Water Research 151 (2019) 447–455

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Systematic evaluation of biomarker stability in pilot scale sewer pipes

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ARTICLE INFO

Article history:
Received 1 October 2018
Received in revised form
11 December 2018
Accepted 16 December 2018
Available online 28 December 2018

Keywords:
Biomarker stability
Gravity sewer
PPCPs
Rising main
Transformation kinetics
Wastewater-based epidemiology

ABSTRACT

Transformation of biomarkers (or their stability) during sewer transport is an important issue for wastewater-based epidemiology (WBE). Most studies so far have been conducted in the laboratory, which usually employed unrealistic conditions. In the present study, we utilized a pilot sewer system including a gravity pipe and a rising main pipe to investigate the fate of 24 pharmaceutical biomarkers. A programmable logic controller was used to control and monitor the system including sewer operational conditions and wastewater properties. Sequential samples were collected that can represent hydraulic retention time (HRT) of up to 8 h in a rising main and 4 h in a gravity sewer. Wastewater parameters and biomarker concentrations were analysed to evaluate the stability and transformation kinetics. The wastewater parameters of the pilot system were close to the conditions of real sewers. The findings of biomarker transformation were also close to real sewer data with seventeen biomarkers reported as stable while buprenorphine, caffeine, ethyl-sulfate, methadone, paracetamol, paraxanthine and salicylic acid degraded to variable extents. Both zero-order and first-order kinetics were used to model the degradation of unstable biomarkers and interestingly the goodness of fit R^2 for the zero-order model was higher than the first-order model for all unstable biomarkers in the rising main. The pilot sewer system simulates more realistic conditions than benchtop laboratory setups and may provide a more accurate approach for assessing the in-sewer transformation kinetics and stability of biomarkers.

1. Introduction

Wastewater-based epidemiology (WBE) is recognised as a complementary approach to traditional surveys in monitoring consumption of, or exposure to substances in the population (ACIC, 2017; Castiglioni et al., 2014; Cyranoski, 2018; EMCDDA, 2018; Ort et al., 2014). Illicit drugs were the main targeted substances in previous WBE studies, but pharmaceutical biomarkers can also be analysed to estimate the real time population and access the population health status (Fattore et al., 2016; Gao et al., 2016; Ghosh et al., 2010; O'Brien et al., 2014). To provide accurate consumption/exposure estimates by WBE, researchers have to use biomarkers whose in-sewer loss is negligible or known (van Nuijs et al., 2018). Therefore, the stability of biomarkers has been raised as an important uncertainty in the early stage of the WBE method development (Castiglioni et al., 2013; van Nuijs et al., 2012) and studies to understand the biomarker transformation in the sewer and in the sample have been carried out in the past decade (McCall et al., 2016a).

Transformation of biomarkers in the sewers is mostly investigated under laboratory conditions. Many laboratory experiments used bulk liquid wastewater in a container to represent the sewer conditions (Ostman et al., 2014; Senta et al., 2014), and other studies utilized sewer reactors that have biofilms (Gao et al., 2017; O'Brien et al., 2017; Ramin et al., 2017; Thai et al., 2014). These studies have found, for example, that the relatively fast degradation of cocaine and 6-monoacetylmorphine compromised their usability as biomarkers in WBE. Hence, their transformation products that are more stable (benzoylecgonine and morphine) were used to estimate consumption of cocaine and heroin (Been et al., 2016; Du et al., 2017). These laboratory studies can sometimes underestimate the transformation due to the lack of sewer biofilms (Baker and Kasprzyk-Hordern, 2011; Senta et al., 2014; van Nuijs et al., 2012) or overestimate the transformation due to higher biofilm area to wastewater volume ratio (A/V) in sewer reactors (Gao et al., 2017;

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https://doi.org/10.1016/j.watres.2018.12.032
0043-1354/

O'Brien et al., 2017). In addition, the impact of sewer operational parameters (pumping frequency, flow speed) can be difficult to replicate in laboratory settings. It is expected that real sewers and pilot sewer systems can overcome the abovementioned limitations to be used to investigate the transformation of biomarkers (Gao et al., 2018; Jelic et al., 2015; Jin et al., 2015; Li et al., 2018).

Real sewers have dynamic operational parameters (such as pumping frequency), diverse dimensions and wastewater compositions depending on the catchment characteristics (Hvitved-Jacobsen et al., 2013). Studying biomarker transformation in a real sewer has the advantage of having the most realistic sewer conditions, but factors that can affect the transformation of chemicals, such as hydraulic retention time (HRT), biofilm area to wastewater volume ratio (A/V) and wastewater pH are usually difficult to monitor and/or control. To our best knowledge, studies on biomarker stability in real sewers have only been conducted in Spain, Switzerland and Australia, three in rising mains (Gao et al., 2018; Jelic et al., 2015; Li et al., 2018), and one in a gravity sewer (McCall et al., 2017). In addition, sampling in the real sewer experiments is usually limited to the start and the end of the pipe, resulting in a limited number of samples and narrow window of HRT, which made it difficult to evaluate the transformation kinetics. For the purpose of studying processes within sewers under realistic but variable and measurable sewer conditions, pilot sewers were developed (Jin et al., 2018; Shypanski et al., 2018). These pilot sewers are sections of real sewer pipes that are fed continuously with wastewater. They can maintain conditions as in real sewers and have the capability of controlling and monitoring parameters such as pumping frequency, flow rate and pH. In addition, multiple sampling points along the pipe can be constructed in the pilot sewers to provide more samples for in-depth investigations.

In this study, we utilized a unique pilot sewer system to evaluate the stability of selected pharmaceutical and personal care (PPCP) biomarkers. The system contains both gravity sewer and rising main pipes and allows on-line control and monitoring of operational parameters and wastewater properties. The aims of this study include: i) characterise the hydraulics and bioactivities in both gravity sewer and rising main; ii) investigate the stability of a suite of PPCPs in a wide therapeutic category; iii) compare the biomarker transformation kinetics between the gravity sewer and rising main of the pilot system as well as with the data previously observed in laboratory conditions and real sewers.

2. Materials and methods

2.1. Chemicals and reagents

Twenty-four PPCP parent and metabolites were selected due to their high use and presence in wastewater with the potential to serve as biomarkers. Additionally, the in-sewer stability of most of those biomarkers have been evaluated in laboratory settings and thus will facilitate the comparison of performances between laboratory and pilot systems for biomarker stability assessment. We investigated acesulfame, atenolol, atorvastatin, buprenorphine, carbamazepine, caffeine, citalopram, cotinine, codeine, ethyl-sulphate (EtS), gabapentin, hydrochlorthiazide, ibuprofen, iopromide, morphine, methadone, paracetamol, nicotine, naproxen, paraxanthine, trans-3′-hydroxycotinine, salicylic acid, tramadol and venlafaxine. The properties of these biomarkers (category, formula, solubility, Log K_{ow}, human excretion profile and structure) are presented in Table S1 and S2.1.

2.2. The pilot sewer system

The pilot system has two configurations, one for a gravity sewer (GS) and one for a rising main (RM) (Fig. 1, Figure S1). Both sewer pipes were made of PVC with a length of 300 m. The system was operated with a programmable logic controller (PLC) that allowed the on-line control of pumping frequency and flow rate. Wastewater was pumped using a Loweara SHE50-12522 2.2kw and a SHE50-16075 7.5 kW 3 phase pump for the gravity line and pressure line respectively. Both pumps were equipped with a Hydrovar variable frequency drive for flow control. Each line was fitted with an inline magnetic resonance flow meter covering the expected flow ranges for each pump (IFM SM2000 (5-600 LPM)). Both GS and RM were conditioned for a year by pre-screened influent wastewater from the Luggage Point wastewater treatment plant (WWTP) in Brisbane, Australia. Pre-tests examining the biofilms in the removable pipe section (Figure S2) indicated that mature biofilms had developed in both GS and RM pipes.

Gravity sewer (GS): The GS pipe has a diameter of 225 mm (A/V of ~27 m^{-1}) with a slope of 0.56%. There is a recirculation pump together with a 250 L recirculation tank that can recirculate the wastewater in a closed circuit. The recirculation mode was achieved by stopping the wastewater feed from the Equalization tank, so there would be no influent flow entering the system and no effluent was discharged. The recirculation mode was used to achieve a longer HRT that is important for kinetic studies and represent the mean residence time in a WWTP catchment. The re-circulation pump was running at 125 L/min and the HRT of the wastewater per circulation circle was approximately 20 min resulting in a 21% filling of the pipe. The online monitoring of the flow tracer rhodamine was conducted with a portable Cyclops®-7 Submersible Rhodamine Sensor coupled with a Cyclops® Explorer. Temperature and pH were measured on-site using a portable pH/temperature meter (TPS Aqua-pH/Temp). Bioactivity indicators including methane, sulfate (SO_4-S) and sulfide (H_2S) were analysed offline. In addition, volatile fatty acids (VFAs), chemical oxygen demand (COD), total suspended solids (TSS) in wastewater samples were also analysed offline. Detailed information is presented in the Supplementary Information (S2.2).

Rising main (RM): The RM pipe has a diameter of 100 mm (A/V of 40 m^{-1}) (Shypanski et al., 2018). The feed pump was programmed to run for 1 min every 1 h at a flow rate of 236 L/min (0.51 m/s) to push the “spiked wastewater plug” approximately 30 m forward in the pipe. There were multiple sampling points in the middle of each 30 m of the pipe, and samples were taken in the sampling points aiming to catch the “spiked wastewater plug” for HRT up to 8 h.

2.3. The properties of wastewater

The wastewater used in this study was the influent of WWTP serving a large urban catchment, so it can be considered as typical domestic wastewater. The temperature was 21–24 °C across all experiments. The pH was stable at around 7.0 across all the experiments, similar to the observation in other studies (Table S2). We were unable to measure dissolved oxygen levels in our GS experiments due to practical reasons. However, under the same re-circulation mode in other experiments, it was in the range of 0.5–2 mg/L which should be comparable to our experiments (Shypanski, 2018). TSS in the GS experiments was 500–800 mg/L with some fluctuation. Volatile suspended solids (VSS) in GS were steady around 500 mg/L, while in the RM, TSS ranged from 300 to 600 mg/L, and VSS was around 200 mg/L (Figure S3). The higher TSS and VSS in GS indicate there was some erosion of the sediments in GS. A detailed comparison of the sewer and wastewater parameters in this study and other studies is summarized in Table S2.

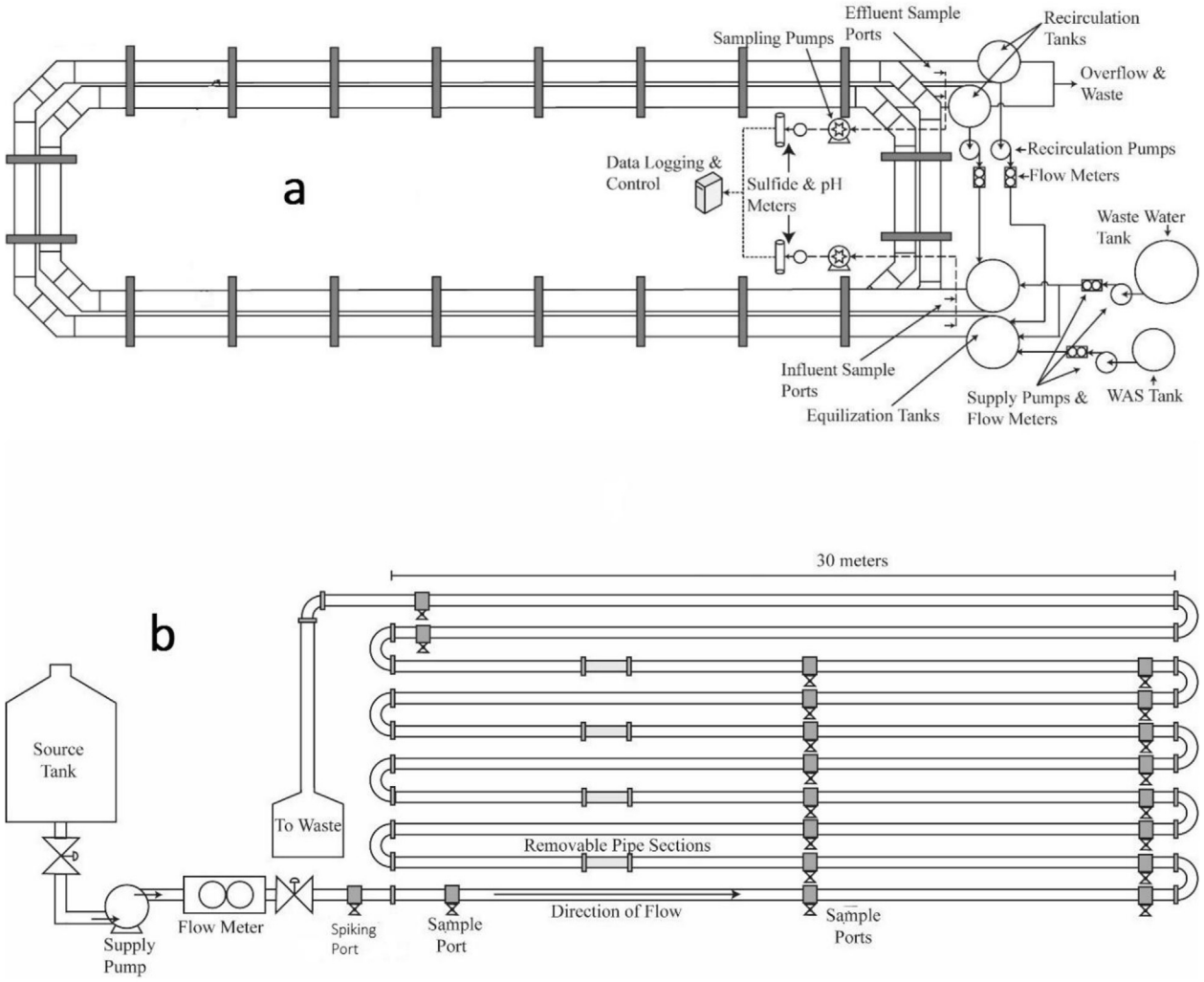

Fig. 1. Layout of the pilot gravity sewer (a) and rising main (b).

2.4. Chemical spiking and sampling

Standards (unlabelled) of the selected biomarkers in methanol were dissolved in fresh wastewater and spiked into the system to achieve quantifiable concentrations and at the same time to remain at a realistic concentration in the upstream of a catchment (Table S4). The HRT for GS and RM, four- and eight-hours respectively, were selected based on the average HRT reported in real sewers in Europe and the US (Kapo et al., 2017; Ort et al., 2014). In the GS experiments, the biomarker mixture, together with the flow tracer rhodamine mixed with raw wastewater, was spiked into the recirculation tank. Every 15 min after spiking, a 100 mL wastewater sample was taken from the recirculation tank until 4 h after spiking. In the RM experiment, 1 L of spiked wastewater was pumped into the system in the first pumping event, using a peristaltic pump synchronized with the major feed pump. The rhodamine probe was moved according to the pumping event, to the sampling port where the spiked wastewater plug was expected, to continuously monitor the real-time rhodamine signals. Samples were taken every 15 min at different sampling points to catch the spiked plug (in the middle of each layer, Fig. 1). The last sample was taken 8 h after the first sample. To avoid the interference of UV light to the stability from the rhodamine sensor, samples were taken before the inlet of rhodamine probe.

2.5. Sample preparation and chemical analysis

Wastewater samples were acidified to pH 2 on site using 2 M HCl immediately after sampling. A ten mL sample was filtered onsite using a regenerated cellulose syringe filter and a 1 mL filtered sample was pipetted into a 2 mL brown glass injection vial. Ten μL of 1 mg/L labelled analogue mixture was added to each 1 mL sample in the injection vial. The samples were frozen after collection and stored in a freezer at −20 °C and were analysed within two weeks. The concentration of biomarkers in the sample was determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) consisting of a Shimadzu Nexera HPLC system (Kyoto, Japan) and a Sciex API 5500 mass spectrometer (Ontario, Canada) equipped with an electrospray (Turbo V) interface. For all analytes except EtS, a 7 μL sample was injected into a 2.6 μm 50 × 2.0 mm Phenomenex Kinetek Biphenyl column (Torrance, CA, USA) run at 45 °C with a flow rate of 0.3 mL/min. A linear gradient of the mobile phase was used, starting at 5% B, ramped to 100% B in 10.0 min, then held at 100% B for 4.5 min followed by equilibration at 5% B for 4.0 min (A = 0.1% formic acid in MilliQ water, B = 0.1% formic acid in methanol). The mass spectrometer was operated in the positive/negative ion switching mode with scheduled multiple reaction-monitoring (sMRM) using nitrogen as the collision gas. Detailed mass spectrometer parameters can be

found in Gao et al. (2017). EtS was analysed by the same LC-MS/MS system with a 1.7 μm 50 × 2.0 mm Phenomenex EVO C18 column (Torrance, CA, USA) run at 45 °C. A flow rate of 0.27 mL/min mobile phase with a linear gradient was used, starting at 0% B, ramped to 100% B in 3.0 min, then held at 100% B for 2.0 min, followed by equilibration at 0% B for 4.0 min (A = 5 mM dihexyl ammonium acetate in MilliQ water, B = 5 mM dihexyl ammonium acetate in methanol). A 50 mm × 2 mm, 3 μm Gemini NX C18 column (Phenomenex) was inserted between the pumps and the autosampler. Detailed mass spectrometer parameters can be found in Gao et al. (2018). The quantification was carried out using internal calibration method with 1/x weighing. Satisfactory correlation coefficient ($r > 0.99$) within the calibration range was achieved from 0.1 to 50 μg/L. Method performance data including accuracy and precision is provided in Table S4.

2.6. Data processing

Transformation was calculated using the concentration (unspiked biomarkers) or concentration ratio of biomarker to rhodamine (spiked biomarkers) in the investigated HRT to their initial value when the experiments started. The detailed calculation method is provided in the **S2.3**. The triplicate transformation results were combined to investigate the transformation. Stable biomarkers in the pilot sewers were defined as having less than 20% loss during the experiments (McCall et al., 2016a). Pearson correlation was applied to the degradation of unstable biomarkers and bioactivity indicators and wastewater parameters. The transformation of unstable biomarkers in the pilot sewers was fitted to both zero-order and first-order kinetics models. The statistical analysis was performed using GraphPad Prism 7.03.

We found that the goodness of fit R^2 is higher in the zero-order model for all the unstable biomarkers (see Table 3 in later section). Therefore, the A/V normalized transformation coefficients K_{bio} ($m \cdot h^{-1}$) was calculated using Equation (1).

$$K_{bio} = \frac{\frac{C_0 - C_j}{t} - K_{ww}}{A/V} \quad (1)$$

K_{bio} is the transformation coefficient in zero-order kinetics, $m \cdot h^{-1}$;

C_0 is the initial concentration of biomarker (for unspiked biomarkers) treated as 100%, or the concentration ratio of biomarker to rhodamine (for spiked biomarkers) treated as 100% at t (0);

C_j is the concentration of biomarker at t (h) relative to the concentration in T0 in percentage or the concentration ratio of biomarker to rhodamine in sample collected at time t (h) relative to the biomarker/rhodamine ratio in t (0);

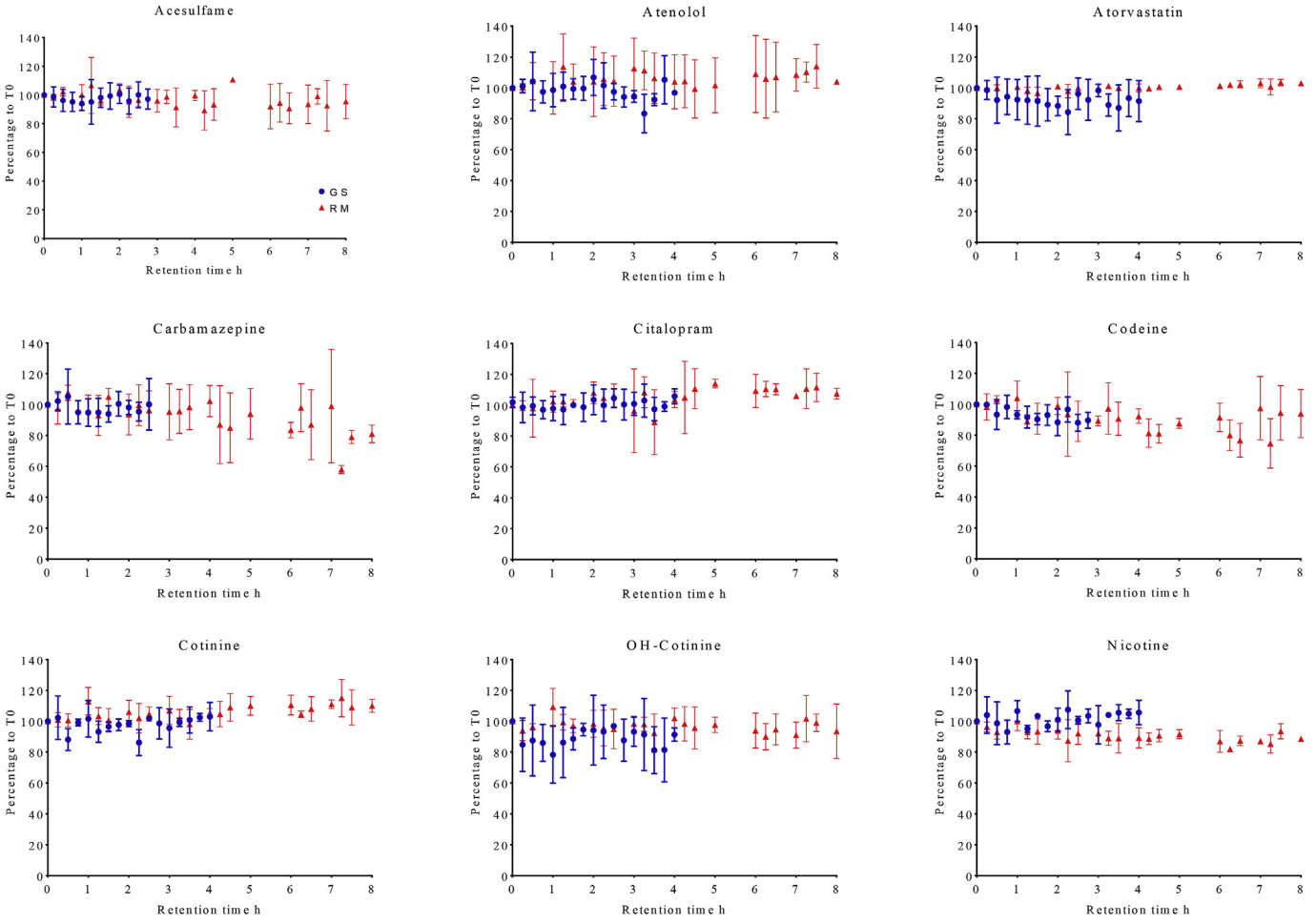

Fig. 2. Profile of stable biomarkers in the pilot sewers (red triangles = RM, blue circles = GS).

K_{ww} is the transformation coefficient in control sewer reactor in zero-order kinetic, h^{-1}.

3. Results and discussion

3.1. Characterization of the pilot sewers and wastewater

In the GS experiments, sulfate concentrations (SO_4-S) remained constant during the 4 h HRT and the sulfide decreased from 17 mgS/L to less than 0.5 mgS/L in the first 2 h (Figure S3). This indicated that the sulfate reducing activity was negligible and some sulfide may have been oxidized to sulfate. In addition, the intensive turbulence created by recirculation accelerated the release of hydrogen sulfide (H_2S) into the sewer atmosphere. The dissolved sulfide concentration in the feed wastewater was attributed to the fact that the head works of the Luggage Point WWTP receives discharges from several large RM. However, no evidence has been identified that such sulfide concentration would inhibit the biological activities. Therefore, the impact of high initial sulfide concentration to the biomarker transformation should be limited (Sharma et al., 2014). There was no significant methane formation and the VFAs decreased by approximately 30%, which indicated that the aerobic and anaerobic bioactivities consumed VFAs. In the RM experiments, in contrast, significant formation of sulfide was observed together with >50% decrease of sulfate, indicating strong sulfate reducing activities. In addition, the formation of approximately 30 mg COD/L methane also suggests strong methanogens activities. The decrease of VFAs was much lower in the RM compared to the GS, suggesting the overall consumption rate of VFAs in strict anaerobic conditions could be slower than in aerobic conditions. There could also be formation of VFAs in RM due to anaerobic fermentation. Activities of sulfate reducing bacteria (1.16 ± 0.45 g S m^{-2} d^{-1}) and methanogens (3.27 ± 0.39 g COD m^{-2} d^{-1}) in the RM were comparable to the laboratory RM reactor and the real RM (Table S2) (Gao et al., 2017; Li et al., 2018; Thai et al., 2014).

3.2. Hydraulic aspects in pilot sewers

In the GS, rhodamine concentrations from the initial spike at 0 h fluctuated substantially in the first 1.5 h (Figure S4). The concentration of the spiked biomarkers also fluctuated during the same period, indicating similar mixing behaviour of spiked rhodamine and biomarkers.

In the RM, there was some degree of diffusion and dispersion for the spiked biomarkers and rhodamine during the transportation from upstream to downstream of the pipes. The mixing and diffusion were mainly driven by the turbulence created by the pumping event, and the upstream plugs (close to the pump) were affected more than the downstream plugs.

3.3. Transformation of biomarkers in pilot sewers

Seven out of twenty-four biomarkers were unstable in the experimental sewer conditions. Seventeen biomarkers were stable including acesulfame, atenolol, atorvastatin, carbamazepine, citalopram, codeine, cotinine, trans-3′-hydroxycotinine, gabapentin, hydrochlorothiazide, ibuprofen, iopromide, morphine, nicotine, naproxen, tramadol and venlafaxine. These biomarkers were also observed to be stable in other studies as indicated in Table S5.

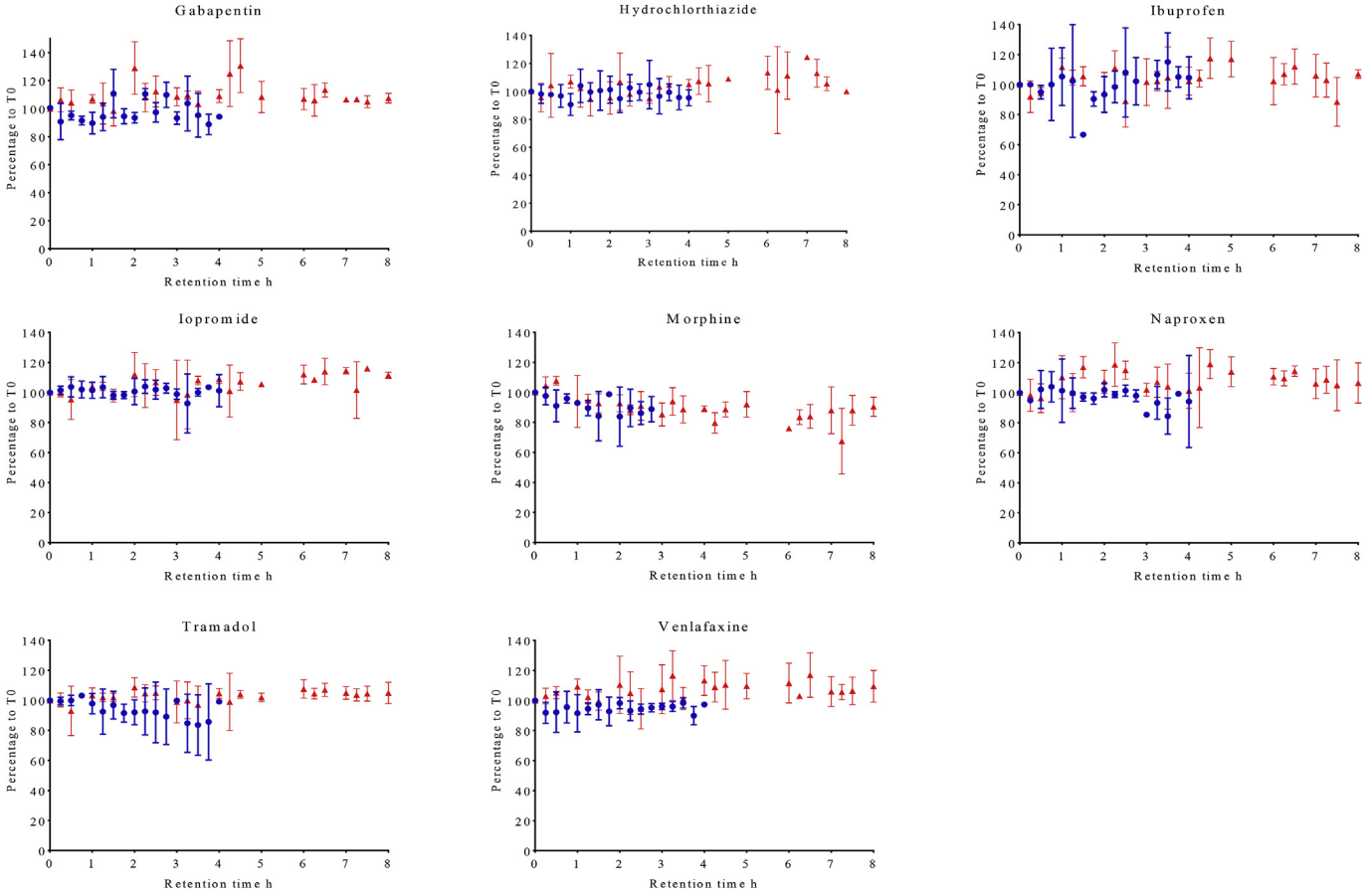

Fig. 2. (*continued*).

Therefore, they can be considered stable in a real catchment if the average HRT in the catchment is comparable to or shorter than the HRT values mentioned in Table S5.

3.3.1. Transformation of biomarkers in the GS

Most of the investigated biomarkers were stable in the GS (Fig. 2). The degradation of seven unstable biomarkers, buprenorphine, caffeine, EtS, methadone, paracetamol, paraxanthine and salicylic acid is shown in Fig. 3. Paracetamol had the highest degradation rate with approximately 50% loss in 4 h with a zero-order transformation coefficient of 0.4185 m h^{-1} followed by methadone and caffeine (Table 1). The loss of biomarkers in the pilot GS is relatively lower compare with GS reactors in the same HRT as demonstrated in Table 2.

Fast degradation has been observed for many of those biomarkers in laboratory batch experiments. The in-sewer loss of biomarkers in the laboratory GS reactor was higher than the pilot GS for all unstable biomarkers in the same HRT. This can be partially attributed to the higher A/V in laboratory GS reactor (65.4 m^{-1} for the GS reactor and ~27 m^{-1} for pilot GS) as shown in Table S5. Although there is both formation and consumption of VFAs in the GS, the overall decrease in VFAs showed high correlation with the degradation of unstable biomarkers (Table S6). Therefore, VFAs can be considered a prediction factor for the degradation of unstable biomarkers. The soluble COD (sCOD), had lower correlations with the degradation of unstable biomarkers, although its decrease has been observed in other studies (McCall et al., 2016b; Ramin et al., 2017). The correlation between the degradation of unstable biomarkers in GS is not as good as in RM, indicating that the transformation of biomarkers in GS could be attributed to more diverse biota in the biofilm.

For a given length and diameter, GSs usually generate much shorter HRT than RMs because there is a minimum flow speed of 0.6 m/s for self-cleaning and the GSs flow is continuous. Therefore, the extent of transformation of biomarker in a single GS pipe can be relatively small due to the short HRT in the pipe. However, this study suggests that for a whole sewer catchment, especially large ones with considerable proportion of GSs (with diverse diameters and A/V), where the average HRT can be several hours, the in-sewer loss cannot be neglected for unstable biomarkers.

Table 1
A/V normalized transformation coefficients K_{bio}.

Biomarker	K_{bio} m·h^{-1}	
	GS	RM
Buprenorphine	0.1193	0.0488
Caffeine	0.1263	0.1923
EtS	0.1185	0.1113
Methadone	0.2322	0.0823
Paracetamol	0.4185	0.1815

Note: Paraxanthine was not calculated due to the lack of K_{ww} in the control reactor; Salicylic acid was not shown since the K_{ww} value in control reactor is higher than the overall K in the pilot system.

3.3.2. Transformation of biomarkers in the RM

The unstable biomarkers observed in GS were also unstable in RM (Fig. 3). Caffeine had the highest loss of 65% over 8 h and a transformation coefficient of 0.1923 m·h^{-1}. EtS lost up to 23% over 5 h HRT in the real RM (Gao et al., 2018), while in the pilot RM, the loss was 19%, which is slightly lower than the real sewer, despite its A/V ratio being 1.5 times higher. Nicotine, cotinine and trans-3′-hydroxycotinine were stable in the pilot RM, in contrast with the observed formation in the real RM. The possible reason is that the feed of the pilot sewers is the influent of the WWTP where the

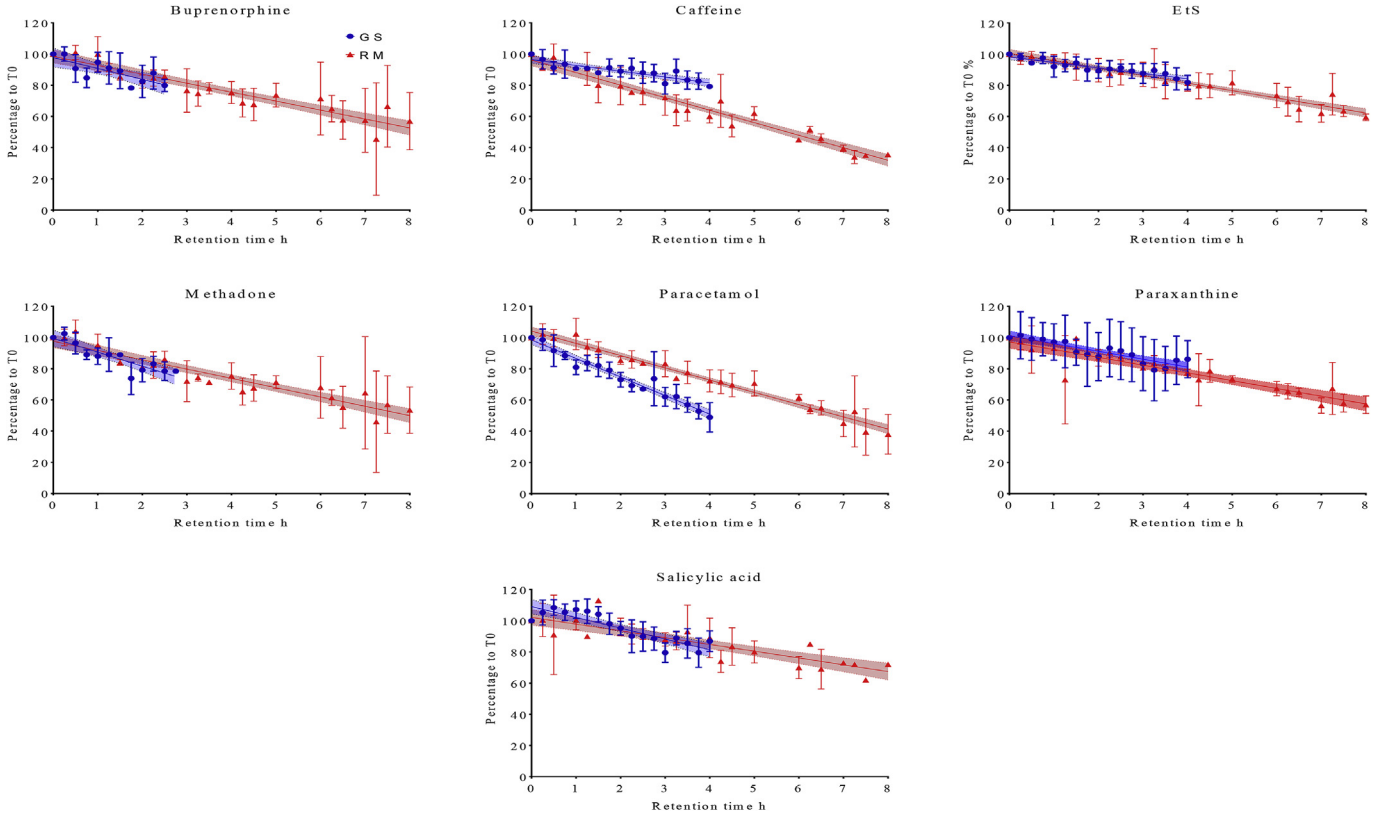

Fig. 3. Transformation of unstable biomarkers in the pilot sewer (the filled area is the 95% confidence interval bands) (red triangles = RM, blue circles = GS).

Table 2
Loss of biomarkers in pilot sewers and laboratory sewer reactors in the same HRT.

	GS pilot	GS reactor	Pilot RM 6 h	RM reactor 6 h
Buprenorphine	18 ± 10%/2 h	26 ± 3%/2 h	32 ± 18%	61 ± 6%
Methadone	21 ± 7%/2 h	25 ± 9%/2 h	31 ± 14%	61 ± 7%
Caffeine	19 ± 7%/3 h	37 ± 6%/3 h	51 ± 4%	94 ± 2%
EtS	12 ± 6%/3 h	27 ± 4%/3 h	29 ± 7%	98 ± 1%
Paracetamol	38 ± 6%/3 h	88 ± 5%/3 h	40 ± 4%	99 ± 1%
Salicylic acid	16 ± 9%/3 h	53 ± 11%/3 h	33 ± 8%	94 ± 3%

amount of conjugates of nicotine metabolites is limited compared to the wastewater in upstream RM monitored by Gao et al. (2018). It also suggested that the significant degradation observed for cotinine and trans-3′-hydroxycotinine in the laboratory RM reactor was an over-estimation (Banks et al., 2018). Formation of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP) was not observed despite the considerable level of methadone degradation. This is in agreement with the observation in laboratory reactors and real sewers (Gao et al., 2017; Li et al., 2018). Similarly with the GSs, within the same HRT in RMs, the overall loss of unstable biomarkers was higher in the reactor than in the pilot RM (Table 3). For most unstable biomarkers, their transformation had a strong Pearson correlation coefficient (>0.9) with each other (Table S7), indicating the transformation of these biomarkers is likely attributable to similar processes. In addition, the degradation of biomarkers also had good Pearson correlation (absolute value) with anaerobic sewer bioactivity indicators such as the methane formation and sulfate reduction, which suggests that the transformation of biomarkers could directly or indirectly relate to the methanogen and sulfate reducing activities.

Some discrepancies with previous studies was noticed, for example, citalopram was observed to have some degradation in the 7.6 km real RM (Jelic et al., 2015), but was stable in the pilot RM for up to 8 h. In the real WWTP catchment, RMs are often only used where the construction of GSs is not feasible. As a result, there is a much higher proportion of GSs than RMs for most of the catchments globally. Nevertheless, this study suggests that the loss of biomarkers in the RMs should be taken into account.

3.4. Transformation kinetics and comparison with previous transformation studies

Most of the biomarker transformations had some level of deviation from both first-order and zero-order kinetics as the goodness of fit R^2 was less than 0.8, especially in GS (Table 3). This could be attributed to the complexity of the mass transfer in the sewers and the relatively short HRT in GS. In GS, only paracetamol has an R^2 value greater than 0.8, and both zero-order and first-order kinetics can describe the degradation well, with R^2 values of 0.96 and 0.86 respectively. In RM, zero-order kinetics have good R^2 (>0.8) for the transformation of buprenorphine, caffeine, ethyl-sulphate, methadone paracetamol and paraxanthine. In contrast, under more controlled laboratory conditions and higher A/V, the R^2 value was much higher in the sewer reactors (Gao et al., 2017; O'Brien et al., 2017; Thai et al., 2014).

In previous real sewer studies, the data obtained were usually not sufficient to establish transformation kinetics. In some cases, e.g. nicotine metabolites, the deconjugation process can also interfere with the degradation assessment (Gao et al., 2018). Overall, we see the comparability of data from this study with data obtained from previous real sewer experiments (Table S5), reflecting the realistic condition of the pilot sewer system used in this study and the advantage of using pilot system for kinetic study. A summary of advantages and disadvantages of different sewer settings is presented in Table S8. If investigating the biomarker stability under realistic and variable sewer conditions is the aim, pilot sewer system is a good platform although the cost to build and maintain the system is much higher than simple laboratory reactors.

3.5. Implications for wastewater-based epidemiology

This study examined the in-sewer stability of selected PPCP biomarkers. The stable biomarkers identified can be further evaluated against the criteria proposed by Daughton (2012). If they meet the other requirements, they can be used for reliable consumption estimations and provide temporal and geographical profiles as well as estimate the real-time population. For unstable

Table 3
Transformation kinetics of unstable biomarkers in pilot sewers and in laboratory sewer reactors.

Biomarker	Pilot GS				Pilot RM			
	Zero-order		First-order		Zero-order		First-order	
	Slope	R^2	half-life h	R^2	Slope	R^2	half-life h	R^2
Buprenorphine	−7.01 ± 1.83	0.62	1.0	0.42	−5.74 ± 0.45	0.88	5.0	0.65
Caffeine	−3.75 ± 0.50	0.78	10.0	0.46	−8.03 ± 0.38	0.96	14.7	0.90
Ethyl-sulphate	−3.53 ± 0.37	0.86	8.59	0.50	−4.78 ± 0.27	0.94	~1012	0.72
Methadone	−8.94 ± 1.48	0.78	1.27	0.62	−5.96 ± 0.45	0.90	4.2	0.69
Paracetamol	−11.9 ± 0.59	0.96	18.43	0.86	−7.86 ± 0.29	0.97	~1461	0.86
Paraxanthine	−5.01 + 0.64	0.80	4.86	0.19	−4.98 + 0.44	0.86	~403	0.62
Salicylic acid	−6.92 + 0.93	0.79	~1048	0.58	−4.34 + 0.54	0.76	~1011	0.58
	GS reactor				RM sewer reactor			
	Zero-order		First-order		Zero-order		First-order	
	Slope	R2	half-life h	R2	Slope	R^2	half-life h	R^2
Buprenorphine	−5.32 ± 0.62	0.92	4.4	0.79	−5.59 ± 1.51	0.7	1.1	0.86
Caffeine	−4.10 ± 0.50	0.92	~2000	0.55	−8.88 ± 1.09	0.92	4.3	0.84
EtS	−8.60 ± 0.55	0.96	3.77	0.96	−15.24 ± 3.00	0.77	1.27	0.90
Methadone	−5.17 ± 0.61	0.92	3.8	0.86	−5.67 ± 1.58	0.68	1.1	0.88
Paracetamol	−8.31 ± 0.96	0.69	1.46	0.92	−6.74 ± 1.20	0.60	0.77	0.99
Paraxanthine	NA		NA		NA		NA	
Salicylic acid	−8.79 ± 0.66	0.85	2.63	0.95	−7.49 + 1.14	0.64	1.3	0.93

Note: sewer reactor data was extracted from Gao et al. (2017), Banks et al. (2018) and O'Brien et al. (2017).

biomarkers, however, if they can meet all the other requirements as Daughton suggested, they can still be used as biomarkers in WBE if catchment specific correction factors can be used. Preferably, such correction factors are derived from modeling work based on the understanding of transformation kinetics and the catchment characteristics (Li et al., 2018; McCall et al., 2017; Ramin et al., 2017).

4. Conclusion

Our study demonstrated that the pilot sewer system is a good platform for the evaluation of biomarker stability. It provides more realistic sewer conditions than laboratory studies, and the operational parameters can be controlled for a kinetic study. Among the biomarkers tested, seventeen were stable, while seven were unstable in both GS and RM, with a lower level of loss compared to the sewer reactor data. In reality, the level of loss of unstable biomarkers is dependent on the proportion of GS and RM in the catchment, and its HRT. In RM, the transformation of biomarkers correlated well with bioactivity indicators including the sulfate reduction, methane generation and VFAs decrease, which could be used as prediction factors for in sewer loss.

Acknowledgements

The Queensland Alliance for Environmental Health Sciences, The University of Queensland gratefully acknowledges the financial support of the Queensland Department of Health. Special thanks to support from Queensland Urban Utilities for the conduct site of the study. Jianfa Gao receives an ARC scholarship (DP150100645). Jiaying Li is funded by China Scholarship Council. Phong K. Thai was funded by the QUT VC Fellowship. Ludwika M. Nieradzik was the recipient of UQI scholarship. Mr. Adam Shypanski acknowledges the scholarship support from the University of Queensland. Guangming Jiang is the recipient of Australian Research Council DECRA Fellowship (DE170100694). Some results reported in this paper were obtained at the Central Analytical Research Facility operated by the Institute for Future Environments (QUT). Access to CARF is supported by through the Science and Engineering Faculty (QUT). Thanks to Dr Berwyck Poad for instrumental support. The Cooperative Research Centre for Water Sensitive Cities is gratefully acknowledged for their funding and research support. Many thanks to Natasha Rossi, Tobias Hesse for helping with the field work. And thanks to Amelia Cossart, Rachel Mackie, Stephanie Hall and Robert Gray for English proof reading.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.12.032.

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