓															
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						$q_m X_{Hw}$
	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}		1	$-\frac{1}{Y_{SRB}}$	$-\frac{1}{Y_{SRB}}$						$-0.5\frac{1-Y_{SRB}}{Y_{SRB}}$					$\mu_{SRB}\frac{S_F+S_A}{S_F+S_A+K_{SRB,S}}\frac{S_{SO4}}{(S_{SO4}+K_{SRB,SO4})}X_{SRB}\alpha_S^{(T-20)}$
	Hydrolysis, rapid			1				-1								$\eta_h k_{h1}(X_{S1}/X_{Hw})/(X_{S1}/X_{Hw}+K_{X1})X_{Hw}\alpha_w^{(T-20)}$
	Hydrolysis, slow			1					-1							$\eta_h k_{h2}(X_{S2}/X_{Hw})/(X_{S2}/X_{Hw}+K_{X2})X_{Hw}\alpha_w^{(T-20)}$
	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		$k_{bio,CJ}C_{CJ}(X_{Hw}+X_{SRB})10^{-3}$

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-COD ratio; M: biomarker molecular weight

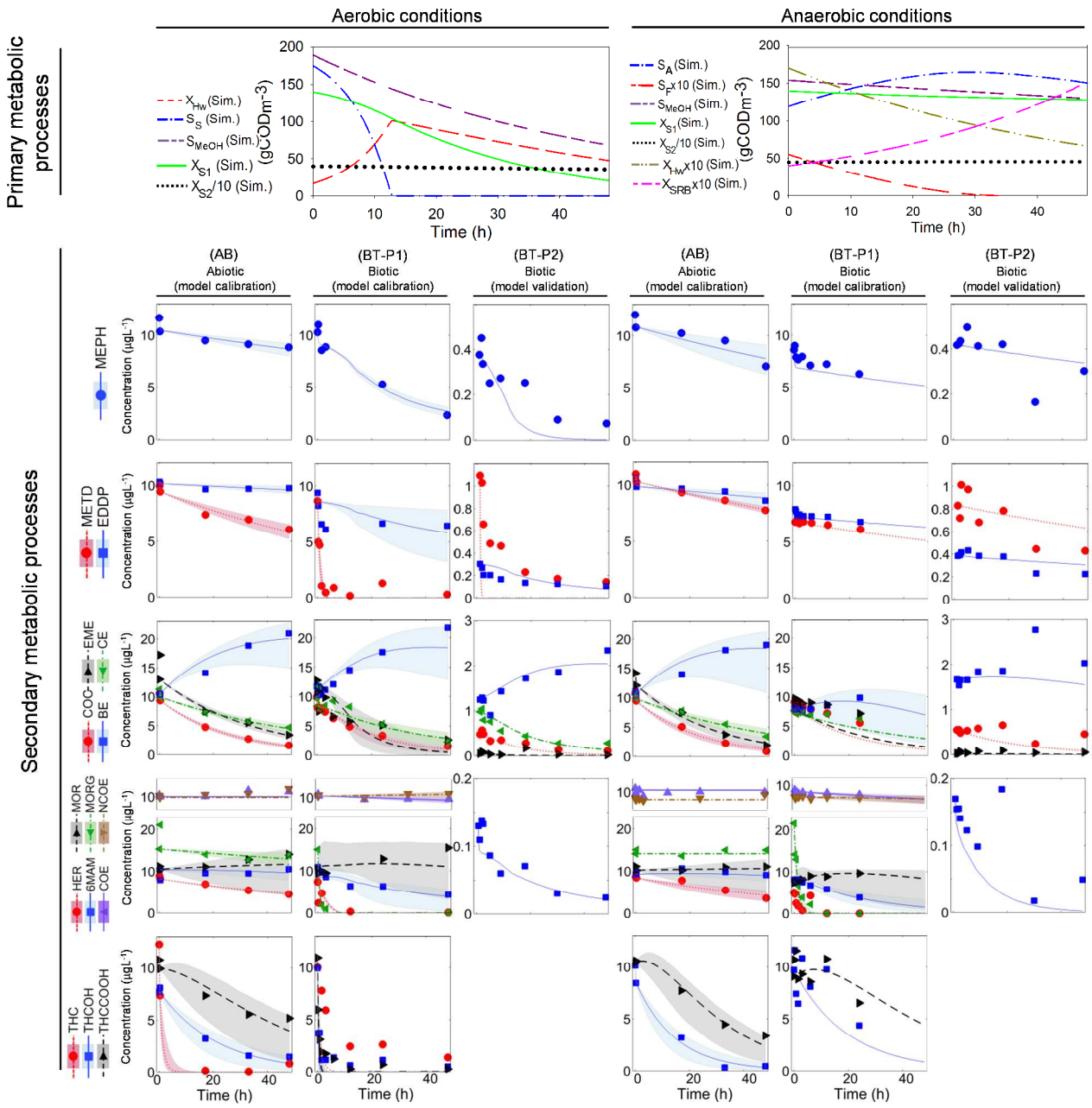

Figure 1. Experimental data and simulation results for primary and secondary metabolic processes under aerobic and anaerobic conditions. *Primary metabolic processes*: aerobic WATS model outputs including heterotrophic biomass X_{Hw}, soluble substrate S_S and rapid, X_{S1}, and slow, X_{S2}, hydrolysable fractions. Anaerobic WATS model outputs, including X_{Hw}, sulfate reducing bacteria, X_{SRB}, fermentation product (VFA), S_F, fermentable substrate, S_A, 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 is used to validate the WATS–ASM-X model. Markers are measured data and lines are simulation results. The shaded area reflects 95% credibility interval of model prediction.

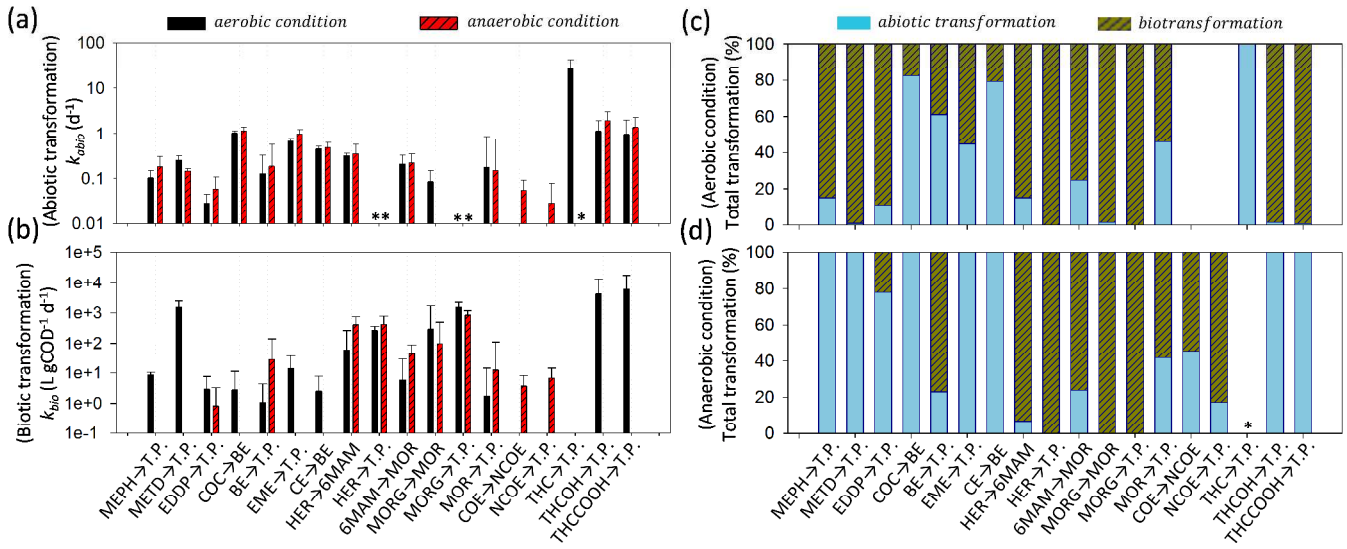

Figure 2. Comparing the effect of aerobic and anaerobic conditions on abiotic transformation (a) and biotransformation rates (b) as well as comparing abiotic transformation with biotransformation under aerobic (c) and anaerobic (d) conditions. Undetermined transformations are indicated with asterisk (*). 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 ASM-X model without WATS.

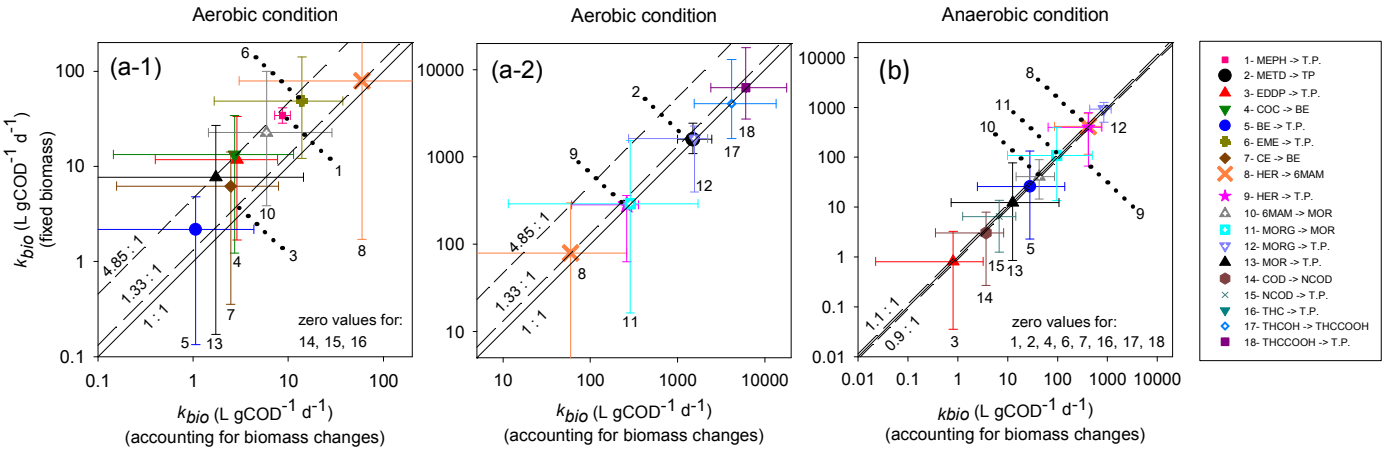

Figure 3. Comparing estimated k_{bio} using WATS–ASM-X considering biomass changes (X axis) with k_{bio} estimated using ASM-X with a fixed biomass fraction (Y axis) under aerobic conditions (a-1 and a-2) and under anaerobic conditions (b). Dashed lines indicate the ratio of estimated parameters.

TOC/Abstract art: