Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater

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Abstract

The quantitative analysis of human urinary metabolites as biomarkers in wastewater streams has been used to estimate the rates of illicit drug use in the wider community. The primary underlying assumption in such studies is that a sample of wastewater is equivalent to a cumulative sample of urine. Drug metabolism in humans is predominantly enzymatically mediated, but these processes are not exclusive to the human body, and are found to occur in the environment and the sewer network. Understanding what happens to drugs and their urinary metabolites in the sewer system between the point of excretion and sampling is particularly important since it is possible that in-sewer transformation may influence final biomarker concentration. The present study uses batch experiments to measure and assess the biotransformation processes of cocaine and its two major human metabolites, benzoylecgonine and ecgonine methyl ester. The activated sludge modelling framework for xenobiotic organic micro-pollutants (ASM-X) is used for model structure identification and calibration. Biotransformation was observed to follow pseudo first-order kinetics. The biodegradation kinetics of cocaine, benzoylecgonine and ecgonine methyl ester is not significantly affected by the availability of dissolved oxygen. Results obtained in this study show that omitting in-pipe biotransformation affects the accuracy of back-calculated cocaine use estimates. This varies markedly depending on the in-sewer hydraulic retention time, total biomass concentration and the relative concentration of each metabolite. However, back-calculated cocaine use estimates derived from wastewater concentrations of benzoylecgonine and ecgonine methyl ester do show very close agreement if ex-vivo biotransformation of these compounds is considered.

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1. Introduction

The measurement of the illicit drugs and their human urinary metabolites in wastewater streams as biomarkers of drug use has been used to estimate the level of illicit drug use in the wider community (Daughton, 2001; Zuccato et al., 2005; Daughton, 2011; Castiglioni et al., 2011; Harman et al., 2011; Khan and Nicell, 2011; Reid et al., 2011a; van Nuijs et al., 2011; Thomas et al., 2012). The technique is based on the principle that a representative sample of wastewater can be assumed to be equivalent to a pooled urine sample from all of the individuals in the wastewater catchment (Daughton, 2001). Pharmacokinetic studies on the drugs of interest establish an accepted urinary excretion rate which defines the amount (percent) of administered drug that is excreted in urine, either unchanged or as a metabolite (Castiglioni et al., 2011; Khan and Nicell, 2011). The measurement of the amount of a drug (or its urinary metabolites) in wastewater then provides the possibility to back-calculate the amount of drug that was originally administered. The approach has been widely applied but there are a number of uncertainties associated with obtaining representative samples, sample analysis, the variability in pharmacokinetics, and the estimation of population size. These uncertainties were thoroughly examined by Lai et al. (2011) and determined to be of the order of 14–24% (relative standard deviation) on estimates of per-capita drug consumption. The authors clearly state, however, that biodegradation or biotransformation of the target compounds in the sewer network was not assessed in their study.

Human drug metabolism is a biochemical process that occurs primarily in the liver, but this process is also evident in all biological tissue (Ding and Kaminsky, 2003). The primary phases of drug metabolism; oxidation, reduction and hydrolysis, are enzymatically mediated processes associated with the cytochrome P450 family of enzymes together with peroxidases and esterases. These processes are not exclusive to the human body and are apparent in the environment and indeed the sewage system (Baker and Kasprzyk-Hordern, 2011). Understanding what happens to illicit drug biomarkers in the sewer system between the point of excretion and sampling is particularly important since it is possible that in-sewer transformation may influence the final biomarker concentration. Since it is already known that partitioning onto particulate material is not a process that significantly affects the concentration of cocaine in sewer (Baker and Kasprzyk-Hordern, 2011; Langford et al., 2011), the in-sewer transformation of cocaine was investigated with a view to improving the accuracy of estimations of drug use through sewage analysis.

The present study uses the activated sludge modelling framework for predicting the fate of xenobiotics in wastewater, ASM-X (Plósz et al., 2010a, 2010b, 2012) to identify and calibrate the simplest process model and estimate the rates of biotransformation of cocaine, benzoylecgonine (BE) and ecgonine methyl ester (EME) in wastewater. Importantly, the experimental methodology proposed in the ASM-X framework can effectively provide environmentally relevant conditions, i.e. trace chemical concentration range and growth substrate concentration. This is because xenobiotics, spiked into targeted batch experiments, contain only chemicals occurring in raw pre-clarified municipal sewage, i.e. no pure reference substances (Plósz et al., 2010b). Furthermore, growth substrates can impact the co-metabolic xenobiotic substrate oxidation process – a compound-specific factor that was found to competitively inhibit, for instance, antibiotics biotransformation in activated sludge (Plósz et al., 2010b). BE is the primary urinary metabolite of cocaine, and the recovery of this compound in urine can account for an average of 13–39% of the initial cocaine dose depending on the route of drug administration – such as intra-nasal or smoking (Ambre, 1985; Castiglioni et al., 2011; Cone et al., 1998, 2003; Khan and Nicell, 2011). Cocaine is also excreted unchanged (accounting for an average 0.5–1.8% of the initial dose depending on the route of administration) and is also hydrolysed to form EME which has average urinary recoveries of 13–21% of the initial cocaine dose depending on the route of drug administration. We note that the selection of the most appropriate biomarker has a significant impact on the resulting back-calculated cocaine use estimate. An assessment of this uncertainty has not yet been reported in literature – an area investigated in this study. Temperature can significantly affect microbial growth, and therefore the uptake of growth substrates (e.g., Grady et al., 1999). Literature references on the effect of temperature on the biotransformation of trace xenobiotic chemicals – most of them considered as non-growth (co-metabolic) substrate – is less well established. Suárez et al. (2010, 2012) assess the impact of a 10 °C reduction (16–26 °C) on the biotransformation rate of sixteen different pharmaceuticals and personal care products. They found that the impact of temperature is inversely proportional to the biotransformation rate coefficient. For chemicals with the biotransformation rate coefficient, $k_{bio} \sim 10 \, \text{L g}^{-1} \, \text{d}^{-1}$ a reduction in temperature can cause an approximately 10% decrease in transformation. For chemicals with $k_{bio} > 100 \, \text{L g}^{-1} \, \text{d}^{-1}$, the same reduction in temperature would account for about 5% decrease in biotransformation rate. For chemicals e.g., sulfamethoxazole, with $k_{bio} \sim 0.1 \, \text{L g}^{-1} \, \text{d}^{-1}$, the same level of reduction can reduce the biotransformation rate coefficient by >50%.

The aims of the present study are (i) to identify the simplest model structures for the biotransformation of cocaine and its two human metabolites in the environmentally relevant concentration range, and to calibrate the models using batch experimental data; (ii) to evaluate the factors that influence biotransformation in sewer networks and (iii) to assess the significance of in-pipe biotransformation and pharmacokinetic parameters in back-calculating cocaine use.

2. Materials and methods

2.1. Batch experiments

Laboratory-scale experiments were performed using sewage collected from the effluent of the primary clarifier in a wastewater treatment plant (WWTP) in Oslo, Norway (VEAS AS) under aerobic and anaerobic conditions. In primary effluents, the dispersion of each single toilet flush is significantly greater...
than at the plant entrance (Ternes and Joss, 2006). Using this source of sewage ensured that environmentally relevant concentrations prevailed in the batch experiments used for model development. This is crucial to identify and to estimate environmentally relevant model structure and parameter values, respectively. The WWTP processes wastewater from approximately 557,000 people with an average flow of 3850 L s⁻¹ (30% of which is industrial). Batch experiments were conducted under both aerobic and anaerobic conditions as previously described (Plósz et al., 2010b). In order to infer a suitable model structure and representative model parameter values, batch experiments were performed using the cocaine, BE and EME already present in raw pre-clarified municipal sewage, i.e. without pure reference substances, as shown by Plósz et al. (2010b). In brief, pre-clarified wastewater aliquots were placed in two large glass reaction vessels (20 L); one maintained under aerobic and the other under anaerobic conditions. The initial biomass concentration Xss was set to 0.4 and 0.5 g L⁻¹ in the aerobic and anaerobic reactor, respectively. Air (for the aerobic reactor) and nitrogen (for the anaerobic reactor) flowed continuously through the reactor contents throughout the course of the experiment. Samples (100 mL) were collected at t = 0, 15, 30, 60, 90, 120 min following the initiation of the experiment, and also at t = 4, 6, 8, 10, 16, 18 and 24 h. The pH and temperature were 7.4 and 21 °C, respectively. The experiment was terminated after 24 h. The samples were quantitatively analysed for cocaine, BE and EME. Samples were also analysed for ammonium, nitrate, nitrite and chemical oxygen demand and COD (data not shown), according to (APHA, 1995) using HACH-Lange test kits and a DR2800 spectrophotometer (HACH, Germany). In the batch experiments, values of the initial readily biodegradable concentration (S) was estimated (data not shown) using soluble COD (0.1 μm membrane filtered) COD concentration data and WWTP effluent soluble COD data, according to (Roeleveld and van Loosdrecht, 2002).

2.2. Biomass used in the batch experiments and estimation of solids concentration in the sewer

The oxidation of cometabolic and readily biodegradable substrates occurs on non-specific (common) oxidase enzyme sites (e.g., Grady Jr. et al., 1999). To assess the biotransformation of cocaine and its metabolites in the sewer network, we assume that microbial catalysis — e.g., dioxygenase enzyme reactions due to the mostly heterotrophic activity, causing ring cleavage (Khunjar et al., 2011) — prevailing in the sewer pipeline and in the activated sludge bioreactors, exhibits the same reaction kinetics. Batch experiments were therefore carried out using the mixed microbial population contained in the pre-clarified wastewater aliquots and in activated sludge that derived from the WWTP (solids retention time, SRT = 16 days). Hvitved-Jacobsen et al. (1998) demonstrate that the predominately heterotrophic microbial growth processes in sewer networks can be described using the Activated Sludge Model Nr.1, ASM 1 (Henze et al., 1987) — a modelling framework used to predict biological nitrogen and COD removal in WWTPs. Microbial growth in the batch experiments was therefore modelled using ASM1. In, for instance, Dutch sewer networks, Flamink et al. (2005) show that the biological activity of suspended biomass (reported daily average with standard deviation $\text{COD}_{\text{suspended}} = 151 \pm 85$ mg L⁻¹) is significant, and, in fact, the relative contribution of the biofilm to the total organic matter conversion is only about 20%. An average total suspended solid concentration of 150 mg L⁻¹ was used in this study (based on operational data). Note that in raw sewage the total suspended solids can contain around 30–35% active biomass in terms of COD compared with 48–50% in activated sludge (Henze et al., 1987). A correction factor was not applied for this, however, because this factor is offset by the additional biocatalysis potential of the biofilm in sewer walls.

2.3. Model simulations and parameter estimation

The software, WEST® (DHI, Denmark) was used to perform model simulations, and to obtain numerical results on xenobiotic fate in the batch experiments. We used the ASM1 (Henze et al., 1987) to simulate biomass growth under aerobic and anoxic conditions in the batch experimental studied. For the ASM-X and the ASM1, we used the parameter values shown in Table 1 and in Spanjers et al. (1998), respectively. Parameter estimation was carried out using trajectory optimisation experiments with the Simplex method, and more information on model calibration is presented by Plósz et al. (2010b).

2.4. Sorption experiment

Solid-liquid partitioning coefficient data $K_D$ [L g Xss⁻¹] are not available in literature for the target analytes of this study. The $pK_a$ values of cocaine, EME and BE (pK$_{a,1}$) are 8.6, 8.5 and 11, respectively. Therefore, $K_D$ varies as a function of pH, and it is crucial to infer parameter values at the environmentally relevant pH level. An additional “abiotic” batch experiment was therefore performed at pH 7.4 (typical pH in the wastewater received by VEAS WWTP) by the addition of HCl (1 M) and NaOH (1 M). Pre-clarified sewage (10 L) was placed in a large glass reaction vessel and the biomass concentration Xss adjusted to 0.5 g L⁻¹. Mercuric chloride (HgCl₂, 100 mg L⁻¹) was then added to sterilize the sewage volume and ensure that biologically mediated transformation could not occur (see Maurer et al., 2007). The resulting sample matrix was continuous stirred throughout the duration of the experiment (60 min). This is somewhat shorter than that used by Maurer et al. (2007) — i.e. 1.5–4 h. According to our preliminary model simulations (data not shown), however, for $K_p$ values between 0.01 and 1 L g Xss⁻¹, this experimental setup allows to reach steady-state equilibrium aqueous concentration within approximately 40 min. Samples (100 mL) were collected at t = 0, 10, 20, 30, 45 and 60 min following the initiation of the experiment and the liquid-phase of each sample quantitatively analysed for the concentration of cocaine, BE and EME over time.

2.5. Analytical measurements

Sample analysis was carried out via previously validated and published methodology (van Nuijs et al., 2011; Reid et al., 2011b; Thomas et al., 2012). In short, samples were spiked...
with deuterated internal standards (1 mL of cocaine-D3, benzoylecgonine-D3, ecgonine methyl ester-D3 at 50 ng/mL in methanol) at the time of collection. Sample cleanup and pre-concentration was via centrifugation (10,000 g for 10 min) followed by solid phase extraction (SPE) using Oasis MCX cartridges (150 cc, 6 cc. Waters, Milford, USA). The cartridges were primed with methanol (5 mL) and then water (10 mL, pH 3.5). Samples were loaded under vacuum and subsequently were primed with methanol (5 mL) and then water (10 mL, pH 3.5) over 6 min. Detection of the analytes was via tandem quadrapole mass spectrometry (Quatro Premier XE, Waters, Milford, USA) with positive electrospray ionization. Lower limits of quantification were 1 ng L⁻¹ for cocaine, BE and EME. Ratios of the molar mass of parent drug to its metabolite concentration in percentage of the drug consumeda.

### Case study

The biodegradation parameters were then retrospectively applied to wastewater data in order to quantify their effects on back-calculated cocaine use from a real-world biomarker study. For comparison, three distinct datasets providing measured concentrations of cocaine, BE and EME in wastewater were included in the case study (Fig. 1, Fig. S1). The patterns of cocaine use are seen to vary with time, day of the week and during regional festivals or celebrations (Harman et al., 2011; Reid et al., 2011a, 2011b) so the three datasets were selected to best represent wastewater flows at differing periods of intensity of cocaine use. The weekday and weekend data are derived from the collection and measurement of time-proportional samples (sampling time period, T_sampling = 6 h) from a period of four weeks in Oslo (Norway). Weekday load is assessed by considering data measured between Saturday and Monday. Details of the catchment and wastewater parameters have been previously published (Reid et al., 2011b). The third data-set is from an intensive sampling campaign that was performed at the same location during a national festival celebration/festival (17 May 2009). Two-hour time-proportional samples (T_sampling = 2 h) were collected in this case. Further information on the case study is shown in the Supporting Information. Importantly, in Fig. 1, the average festival cocaine concentration (209 ng L⁻¹) is about double of the normal weekend value, whereas the festival BE concentration (average value: 301 ng L⁻¹) is approximately the same as during the weekends. The impact of this variation in the occurrence is further discussed later in the paper.

### Table 1 – Information on cocaine, BE and EME as well as on the model parameter values.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS registry #</td>
<td>Molar weight</td>
<td>g mole⁻¹</td>
<td>Cocaine</td>
</tr>
<tr>
<td>R_i</td>
<td>Ratio of the molar mass of parent drug to its metabolite</td>
<td>(-)</td>
<td>50-36-2</td>
</tr>
<tr>
<td>E_i</td>
<td>Average and the range of urine concentration in percentage of the drug consumeda</td>
<td>(%)</td>
<td>1.2 (0.08–0.15)</td>
</tr>
<tr>
<td>Kinetic model parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{Det,AN}</td>
<td>De-sorption rate coefficient for C_{SL}</td>
<td>(d⁻¹)</td>
<td>200⁸</td>
</tr>
<tr>
<td>K_0</td>
<td>Half-saturation coefficient for dissolved oxygen</td>
<td>(mg L⁻¹)</td>
<td>0.2b</td>
</tr>
<tr>
<td>K_{AN,AN}</td>
<td>Aerobic-anoxic solids-liquid sorption coefficient</td>
<td>(L g X̅̅̅_{SL})</td>
<td>0.84a</td>
</tr>
<tr>
<td>Aerobic process parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{Det,Ox}</td>
<td>Aerobic biotransformation rate coefficient for C_{Ox}</td>
<td>(L g X̅̅_{Ox} d⁻¹)</td>
<td>–</td>
</tr>
<tr>
<td>k_{Bio,Ox}</td>
<td>Aerobic biotransformation rate coefficient for C_{Ox}</td>
<td>(L g X̅̅_{Ox} d⁻¹)</td>
<td>22a</td>
</tr>
<tr>
<td>Anaerobic process parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{Det,AN}</td>
<td>Anaerobic biotransformation rate coefficient for C_{AN}</td>
<td>(L g X̅̅_{AN} d⁻¹)</td>
<td>–</td>
</tr>
<tr>
<td>k_{Bio,AN}</td>
<td>Anaerobic biotransformation rate coefficient for C_{AN}</td>
<td>(L g X̅̅_{AN} d⁻¹)</td>
<td>22a</td>
</tr>
</tbody>
</table>

a Parameter values estimated using the measured batch experimental data.
b ASM1 parameter values according to Henze et al. (1987).
c Parameter value ranges reported by Plośz et al. (2010b).
d Data reported by Khan and Nicell (2011).
For the excretion rate of a given drug residue (i), denoted as $E_i$, we use average values based on the range of data reported in literature (Table 1), i.e. we assume an even probability for all the various methods of drug administration. For each drug residue (parent drug and metabolites), the used cocaine mass ($M_{COC}$), residue (parent drug and metabolites), the used cocaine mass ($M_{COC}$), the total biomass concentration ($X_{SS}$) in Eq. (11).

Step 2. Back-calculation of the $C_{BE}(t = 0)$ concentration using the two- or six-hourly measured $C_{COC}(t = t_{Sampling})$ and $C_{BE}(t = t_{Sampling})$ concentration data, the $t_{Sampling}$ value obtained in Step 1 and the total biomass concentration ($X_{SS}$) in Eq. (11).

Step 3. Back-calculation of the $C_{EME}(t = 0)$ concentration using the six-hourly measured $C_{EME}(t = t_{Sampling})$ concentration data, the $t_{Sampling}$ value obtained in Step 1 and the $X_{SS}$ value in Eq. (13).

Step 4. Substituting the six-hourly concentration data obtained in Step 2 and Step 3 into Eq. (1) as well as $R_i, E_i, t_{Sampling}, P$ and $Q$, yielding the back-calculated load of cocaine for each sampling period (for $R_i$, $E_i$ parameter values see Table 1).

Step 5. Calculation of the daily load of cocaine consumption using results obtained in Step 4.

Step 6. Calculation of the average weekday cocaine consumption rate using data obtained in Step 5 for periods between Tuesday and Friday.

Step 7. Calculation of the average weekend cocaine consumption rate using data obtained in Step 5 for periods between Saturday and Monday.

Step 8. Variability of cocaine daily consumption rates are assessed by calculating the standard deviation of daily rate values obtained in Step 4.

2.8. Method developed to estimate the in-sewer hydraulic retention time ($\tau$)

The back-calculation method presented in this paper takes into account the biodegradation of cocaine and its metabolites in the sewer network. The in-sewer hydraulic retention time ($\tau$) needs to be estimated in order to back-calculate cocaine use based on the concentration data measured at the influent to the WWTP. Based on WWTP operational experience we use an average hydraulic retention time of 7 h (including the primary settler) for calculations in the present case-study. Sewer catchment models, e.g., MIKE Urban (DHI, Denmark), can make very precise approximation of the hydraulic behaviour of sewer networks; however, they may not be available for every catchment area. The lack of a suitable sewer network model would require engineers and authorities to use simpler estimation methods. In this chapter, we present a novel method that we developed to approximate $\tau$.

Under the assumption of stationarity and that the relationship between the average dry weather flow and the flow when the pipes are full-running is constant throughout the system, the hydraulic retention time in the system can be estimated based solely on the typical average retention time and the current flow to the WWTP by using the Bretting empirical formula for stationary flow in part-full pipes (Bretting, 1960):

$$Q/Q_f = 0.46 - 0.5 \cdot \cos \left( \frac{\pi \cdot X}{d} \right) + 0.04 \cdot \cos \left( 2 \cdot \pi \cdot y/d \right),$$  \label{eq:2}

where $Q/Q_f$ is the current flow relative to the full-running pipe flow and $y/d$ [m m$^{-1}$] is the water level in the pipe relative to the pipe diameter. Since the velocity of the water in a pipe can be calculated as $Q/A$, where $Q$ [L s$^{-1}$] is the flow and the wetted area $A$ [m$^2$] is the cross sectional area of the flow, the hydraulic...
retention time is proportional to A/Q. Therefore the fraction \( \tau / \tau_r \), where \( \tau \) is the hydraulic retention time when the pipes are full running, can be calculated from \( Q/Q_r \) when the corresponding wetted areas are known. The relationship between the relative filling of a circular pipe, \( y/d \), and the fraction \( A_{rel} = A/A_r \), where \( A_r \) is the full cross sectional area of the pipe, can be determined by simple geometrics to:

\[
A_{rel} = \frac{\theta - \sin \theta}{2 - \pi} \quad \text{where} \quad \theta = 2 \cdot \arccos \left( 1 - 2 \cdot \frac{y}{d} \right)
\]  

(3)

given that the pipe is less than half full. Using the above information it is possible to relate the relative flow in a pipe with a corresponding relative retention time. A simple two parameter power model fit very well to data series of \( Q/Q_r \) calculated using Bretting’s formula on the relative filling, \( y/d \), against the corresponding relative hydraulic retention time \( \tau / \tau_r \), calculated as \( (A_{rel}+Q) / Q \), see Fig. S2 in Supporting Information. From this it can be approximated that \( \tau \) is proportional to the flow in the power \(-0.29\):

\[
\tau = \left( \frac{Q}{Q_{average}} \right)^{-0.29}
\]  

(4)

Making the assumption that the relationship between the actual flow and the full-running flow \( Q/Q_r \) is approximately the same all over the system the above relation leads to the following useful formula:

\[
\tau = \left( \frac{Q}{Q_{average}} \right)^{-0.29} \cdot \tau_{average}
\]  

(5)

where \( Q \) is the current flow, \( \tau_{average} \) is the average hydraulic retention time under normal circumstances and \( Q_{average} \) is the corresponding flow (dry weather flow). Based on a simple statistical analysis of flow data, we use an average value of \( Q_{average} = 3850 \text{ L s}^{-1} \) and an estimated \( \tau_{average} = 7 \text{ h} \), meaning that the formula for calculating the in-sewer hydraulic retention time becomes:

\[
\tau = \left( \frac{Q}{3850 \text{ L s}^{-1}} \right)^{-0.29} \cdot 7 \text{ h}
\]  

(6)

3. Results

3.1. Sorption

The solid–liquid partitioning coefficients \( K_D [\text{L g } X_{SS}^{-1}] \) (see Table 1) were calculated according to

\[
K_D = \frac{C_{LI, t=0 \text{ min}} - C_{LI, t=60 \text{ min}}}{X_{SS} C_{LI, t=60 \text{ min}}},
\]  

(7)

where the aqueous phase concentration is denoted as \( C_{LI} \), measured in the liquid phase at \( t = 0 \) and 60 min time elapsed since the start-up of the experiment. \( X_{SS} \) denotes the biomass concentration. In the model simulations, solid–liquid partitioning is described by two independent differential equations for de-sorption and sorption processes, and further information on the conceptual approach are presented by Plośz et al. (2010b). The \( K_D \) data obtained for cocaine and EME are 0.84 and 0.3 L g \( X_{SS}^{-1} \) respectively (Table 1). The equivalent parameter for BE was found to be comparatively low (0.02 L g \( X_{SS}^{-1} \)).

3.2. Biotransformation of cocaine (COC)

In Fig. 2a, we approximate the measured cocaine concentration \( (C_{COC}) \) using the pseudo first-order kinetic expression,

Fig. 2 – Aqueous concentration values measured (symbols) and simulated (solid line) in the aerobic (red circle) and anaerobic (blue triangle) batch tests – cocaine (A) benzoylcegonine (B) and ecgonine methyl ester (C) – plotted as function of time elapsed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
\[
\frac{dC_{\text{COC}}}{dt} = -k_{\text{Bio, COC}} \cdot C_{\text{COC}} \cdot X_{SS}.
\]

\[
C_{\text{BE}}(t = 0) = C_{\text{BE}}(t = r) - \kappa_{\text{Dec, COC}} \cdot C_{\text{COC}}(t = 0) \left( e^{-k_{\text{Bio, BE}} \cdot \frac{r}{X_{SS}}} + \frac{e^{-k_{\text{Bio, BE}} \cdot \frac{r}{X_{SS}}}}{\kappa_{\text{Dec, COC}} - k_{\text{Bio, BE}}} \right) \cdot \frac{1}{e^{-k_{\text{Bio, BE}} \cdot \frac{r}{X_{SS}}}}.
\]

where \( \kappa_{\text{Dec, COC}} = k_{\text{Dec, COC}} \cdot X_{SS} \) and \( k_{\text{Bio, BE}} = k_{\text{Bio, BE}} \cdot X_{SS} \).

The value of the biotransformation rate coefficient \( (k_{\text{Bio, COC}}) \), estimated according to Plósz et al. (2010b), is \( 22 \text{ L g}^{-1} X_{SS} \text{ d}^{-1} \) (Table 1) under both aerobic and anaerobic conditions. In other words, our experimental data shows no significant difference between the biotransformation kinetics of cocaine under different redox conditions. The theoretical upstream concentration of cocaine \((t = 0)\) based on measurements made at a downstream sampling point (characterised by an insewer hydraulic retention time, \(t = r\)) is therefore given by

\[
C_{\text{COC}}(t = 0) = \frac{C(t = r)}{e^{-k_{\text{COC}} \cdot \frac{r}{X_{SS}}}} \text{ where } k_{\text{COC}} = k_{\text{Bio, COC}} \cdot X_{SS}
\]

3.3. Biotransformation of benzoylecgonine (BE)

Benzoylecgonine is the demethylation product of cocaine. Enzymatic demethylation of cocaine occurs during human metabolism (Maurer et al., 2006), but BE is also formed as a product of the transformation of cocaine in wastewater (Baker and Kasprzyk-Hordern, 2011). The concentration of BE in the sewer system is therefore a function of not only the amount of BE excreted in urine, but also a function of the concentration of unmetabolised cocaine. As such, the pseudo first-order kinetic equation (Eq. (10)), which is derived from results of the batch experiments (Fig. 2b) describes the biodegradation of BE in the sewer and also accounts for the formation/retransformation of BE via cocaine biotransformation according to

\[
\frac{dC_{\text{BE}}}{dt} = \left( k_{\text{Bio, COC}} \cdot \frac{M_{\text{BE}}}{M_{\text{COC}}} \cdot C_{\text{COC}} - k_{\text{Bio, BE}} \cdot C_{\text{BE}} \right) \cdot X_{SS}.
\]

In Table 1, the BE parent compound formation rate is denoted as \( k_{\text{Dec, COC, BE}} \), and its value is obtained by multiplying \( k_{\text{Bio, COC}} \) values with the molar ratio, \( M_{\text{BE}}M_{\text{COC}} = 0.954 \). Values of the maximum oxidation rate of BE \( (k_{\text{Bio, BE}}) \) obtained under aerobic and anaerobic conditions are not significantly different, and both were obtained around \( 8 \text{ L g}^{-1} X_{SS} \text{ d}^{-1} \) (Table 1).

To estimate the theoretical (upstream) concentration of BE at \( t = 0 \) based on observations made in a downstream sampling point \( C(t = r) \), we use the analytical solution of the Eq. (10), a two-compartment cascade differential equation system, for which we also consider the superposition principle, yielding

\[
C_{\text{BE}}(t = 0) = \frac{C_{\text{BE}}(t = r)}{e^{-k_{\text{BE}} \cdot \frac{r}{X_{SS}}}} \text{ where } k_{\text{BE}} = k_{\text{Bio, BE}} \cdot X_{SS}
\]

3.4. Biotransformation of eucgonine methyl ester (EME)

EME can be formed as a product of the biotransformation of cocaine in wastewater. The impact of this pathway on the EME concentration in wastewater, however, is unclear. Therefore, we carried out an assessment, in which we considered EME formation and biotransformation described analogously to BE (see Supporting Information). Results obtained, shown in Fig. S3, indicate that the biotransformation of cocaine to eucgonine-methylester is a minor/negligible pathway at the environmentally relevant biomarker concentration levels. Consequently, we assume that the cocaine-to-EME pathway cannot significantly influence the back-calculated drug use. Additionally, the batch experimental data indicate that there is no significant difference between the rates of aerobic and anaerobic biotransformation of EME. The reaction follows pseudo first-order kinetics described by the following equation

\[
\frac{dC_{\text{EME}}}{dt} = \left( k_{\text{Bio, EME}} \cdot \frac{M_{\text{EME}}}{M_{\text{COC}}} \cdot C_{\text{COC}} - k_{\text{Bio, EME}} \cdot C_{\text{EME}} \right) \cdot X_{SS}.
\]

The maximum biotransformation rate \( (k_{\text{Bio, EME}} = 8.4 \text{ L g}^{-1} X_{SS} \text{ d}^{-1}) \) is obtained under both aerobic and anaerobic conditions. To estimate the theoretical (upstream) concentration of EME at \( t = 0 \) based on observations made in a downstream sampling point \( C(t = r) \) we use the formula

\[
C_{\text{EME}}(t = 0) = \frac{C_{\text{EME}}(t = r)}{e^{-k_{\text{EME}} \cdot \frac{r}{X_{SS}}}} \text{ where } k_{\text{EME}} = k_{\text{Bio, EME}} \cdot X_{SS}
\]

As such, the highest biotransformation rate value was obtained for cocaine, and significantly lower and very similar biotransformation rate values were observed with BE and EME. With regard to the parameters obtained for cocaine and EME biotransformation kinetics, we understand that the scarcity of anaerobic data (no measurements after 0.1 day) can be a source of uncertainty that can significantly influence the inferred parameter values and we highlight that further experimental verification of these values may be required in the future.

4. Discussion

The loss of the selected drug biomarkers in the sewer network due to biotransformation is impacted by numerous factors.
Those evaluated in this study are (i) the availability of dissolved oxygen (explicit observations are shown in Fig. 2) and readily biodegradable (growth) substrates, (ii) the relative cocaine concentrations and their impact on back-calculated BE concentration; (iii) the total biomass concentration catalysing biochemical reactions; and (iv) the average hydraulic retention time in sewer networks. For the evaluation, we developed a simple model to approximate hydraulic residence time in sewer networks. For the evaluation, we developed a simple model to approximate hydraulic residence time in sewer networks, \( \tau \), as a function of the flow rate measured at the discharge point of this network (Eq. (5)).

Based on results presented by Suarez et al. (2012), we assume that the \( k_{\text{bio}} \) values, obtained at room temperature in this study (8–22 L g \(^{-1} \)) are decreased by only 5–10%, at the lower aqueous temperature, prevailing in the sewer. In our assessment, therefore we consider this effect negligible.

4.1. Impact of the availability of dissolved oxygen and readily biodegradable (growth) substrates in aqueous phase

Our results suggest that the assessment of re-aeration and the prediction of dissolved oxygen concentrations would not be required to decrease the level of uncertainty with respect to cocaine use estimates derived from measurements of cocaine, BE and EME in wastewater. Additionally, results shown in Fig. 2 and simulation results (not shown) obtained on \( S_5 \) uptake in the batch reactors using ASM1, indicate that the readily biodegradable substrate content of pre-clarified sewage does not significantly influence (inhibit or enhance) the cocaine, BE and EME chemicals biotransformation. Previous studies, carried out in the ASM-X framework, show that, depending on the molecular structure, \( S_6 \) can also competitively inhibit (selected antibiotics – Plośz et al., 2010a) or enhance (diclofenac, carbamazepine – Plośz et al., 2012) trace chemical biotransformation in the ng L\(^{-1} \) and low \( \mu \text{g} \) L\(^{-1} \) range.

4.2. Impact of the relative concentrations of the cocaine biomarkers

Fig. 3a presents model simulation results obtained using the measured concentration values derived from the case study. We show the average percentage reduction in the concentration of the three biomarkers during transport through the sewer network (from excretion to sample-collection). For the model prediction, we use the biokinetic models identified and calibrated using data shown in Fig. 2. In the Eq. (11) and Eq. (13), \( \tau \) is calculated for each sampling period using 7-h average in-sewer hydraulic retention time in Eq. (5). For cocaine, the average in-sewer reduction in concentration due to biotransformation was determined to be −60%. Biotransformation of EME during the same 7-h period was determined to be around −29%. Data in Fig. 3a for BE is somewhat more complicated because the back-calculated BE concentration depends not only on the biodegradation rate of this compound, but also on the amount of cocaine that is transformed to BE in the sewer. According to simulation results shown in Fig. 3a, the biotransformation of BE is completely offset by the formation of the compound as a result of in-pipe degradation of cocaine, and, for weekdays and weekends, there is some (average value: +18%) of increase in BE concentration at the point of sampling. During the period of the national celebration/festival, the measured concentration of cocaine (see Fig. 1) was sufficiently elevated to result in a +60% net-increase in the concentration of BE (see in Fig. 3a) during the 7-h average retention time in the sewer network. These simulation results suggest that biotransformation processes can result in significant alterations in wastewater biomarker concentration in the sewer network considered in this assessment.
4.3. Impacts of total biomass and in-sewer hydraulic retention time

The average flow-rate and biomass concentration can vary considerably in sewer networks. The scale of the impact of such variations is demonstrated in Fig. 3b that presents data from the case study in terms of the average percentage change in the concentration of the three biomarkers plotted as a function of the hydraulic residence time range (+30%–33%), i.e. 4.7–9.1 h. The error introduced by the uncertainty derived from the biomass concentration data is represented by the standard deviation value which was calculated using ten randomly selected total biomass concentration values in the selected parameter range (±10%), 135–165 mg L\(^{-1}\). In Fig. 3b, a ±10% uncertainty in the flow-rate data is indicated by the red lines, and results show the same degree of error, caused by uncertainties in solids concentration and hydraulic retention time, in the estimates of biomarker concentration. We note that this is also obvious from e.g., Eq. (9).

According to the data shown in Fig. 3b, the loss of cocaine as a function of hydraulic retention time is ~47 to ~68%. It is also for cocaine that we observe an insignificant error associated with the uncertainty in biomass and sewer hydraulic parameters (max. ±4% for cocaine considering the +30%–33% uncertainty range for flow conditions). It should also be remembered that uncertainty associated with the measured cocaine concentration is important because this uncertainty can propagate and affect the interpretation of BE data. Based on Eq. (11), this impact can be significant, given the comparably low \( k_{\text{Bio,WE}} \) (see Table 1). The weekday/weekend BE concentration increase obtained is 9%–82%. As to the festival BE data, the concentration increase obtained is between 16 and 135%. For EME, the concentration change difference caused by uncertainties in biomass concentration, obtained at the minimum and maximum flow rate values, is negligible (maximum ±3% of the average \( C(t = t) \)). This error is considerably lower than the estimated absolute value of EME concentration loss in the sewer (i.e. 21–36%) calculated for the in-sewer hydraulic retention time range considered in this study. These results imply that illicit drug use estimates derived from measurements of biomarkers in wastewater could benefit from effective approximation of and consideration of errors associated with the hydraulic retention time and solids concentration data.

4.4. Impact of biotransformation on cocaine consumption estimates

The present study finds that the omission of in-sewer biotransformation can introduce significant error to the concentration data used for the estimation of cocaine use. In Fig. 4, daily average cocaine use rate \( (\bar{r}_{COC}) \) estimates — calculated using the measured biomarker data (Fig. 1) — are plotted with and without the consideration of in-sewer biotransformation of the biomarkers (with the average hydraulic residence time of 7 h and total biomass concentration of 150 mg L\(^{-1}\)). For the weekday and weekend data, error bars indicate the variability of use estimates (in-sewer biotransformation accounted for) based on the month-long dataset. The percentage intervals of underestimation of consumption due to omitting in-sewer biotransformation are shown with dashed lines.

Results plotted in Fig. 4 suggest that the impact of omitting in-sewer biotransformation on the accuracy of back-calculated cocaine consumption estimates varies markedly depending on the relative concentration levels of each of the metabolites at any given time. If biotransformation is ignored when estimating cocaine use, based on BE the degree of overestimation obtained is between 2% and 10%. During the festival period, however, omitting biotransformation results in an overestimation of cocaine use by approximately 50%, due to the effect of an increase in the relative cocaine concentration in wastewater (see Fig. 1). For EME, the degree of underestimation is around 50% during the weekday and weekend periods, but jumps to around 110% during the festival period.

Cocaine use estimates based on both EME and BE during all time periods (weekday, weekend or festival) do, however, show a very close agreement if biotransformation is considered. For weekdays, the estimated average daily cocaine use rate is around 0.28 g d\(^{-1}\) 1000 PE\(^{-1}\). Weekends can be characterised, on average, with a rate of 0.54 g d\(^{-1}\) 1000 PE\(^{-1}\). For the festival period, the cocaine use rate value obtained is comparable to that obtained for the weekday period — i.e. 0.29 g d\(^{-1}\) 1000 PE\(^{-1}\).

In Fig. 4b, we show the weekly distribution of use rate values, obtained based on both EME and BE, for the month-long sampling and for the festival periods. For each day, close correspondence of BE- and EME-based back-calculated consumption estimates is demonstrated, again, only if biotransformation is considered. The order of days, sorted from the highest to the lowest average back-calculated consumption \( (g d^{-1} 1000 \text{ PE}^{-1} \times \% \text{ of weekly total}) \) obtained, is Sunday (0.63–23%), Monday (0.62–22%), Tuesday (0.39–14%), Saturday (0.37–13%), Wednesday/Thursday/Friday (0.25–9%). In the festival period, consumption rates obtained, based on the BE- and EME-data, are not significantly different from the corresponding week day (Friday and Saturday) values.

4.5. Impact of pharmacokinetic parameters on cocaine use estimates

An additional source of uncertainty is the parameter used for the percentage recovery of the initial drug dose in urine (\( E_\text{u} \)). Here, we present a preliminary assessment of the effect of this on back-calculation results. To estimate the rate of cocaine use in Fig. 4, we assumed an even probability for the various methods of drug administration (i.e. average \( E_\text{u} \) values calculated based on the ranges reported in literature, see Table 1). However, in order to further assess the impact of \( E_\text{u} \) on the back-calculated estimates, we tested the full range of published \( E_{\text{BE}} \) and \( E_{\text{EME}} \) values (Table 1) and subsequently identified the \( E_{\text{BE}} - E_{\text{EME}} \) pairs that give equal cocaine-use estimates when biotransformation is considered.

The lowest estimated rate of cocaine use was obtained with the pairing of \( E_{\text{BE}} = 31\% \) and \( E_{\text{EME}} = 21\% \) values. The highest estimates are obtained using \( E_{\text{BE}} = 21\% \) and \( E_{\text{EME}} = 13\% \). The range between these two extremes accounts for a total uncertainty in back-calculated cocaine-use estimates of between ~21% and ~15%. This uncertainty can be significantly reduced if information is available on the most common route of cocaine administration in the study area, and the most appropriate excretion rate is applied accordingly. A review of
the published pharmacokinetic studies (Khan and Nicell, 2011) shows that for intra-nasal cocaine the correct excretion parameters are $E_{EME} = 21\%$ and $E_{BE} = 31\%$. Cocaine use in Oslo is dominated by intra-nasal administration, so the excretion kinetics following this route of administration may be more appropriate for Oslo than the equal probability scenario. The $E_{BE}$ - $E_{EME}$ pair evaluated for the lowest possible rate of cocaine use in the catchment is in very close agreement with parameters shown by Khan and Nicell (2011).

5. Conclusions

- The primary underlying assumption in wastewater studies of drug-use is that a sample of wastewater is equivalent to a cumulative sample of urine. However, metabolism and biotransformation processes are not exclusive to the human body and were found to occur in the wastewater network.
- Biotransformation of cocaine metabolites in wastewater was observed to follow pseudo first-order kinetics, and does not appear to be significantly affected by the availability of dissolved oxygen and readily biodegradable growth substrates.
- Cocaine is transformed to BE in wastewater, so the measured concentration of BE and the rate of change of this concentration are strongly affected by the relative concentration of cocaine. Biotransformation of BE in wastewater would be expected to result in the loss of BE over time, however, elevated cocaine-loads can produce a net-growth of BE over time because the rate of formation of this

Fig. 4 – Daily average cocaine consumption rate data calculated using estimated $C(t = 0)$ values for BE and EME human metabolites plotted against values calculated based on the measured $C(t = 1)$ data for weekend, weekday and festival periods (A); weekly distribution of daily average cocaine consumption rate data calculated using estimated $C(t = 0)$ values for metabolites plotted against values calculated based on the measured $C(t = 1)$ data (B). Error bars indicate the variability of weekday and weekend consumption rate estimates calculated using the month-long dataset. Dashed lines indicate the percentage under- and overestimation due to the omission of in-pipe biotransformation.
metabolite (from in-sewer transformation of cocaine) is higher than the rate of loss.

- Cocaine, BE and EME are all urinary excretion products of cocaine. The measureable concentrations of each of these compounds in wastewater is a function of both their respective excretion rates (as a fraction of the initial cocaine dose), and their rates of biotransformation in wastewater. Back-calculated estimates of cocaine use based on all three compounds are in good agreement, but only when biotransformation is considered.

- The impact of the omission of in-sewer biotransformation on the accuracy of back-calculated cocaine consumption estimates varies markedly depending on the in-sewer hydraulic retention time, total solids concentration and on the relative concentration levels of each of the metabolites. The exclusion of biotransformation can lead to significant underestimation of drug use using the EME data in weekdays, weekends and festival (between −50 and −100%). Additionally, the omission of biotransformation can result in significant overestimation (±50%) of cocaine use from BE-based calculation in the festival period.

- The pharmacokinetic parameter, $E_{\text{BE}}$, used to approximate the rate of metabolism and excretion for each of the various drug metabolites in urine can impact the back-calculated drug use rate. In this study, the excretion ratios used represent a scenario whereby equal probability is given for the various ways cocaine can be consumed. Results obtained in a preliminary assessment show that the lowest cocaine use rates are obtained using $E_{\text{BE}} = 31\%$ and $E_{\text{EME}} = 21\%$ values. The highest rate values are obtained using $E_{\text{BE}} = 21\%$ and $E_{\text{EME}} = 13\%$. For the weekday/festival and the weekend periods, depending on the excretion ratios used, the back-calculated cocaine consumption rates under- and over-estimates average values obtained in the even-probability scenario by approximately −21% and +15%, respectively.

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**Appendix A. Supplementary information**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2012.12.034.

**REFERENCES**


