



Organic micropollutant removal in full-scale rapid sand filters used for drinking water treatment in The Netherlands and Belgium

Camilla Di Marcantonio^a, Cheryl Bertelkamp^b, Nikki van Bel^b, Tessa E. Pronk^b, Peer H.A. Timmers^b, Paul van der Wielen^{b,c}, Andrea M. Brunner^{b,*}

^a Sapienza University of Rome, Department of Civil, Constructional and Environmental Engineering (DICEA), Rome, Italy

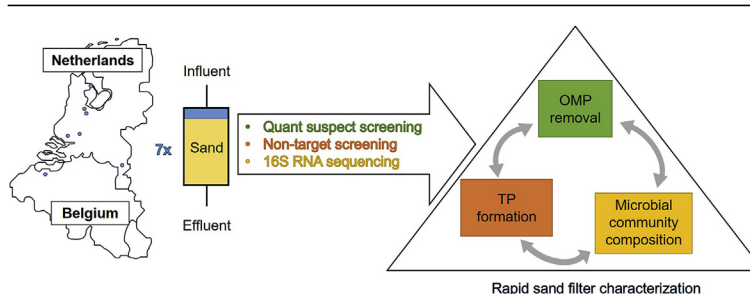
^b KWR Water Research Institute, P.O. Box 1072, 3430, BB, Nieuwegein, the Netherlands

^c Laboratory of Microbiology, Wageningen University & Research, Stippeneng 4, 6708WE, Wageningen, the Netherlands

HIGHLIGHTS

- 27% of OMPs detected in RSF influents removed during filtration.
- Structure of ten transformation products from RSF effluents elucidated.
- Two novel transformation products identified using BioTransformer prediction.
- Microbial community similar in influent and effluent, different in filter medium.
- Overall OMP removal efficiencies not related to microbial community composition.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 7 April 2020

Received in revised form

19 June 2020

Accepted 5 July 2020

Available online 12 July 2020

Handling Editor: Chennai Guest Editor

Keywords:

Organic micropollutants
Drinking water treatment
Rapid sand filtration
Non-target screening
Microbial community composition analysis
Transformation products

ABSTRACT

Biological treatment processes have the potential to remove organic micropollutants (OMPs) during water treatment. The OMP removal capacity of conventional drinking water treatment processes such as rapid sand filters (RSFs), however, has not been studied in detail. We investigated OMP removal and transformation product (TP) formation in seven full-scale RSFs all treating surface water, using high-resolution mass spectrometry based quantitative suspect and non-target screening (NTS). Additionally, we studied the microbial communities with 16S rRNA gene amplicon sequencing (NGS) in both influent and effluent waters as well as the filter medium, and integrated these data to comprehensively assess the processes that affect OMP removal. In the RSF influent, 9 to 30 of the 127 target OMPs were detected. The removal efficiencies ranged from 0 to 93%. A data-driven workflow was established to monitor TPs, based on the combination of NTS feature intensity profiles between influent and effluent samples and the prediction of biotic TPs. The workflow identified 10 TPs, including molecular structure. Microbial community composition analysis showed similar community composition in the influent and effluent of most RSFs, but different from the filter medium, implying that specific microorganisms proliferate in the RSFs. Some of these are able to perform typical processes in water treatment such as nitrification and iron oxidation. However, there was no clear relationship between OMP removal efficiency and microbial

Abbreviations: BMIT, biotransformer metabolite identification tool; BMPT, biotransformer metabolite prediction tool; CL, confidence level; FC, fold change; FISH, fragment ion search; IS-eq, conc. internal standard equivalent concentration; LC HR MS/MS, liquid chromatography coupled to high-resolution tandem mass spectrometry; MW, molecular weight; MS, mass spectrometry; NTS, non target screening; OMP, organic micro-pollutant; OTU, operational taxonomic unit; PC, parent compounds; RSF, rapid sand filter; RT, retention time; TP, transformation product.

* Corresponding author.

E-mail address: andrea.brunner@kwrwater.nl (A.M. Brunner).

<https://doi.org/10.1016/j.chemosphere.2020.127630>

0045-6535/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

community composition. The innovative combination of quantitative analyses, NTS and NGS allowed to characterize real scale biological water treatments, emphasizing the potential of bio-stimulation applications in drinking water treatment.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

1.1. OMP removal in drinking water treatment with biological filtration processes

Organic micropollutants (OMPs) cover a large and diverse number of compounds such as pharmaceuticals, pesticides, personal care and industrial waste products as well as their transformation products (TPs). They have been detected in the lower ng/L – µg/L range in several drinking water sources in the Netherlands, Belgium, and worldwide (de Jongh et al., 2012; Wilkinson et al., 2017). Commonly applied treatment processes for OMP removal are membrane filtration, (advanced) oxidation techniques and/or activated carbon filtration. Although these processes are effective for a wide array of compounds, they are also energy intensive and require the use of chemicals.

Biological processes such as riverbank filtration, dune filtration, rapid and slow sand filtration and activated carbon filtration (ACF) may be a promising alternative with respect to their ability to remove OMPs without extensive demand for energy and chemicals (Abu Hasan et al., 2020; Alidina et al., 2014a; Bertelkamp et al., 2017; Vandermaesen et al., 2016; Zearley and Summers, 2012). A further advantage of these biological processes are that they are present in existing drinking water treatment plants. Rapid sand filters for example have the additional advantage that they are often already present in conventional drinking water treatment plants to remove (in)organic compounds and residual particles remaining after upstream processes such as aeration or coagulation/sedimentation (Cakmakci et al., 2008; Clasen, 1998; Gude et al., 2018). Although not specifically designed for the removal of OMPs, RSFs seem to have the capability to (partially) degrade a number of different OMPs (Hedegaard and Albrechtsen, 2014; Shimabuku et al., 2019; Zearley and Summers, 2012). For instance, Zearley and Summers (2012) investigated OMP removal in a laboratory-scale column filled with sand from a full-scale drinking water treatment plant. More than 30% of OMPs dosed into the feed of the column were degraded by more than 50%. Also, Hedegaard and Albrechtsen (2014) demonstrated the removal of the specific OMPs MCPP, bentazone, glyphosate and *p*-nitrophenol in microcosms with sand obtained from three different full-scale groundwater filters, and Shimabuku et al. (2019) the removal of MIB and 2,4-D in a column filled with sand obtained from a full-scale bio-filter. Although these studies demonstrated the capacity of RSFs to remove OMPs, they were limited to laboratory-scale set-ups and used synthetic feed water. Since substrate concentration and composition has been reported to play an important role in shaping the microbial population and thus OMP removal (Alidina et al., 2014b), it remained to be shown how well the results of these lab-scale studies would translate to full-scale RSFs.

1.2. Microbial populations in RSF

Previous studies indicated that OMP removal could vary between different types of biological filtration processes, as well as between similar filters of different drinking water treatment plants (Bertelkamp et al., 2017). Since a number of studies have reported

that for most OMPs biodegradation is the most important removal mechanism (Bertelkamp et al., 2014; Maeng et al., 2011b; Regnery et al., 2015), it is likely that the differences in OMP removal efficiency are caused by differences in the microbial population present in these systems. Although knowledge about the microorganisms involved in OMP removal is limited, bacterial genera such as *Rhodococcus*, *Acidovorax*, *Mesorhizobium*, *Sphingomonas*, *Chryseobacterium*, *Methylophilus*, *Mesorhizobium*, *Termonas*, *Paracoccus*, *Bacillus* (species *B. thuringiensis*), *Rhizobium*, and *Sinorhizobium* have been shown to degrade certain OMPs, such as pesticides, PCBs, and PAHs (Jabeen et al., 2015; Keum et al., 2006; Kumar et al., 2012; Mandal et al., 2013; Ning et al., 2010; Ohtsubo et al., 2006; Poonthirigpun et al., 2006; Tu et al., 2011; Yam et al., 2010; Zhang et al., 2011). In addition, ammonia oxidizing bacteria (AOBs) have been reported to play an important role in the degradation of OMPs in wastewater treatment processes (Margot et al., 2016; Park et al., 2017). However, these studies used other inocula than RSF and were mostly limited to laboratory-scale studies. The microbial community composition of RSFs in relation to OMP removal thus remains largely unknown.

1.3. Transformation product formation

OMP concentrations in source water for drinking water production are usually in the ng – µg/L range. Since these concentrations are probably too low for bacteria to use them as a sole carbon source for growth, it is likely that (part of the) OMPs are co-metabolically degraded, resulting in TP formation (Alidina and Li, 2014) (Alidina et al., 2014b; Maeng et al., 2011a; Rauch-Williams et al., 2010). Biodegradation of OMPs resulting in the formation of transformation products (TPs) has become a concern in drinking water treatment since the number of TPs and their molecular structure are often unknown, and TP toxicity can be similar or higher than the parent compound's (PC) (Li et al., 2017).

Several studies have described TP formation during wastewater treatment and their environmental risk assessment (Bletsou et al., 2015; Schollée et al., 2018; Schymanski et al., 2015). However, studies on TP monitoring in drinking water treatment are limited; they mainly focused on abiotic drinking water treatment processes such as advanced oxidation and laboratory scale experiments (Brunner et al., 2019; Hübner et al., 2015; Postigo and Richardson, 2014). Whereas biological processes have been shown to (partially) remove OMPs, possible TP formation during these drinking water treatment processes remains to be elucidated (Benner et al., 2013).

A major challenge in identifying TPs in drinking water treatment is the low concentration of both PCs and TPs in full-scale studies. Moreover, there is not necessarily a one-to-one relationship between PC decrease and TP formation, one PC can form multiple TPs and also the same TP can be formed from various PCs. Additionally, also partial PC removal can cause an increase of a TP to a low, but toxicologically relevant concentration. To avoid these challenges, studies typically focused on laboratory experiments at elevated concentrations (Brunner et al., 2019; Kaiser et al., 2014) which might be less representative for full-scale operated drinking water treatment processes. However, only full-scale experiments that

monitor PC removal and TP formation can provide information on the actual potential of RSFs for drinking water treatment.

1.4. Non-target screening for the identification of unknown unknowns

As the TPs' molecular structures are often unknown, their identification requires an analytical method of detection that can identify compounds for which no previous knowledge is available, such as non-target screening (NTS) based on liquid chromatography (LC) coupled to high-resolution tandem mass spectrometry (HR MS/MS). NTS allows the analysis of target compounds suspects, i.e. compounds that are expected to be present in a sample and unknown compounds in a single analytical run (Bletsou et al., 2015). However, as a single run can result in thousands of so called features, i.e. mass and retention time pairs associated with a signal intensity, a prioritization step is needed to limit the number of unknown peaks to be identified (Schollée et al., 2018).

The computational workflows to prioritize TPs from NTS data follow two general strategies; the first is a true NTS strategy that considers all detected features and treats them based on temporal, spatial, or process-related connections (Bletsou et al., 2015; Schollée et al., 2016). The second strategy is based on suspect screening and relies on the prediction of possible TPs through computational tools (Djoumbou-Feunang et al., 2019; Ellis et al., 2008; Li et al., 2017). The predicted potential TPs from a suspect list can then be searched for in the NTS data, based on accurate mass matches. Finally, with both strategies, the structures of the prioritized features, i.e. potential TPs, are elucidated based on the match of mass spectrometric information of the full scan (MS1) and fragmentation spectra (MS2), using spectral libraries or *in silico* fragmentation tools (Hollender et al., 2017). While workflows combining both strategies have been described previously for full-scale waste water treatment plants and lab-scale drinking water treatment processes, to date they have not been applied to full-scale drinking water treatment.

2. Objectives

Insight in the OMP removal capacity of full-scale RSFs, including the formed TPs and the microbial species involved is needed to understand why certain OMPs are removed or partially degraded while others persist in similar biological filtration processes. Based on this information OMP removal in RSFs could then be optimized.

This study investigated OMP removal, TP formation and microbial community composition and the interdependencies of the three in seven full-scale RSFs from drinking water treatment plants in The Netherlands and Belgium. All investigated RSFs use surface water as a source. OMP removal was assessed for 127 OMPs based on quantitative suspect screening. Based on the NTS analysis, OMP removal and TP identification was achieved through a combined data-driven approach that used feature intensity profiles for prioritization and suspect screening for PC identification, TP prediction with the prediction tool BioTransformer, suspect screening for predicted TPs and structural elucidation of suspect TP matches. Ultimately, based on occurrence and abundance, microbial communities were linked to target and NTS data for a comprehensive characterization of RSF based water treatment.

3. Materials and methods

3.1. Rapid sand filter locations and sampling

Seven RSFs from different full-scale drinking water treatment plants, using surface water as source water, in The Netherlands and

Belgium (operational parameters provided in Table SI 1) were sampled in May 2018, during two consecutive days. Influent water, effluent water and filter medium from the top of the filter-bed were sampled at all RSFs. At each drinking water treatment plant, the filter with the longest run time was selected because of expected highest and stable biomass concentrations. For sampling of the filter material, the filter was temporarily taken out of operation and the water level was lowered to the height of the filter bed. About 1 kg of the filter material, the top layer of the RSF (including the Schmutzdecke), was sampled using a stainless steel beaker which was flushed with drinking water, disinfected with chlorine and flushed again with drinking water before each sample was taken. The filter material was transferred to a sterile bottle and kept on ice until the analyses were performed within 24 h. Microorganisms were released from the filter material by low energy sonification with 40 kHz for 2 min in sterile, unchlorinated drinking water (sterilization by autoclave) (Magic-Knezev and van der Kooij, 2004). For LC-HRMS analyses, 1 L of influent and effluent water was sampled in dark glass bottles flushed with distilled water. To determine the microbial community composition, 2 L of influent and effluent was sampled in sterile bottles and kept on ice until further analyses, which were performed within 24 h.

A second sampling campaign was performed in September 2018 to assess OMP removal rates. Since the results were largely similar to those of May 2018, only the latter are discussed in this paper.

3.2. LC-HRMS analyses and quantitative suspect screening

NTS based on LC-HR MS/MS was carried out as described previously (Brunner et al., 2020) with the distinction that the mass range in the full scan was 80–800 *m/z* for acquisition. Suspects were quantified by means of a one-point calibration using the Xcalibur data processing program (ThermoFisher Scientific). Suspect calibrants used for quantification are listed in Table SI 3. OMP removal was defined based on influent OMP concentrations; at influent OMP concentrations less than 0.1 µg/L at least 50% of the OMP had to be removed, and at influent OMP concentrations larger than 0.1 µg/L at least 30% of the OMP had to be removed. However, this definition might underestimate the OMP removal efficiencies.

3.3. Molecular fingerprints, biodegradability and hydrophobicity of detected OMPs

OMP detected with the quantitative suspect screening were clustered based on their molecular fingerprints, i.e. mathematical representations of the structures of the molecules. The extended versions of path-based hashed molecular fingerprints which take into account rings systems were used in the R package "rcdk". The fingerprint similarity was calculated based on the Tanimoto distance using the R package "fingerprint".

Biodegradability was predicted using the biodegradation probability program (BIOWIN) and two different models (a) non-linear model prediction and (b) ultimate biodegradation timeframe prediction according to the European technical guidance on risk assessment (European Commission, 2003). An OMP was considered *Readily Biodegradable* if the probability from model a) was >0.5 and the persistence scoring from model b) was >2.2.

3.4. NTS data analysis

LC-HRMS/MS data was analysed in Compound Discoverer 3.0 (Thermo Fisher Scientific, San Jose, USA), including peak picking, feature building, and MS1 and MS2 based suspect screening. The summary of data processing parameters and Compound Discoverer 3.0 settings are reported in the SI (Table SI 2). The feature output,

i.e. mass and retention time pairs with intensities reported as area under the curve for every sample, was imported into R for further data analysis. Principal Component Analysis (PCA) was performed using the R package “FactoMineR” (Lê et al., 2008). Statistical differences between feature intensities of influent and effluent samples were assessed with the Student’s t-test using the R package “Stats”. The difference was considered statistically significant if the p-value was <0.05.

To reduce feature numbers, features a) which did not have an associated MS2 spectrum, b) to which no elemental formula was assigned in Compound Discoverer or c) that did not exceed at least 10x the blank signal intensity in any sample were removed prior to suspect screening.

3.5. Workflow for the identification and structural elucidation of transformation products in NTS data

A data-driven workflow was developed for the identification of TPs based on feature treatment profiles and TP prediction, followed by a wide-scope suspect screening, exceeding the quantitative screening described in 2.2. The workflow is schematically depicted in Fig. 1 and consists of three main phases: 1) PC identification (blue squares), 2) TP prediction (green ellipse) and 3) TP identification (green squares). Lists of PCs detected with the quantitative (trapeze and red font) and the wide-scope suspect screening (squares) were both used to predict TPs to evaluate the advantage in using NTS data for the selection of PCs.

3.5.1. Parent compound identification

PC identification was performed with a wide-scope suspect screening in Compound Discoverer. To this end, NTS features were searched for matches in monoisotopic mass (MS1) and - if available - fragmentation spectra (MS2) in different databases in the following order of priority: 1) in-house suspect list of the 127 water relevant chemicals that were also quantified (Table SI 3, MS1 and MS2 based identification); 2) the mass spectral library mzCloud (www.mzcloud.org, MS1 and MS2 based identification); 3) the chemical structure databases EAWAG Biocatalysis/Biodegradation Database, EPA DSSTox, and EPA Toxcast via ChemSpider (MS1 based identification (Little et al., 2012)).

For features that matched a suspect, their concentration relative to the internal standard (IS) atrazine-d₅ was calculated and

expressed as Internal Standard equivalent concentration (IS-eq conc.).

$$IS\ eq\ conc. = \frac{A_f}{A_{IS}} \cdot C_{IS} \quad (1)$$

where A_f is the peak area of the unknown feature, A_{IS} is the peak area of the IS (atrazine-d₅) and C_{IS} is the concentration of IS spiked in each sample (1 µg/L).

The change in the intensity between influent and effluent samples was determined and expressed as base-2 logarithm of the fold change (log₂FC).

$$\log_2 FC = \log_2 \frac{A_E}{A_I} \quad (2)$$

where the fold change between influent and effluent (FC) is expressed as base-2 logarithm of the ratio of peak area of the feature in the effluent (A_E) and in the influent (A_I). The log₂FC is negative if the intensity of the feature decreases during the treatment (removal) and it is positive if the intensity increases (formation). Identified suspects were classified as PCs if they met the following criteria:

- the influent IS-eq conc. exceeded 10 ng/L in at least one location;
- a minimum removal of 10% which corresponds to a log₂FC smaller than −0.152 was observed in at least in one location.

For all features classified as PCs, feature identification was attempted using MS1 and MS2 information. A confidence level (CL) for the confidence of the identification according to Schymanski et al. (2014) was assigned through visual inspection of the match between experimental fragmentation spectra and *in silico* predicted spectra through Fragment Ion search (FISH) coverage calculation in Compound Discoverer. The five defined CLs range from the highest level of confidence CL1, at which the chemical structure is confirmed with a reference standard, to CL5 the lowest level at which merely the exact mass of the compound is known. CL3 refers to one or more tentative suspect candidates, CL2 to a probable structure by library or diagnostic evidence. Both are assigned based on MS1 and MS2 information. If CL3 or 2 was reached by more than one suspect candidate, all candidates were

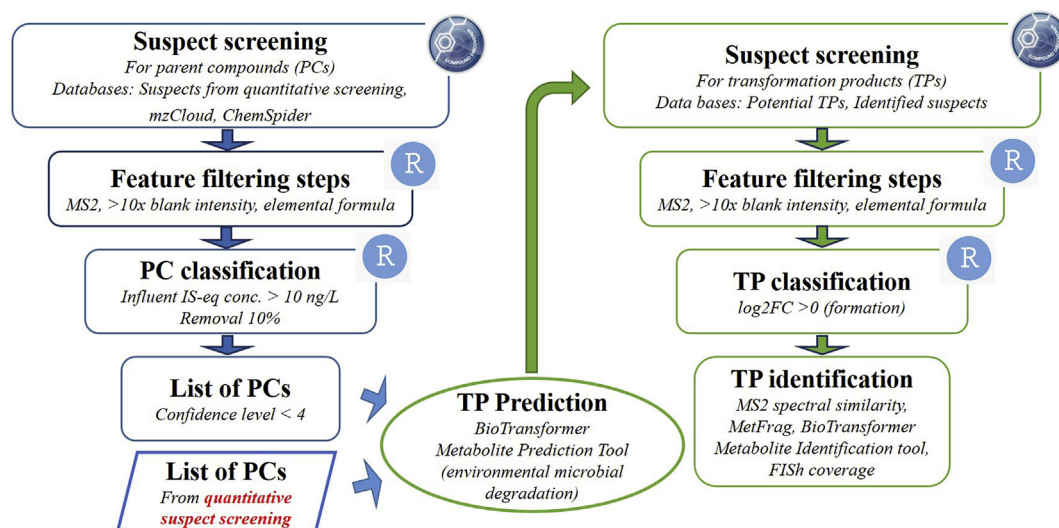


Fig. 1. Workflow for TP identification from NTS data.

used in the TP prediction step. For PC features for which a corresponding TP was detected, further steps to confirm the identification were performed, following the same procedure described in section 2.5.3.

3.5.2. Transformation product prediction

Prediction of TPs was performed using the environmental microbial module of the metabolism prediction tool (BMPT) in BioTransformer, an open access web service for *in silico* metabolism prediction and metabolite identification (Djombou-Feunang et al., 2019). The TPs of the OMPs previously defined as PCs were predicted using the SMILES of the PCs as input and a set of rules provided by the EAWAG-BBD/PPS system for prediction (Ellis et al., 2008). The resulting suspect list of predicted TPs consisted of an elemental formula, monoisotopic mass and International Chemical Identifier (InChI) as structural identifier. The name used to identify the molecules was defined merging the CAS of the PC and the metabolite ID proposed by BioTransformer (e.g. 116,459-29-1-BTM00004).

3.5.3. Transformation product identification

TP identification was achieved through a second suspect screening of the NTS data. The suspect list consisted of both the identified suspects and the TPs predicted with BioTransformer. Matched features were filtered for increasing intensity from influent to effluent, i.e. positive log₂FC, which indicates TP formation in at least one location. To increase the level of confidence of identification (Schymanski et al., 2014), the metabolite identification tool (BMIT) was used in BioTransformer; BMIT performed the metabolite identification based on PubChem suspect screening using as input the chemical structure of the starting molecule (the PC) and the mass or molecular formula of the metabolite (obtained from BMPT). To structurally elucidate the detected suspects, their fragmentation spectra were compared to mzCloud library and/or *in silico* spectra generated with MetFrag (Ruttkies et al., 2016) and Compound Discoverer FiSH. To confirm the tentatively identified TPs, comparison with the mass and retention time (RT) of a reference standard was performed. Furthermore, the spectral similarity between PC and TP spectra was calculated using the R package "OrgMassSpecR" (<http://OrgMassSpec.github.io/>). Head-to-tail plots of the mass spectra were generated and a similarity score was calculated as the dot product between the aligned intensity vectors of the two spectra.

3.6. DNA extraction and microbial community analysis

After sampling, influent and effluent water, sonicated filter material as well as negative control samples consisting of ultrapure water were filtered over 0.2 µm polycarbonate filters (Sartorius) and stored at −20 °C until further processing. DNA isolation was performed with the DNeasy PowerBiofilm Kit (Qiagen) according to the manufacturer's instructions. For DNA amplification, the V4 variable region of the 16S rRNA genes was amplified from all samples, commercially available Mock communities (Zymo research, Irvine, CA) and negative controls with primers 515 F and 806 R (Caporaso et al., 2010) using the KAPA HiFi polymerase (KAPABiosystems). The DNA concentrations of PCR products were measured using QuBit (ThermoFisher Scientific, Waltham, MA) and amplicons were barcoded, pooled in equimolar DNA concentrations and sequenced on an Illumina MiSeq platform according to the manufacturer's instructions, with 20% PhiX Control v3 (Illumina, San Diego, CA). From the raw sequence data (2 × 250 bp paired-end reads), quality-filtered sequences were clustered into Operational Taxonomic Units (OTUs) with a 97% identity cut-off using the mothur pipeline (Schloss et al., 2009). Taxonomic identification was

performed against the Silva SSU 16S rRNA gene database (version 132; Quast et al., 2013) and only prokaryotic reads were retained in the dataset. Data analysis was performed in R studio version February 1, 1335 (R studio Inc., Boston, MA) using the R package "Ampvis2" (Andersen et al., 2018).

3.7. Correlation analysis between OTUs and micropollutant increase or decrease

Correlations between individual OTU abundance and NTS features were assessed using correlation analysis. To limit noise and false positives for inclusion, OTUs were required to have >0.15% total read abundance in at least one sample and at least 25% of this amount in at least two other samples. In addition, OTUs were filtered for positive Spearman correlation of at least 0.8 with any measured feature in the influent.

NTS features were filtered based on their difference between influent and effluent of RSF locations. Features with less than 10 ng/L IS-equivalent intensity difference between the highest and lowest decrease in RSF locations were omitted. In addition, NTS features that did not have a difference of 15% of that value in at least two separate RSF locations were also omitted.

A Spearman correlation between the feature intensity difference between influent and effluent and OTU abundance was calculated for each possible pair of selected NTS feature and OTU over all 7 RSF locations. Pairs of features and OTUs with a correlation (negative or positive) of at least 0.9 were selected. This correlation is stricter than used in the selection criteria for OTUs, because we want to limit noise as much as possible. For the NTS features of correlated pairs, feature peak shape was manually assessed and noise features introduced by faulty peak picking discarded. MS2 information based identification was attempted for the NTS feature of the remaining pairs.

4. Results and discussion

4.1. OMP removal in full-scale rapid sand filters in The Netherlands and Belgium

Since the source waters and process configurations were different between the seven RSF locations, the initial OMP levels and the resulting OMP removal rates varied significantly (Table SI 6). Most OMPs (97 of 127 OMPs) were not detected in the influent water, the other 30 OMPs were detected in the influent waters and 8 of these OMPs showed a substantial removal (Fig. 2) in one of the full-scale RSFs (corresponding to 27% of the detected OMPs). Corresponding to these results from May 2018, similar OMP removal rates were detected in a second sampling round in October 2018 (Table SI 6). These findings confirm the results of other studies that demonstrated OMP removal in laboratory-scale RSF (Hedegaard and Albrechtsen, 2014; Shimabuku et al., 2019; Thomas L. Zearley and Summers, 2012). It should be noted that the results of the current study do not reflect the full OMP removal potential of the RSFs, since removal of OMPs that are not detected in the influent or only in very low concentrations cannot be assessed. The true OMP removal potential of RSFs might thus be higher.

In the influent of locations 1 and 6, 10 or less OMPs could be detected on average. A possible explanation is the source water of the drinking water treatment plant and the position of the RSF in the treatment plant configuration. The RSF of location 1 is preceded by river bank filtration, a process known to be able to degrade a wide variety of OMPs (Hamann et al., 2016). The RSF of location 6 is fed mainly with surface water from a protected area, for which the OMP concentration and the number of OMPs is expected to be lower than for most surface waters. The influents of locations 4, 5

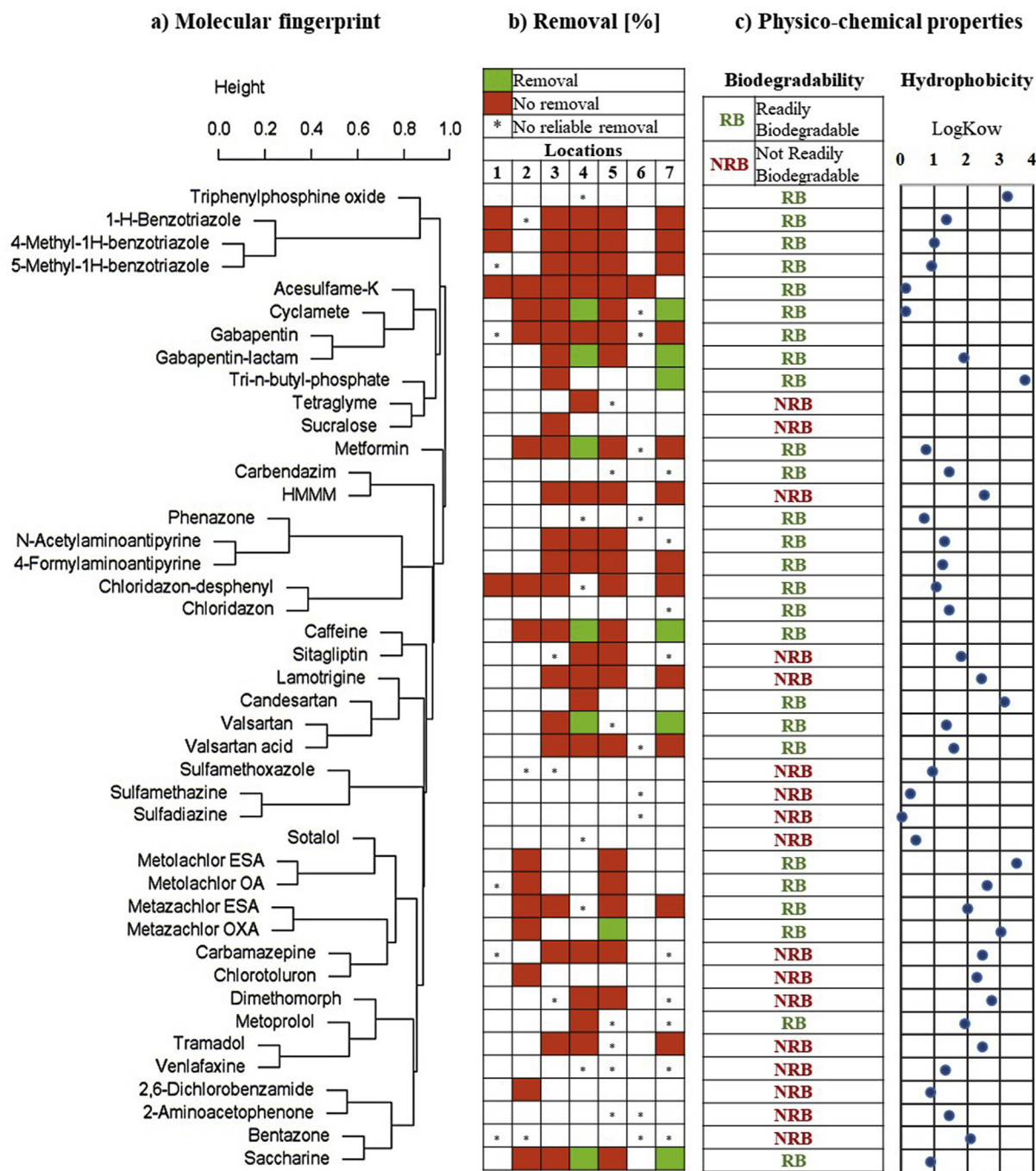


Fig. 2. OMPs detected in RSF through suspect screening. A) OMPs clustered based on molecular fingerprint; b) OMP removal in the different RSFs; c) OMP properties: and biodegradability (BIOWIN) and octanol-water partition coefficient (LogKow) (Chemistry Dashboard, OPERA model). Red: OMP not removed. Green: OMP removed (OMP removal considered to be substantial if (1) at influent OMP concentrations of <0.1 µg/L at least 50% of the OMP was removed, or 2) if, at influent OMP concentrations of >0.1 µg/L, at least 30% of the OMP was removed.) White with *: OMP detected in influent, but no reliable removal could be demonstrated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and 7 were characterized by the highest number of OMPs (29, 30 and 27 OMPs, respectively). This could be expected since these RSFs are located closest to the water intake and are only preceded by coagulation/sedimentation having a minor effect on OMP removal. In addition, RSFs 4 and 7 also removed most of the OMPs.

Two categories in OMP removal by RSFs are distinguished: 1) OMPs that are removed, and 2) OMPs that were not removed. In addition, it should be mentioned that some OMPs were detected in the influent but in concentrations close to the detection limit

(white boxes with * in Fig. 2). This means that a reliable removal could not be demonstrated, but it cannot be stated that the filter is not able to remove these OMPs.

Several OMPs belonged to the first category and were biologically degraded by the RSFs: metformin, cyclamate, tri-*n*-butyl-phosphate, gabapentin-lactam, saccharin, metazachlor-OXA, valsartan and caffeine. Cyclamate, gabapentin-lactam, saccharine, valsartan and caffeine show considerable removal in two RSF (location 4 and 7). This is in line with a number of studies that

demonstrated removal of these compounds in biological processes. Caffeine showed good removal in several lab-scale drinking water filter processes (Bogunovic et al., 2017; D'Alessio et al., 2015). Tran et al. (2015) demonstrated removal till below the detection level for saccharine and cyclamate after biological treatment (Modified Ludzack-Ettinger (MLE) process) followed by conventional sedimentation or MBR in a full-scale waste water treatment plant. Hermes et al. (2019) reported high removal (70–99%) of saccharine, gabapentin-lactam, valsartan and caffeine in a laboratory-scale sequential biofiltration system. This is in contradiction to Foolad et al. (2015) who reported limited removal (12–15%) of saccharine and cyclamate in laboratory-scale soil columns. These discrepancies are most likely caused by differences in the experiment set-up (e.g. water quality, filter media).

Other OMPs such as metformin, tri-*n*-butyl-phosphate and metazachlor-OXA showed considerable removal at one of the locations, but persistent behaviour at other locations. It is possible that only the microbial population in the filters at this specific location was capable of expressing the appropriate enzymes, but further research is required to confirm this hypothesis. While the biological removal of metformin in waste water treatment plants has been reported in several studies (Briones et al., 2018; Poursat et al., 2019; Zeng et al., 2015), studies investigating metformin removal in drinking water treatment plants are limited. Scheurer et al. (2012) demonstrated with laboratory-scale batch tests that, in contrast to chlorination, flocculation and activated carbon filtration (bacteria inactivated with sodium azide) were ineffective for metformin removal. By monitoring full-scale waterworks they showed that riverbank filtration and artificial recharge could completely remove metformin. This contradicts with other studies reporting effective metformin removal in batch experiments representative of GAC filtration in a drinking water treatment plant (Piai et al., 2020). The differences in metformin removal with activated carbon can be attributed to the use of sodium azide in the study of Scheurer et al. (2012) which created abiotic conditions in the filter.

Nakamura et al. (1991) reported some removal of tri-*n*-butyl-phosphate in a conventional waste water treatment plant and effective removal with powdered activated carbon, but no removal with dune infiltration or standard drinking water treatment techniques such as settling/coagulation/flocculation/rapid sand filtration (Sheldon and Hites, 1979).

To the best of the authors' knowledge this is the first study that demonstrates metformin and tri-*n*-butyl-phosphate removal in a full-scale RSF of a drinking water treatment plant.

A number of OMPs were placed in the second category: acesulfame-K, 1-*H*-benzotriazole, 4-methyl-1-*H*-benzotriazole, 5-methyl-1-*H*-benzotriazole, valsartan acid, metformin and HMMM. These OMPs were detected in the influent of four or more of the RSFs but did not show considerable removal in these filters. Acesulfame-K, 1-*H*-benzotriazole, 4-methyl-1-*H*-benzotriazole, 5-methyl-1-*H*-benzotriazole and HMMM did not show removal in any of the investigated filters. The persistence of acesulfame-K is in line with its use as anthropogenic marker (Kahl et al., 2018). This suggests that some OMPs cannot be biologically degraded in RSFs. However, there are also studies demonstrating acesulfame-K can be biodegraded (Castronovo et al., 2017; Hellauer et al., 2018; Kahl et al., 2018). This shows that OMPs that are not removed in a RSF (or in a biological process in general) are not necessarily persistent, but cannot be degraded under the prevailing conditions.

Besides variations in OMP removal capacity between different RSFs, there were also large differences in the type of OMPs removed within the RSFs. However, these differences could not be explained by the molecular structure of the OMP, expressed as extended molecular fingerprints. No clear trends could be observed between

removal and OMP structure, hydrophobicity and predicted biodegradability, see Fig. 2. This suggests that filter-specific conditions such as influent water quality, filter layout, operational conditions and microbial communities present in the filter lead to differences in OMP removal efficiencies.

4.2. Monitoring of changes in water quality in full-scale rapid sand filters in The Netherlands and Belgium based on NTS data

In total, 534 features were detected in the NTS data across all samples. For most RSFs, differences in features between influent and effluent samples were smaller than those between the production locations, as revealed by the PCA visualized in Fig. 3a. This is in agreement with the microbial data from the same samples (section 3.4). Overall, features were characterized by low signal intensities, confirming the low contamination level of the samples detected with the target analyses (Fig. 3b). Both the highest (feature area $\sim 1 \times 10^5$) and lowest feature intensities were detected at locations 1 and 6. The intensity of most features at these locations were below 100. Locations 4 and 5 showed many features with high signal intensities. At locations 1, 2 and 3, the distribution of the feature intensities between influent and effluent was not statistically different (p -value > 0.05 ; Student's t -test), suggesting that these RSFs do not significantly change the OMP concentrations. This is in line with the quantitative data. In contrast to locations 1, 2 and 3, locations 4 to 7 showed a significant difference between influent and effluent samples (p -value < 0.01). RSFs at locations 4, 5 and 7 are treating surface water that has not been extensively pre-treated. This water seems to contain OMPs that have a high biodegradation potential, as these are partially removed by the RSFs. In contrast, location 6 receives water from a protected area with low OMP concentrations, as illustrated in Fig. 2 for the quantified target OMPs. Correspondingly, the NTS feature intensities of this water were overall low, with most feature intensities ranging from 10^1 to 10^2 (Fig. 3b). However, while the concentrations of the target OMPs in the feed were too low to determine removal, the NTS data did reveal a significant decrease in feature intensities between influent and effluent water. This interesting finding stresses the importance of NTS for comprehensive monitoring of chemical water quality changes induced water treatment steps. An increase in the number of low intensity features in the effluent was also observed in locations 5 and 7, graphically represented by a downward shift of the effluent distribution (Fig. 3b). At all locations, the lowest intensities were found for hydrophobic features, i.e. at a RT of around 20 min (dark blue points). Interestingly, the most abundant intensities, i.e. the intensity range that most features exhibited in a given sample which differed by sample, were found at a RT of around 10 min (green points).

4.2.1. Detection of parent compounds and transformation products

Of all 534 detected NTS features, 90 exceeded an IS-eq conc. of 10 ng/L in at least one influent sample, and at the same time showed 10% removal during the treatment. These features were classified as PC (as showed in Fig. 4b). Using the same criteria, 14 of the 127 OMPs that were quantified with the suspect screening were defined as PCs.

444 and 63 TPs were predicted from the NTS and suspect screening PCs, respectively, using BioTransformer. 140 NTS features showed an increasing intensity from influent to effluent in at least one location. 15 of these matched the suspect list of predicted TPs based on accurate mass. Only one of the NTS features matched the TP suspect list predicted from the quantitative PCs, emphasizing the importance of NTS to monitor TPs.

The list of detected TP features is reported in Table 1. The first

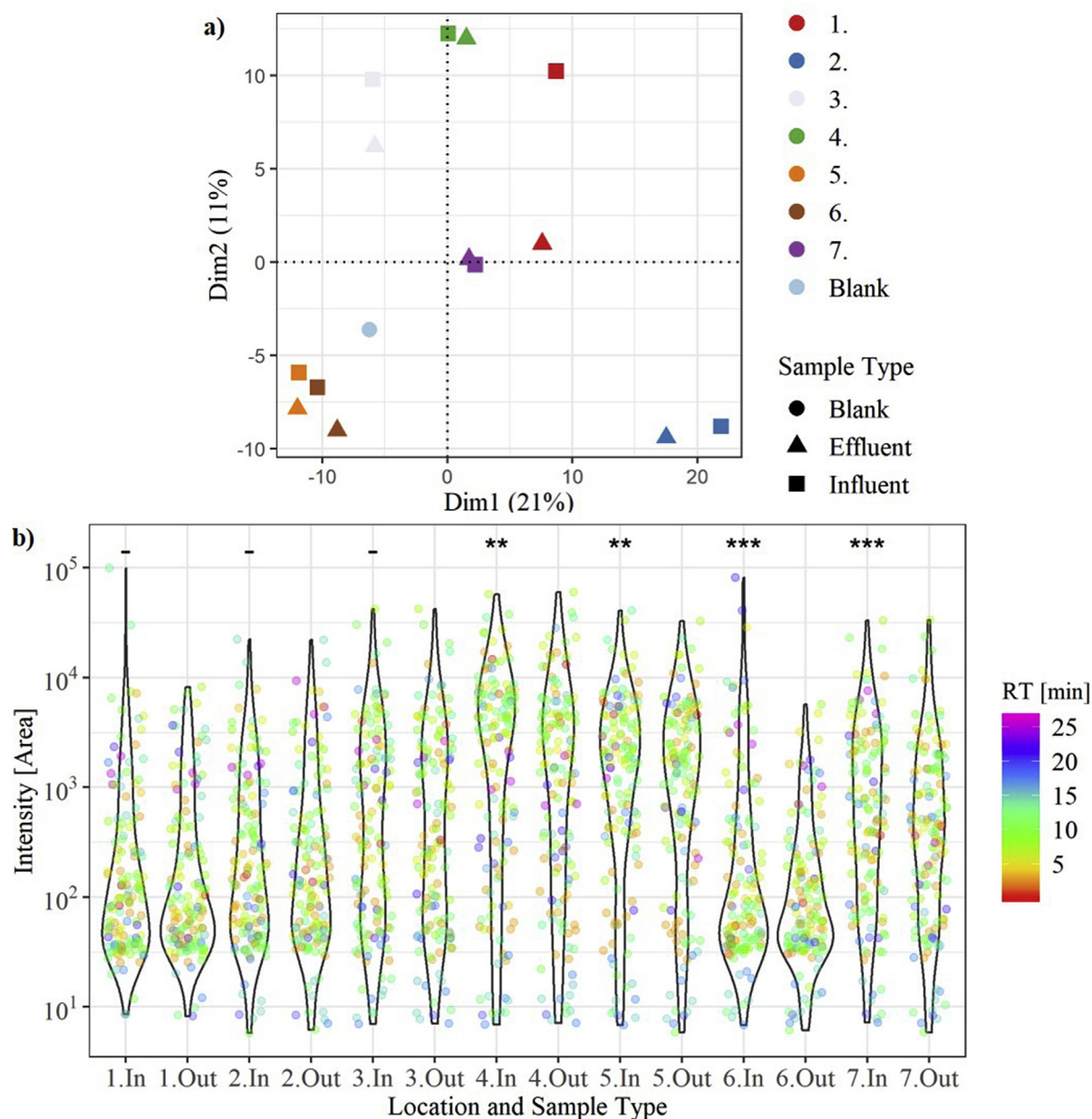


Fig. 3. a) Score plot of the two main PCA dimensions of the NTS features. Samples are colored according to their location and shaped according to the sample type; b) Distribution of feature intensities detected in influent and effluent samples of RSF per location, colored based on RT. The p-values between influent and effluent obtained with the Student's t-test are indicated by * for <0.05 significance and ** for <0.01 *** < 0.0001. — indicates no significant difference.

row of the table contains the feature which matched TP suspect lists from both target and non-target screening (feature ID: 267.18,335/9.2).

4.2.2. Structural elucidation of transformation products and their parent compounds

Through the identification step of the developed workflow, a CL, structure and name were assigned to both TPs and PCs. As highlighted in Table 1, ten out of fifteen features were identified with a confidence level range from 1 to 3, and then considered successfully identified: two features were identified with CL equal to 1, five with CL 2 and three with CL 3 (the other five features were identified with CL ≥ 4).

Eight of these features were known compounds; their structures were listed in one of the used databases, i.e. mzCloud, EAWAG

Biocatalysis/Biodegradation Database, EPA DSSTox, EPA Toxcast and/or PubChem. For the remaining two features, named 2372-82-9-BTM00001 and 120,013-45-8-BTM00001, the experimental spectra matched the *in silico* predicted structures proposed by BMPT (CL = 2). However, these compounds were missing from all databases and no information could be found in the literature. Details on the identification parameters for confidence level determination for TPs and PCs are provided in SI (Tables SI 4 and SI 5).

Five out of the ten identified TPs could be linked to their PCs. For the other five, the fragmentation spectrum of the TP feature achieved a better match with the spectral library or *in silico* predicted spectrum of another compound than that proposed by BMPT. For instance, feature 245.11631/7.09 was identified as N-Acetylaminopyrene with CL1, based on the comparison of its mass and

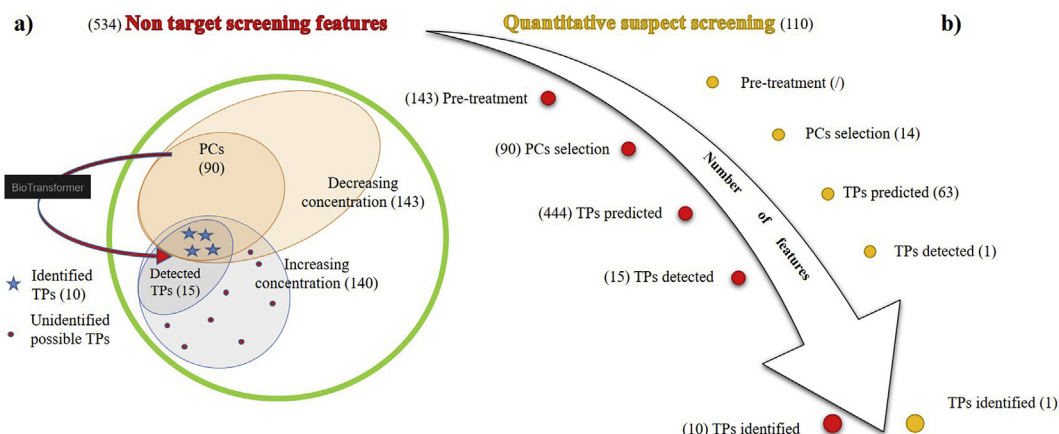


Fig. 4. a) Venn diagram: logical relations between the steps of the proposed workflow. b) Application of the proposed workflow to the experimental dataset obtained in wide-scope and quantitative suspect screening, reported in the left and right side of the figure respectively. The number of features or compounds selected in each step are given in brackets.

Table 1

List of the proposed elemental formulas and structures of the detected TP features derived from the prediction with Biotransformer, and their corresponding parent compounds (if detected). The asterisk indicates the feature was predicted from both quantitative suspect screening and NTS. CL = confidence level; RT = retention time; MW = molecular weight.

Transformation Products					Parent Compounds				
FeatureID (MW / RT)	Formula	CL	Structure	Proposed name	FeatureID (MW / RT)	Formula	CL	Structure	Proposed name
267.18335 / 9.2*	C ₁₅ H ₂₅ NO ₃	1		Metoprolol	/	/	/	/	/
245.11631 / 7.09	C ₁₃ H ₁₅ N ₃ O ₂	1		N-Acetylaminoantipyrine	/	/	/	/	/
298.29815 / 12.61	C ₁₈ H ₃₅ N ₂ O	2		2372-82-9-BTM00001	299.33003 / 12.619	C ₁₈ H ₄₁ N ₃	2		Laurylamine dipropylendiamine
139.09953 / 13.041	C ₈ H ₁₃ NO	2		Tropinone	141.11517 / 11.724	C ₈ H ₁₅ NO	2		Tropine
236.116 / 5.091	C ₁₂ H ₁₆ N ₂ O ₃	2		Phe-Ala	/	/	/	/	/
349.20439 / 17.402	C ₂₃ H ₂₇ NO ₂	2		120013-45-8-BTM00001	363.22002 / 18.529	C ₂₄ H ₂₉ NO ₂	2		Dehydrodeoxy donepezil
169.11015 / 8.457	C ₉ H ₁₅ NO ₂	2		(2S,3aR,7aS)-Octahydro-1H-indole-2-carboxylic acid3	/	/	/	/	/
267.147 / 6.96	C ₁₄ H ₂₁ NO ₄	3		Atenolol acid or Metoprolol acid	/	/	/	/	/
192.12621 / 5.094	C ₁₁ H ₁₆ N ₂ O	3		(S)-2-amino-N-ethyl-3-phenylpropionamide	236.116 / 5.091	C ₁₂ H ₁₆ N ₂ O ₃	2		Phe-Ala
141.11517 / 11.724	C ₈ H ₁₅ NO	3		Tropine	289.16775 / 10.108	C ₁₇ H ₂₃ NO ₃	2		Atropine
139.09953 / 7.441	C ₈ H ₁₃ NO	4		1-cyclopropyl-4-piperidone	/	/	/	/	/
662.19414 / 14.693	C ₃₄ H ₃₄ N ₂ O ₁₀ S	4	/	/	/	/	/	/	/
201.1729 / 10.52	C ₁₁ H ₂₃ NO ₂	4	/	/	/	/	/	/	/
187.15703 / 7.586	C ₁₀ H ₂₁ NO ₂	4	/	/	/	/	/	/	/
265.18892 / 10.44	C ₁₄ H ₂₁ NO ₄	4	/	/	/	/	/	/	/

retention time to those of a reference standard, and the high spectral similarity with the corresponding mzCloud entry. The assignment of structures of the three unknown metabolites predicted by Biotransformer, i.e. 14761-40-1-BTM00002, 4875-49-4-BTM00003, and 4875-49-4-BTM00002, to this feature was less

confident (CL $\geq$ 2). Consequently, no link between TP and PC could be established. Moreover, most of the TPs were also selected as PCs. While this may seem like a paradox, it could be due to the varied water sources and water pre-treatments applied resulting in a change of behaviour between the studied RSFs. Alternatively, this

could be related to the interaction of the (mixture of) contaminant and bacterial community.

Lastly, the spectral similarity between PC and respective TP was assessed for the five features that could be linked, expressed as a similarity score and visualized in a head-to-tail plot (Fig. SI 1). The pairs Dehydrodeoxy donepezil - 120,013-45-8-BTM00001, Phe-Ala - (S)-2-amino-N-ethyl-3-phenylpropionamide, and Laurylamine dipropylenediamine - 2372-82-9-BTM00001 showed the highest similarities, with scores of 0.89, 0.48, 0.26, respectively.

For the five features with $CL \geq 4$, the *in silico* predicted spectra based on the structure of the predicted TP and the experimental fragmentation spectra did not match. As the structure could not be confirmed, the structures of the predicted TPs were thus rejected, and only an elemental formula could be assigned to the features. Based on the feature intensity trend that is increasing from influent to effluent, these features are assumed to be TPs, however, the associated parent compounds remain unknown.

4.3. Evaluation of the proposed workflow

The feature intensity profiles between influent and effluent samples from full-scale RSFs show that for some features the intensity decreases and increases depending on the treatment facility, which causes an overlap between the PC and TP sets (Fig. 4a). The same compound can thus behave in opposite ways in different filters, as was for instance observed for metoprolol. PCs are expected to be transformed into TPs through a specific process, nevertheless this study showed that the same compound can behave as PC (decreasing intensity during the treatment) or TP (increasing intensity) depending on the RSF. Moreover, the feature intensities and the differences between influent and effluent were overall very low (IS-eq conc. below 0.35 $\mu\text{g/L}$) which complicated the identification of TPs and the respective transformation process.

In order to validate the developed workflow, we applied it to the well-studied biotic TP Gabapentin-lactam (Henning et al., 2018). Gabapentin-lactam is a derivative of the anti-convulsant agent Gabapentin which was also present in the PC list. In the NTS data, Gabapentin-lactam was defined as PC because it was detected in the influent sample of location 4 and was removed with 46%. In contrast, at locations 2 and 3 the intensity of Gabapentin-lactam increased in the effluent samples (31% and 19%, respectively). However, the compound was not identified as a TP with the developed workflow as it was not predicted by BMPT as a TP of Gabapentin. This example showed that while BioTransformer is a useful tool, as 10 of the 464 TPs it predicted could be identified in the RSF samples, it cannot be considered comprehensive. Consequently, the performance of the developed workflow is limited by the prediction capacity of BioTransformer; TPs that are not predicted cannot be identified. In Fig. 4a unidentified TPs are represented by red dots.

The data-driven workflow presented here, based on the combination of feature intensity profiles between influent and effluent samples and the prediction of biotic TPs, allowed to identify TPs in LC-HRMS based NTS data in full-scale drinking water treatment plants. The structures of 10 of the 15 TPs detected were elucidated with confidence levels ranging from 1 to 3. Among them, 8 TPs were identified as known compounds based on the match of several databases (mzCloud, ChemSpider, Norman network SusDat, PubChem); two of them were novel. The concentration of detected TPs was low, however, an (eco)toxicological assessment is necessary to determine whether these compounds present a risk at these contamination levels. While the developed approach was effective for TP identification in the seven full-scale drinking water treatments, it requires improvement to reduce the manual work for assigning confidence levels based on structural similarity. This is

particularly important when studies include more treatment trains, locations and/or samples with higher levels of contamination, such as samples from wastewater treatment. Moreover, BioTransformer proved to be useful, but not comprehensive for the prediction of possible TPs resulting from a list of PCs.

4.4. Characterization of microbial communities in rapid sand filters

The microbial communities of influent waters, filtration medium materials and effluent waters of all RSFs were analysed using 16S rRNA gene amplicon sequencing. Results showed that microbial communities of influent and effluent waters were different from the microbial communities in the sand and anthracite filter material at five of the seven locations (Fig. 5). The material of the filter medium selected for a different community composition, probably due to attachment and growth of other microorganisms than those that are dominant in the water phase. Location 1 showed a different microbial community compared to the other locations, possibly because the RSF is preceded by river bank filtration. In this step the RSF is fed with anoxic river bank filtrate. Location 1 also showed a similar microbial community on the filter material and in the influent and effluent of the RSF. This could indicate that the filter material of location 1, that consists of anthracite, is not selecting for a different microbial community due to lack of growth and attachment, as contrary to sand as carrier material.

The dominant OTUs of the microbial communities in most influent water samples from the RSFs were different from the filter media materials in the sand filters and consisted of OTUs that relate, amongst others, to *Actinobacteria* hgcl-clade, *Burkholderiaceae*, *Sporichthyaceae*, *Polynucleobacter*, *Flavobacterium*, "*Candidatus* Planktophila", *Sediminibacterium*. Bacterial species belonging to most of these groups have been detected in freshwater habitats (Jezbera et al., 2011, 2009; Kang et al., 2014; Llió et al., 2014; Newton et al., 2011), which explains their presence in the intake surface waters of the sampled locations. The dominant microbial community on the sand and anthracite materials contained some of the OTUs that were present in the influent, but also OTUs that seemed specific for the media materials, especially for sand as carrier material. These microorganisms were in most cases also present in the effluent of the RSFs, which implied that they were growing on the carrier material and continuously flushed out of the RSF with the water. This has also been observed in other studies on the microbial communities of rapid sand filters (Lautenschlager et al., 2014; Oh et al., 2018; Pinto et al., 2012). The dominant OTUs on these filter media materials entailed bacterial groups that were probably performing nitrification processes such as *Nitrospira* (ammonia/nitrite oxidizer), *Gemmata* (anaerobic ammonium oxidizer; anammox) and other *Planctomycetacia* (class that contains species that perform anammox) such as *Pirellula*. The aerobic ammonium oxidizer *Nitrosomonas*, was only present in the RSFs at locations 1 and 6, suggesting that at most other locations the *Nitrospira* performed complete nitrification alone and belonged to the Comammox bacteria (Daims et al., 2015). This was also found in another study on RSF microbial communities where *Nitrospira* were more overrepresented than *Nitrosomonas* and *Nitrobacter* (Oh et al., 2018). Previous studies have shown that these Comammox bacteria can be present in RSFs that are used in drinking water treatment of groundwater (Fowler et al., 2018; Tatari et al., 2017). The presence of the anaerobic anammox bacteria seems surprising in fully oxygenated RSFs, but anaerobic microorganisms have been found more often in these systems, suggesting the presence of anoxic microniches (Gülay et al., 2016). For most of the other groups that were dominantly present in the media material of the RSFs, little is known on their occurrence and metabolism. The limited information available suggests that the abundance of *Acidobacteria*

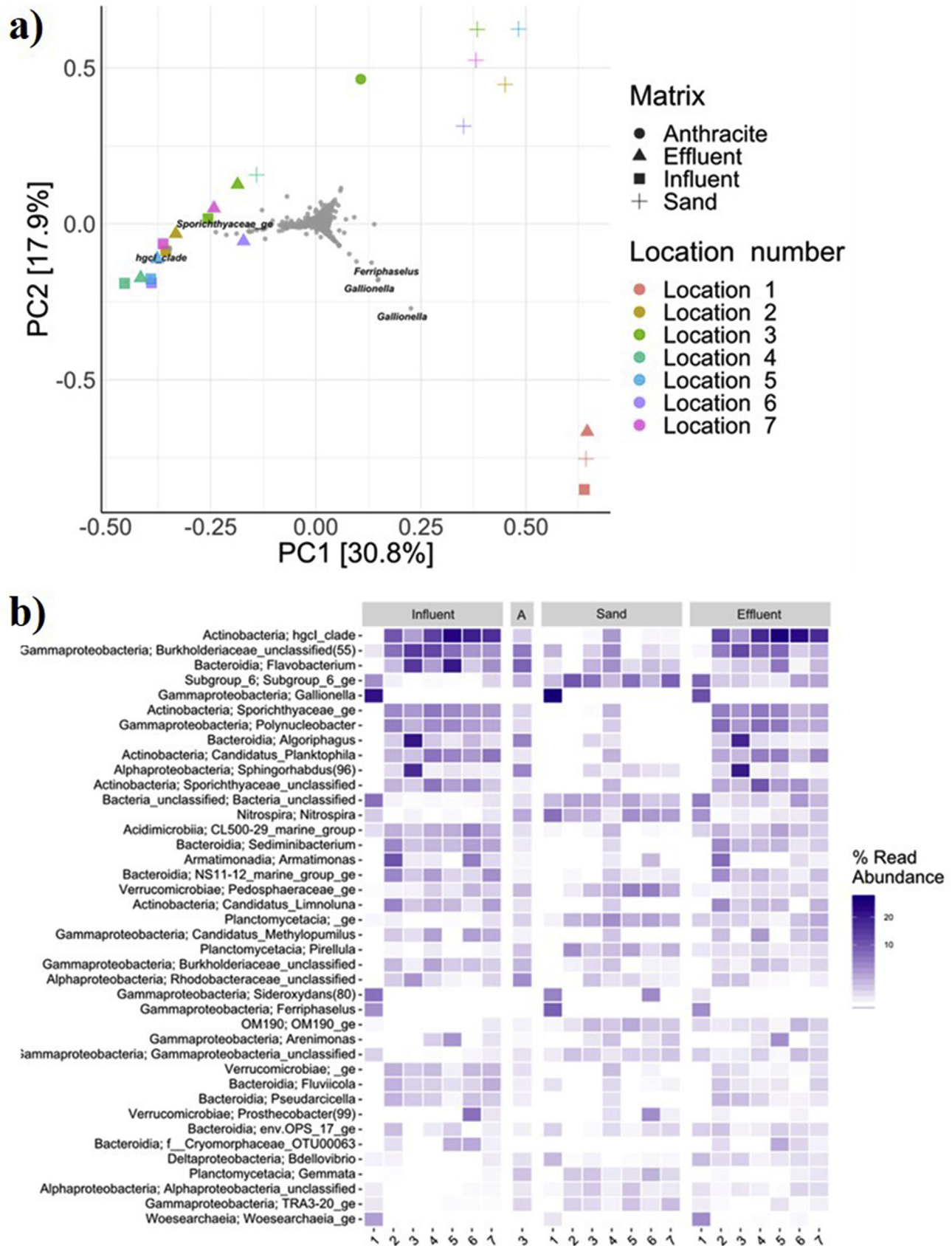


Fig. 5. a) Principal component analysis (PCA) that shows Bray-Curtis distance ordination of 16S rRNA gene amplicon sequencing data of all samples of RSF material (sand or anthracite) and influent and effluent waters. The PCA was constructed after removing low abundance OTUs (relative abundance <0.01% in any sample) and Hellinger transformation on the absolute abundances. Data point shapes represent matrix type (sand, anthracite, effluent, influent) and colours represent the location number (see Table SI 1). b) Heatmap of 16S rRNA gene amplicon sequencing data from samples of all locations of the RSFs. Shown are the average relative abundances (%) of the top 40 most abundant OTUs in influent, anthracite (A), sand, and effluent samples from the different locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Subgroup 6 appears to be correlated to changes in pH, nutrient and ion concentrations (Kielak et al., 2016). Furthermore, some members of the *Planctomycetes* clade OM190 seem to be distantly related to anammox bacteria and were found to be correlated to dissolved organic carbon (DOC), leading to the speculation that these are major contributors to autotrophic dissolved organic carbon production (Ye et al., 2016) and are possibly associated to macroalgae (Lage and Bondoso, 2014). However, OTUs observed in our study might belong to other bacterial species within these groups and could behave differently than observed in these studies. The media material of the RSF of location 1 did not contain the specific microorganisms that were present in the media materials of other RSFs. *Gallionella*, *Sideroxydans* and *Ferriphaselus* were dominantly present in the samples from location 1. The dominance of these genera was the main difference to all other locations (Fig. 5). These three genera consist of species mostly involved in iron oxidation. The presence of these genera in the RSF at location 1 is not surprising, since these filters are fed with anoxic river bank filtrate which is rich in Fe(II).

Overall, the microbial community composition on sand and anthracite in RSFs differed in most cases from the community composition observed in influent waters, indicating that distinct clades of microorganisms grow on the sand and/or anthracite grains of RSFs. Bacteria were identified that are known to be involved in iron oxidation or nitrification. However, the metabolism of most of the other microorganisms identified in the RSFs remains unknown.

OMP removal efficiencies (Fig. 2) and formation of transformation products (Fig. 3) did not seem to be related to the differences in microbial community composition of the locations (Fig. 5a). This could imply that OMP removal occurred via co-metabolism of the dominant microorganisms present in all RSFs or by members of the microbial communities that were present in low relative abundance. Since OMP concentrations in surface water are generally much lower (ng - µg/L) than the concentration of organic matter (mg/L), several studies suggested co-metabolic degradation of OMPs to be more likely than metabolic degradation (Alidina et al., 2014a; Maeng et al., 2011a; Rauch-Williams et al., 2010). Some studies demonstrated that ammonia oxidizing bacteria (AOB) were involved in co-metabolic degradation of OMPs in wastewater treatment processes (e.g. nitrifying activated sludge, membrane bioreactor, granular activated carbon) (Men et al., 2017; Park et al., 2017; Rattier et al., 2014; Xu et al., 2016). The presence of AOB in RSFs of the current study could indicate their involvement in OMP biodegradation in RSFs. Alternatively, OMP removal by physico-chemical processes such as adsorption to the filter sand or NOM present in the surface water (Delgado-Moreno et al., 2010) cannot be excluded. Future research in controlled laboratory-scale experiments is required to test the aforementioned hypotheses.

Due to the large number of variables in the full-scale RSFs such as influent water quality and process conditions, and the varying numbers and concentrations of OMPs present in the influents, it was not feasible to identify bacterial genera that could be correlated to (specific) OMP removal. Future research with controlled laboratory-scale experiments is required to reveal the microorganisms involved in OMP degradation. Once the responsible micro-organism(s) is/are identified, their presence in full-scale RSFs can be investigated to confirm the results obtained with the laboratory-scale experiments.

4.4.1. Correlating microbial communities, OMPs and TPs

Information on biological degradation of OMPs, in particular on the involved microorganisms in RSFs is scarce. This is largely due to the fact that the potential of RSFs to remove OMPs has only been recognized in recent years. This study indicates that RSFs at some

drinking water treatment plants treating surface water are capable of removing a number of OMPs to a significant extent (location 7 and 4), while RSFs at other treatment plants show no removal (location 1, 2, 3 and 6). OMP removal in RSFs is thus specific for each drinking water treatment plant. As the microbial populations of the RSFs are comparable on genus level, the removal seems to be correlated to, amongst others, the amount and type of OMPs present in the feed water. Correspondingly, the NTS data reflected this finding, with the same compounds behaving as TPs or as PCs according to the location.

OTUs correlating with feature decrease or increase in RSF locations are possible candidates involved in OMP removal through transformation processes. To further the understanding of biological OMP degradation in RSFs, correlations between the abundance of individual OTUs and decrease or increase of individual features were analysed.

The correlation analysis between the OTUs and features resulted in six correlating validated features and OTUs (Fig. SI 3). There were negative correlations, implying the OTU in the pair is involved in degrading the feature in the pair, and one positive correlation, implying the OTU is involved in the formation of the feature as a transformation product. Negative correlations were found between 169.11015/8.457 with OTU00391 (uncultured *Microscillaceae*), between 169.11015/8.457 with OTU00172 (*Planctomycetes* clade OM190), between 267.147/6.96 and 398.24355/21.503 with OTU00723 (*Pedospaeraceae*), and between 662.19414/14.693 with OTU00129 (*Acidobacteria* Subgroup 17). A positive correlation was found between 255.00778/9.113 with OTU00341 (Phylum "*Candidatus* *Eremiobacterota*" WPS-2). The five OTUs related to these pairs of interest could not be classified further than the phylum or family level. Literature on these clades is scarce, especially concerning their role in OMP removal. The paired OTUs were most abundant in the filter material of the RSFs (Fig. SI 2), and less abundant in the influent. When the correlation between the feature and the OTU is an indication that these OTUs are involved in degradation of OMPs, the degradation mostly occurs within the biofilm that is attached to the media material, and not by bacteria present in the water. OTU00129 with 662.19414/14.693 was of specific interest, because the OTU in this specific pair increases with increasing influent concentration of the feature and feature intensity declines more between influent and effluent with high abundance of this OTU. This makes OTU00129 a likely candidate that is involved in degradation of this feature and is, thus, worthwhile pursuing in isolation studies. OTU00129 belongs to *Acidobacteria* Subgroup 17. Little is known about this clade of *Acidobacteria*; it occurs in many different types of pasture soils and is negatively correlated to soil acidity (Navarrete et al., 2015). In addition, it showed negative correlations with many carbon degrading gene families in a microarray study (Chaves et al., 2019).

The little information available on the metabolism and ecology of the detected microorganisms rendered it difficult to assess the validity of OTU-feature pairs. The OTUs that correlated with an increase or decrease of a feature in RSF locations can be considered candidate pairs, and their relationship will have to be confirmed in controlled experiments. Afterwards, these microorganisms could be specifically enriched during controlled cultivation in order to test their capacity to degrade certain OMPs. Together these methods would then not only provide more insight into RSF based OMP removal but could also be used as starting points for bio-augmentation/bio-stimulation applications in drinking water treatment.

5. Conclusions

Here, we combined data from quantitative suspect screening,

NTS and NGS analyses from seven full-scale RSFs in a proof-of-principle study to show that we can: (1) assess OMP removal capacity, (2) identify TPs, (3) provide insight in differences/similarities between microbial community composition in different RSFs, and (4) correlate TP formation to specific OTUs with this combination of methods. The approach proved successful and should be seen as an important first step towards a better understanding of OMP removal in full-scale drinking water treatment filters, specifically RSFs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the Joint Research Program of the Dutch and Belgian drinking water companies. The authors would like to thank the Dutch and Belgian drinking water companies, in particular Luk van Lokeren, Ruud Kolpa, René van der Aa and Sander Nugteren for critical reading, and the KWR Laboratory of Microbiology and the KWR Chemical Laboratory for their contribution.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.127630>.

References

- Abu Hasan, H., Muhammad, M.H., Ismail, N.I., 2020. A review of biological drinking water treatment technologies for contaminants removal from polluted water resources. *J. Water Process Eng.* 33, 101035. <https://doi.org/10.1016/j.jwpe.2019.101035>.
- Alidina, M., Li, D., 2014. Investigating the role for adaptation of the microbial community to transform trace organic chemicals during managed aquifer recharge. *Water Res.* 6, 172–180. <https://doi.org/10.1016/j.watres.2014.02.046>.
- Alidina, M., Li, D., Drewes, J.E., 2014a. Investigating the role for adaptation of the microbial community to transform trace organic chemicals during managed aquifer recharge. *Water Res.* 56, 172–180. <https://doi.org/10.1016/j.watres.2014.02.046>.
- Alidina, M., Li, D., Ouf, M., Drewes, J.E., 2014b. Role of primary substrate composition and concentration on attenuation of trace organic chemicals in managed aquifer recharge systems. *J. Environ. Manag.* 144, 58–66. <https://doi.org/10.1016/j.jenvman.2014.04.032>.
- Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R Package to Analyse and Visualise 16S rRNA Amplicon Data. <https://doi.org/10.1101/299537> bioRxiv (preprint) 299537.
- Benner, J., Helbling, D.E., Kohler, H.P.E., Wittebol, J., Kaiser, E., Prasse, C., Ternes, T.A., Albers, C.N., Aamand, J., Horemans, B., Springael, D., Walravens, E., Boon, N., 2013. Is biological treatment a viable alternative for micropollutant removal in drinking water treatment processes? *Water Res.* 47, 5955–5976. <https://doi.org/10.1016/j.watres.2013.07.015>.
- Bertelkamp, C., Hofman-Caris, R., Siegers, W.G., Beerendonk, E.F., Nugteren, S., 2017. Biodegradation of pyrazole in rapid sand filters. *Conf. Proc. 10th Micropol Ecotax Conf. IWA* 17–20.
- Bertelkamp, C., Reungoat, J., Cornelissen, E.R., Singhal, N., Reynisson, J., Cabo, A.J., van der Hoek, J.P., Verliefe, A.R.D., 2014. Sorption and biodegradation of organic micropollutants during river bank filtration: a laboratory column study. *Water Res.* 52, 231–241. <https://doi.org/10.1016/j.watres.2013.10.068>.
- Bletsou, A.A., Jeon, J., Hollender, J., Archontaki, E., Thomaidis, N.S., 2015. Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. *TrAC Trends Anal. Chem. (Reference Ed.)* 66, 32–44. <https://doi.org/10.1016/j.trac.2014.11.009>.
- Bogunovic, M., Knezevic, V., Simeunovic, J., Teodorovic, I., Ivancev-Tumbas, I., 2017. Biodegradation of a mixture of benzophenone, benzophenone-3, caffeine and carbamazepine in a laboratory test filter. *J. Serb. Chem. Soc.* 82, 1445–1459. <https://doi.org/10.2298/JSC170717105B>.
- Briones, R.M., Zhuang, W.Q., Sarmah, A.K., 2018. Biodegradation of metformin and guanyurea by aerobic cultures enriched from sludge. *Environ. Pollut.* 243, 255–262. <https://doi.org/10.1016/j.envpol.2018.08.075>.
- Brunner, A.M., Bertelkamp, C., Dingemans, M.M.L., Kolkman, A., Wols, B., Harmsen, D., Siegers, W., Martijn, B.J., Oorthuizen, W.A., ter Laak, T.L., 2020. Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment. *Sci. Total Environ.* 705, 135779. <https://doi.org/10.1016/j.scitotenv.2019.135779>.
- Brunner, A.M., Vughs, D., Siegers, W., Bertelkamp, C., Hofman-Caris, R., Kolkman, A., ter Laak, T., 2019. Monitoring transformation product formation in the drinking water treatments rapid sand filtration and ozonation. *Chemosphere* 214, 801–811. <https://doi.org/10.1016/j.chemosphere.2018.09.140>.
- Cakmakci, M., Koyuncu, I., Kinaci, C., 2008. Effects of iron concentrations, filter hydraulic loading rates, and porosities on iron removal by rapid sand filtration. *Environ. Eng. Sci.* 25, 669–676. <https://doi.org/10.1089/ees.2007.0060>.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez-Pena, A., Goodrich, J.K., Gordon, J.L., Huttley, G.A., Scott, T.K., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, A.C., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 1–12. <https://doi.org/10.1038/nmeth.f.303.QIIME>.
- CastroNovo, S., Wick, A., Scheurer, M., Nödler, K., Schulz, M., Ternes, T.A., 2017. Biodegradation of the artificial sweetener acesulfame in biological wastewater treatment and sandfilters. *Water Res.* 110, 342–353. <https://doi.org/10.1016/j.watres.2016.11.041>.
- Chaves, M.G., Silva, G.G., Rossetto, R., Edwards, R.A., Tsai, S.M., Navarrete, A.A., 2019. Acidobacteria subgroups and their metabolic potential for carbon degradation in sugarcane soil amended with vinasse and nitrogen fertilizers. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.01680>.
- Clasen, J., 1998. Efficiency control of particle removal by rapid sand filters in treatment plants fed with reservoir water: a survey of different methods. *Water Sci. Technol.* 37, 19–26. [https://doi.org/10.1016/S0273-1223\(98\)00005-5](https://doi.org/10.1016/S0273-1223(98)00005-5).
- D'Alessio, M., Yoneyama, B., Kirs, M., Kisan, V., Ray, C., 2015. Pharmaceutically active compounds: their removal during slow sand filtration and their impact on slow sand filtration bacterial removal. *Sci. Total Environ.* 524 (525), 124–135. <https://doi.org/10.1016/j.scitotenv.2015.04.014>.
- Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by a Nitrospira bacteria. *Nature* 528, 504–509. <https://doi.org/10.1038/nature16459.Complete>.
- de Jongh, C.M., Kooij, P.J.F., de Voogt, P., ter Laak, T.L., 2012. Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. *Sci. Total Environ.* 427, 70–77. <https://doi.org/10.1016/j.scitotenv.2012.04.010>. –428.
- Delgado-Moreno, L., Wu, L., Gan, J., 2010. Effect of dissolved organic carbon on sorption of pyrethroids to sediments. *Environ. Sci. Technol.* 44, 8473–8478. <https://doi.org/10.1021/es102277h>.
- Djombou-Feunang, Y., Fiamoncini, J., Gil-de-la-Fuente, A., Greiner, R., Manach, C., Wishart, D.S., 2019. BioTransformer: a comprehensive computational tool for small molecule metabolism prediction and metabolite identification. *J. Cheminf.* 11, 1–25. <https://doi.org/10.1186/s13321-018-0324-5>.
- Ellis, L.B.M., Gao, J., Fenner, K., Wackett, L.P., 2008. The University of Minnesota pathway prediction system: predicting metabolic logic. *Nucleic Acids Res.* 36, 427–432. <https://doi.org/10.1093/nar/gkn315>.
- European Commission, 2003. Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, vol. 337. <https://doi.org/10.1002/mp.12308>. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the Europ. Off. Publ. Eur. Communities, Luxemb.
- Foolad, M., Ong, S.L., Hu, J., 2015. Transport of sewage molecular markers through saturated soil column and effect of easily biodegradable primary substrate on their removal. *Chemosphere* 138, 553–559. <https://doi.org/10.1016/j.chemosphere.2015.07.008>.
- Fowler, S.J., Palomo, A., Dechesne, A., Mines, P.D., Smets, B.F., 2018. Comammox Nitrospira are abundant ammonia oxidizers in diverse groundwater-fed rapid sand filter communities. *Environ. Microbiol.* 20, 1002–1015. <https://doi.org/10.1111/1462-2920.14033>.
- Gude, J.C.J., Joris, K., Huysman, K., Rietveld, L.C., van Halem, D., 2018. Effect of supernatant water level on as removal in biological rapid sand filters. *Water Res.* X 1, 100013. <https://doi.org/10.1016/j.wroa.2018.100013>.
- Gülai, A., Musovic, S., Albrechtsen, H.J., Al-Soud, W.A., Sørensen, S.J., Smets, B.F., 2016. Ecological patterns, diversity and core taxa of microbial communities in groundwater-fed rapid gravity filters. *ISME J.* 10, 2209–2222. <https://doi.org/10.1038/ismej.2016.16>.
- Hamann, E., Stuyfzand, P.J., Greskowiak, J., Timmer, H., Massmann, G., 2016. The fate of organic micropollutants during long-term/long-distance river bank filtration. *Sci. Total Environ.* 545 (546), 629–640. <https://doi.org/10.1016/j.scitotenv.2015.12.057>.
- Hedegaard, M.J., Albrechtsen, H.J., 2014. Microbial pesticide removal in rapid sand filters for drinking water treatment - potential and kinetics. *Water Res.* 48, 71–81. <https://doi.org/10.1016/j.watres.2013.09.024>.
- Hellauer, K., Karakurt, S., Sperlich, A., Burke, V., Massmann, G., Hübner, U., Drewes, J.E., 2018. Establishing sequential managed aquifer recharge technology (SMART) for enhanced removal of trace organic chemicals: experiences from field studies in Berlin, Germany. *J. Hydrol.* 563, 1161–1168. <https://doi.org/10.1016/j.jhydrol.2017.09.044>.

- Henning, N., Kunkel, U., Wick, A., Ternes, T.A., 2018. Biotransformation of gabapentin in surface water matrices under different redox conditions and the occurrence of one major TP in the aquatic environment. *Water Res.* 137, 290–300. <https://doi.org/10.1016/j.watres.2018.01.027>.
- Hermes, N., Jewell, K.S., Schulz, M., Müller, J., Hübner, U., Wick, A., Drewes, J.E., Ternes, T.A., 2019. Elucidation of removal processes in sequential biofiltration (SBF) and soil aquifer treatment (SAT) by analysis of a broad range of trace organic chemicals (TOCs) and their transformation products (TPs). *Water Res.* <https://doi.org/10.1016/j.watres.2019.114857>, 114857.
- Hollender, J., Schymanski, E.L., Singer, H.P., Ferguson, P.L., 2017. Nontarget screening with high resolution mass spectrometry in the environment: ready to go? *Environ. Sci. Technol.* 51, 11505–11512. <https://doi.org/10.1021/acs.est.7b02184>.
- Hübner, U., von Gunten, U., Jekel, M., 2015. Evaluation of the persistence of transformation products from ozonation of trace organic compounds – a critical review. *Water Res.* 68, 150–170. <https://doi.org/10.1016/j.watres.2014.09.051>.
- Jabeen, H., Iqbal, S., Anwar, S., 2015. Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a novel rhizobial strain *Mesorhizobium* sp. HN3. *Water Environ. J.* 29, 151–160. <https://doi.org/10.1111/wej.12081>.
- Jezbera, J., Jezberová, J., Brandt, U., Hahn, M.W., 2011. Ubiquity of Polynucleobacter necessarius subspecies asymbioticus results from ecological diversification. *Environ. Microbiol.* 13, 922–931. <https://doi.org/10.1111/j.1462-2920.2010.02396.x>.
- Jezbera, J., Sharma, A.K., Brandt, U., Doolittle, W.F., Hahn, M.W., 2009. “Candidatus Planktophila limnetica”, an actinobacterium representing one of the most numerically important taxa in freshwater bacterioplankton. *Int. J. Syst. Evol. Microbiol.* 59, 2864–2869. <https://doi.org/10.1099/ijs.0.010199-0>.
- Kahl, S., Kleinstaub, S., Nivala, J., Van Aerden, M., Reemtsma, T., 2018. Emerging biodegradation of the previously persistent artificial sweetener acesulfame in biological wastewater treatment. *Environ. Sci. Technol.* 52, 2717–2725. <https://doi.org/10.1021/acs.est.7b05619>.
- Kaiser, E., Prasse, C., Wagner, M., Bröder, K., Ternes, T.A., 2014. Transformation of oxcarbazepine and human metabolites of carbamazepine and oxcarbazepine in wastewater treatment and sand filters. *Environ. Sci. Technol.* 48, 10208–10216. <https://doi.org/10.1021/es5024493>.
- Kang, H., Kim, H., Lee, B. Il, Joung, Y., Joh, K., 2014. Sediment bacterium goheungense sp. nov., isolated from a freshwater reservoir. *Int. J. Syst. Evol. Microbiol.* 64, 1328–1333. <https://doi.org/10.1099/ijs.0.055137-0>.
- Keum, Y.S., Seo, J.S., Hu, Y., Li, Q.X., 2006. Degradation pathways of phenanthrene by *Sinorhizobium* sp. C4. *Appl. Microbiol. Biotechnol.* 71, 935–941. <https://doi.org/10.1007/s00253-005-0219-z>.
- Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. *Front. Microbiol.* 7, 1–16. <https://doi.org/10.3389/fmicb.2016.00744>.
- Kumar, R., Singh, B., Gupta, V.K., 2012. Biodegradation of fipronil by paracoccus sp. in different types of soil. *Bull. Environ. Contam. Toxicol.* 88, 781–787. <https://doi.org/10.1007/s00128-012-0578-y>.
- Lage, O.M., Bondoso, J., 2014. Planctomycetes and macroalgae, a striking association. *Front. Microbiol.* 5, 1–9. <https://doi.org/10.3389/fmicb.2014.00267>.
- Lautenschlager, K., Hwang, C., Ling, F., Egli, T., Liu, W., Boon, N., Oliver, K., Hammes, F., 2014. Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. *Water Res.* 2, 40–52. <https://doi.org/10.1016/j.watres.2014.05.035>.
- Lé, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. *J. Stat. Software* 25, 1–18. <https://doi.org/10.18637/jss.v025.i01>.
- Li, Z., Kaserzon, S.L., Plassmann, M.M., Sobek, A., Gómez Ramos, M.J., Radke, M., 2017. A strategic screening approach to identify transformation products of organic micropollutants formed in natural waters. *Environ. Sci. Process. Impacts* 19, 488–498. <https://doi.org/10.1039/c6em00635c>.
- Little, J.L., Williams, A.J., Shchenichov, A., Tkachenko, V., 2012. Identification of “known unknowns” utilizing accurate mass data and chemspider. *J. Am. Soc. Mass Spectrom.* 23, 179–185. <https://doi.org/10.1007/s13361-011-0265-y>.
- Llirós, M., Inceoglu, Ö., García-Armisen, T., Anzil, A., Leporcq, B., Pigneur, L.M., Viroux, L., Darchambeau, F., Descy, J.P., Servais, P., 2014. Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. *PLoS One* 9, 1–27. <https://doi.org/10.1371/journal.pone.0116145>.
- Maeng, S.K., Sharma, S.K., Abel, C.D.T., Magic-Knezev, A., Amy, G.L., 2011a. Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: batch and column studies. *Water Res.* 45, 4722–4736. <https://doi.org/10.1016/j.watres.2011.05.043>.
- Maeng, S.K., Sharma, S.K., Lekkerkerker-Teunissen, K., Amy, G.L., 2011b. Occurrence and fate of bulk organic matter and pharmaceutically active compounds in managed aquifer recharge: a review. *Water Res.* 45, 3015–3033. <https://doi.org/10.1016/j.watres.2011.02.017>.
- Magic-Knezev, A., van der Koij, D., 2004. Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment. *Water Res.* 38, 3971–3979. <https://doi.org/10.1016/j.watres.2004.06.017>.
- Mandal, K., Singh, B., Jariyal, M., Gupta, V.K., 2013. Microbial degradation of fipronil by *Bacillus thuringiensis*. *Ecotoxicol. Environ. Saf.* 93, 87–92. <https://doi.org/10.1016/j.ecoenv.2013.04.001>.
- Margot, J., Lochmatter, S., Barry, D.A., Holliger, C., 2016. Role of ammonia-oxidizing bacteria in micropollutant removal from wastewater with aerobic granular sludge. *Water Sci. Technol.* 73, 564–575. <https://doi.org/10.2166/wst.2015.514>.
- Men, Y., Achermann, S., Helbling, D.E., Johnson, D.R., Fenner, K., 2017. Relative contribution of ammonia oxidizing bacteria and other members of nitrifying activated sludge communities to micropollutant biotransformation. *Water Res.* 109, 217–226. <https://doi.org/10.1016/j.watres.2016.11.048>.
- Nakamura, A., 1991. Tri-n-butyl phosphate. *Environ. Health Crit.* 112, 1–80. WHO, Geneva.
- Navarrete, A.A., Venturini, A.M., Meyer, K.M., Klein, A.M., Tiedje, J.M., Brendan, B.J., Nüsslein, K., Tsai, S.M., Rodrigues, J.L.M., 2015. Differential response of Acidobacteria subgroups to forest-to-pasture conversion and their biogeographic patterns in the western Brazilian Amazon. *Front. Microbiol.* 6, 1–10. <https://doi.org/10.3389/fmicb.2015.01443>.
- Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. Rev.* 75, 14–49. <https://doi.org/10.1128/MMBR.00028-10>.
- Ning, J., Bai, Z., Gang, G., Jiang, D., Hu, Q., He, J., Zhang, H., Zhuang, G., 2010. Functional assembly of bacterial communities with activity for the biodegradation of an organophosphorus pesticide in the rape phyllosphere. *FEMS Microbiol. Lett.* 306, 135–143. <https://doi.org/10.1111/j.1574-6968.2010.01946.x>.
- Oh, S., Hammes, F., Liu, W.-T., 2018. Metagenomic characterization of biofilter microbial communities in a full-scale drinking water treatment plant. *Water Res.* 128, 278–285. <https://doi.org/10.1016/j.watres.2017.10.054>.
- Ohtsubo, Y., Goto, H., Nagata, Y., Kudo, T., Tsuda, M., 2006. Identification of a response regulator gene for catabolite control from a PCB-degrading β -proteobacteria. *Acidovorax* sp. KKS102. *Mol. Microbiol.* 60, 1563–1575. <https://doi.org/10.1111/j.1365-2958.2006.05197.x>.
- Park, J., Yamashita, N., Wu, G., Tanaka, H., 2017. Removal of pharmaceuticals and personal care products by ammonia oxidizing bacteria acclimated in a membrane bioreactor: contributions of cometabolism and endogenous respiration. *Sci. Total Environ.* 605–606, 18–25. <https://doi.org/10.1016/j.scitotenv.2017.06.155>.
- Piai, L., Blokland, M., van der Wal, A., Langenhoff, A., 2020. Biodegradation and adsorption of micropollutants by biological activated carbon from a drinking water production plant. *J. Hazard Mater.* 388 <https://doi.org/10.1016/j.jhazmat.2020.122028>.
- Pinto, A.J., Xi, C., Raskin, L., 2012. Bacterial community structure in the drinking water microbiome is governed by filtration processes. *Environ. Sci. Technol.* 46, 8851–8859. <https://doi.org/10.1021/es302042t>.
- Poonthiripun, S., Pattaragulwanit, K., Paengthai, S., Kriangkripiat, T., Juntongjin, K., Thaniyavarn, S., Petsom, A., Pinphanichakarn, P., 2006. Novel intermediates of acenaphthylene degradation by *Rhizobium* sp. strain CU-A1: evidence for naphthalene-1,8-dicarboxylic acid metabolism. *Appl. Environ. Microbiol.* 72, 6034–6039. <https://doi.org/10.1128/AEM.00897-06>.
- Postigo, C., Richardson, S.D., 2014. Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment. *J. Hazard Mater.* 279, 461–475. <https://doi.org/10.1016/j.jhazmat.2014.07.029>.
- Poursat, B.A.J., van Spanning, R.J.M., Braster, M., Helmus, R., de Voigt, P., Parsons, J.R., 2019. Biodegradation of metformin and its transformation product, guanyurea, by natural and exposed microbial communities. *Ecotoxicol. Environ. Saf.* 182, 109414. <https://doi.org/10.1016/j.ecoenv.2019.109414>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, 590–596. <https://doi.org/10.1093/nar/gks1219>.
- Rattier, M., Reungoat, J., Keller, J., Gernjak, W., 2014. Removal of micropollutants during tertiary wastewater treatment by biofiltration: role of nitrifiers and removal mechanisms. *Water Res.* 54, 89–99. <https://doi.org/10.1016/j.watres.2014.01.030>.
- Rauch-Williams, T., Hoppe-Jones, C., Drewes, J.E., 2010. The role of organic matter in the removal of emerging trace organic chemicals during managed aquifer recharge. *Water Res.* 44, 449–460. <https://doi.org/10.1016/j.watres.2009.08.027>.
- Regnery, J., Barringer, J., Wing, A.D., Hoppe-Jones, C., Teerlink, J., Drewes, J.E., 2015. Start-up performance of a full-scale riverbank filtration site regarding removal of DOC, nutrients, and trace organic chemicals. *Chemosphere* 127, 136–142. <https://doi.org/10.1016/j.chemosphere.2014.12.076>.
- Ruttkies, C., Schymanski, E.L., Wolf, S., Hollender, J., Neumann, S., 2016. MetFrag relaunched: incorporating strategies beyond in silico fragmentation. *J. Cheminf.* 8, 1–16. <https://doi.org/10.1186/s13321-016-0115-9>.
- Scheurer, M., Michel, A., Brauch, H.J., Ruck, W., Sacher, F., 2012. Occurrence and fate of the antidiabetic drug metformin and its metabolite guanyurea in the environment and during drinking water treatment. *Water Res.* 46, 4790–4802. <https://doi.org/10.1016/j.watres.2012.06.019>.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.C., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. <https://doi.org/10.1128/AEM.01541-09>.
- Schollée, J.E., Bourgin, M., von Gunten, U., McDardell, C.S., Hollender, J., 2018. Nontarget screening to trace ozonation transformation products in a wastewater treatment train including different post-treatments. *Water Res.* 142, 267–278. <https://doi.org/10.1016/j.watres.2018.05.045>.
- Schollée, J.E., Schymanski, E.L., Hollender, J., 2016. Statistical approaches for LC-HRMS data to characterize, prioritize, and identify transformation products from water treatment processes. *ACS (Am. Chem. Soc.) Symp. Ser.* 45–65. <https://doi.org/10.1021/bk-2016-1241.ch004>.
- Schymanski, E.L., Avak, S.E., Loos, M., Hollender, J., 2015. Prioritizing unknown

- transformation products from biologically- treated wastewater using high-resolution mass spectrometry, multivariate statistics, and metabolic logic. *Anal. Chem.* 87, 12121–12129. <https://doi.org/10.1021/acs.analchem.5b02905>.
- Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* 48, 2097–2098. <https://doi.org/10.1021/es5002105>.
- Sheldon, L.S., Hites, R.A., 1979. Sources and movement of organic chemicals in the Delaware River. *Environ. Sci. Technol.* 13, 574–579. <https://doi.org/10.1021/es60153a018>.
- Shimabuku, K.K., Zearley, T.L., Dowdell, K.S., Summers, R.S., 2019. Biodegradation and attenuation of MIB and 2,4-D in drinking water biologically active sand and activated carbon filters. *Environ. Sci. Water Res. Technol.* 5, 849–860. <https://doi.org/10.1039/C9EW00054B>.
- Tatari, K., Musovic, S., Gülay, A., Dechesne, A., Albrechtsen, H.-J., Smets, B.F., 2017. Density and distribution of nitrifying guilds in rapid sand filters for drinking water production: dominance of *Nitrospira* spp. *Water Res.* 127, 239–248. <https://doi.org/10.1016/j.watres.2017.10.023>.
- Tran, N.H., Gan, J., Nguyen, V.T., Chen, H., You, L., Duarah, A., Zhang, L., Gin, K.Y.H., 2015. Sorption and biodegradation of artificial sweeteners in activated sludge processes. *Bioresour. Technol.* 197, 329–338. <https://doi.org/10.1016/j.biortech.2015.08.083>.
- Tu, C., Teng, Y., Luo, Y., Li, X., Sun, X., Li, Z., Liu, W., Christie, P., 2011. Potential for biodegradation of polychlorinated biphenyls (PCBs) by *Sinorhizobium meliloti*. *J. Hazard Mater.* 186, 1438–1444. <https://doi.org/10.1016/j.jhazmat.2010.12.008>.
- Vandermaesen, J., Horemans, B., Degryse, J., Boonen, J., Walravens, E., Springael, D., 2016. Mineralization of the common groundwater pollutant 2,6-dichlorobenzamide (BAM) and its metabolite 2,6-dichlorobenzoic acid (2,6-DCBA) in sand filter units of drinking water treatment plants. *Environ. Sci. Technol.* 50, 10114–10122. <https://doi.org/10.1021/acs.est.6b01352>.
- Wilkinson, J., Hooda, P.S., Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: an overarching review of the field. *Environ. Pollut.* 231, 954–970. <https://doi.org/10.1016/j.envpol.2017.08.032>.
- Xu, Y., Yuan, Z., Ni, B.J., 2016. Biotransformation of pharmaceuticals by ammonia oxidizing bacteria in wastewater treatment processes. *Sci. Total Environ.* 566 (567), 796–805. <https://doi.org/10.1016/j.scitotenv.2016.05.118>.
- Yam, K., van der Geize, R., Eltis, L., 2010. *Biology of Rhodococcus*, Microbiology Monographs. Springer Berlin Heidelberg, Berlin, Heidelberg. <https://doi.org/10.1007/978-3-642-12937-7>.
- Ye, Q., Wu, Y., Zhu, Z., Wang, X., Li, Z., Zhang, J., 2016. Bacterial diversity in the surface sediments of the hypoxic zone near the Changjiang Estuary and in the East China Sea. *Microbiol.* 5, 323–339. <https://doi.org/10.1002/mbo3.330>.
- Zearley, Thomas L., Summers, R.S., 2012. Removal of trace organic micropollutants by drinking water biological filters. *Environ. Sci. Technol.* 46, 9412–9419. <https://doi.org/10.1021/es301428e>.
- Zeng, X., Liu, Z., He, L., Cao, S., Song, H., Yu, Z., Sheng, G., Fu, J., 2015. The occurrence and removal of organophosphate ester flame retardants/plasticizers in a municipal wastewater treatment plant in the Pearl River Delta, China. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.* 50, 1291–1297. <https://doi.org/10.1080/10934529.2015.1055158>.
- Zhang, S.Y., Wang, Q.F., Wan, R., Xie, S.G., 2011. Changes in bacterial community of anthracene bioremediation in municipal solid waste composting soil. *J. Zhejiang Univ. - Sci. B* 12, 760–768. <https://doi.org/10.1631/jzus.B1000440>.