Supporting information for: Occurrence of xenobiotics in grey water and removal in three biological treatment systems

Lucía Hernández Leal,^{*,†,‡} Niina Vieno,^{‡,¶} Hardy Temmink,^{†,‡} Grietje Zeeman,[†]
and Cees Buisman^{†,‡}

*Section Environmental Technology, Wageningen University, P.O. Box 8129, 6700EV Wageningen,
The Netherlands, and Wetsus, Centre of Excellence for Sustainable Water Technology, P.O. Box
1113, 8900CC Leeuwarden, The Netherlands*

E-mail: lucia.hernandez@wetsus.nl

Introduction

The chemical structures of studied xenobiotics can be seen in Figure S1.

^{*}To whom correspondence should be addressed

[†]ETE, WUR

[‡]Wetsus

[¶]WANDER Nordic Water and Materials Institute/Prizztech Ltd, Kalliokatu 10B, FI-26100 Rauma, Finland

Materials and methods

Operational parameters of the lab-scale grey water treatment systems

Table S1 describes the operation of the grey water treatment systems used in this study. A more extensive description of these systems is available elsewhere (1).

In brief, the systems were operated at steady state during the 6 months of sampling.

Table S1: Operational parameters of grey water treatment systems

		aerobic	anaerobic	anaerobic + aerobic
Volume	L	3.6	5	3.6 + 5
HRT	h	11.7±1.1	12.3±1.8	13.2±2
SRT	d	15	392	97 (an) 379 (a)
COD removal	%	90±7	51±13	89±3
SLR	kgCOD(kg VSS) ⁻¹ d ⁻¹	0.29±0.07	0.12±0.04	0.23±0.08 (an) 0.6±0.3 (a)
Sludge conc.	g VSS L ⁻¹	5.5±1.1	12.5±2.4	12.7±4.3 (an) 3.3±1.1 (a)

A schematic description of the sample preparation for LC-MS/MS analysis is shown in Figure S2 and the preparation procedure of GC-MS is shown in Figure S3.

LC-MS/MS analysis

LC separation

The LC separations were performed on the LC (Agilent Technologies 1200 series) consisting of a binary pump, a vacuum degasser, a thermostated autosampler and a thermostated column oven. Analytes were separated on Eclipse XDB-C18, Rapid Resolution HT column (4.6 x 50 mm, 1.8 μ m, Agilent) equipped with a guard column (Agilent Eclipse XDB-C18, 4.6 x 12.5 mm, 5 μ m). In a direct injection method, the eluent flow rate was 0.5 mL/min, injection volume was 2 μ L and

Figure S2: Sample preparation procedure for analysis with LC-MS/MS in direct injection and inline SPE

Figure S3: Sample preparation procedure for analysis with GC-MS with stir bar sorptive extraction

the column oven was kept at 30 °C. The column elution program is presented in Table S2.

Table S2: LC elution program of direct injection method.

Time (min)	% acetonitrile	% HCOOH (0.1%)
0	5	95
12	100	0
16.5	100	0
17	5	95
26.5	5	95

In addition to the direct injection, samples were concentrated using solid phase extraction. The LC was equipped with a quaternary pump and a column-switching valve that allowed the solid phase extraction to be done in-line with the analytical separation. Solid phase extraction was performed with an Oasis HLB extraction cartridge (2.1 x 20 mm). The analytes were loaded into the cartridge at a flow rate of 0.5 mL/min using aqueous solution (10 mM NH₄COOH + 0.1% HCOOH) and eluted with the gradient run of the analytical column at flow rate of 0.5 mL/min

(Table S3). Column oven was kept at 30 °C and injection volume depended on the sample matrix. Samples from the aerobic effluents was injected at a volume of 175 μ L and grey water samples as well as the anaerobic effluent samples was inject at a volume of 58 μ L.

Table S3: LC Elution program of in-line solid phase method.

Time (min)	% acetonitrile	% HCOOH (0.1%)
0	5	95
6	5	95
18	100	0
26.5	100	0
27	5	95
34	5	95

Mass spectrometry

The flow from the LC column was transferred to a triple-quadrupole mass spectrometer (Agilent Technologies 6410 TripleQuad LC/MS) equipped with an electrospray ionization source. Nitrogen was used as the desolvation gas at the source at a flow rate of 10 L/h. Nebulizer pressure was kept at 50 psi and the capillary voltage was 4000 V. The source temperature was 320 °C. Nitrogen was used as the collision gas. Positive ions of the analytes were acquired in multiple reaction monitoring mode. The optimized MS/MS parameters are presented in Table S4.

Table S4: The optimized MS/MS parameters for LC analytes.

Compound	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (eV)
PBSA	275.1	194.2	190	33
BP3	229.1	151.0	115	15
Avobenzene	311.2	135.0	115	18
BaCl (1)*	304.3	91	140	32
BaCl (2)*	332.3	91	140	35

* Benzalkonium chloride was present at two homologs

Quality parameters

For all three matrices (grey water, aerobic matrix and anaerobic matrix), recoveries were determined by spiking them with a stock solution containing all the analytes and determined as described above. For all the matrices, the spiking level was 100 $\mu\text{g/L}$ when the direct injection method was used. For in-line SPE method, grey water and anaerobic matrix samples were spiked at a level of 1 $\mu\text{g/L}$ and the aerobic effluent at a level of 0.5 $\mu\text{g/L}$. Recovery samples were done at triplicates. Limit of quantifications (LOQ) were determined using a method called the Target RSD (2). The LOQ was determined at RSD 10 %.

GC/MS analyses

The analytes were desorbed from the stir bars in a thermal desorption (TD) unit and then trapped into cooled injection system (CIS) unit. Then, temperature was raised gradually and the analytes were transferred into the GC column. Initial temperature of the TD unit was 30°C and it was increased to 280°C at 720°C/min and held at this temperature for 5 minutes. During desorption, CIS unit was kept at a temperature of -100°C. After desorption, the temperature was increased to 300°C at 12 °C/s and held at this temperature for 10 minutes. The analytical GC column (HP-5ms, 30m x 0.25mm x 0.25 μm , Agilent) was kept at a temperature of 60°C for the first 2 minutes and then ramped into 280°C at 15°C/min and held at this temperature for 5 minutes. The analytes were detected at selected ion mode and the ions are presented in Table S5.

Quality parameters

To determine the recoveries, the matrix samples were spiked with the analytes at different concentrations. Grey water samples were spiked at concentrations between 2 and 25 $\mu\text{g/L}$ and the other two matrices at 1 to 10 $\mu\text{g/L}$ depending on the analyte. The samples were analysed as described

Table S5: Ions selected for monitoring in GC/MS analysis.

Compound	SIM (m/z)
Methylparaben [*]	121
Ethylparaben [*]	121
Propylparaben [*]	138
Butylparaben [*]	138
EHMC	178
2EHS	120
4MBC	254
Octocrylene	249
Galaxolide	243
Tonalide	243
Hexyl cinnamic aldehyde	129
Nonylphenol [*]	135
Triclosan [*]	288
Bisphenol-A [*]	213
Tonalide-d3	246
Bisphenol-A-d16 [*]	224
Benzophenone-d5	187

^{*} Acyl-derivatives of the analytes

above and all the measurements were done in triplicates.

Results and discussion

Recoveries and limits of quantification

Methyl-, ethyl-, propyl- and butyl- parabens and bisphenol-A were very well recovered in all matrix samples, which are grey water, aerobic effluent and anaerobic effluent (Table S6). Triclosan, galaxolide, tonalide, HCA, 2EHS and 4MBC were relatively well recovered (50–100%) in all matrices, except the recovery of triclosan in grey water (38% recovery) and tonalide and galaxolide in the anaerobic matrix (36% and 50%, respectively). In general, a slightly lower recovery was achieved in the anaerobic matrix. Although the recovery for nonylphenol was rather low, it was consistently around 20–30% in all matrices. EHMC showed a rather high recovery of 83% in grey

water, but significantly lower in the effluents (32% for aerobic and 38% for anaerobic matrix). Because of the high limits of quantification (LOQs) for methyl and ethylparaben in grey water, these compounds could not be quantified in any of the grey water samples. The recoveries of octocrylene were low and varied significantly, and it was difficult to obtain a calibration curve with a correlation factor of at least 0.99. This resulted in rather high LOQs and in combination with the low recoveries (Table S6) implied that octocrylene could only be determined semi-quantitatively in the grey water and effluent samples.

Table S6: Recoveries (rec.) and limits of quantification (LOQ) of xenobiotics in grey water, aerobic and anaerobic matrices

	Grey water matrix		Aerobic matrix		Anaerobic matrix	
	rec.	LOQ	rec.	LOQ	rec.	LOQ
	(%)	($\mu\text{g/L}$)	(%)	($\mu\text{g/L}$)	(%)	($\mu\text{g/L}$)
Methylparaben	63	3.19	111	0.90	78	1.29
Ethylparaben	77	1.04	129	0.31	108	0.37
Propylparaben	86	0.23	126	0.08	115	0.09
Butylparaben	88	0.09	125	0.03	118	0.03
EHMC	83	0.10	32	0.13	38	0.11
2EHS	94	0.09	70	0.06	63	0.06
4MBC	87	0.09	100	0.04	61	0.07
PBSA	70	0.06	65	0.02	70	0.06
BP3	49	0.06	83	0.01	47	0.06
Avobenzone	28	0.14	65	0.12	28	0.14
Octocrylene	38	0.53	29	0.34	59	0.17
Galaxolide	83	0.24	77	0.13	36	0.28
Tonalide	84	0.24	70	0.14	47	0.21
HCA	95	0.21	79	0.13	50	0.20
BaCl	70	1.42	26	2.3	79	1.27
Nonylphenol	20	0.41	23	0.17	28	0.14
Triclosan	38	0.21	98	0.04	64	0.06
Bisphenol-A	96	0.08	110	0.04	131	0.03

Recoveries and LOQs for LC-MS/MS compounds shown in Table S6 correspond to the in-line SPE mode (with the exception of BaCl, for which direct injection was used for all measurements). With this method lower LOQs could be achieved compared to the direct injection method. With the method of direct injection, recoveries were above 90% for all compounds except for BaCl in the aerobic matrix, which had a consistent low recovery of 30% (data not shown). BaCl could

not be analyzed with in-line SPE and therefore the LOQ for this compound was rather high ($2.3 \mu\text{gL}^{-1}$). As a result, BaCl could not be quantified in any of the aerobic sample.

Implications of grey water reuse or discharge

Table S7 shows the calculated estrogenicity of grey water and effluents based on YES bioassays.

Table S7: Estrogenic potency of grey water and effluent expressed in 17β estradiol equivalents (ng/L), $\text{IC}_{50} = 57.2 \text{ ng/L}$. All values calculated based on YES assay data from literature (3)

	average	min	max
Grey water	0.70	0.12	2.64
Aerobic	0.07	0.01	0.21
Anaerobic	0.72	0.20	2.01
Anaerobic + Aerobic	0.09	0.01	0.26

References

- (1) Hernández Leal, L.; Temmink, H.; Zeeman, G.; Buisman, C. J. N. Comparison of Three Systems for Biological Greywater Treatment. *Water* **2010**, 2, 155–169.
- (2) Vial, J.; Le Mapihan, K.; Jardy, A. What is the best means of estimating the detection and quantification limits of a chromatographic method? *Chromatographia* **2003**, 57, S303–S306, Suppl. S.
- (3) Miller, D.; Wheals, B. B.; Beresford, N.; Sumpter, J. P. Estrogenic activity of phenolic additives determined by an in vitro yeast bioassay. *Environ. Health Perspect.* **2001**, 109, 133–138.