Microbial degradation pathways of the herbicide bentazone in filter sand used for drinking water treatment

Hedegaard, Mathilde Jørgensen; Prasse, Carsten; Albrechtsen, Hans-Jørgen

Published in:
Environmental Science: Water Research & Technology

Link to article, DOI:
[10.1039/C8EW00790J](https://doi.org/10.1039/C8EW00790J)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Hedegaard, M. J., Prasse, C., & Albrechtsen, H-J. (2019). Microbial degradation pathways of the herbicide bentazone in filter sand used for drinking water treatment. *Environmental Science: Water Research & Technology*, 5(3), 521-532. <https://doi.org/10.1039/C8EW00790J>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Royal Society of Chemistry Editorial system for Environmental Science: Water Research & Technology

Manuscript Draft

Title: Microbial degradation pathways of the herbicide bentazone in filter sand used for drinking water treatment

Article Type: Full Paper

Keywords: bentazone; degradation; transformation product; drinking water; rapid sand filter; methanotrophs

Authors: Mathilde J. Hedegaard¹, Carsten Prasse², Hans-Jørgen Albrechtsen¹

Affiliations: ¹ DTU Environment, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; ² Department of Environmental Health and Engineering, Johns Hopkins University, Baltimore, MD 21218, United States

Corresponding Author: Mathilde Hedegaard

Address: DTU Environment, Technical University of Denmark, Building 113, DK-2800 Kgs. Lyngby, Denmark

Email-address: mjhe@env.dtu.dk

Telephone: (+45) 4525 1478

Fax: (+45) 4593 2850

Abstract

The herbicide bentazone is used extensively worldwide, and it is frequently detected in groundwater sources used for drinking water production. Previously, bentazone has been shown to be biodegraded in filter sand from biological rapid sand filters at various waterworks. This untapped potential could be an inexpensive and sustainable alternative for the removal of trace organic contaminants. To study the fate of bentazone in sand filters and to identify associated risks, degradation pathways in filter sand were identified and the toxicity of identified transformation products was evaluated using quantitative structure–activity relationship (QSAR) modelling. Bentazone degradation was investigated in microcosm experiments with filter sand, effluent water and bentazone at elevated (5 mg L^{-1}) and environmentally relevant concentrations ($<10 \text{ } \mu\text{g L}^{-1}$). The investigations at elevated concentrations revealed up to 10 transformation products, suggesting three main biotransformation pathways: 1) oxidation of the isopropyl-moiety to the corresponding carboxylic acid, 2) oxidation of the aromatic ring leading to ring cleavage and subsequent decarboxylation reactions, and 3) N-methylation followed by oxidation to a carboxylic acid. At environmentally relevant concentrations, 92% of the initial bentazone was removed within 13 days, and at this point only one transformation product, carboxy-bentazone, could be detected in the water. QSAR-models considering both human and environmentally relevant endpoints showed that degradation in filter sand led to a detoxification of bentazone. Initial oxidation processes followed by further degradation, and partial mineralization highlights the relevance of *both* methanotrophs and heterotrophs for the bentazone degradation in rapid sand filters.

1. Introduction

Groundwater represents a major drinking water source worldwide (1), and can contain organic and inorganic compounds, such as methane, hydrogen sulfide, ammonium, iron (Fe^{2+}) and manganese (Mn^{2+}). Therefore, the water treatment often includes aeration to strip-off methane and hydrogen sulfide, and to add oxygen, followed by rapid sand filtration, where physicochemical and biological processes remove e.g. iron, manganese, ammonium, and residual methane (2). However, this process is not designed to eliminate organic trace contaminants that are detected with increasing frequency in groundwater due to anthropogenic activities. Among these, pesticides are some of the most commonly detected contaminants due to their extensive use, high mobility and persistence (3). For example, pesticides were detected in 49.5% of the groundwater monitoring wells in Denmark during the period 1990-2015 (4). In the European Union (EU) the guideline value for pesticides in drinking water is based on a zero tolerance policy and is $0.1 \mu\text{g L}^{-1}$ for a single compound, or $0.5 \mu\text{g L}^{-1}$ for the sum of all pesticides (5). The same guideline applies for transformation products which have pesticidal effects or pose a risk for humans or the environment, while transformation products without these properties can be accepted even at concentrations higher than $10 \mu\text{g L}^{-1}$ (6).

One of these pesticides is bentazone (for IUPAC definitions see Table S1 in the electronic Supplementary Information (SI)), a broad-spectrum herbicide which is used extensively worldwide (7). Bentazone was the second-most frequently detected pesticide in active waterworks abstraction wells in Denmark (3.3% of 6067 wells) during 1992-2015 (4). When the pesticide concentration exceeds the guideline value, the abstraction well is often closed, since water treatment processes such as granular activated carbon (GAC) (8) or advanced oxidation processes (9) are generally not used.

In agricultural soils bentazone can be degraded by aerobic microbial processes with an average half-life of 11 days, mainly to 6-OH-bentazone and 8-OH-bentazone, but also to 2-amino-N-propan-2-ylbenzamide (AIBA) (10,11) and *N*-methyl-bentazone (12). Bentazone is mobile in aquifers (13) and highly recalcitrant (no degradation observed in periods of up to 371 days) (14,15) and can therefore easily be transported from the application site through aquifers to groundwater abstraction wells.

Although rapid sand filters are not designed for biological removal of trace organic contaminants such as pharmaceuticals and pesticides, their potential to degrade such compounds has been demonstrated in previous studies (3,16–18). Rapid sand filters could thus be an inexpensive and sustainable alternative to granular activated carbon (GAC) or advanced oxidation processes (17). Since pesticide degradation in full-scale waterworks would occur at very low concentrations ($< 0.1 \mu\text{g/L}$), it is challenging to monitor the formation of transformation products and degradation pathways in real systems. However, to take advantage of the pesticide degradation and to identify associated risks, it is imperative to understand the degradation processes and pathways, and to evaluate the toxicity of transformation products. Rapid degradation of bentazone was recently observed in filter sand from a biological rapid sand filter receiving methane-rich groundwater (19). Investigations of bentazone degradation with a methanotrophic culture, enriched from this rapid sand filter revealed transformation to several hydroxylated dead-end transformation products, more

specifically 6-OH-, 8-OH-, isopropyl-OH- and di-OH-bentazone (20). As oppose to previous studies with enrichment cultures, methane is generally stripped off by aeration prior to sand filtration, and filter sand harbors complex microbial communities, including *e.g. both* methanotrophs and heterotrophs (21). Biodiversity of microbial communities affects degradation of trace contaminants and is essential for full mineralization during co-metabolic degradation (3,22,23). To our knowledge, has neither the fate of bentazone nor the formation of transformation products in filter sand from real sand filters been investigated up to now, and it is not yet known whether biological removal of bentazone in filter sand could induce unwanted dead-end transformation products in the system.

Thus, the aims of this study were 1) to identify relevant bentazone degradation pathways in filter sand from real rapid sand filters, where complex microbial communities including *both* methanotrophs and heterotrophs could contribute to the degradation, 2) to estimate the toxicity of observed transformation products by quantitative structure–activity relationship (QSAR) modelling. Furthermore, by using this as prioritization strategy we aimed 3) to evaluate the risk connected with the most relevant transformation products in filter sand from rapid sand filters (at environmentally relevant concentrations, $\mu\text{g L}^{-1}$) and compare it to the risk of bentazone.

2. Materials and methods

2.1 Experimental overview

Two different set-ups with repeated experiments were used to investigate degradation. For detailed information of conditions in each experiment, see Table 1;

Formation of Transformation Products in filter sand (FTP1 and FTP2). The aim was to identify transformation products formed during the degradation of bentazone in filter sand using elevated concentrations (5 mg L^{-1}), and to compare this to transformation at environmentally relevant concentrations ($10 \mu\text{g L}^{-1}$ and $1 \mu\text{g L}^{-1}$).

Degradation of known Bentazone Metabolites (BM1 and BM2). The aim was to investigate the removal rate and transformation product formation during degradation of 6-OH- and 8-OH-bentazone in contact with filter sand, and to compare this with degradation of bentazone (compound concentrations: $0.3\text{--}1.0 \text{ mg L}^{-1}$).

2.2 Waterworks

Sjælsø waterworks Plant II, Nordvand A/S, Hørsholm, Denmark, has a capacity of $400\text{--}1000 \text{ m}^3 \text{ h}^{-1}$. The waterworks is described by Hedegaard and Albrechtsen (19) and receive anaerobic groundwater with high concentrations of methane and hydrogen sulphide (19). The water treatment includes aeration by an intensive tray aerator (INKA chamber) followed by filtration in first primary and then secondary rapid sand filters, but without any disinfection. No pesticides were detected in the abstracted raw water wells, and the water quality of the treated water fully complied with the EU drinking water guidelines (19). Filter sand (grain size: $3.0\text{--}5.0 \text{ mm}$) from the primary filters top layer (10 cm) was collected by an aluminium bucket on an extendable shaft

which had been disinfected with 1% hypochloride. Water was collected from the inlet to the clean water tanks.

2.3 Chemicals

Mineralization and removal of bentazone at environmentally relevant concentrations (1-8.3 $\mu\text{g L}^{-1}$) was investigated using [carbonyl- ^{14}C]-bentazone and [benzene ring- ^{14}C]-bentazone (Izotop, Institute of Isotopes Co., Ltd., Hungary). The radiochemical purity of [carbonyl- ^{14}C]-bentazone was 100% (chemical purity 99.77%) and 99% (chemical purity 97.92%) for [benzene ring- ^{14}C]-bentazone according to the manufacturer. Stock solutions of these compounds were prepared in sterile MilliQ water and the concentration was verified by HPLC-DAD within one month prior to the experiment.

Bentazone (chemical purity 99.1%, Dr. Ehrenstorfer GmbH), 6-OH- and 8-OH-bentazone (both with a chemical purity of 97%, TRC, Toronto Research Chemicals Inc., Ontario, Canada) were all dissolved in MilliQ water at least one day prior to the experiment.

Table 1 Overview of conditions in the four experiments. Hydrogenchloride (HCl), liquid scintillation chromatography (LSC).

Experiment	Unit	FTP1*		FTP2*				BM1**	BM2**
		High conc.	Low conc.	High conc.	Low conc.	Low conc.	Low conc.		
Microcosms	no.	1	2	1	2	4	3	12	12
Controls, autoclaved	no.	1	1	-	1	1	1	4	4
Controls, water only	no.	-	-	-	-	-	-	-	4
Blanks*	no.	1	-	-	1	-	-	-	2
Filter sand	(g)	100	100	100	100	100	100	100	50
Water	(mL)	100	100	100	100	100	100	100	150
Incubation temp	(°C)	18-23	18-23	10	10	10	10	18-23	10
Non labelled bentazone	(mg L^{-1})	5	-	5	0.01	-	-	0.5	1
^{14}C -Carbonyl-bentazone	($\mu\text{g L}^{-1}$)	-	8.3	-	-	1	-	-	-
^{14}C -Ring-bentazone	($\mu\text{g L}^{-1}$)	-	-	-	-	-	1	-	-
6OH-bentazone	(mg L^{-1})	-	-	-	-	-	-	0.5	1
8OH-bentazone	(mg L^{-1})	-	-	-	-	-	-	0.5	1
Sample conservation		Frozen	HCl	Frozen	Frozen	HCl	HCl	Acetic acid	Acetic acid
Analysis by		High-res-MS	LSC	High-res-MS	LC/MS/MS	LSC	LSC	HPLC-DAD	HPLC-DAD

*without any compounds, with water only or water and filter sand

Formation of transformation products in filter sand (FTP1 and FTP2)

Degradation of known bentazone metabolites (BM1 and BM2)

2.4 Microcosms and sampling

Bentazone degradation in the filter sand was investigated in a number of microcosm experiments (Table 1 is providing an overview of these experiments). Within two hours after collecting effluent water and filter sand at the waterworks, filter material and water was transferred to 300 mL serum bottles, which had been acid-washed and heated to 555°C for 12 hours. Controls with filter sand were autoclaved three times (20 min, 1 bar and 121°C, the microcosms cooled for approx. 30 min to below 80°C before autoclaving was repeated). To ensure aerobic conditions, atmospheric air was added to the water overnight and the microcosms were subsequently closed with autoclaved Teflon coated rubber stoppers and aluminium caps. Bentazone was added with a syringe by a needle through the rubber stopper.

In the experiments used for the identification of transformation products in filter sand (FTP1 and FTP2 – see Table 1), bentazone degradation and transformation products were investigated at elevated concentrations (5 mg L^{-1}) using high-resolution mass spectrometry (for methods see below), and the obtained results were compared to experiments performed at environmentally relevant concentrations (10 $\mu\text{g L}^{-1}$

(LC/MS/MS) and $1 \mu\text{g L}^{-1}$ (^{14}C -bentazone)). By combining results from different methods it was possible to investigate bentazone degradation pathways at high concentrations and compare it to degradation at low concentration. Since methanotrophic degradation of bentazone can lead to accumulation of 6-OH- and 8-OH-bentazone (20), it was investigated at high concentrations ($0.3\text{--}1.0 \text{ mg L}^{-1}$ (HPLC-DAD)) whether these bentazone transformation products could be degraded in filter sand or be dead-end products (BM1 and BM2).

Experiment FTP1 and BM1 (Table 1) were screening experiments, and were conducted at room temperature ($18\text{--}23^\circ\text{C}$). Data from these experiments were mainly used to confirm observations in subsequent experiments. After these experiments the method was adjusted before running FTP2 and BM2 (Table 1), for instance the filter sand/water ratio was reduced and more samples were collected in the initial phase of experiment BM2, to detect 6-OH- and 8-OH-bentazone removal. In FTP2 additional microcosms were included to compare bentazone removal and transformation by different analytical approaches (High-res-MS, LC/MS/MS and ^{14}C -carbonyl- and ^{14}C -ring-bentazone). In addition, experiment FTP2 and BM2 were run at 10°C - similar to the temperature at the waterworks.

Microcosms were incubated in the dark on a laboratory shaker ($100\text{--}120 \text{ rpm}$) and the pH remained at $7.5\text{--}8$ during the experiments. Oxygen concentration was measured in the water phase every 2-3 days and pure oxygen was added to the headspace when the oxygen saturation decreased to below 50%.

Water samples were collected over time with a syringe by a needle through the rubber stopper. All collected water samples were replaced by an equivalent volume of sterile atmospheric air. The collected water samples were filtered through a $0.22 \mu\text{m}$ nylon GF filter (Frisenette Aps, Q-max[®] GPF Syringe Filters, diameter 25 mm). Experiments confirmed that bentazone did not adsorb to the filters.

To extract adsorbed bentazone, 6-OH- and 8-OH-bentazone from the filter sand, the remaining water was decanted from microcosms after experiment BM2. A volume of 20 mL methanol (chemical purity $\geq 99.9 \%$, Sigma-Aldrich) was added and microcosms with filter sand were extracted for 4 hours at a shaker. Then 10 mL of the methanol was sampled by a syringe with a needle. The remaining methanol was discharged, and another 20 mL methanol was added and the microcosms were left on the laboratory shaker for 11 days. 10 mL of the methanol was sampled, filtered through $0.22 \mu\text{m}$ nylon GF filter (see above) and transferred to 20 mL glass vials, where the methanol was stripped off by N_2 . 2 mL MilliQ water was added to glass vials and within a week it was analysed by HPLC-DAD (see below).

2.5 Analytical approaches

2.5.1 Oxygen measurements

To document aerobic conditions during the experiments, oxygen concentrations were measured in the water phase by Oxygen-Sensitive Minisensors and a Fibox 3 – a fibre optic oxygen meter (Loligo Systems ApS). The measurement was based on a two-point calibration, and the limit of detection was 0.01% oxygen saturation.

2.5.2 Bentazone and OH-bentazone measurements by HPLC-DAD

Removal of bentazone, 6-OH- and 8-OH-bentazone at elevated concentration ($> 0.3 \text{ mg L}^{-1}$) was measured by HPLC-DAD. Acetic acid was added to the water samples (that had been stored frozen) prior to injection on an Ultimate 3000 HPLC-DAD system (Thermo Scientific) (for more information see SI).

2.5.3 Identification of bentazone transformation products by high-resolution MS

Water samples collected during experiments with elevated bentazone concentrations (5 mg L^{-1}) were analysed for transformation products by high-resolution mass spectrometry using a hybrid quadrupole time-of-flight mass spectrometer (QTOF-MS, SCIEX TripleTOF 5600, Darmstadt, Germany) coupled to an Agilent 1260 HPLC system (degasser, binary high-pressure gradient pump, auto sampler and column oven; all Agilent Technologies, Waldbronn, Germany) with an electrospray ionization (ESI) interface. All samples were analysed in negative ESI mode as initial experiments indicated no additional transformation products detection in positive mode (for more information see SI).

2.5.4 LC/MS/MS analysis

Removal of bentazone and detection of transformation products at low concentrations ($< 10 \text{ } \mu\text{g L}^{-1}$) was analyzed by Liquid Chromatography-tandem Mass Spectrometry (LC/MS/MS) analysis using multi-reaction monitoring (MRM) mode. The LC/MS/MS system consisted of an Agilent 1260 HPLC system coupled to an Agilent 6410 triple quadrupole tandem mass spectrometer (all Agilent Technologies). Main fragments used for detection of transformation products in MRM mode were obtained by injection of a sample from experiments at elevated bentazone concentrations (used for identification of transformation products by high-resolution mass spectrometry (HRMS)) using product ion scans (Table S2) (for more information see SI).

2.5.5 ^{14}C -bentazone measurements

The removal and mineralization of bentazone at trace concentrations ($< 1 \text{ } \mu\text{g/L}$) was investigated using ^{14}C -bentazone. The ^{14}C -activity of bentazone in the water phase and the produced $^{14}\text{CO}_2$ from bentazone mineralization was quantified by a double vial system, where produced $^{14}\text{CO}_2$ in the water was stripped off by acidification and captured by a base trap (1 mL 2 M NaOH). The base traps were set up as described by Janniche et al. (24) and the ^{14}C -activity was quantified by a liquid scintillation counter (Hidex 300 SL, 1414 Liquid Scintillation Counter, MikroWin 2000 software). The concentration at a given sampling time was expressed as a fraction of the initial concentration and corrected for the removed mass during sampling (given as $^{14}\text{C}/^{14}\text{C}_0$ (%)).

2.6 Hazard screening of transformation products

To gain toxicological information on the bentazone transformation products they were screened using *in silico* (quantitative structure-activity relationship; QSAR) models. The screening included commercial, free and DTU-developed QSAR-models in the EPI Suite v4.11 (<https://www.epa.gov/tsca-screening-tools/download-epi-suite-tm-estimation-program-interface-v411>), MultiCASE CASE Ultra 1.4.6.6, Leadscape Predictive Data Miner, a component of Leadscape Enterprise version 3.1.1- (<http://www.leadscope.com/> (accessed in the period February to September 2017)), and the Danish (Q)SAR database (<http://qsar.food.dtu.dk/>).

Although hazard-related QSAR-models provide categorical outputs (positive or negative) rather than predicted potencies, thresholds are inherently part of the endpoint definitions in the models. In this screening, possible exposure levels for the transformation products were not compared to the effect thresholds of the models. The analysis included predictions from 98 QSAR models.

2.7 Statistics

The data was treated with the statistical software GraphPad Prism 5.

3. Results and discussion

3.1 Identification of transformation products at high concentration (FTP1 and FTP2)

To investigate the fate of bentazone in the filter sand, experiments with high bentazone concentration (5 mg L⁻¹) (FTP1 and FTP2) were used to screen for transformation products by generating detectable amounts, allowing identification of the chemical structures. In total, 10 transformation products were observed after 28 days (experiment FTP1) and five of these were detected after 14 days in experiment FTP2 (Table S3 in SI). Tentative chemical structures of transformation products were obtained using exact mass information (TP number refers to the molecular weight of the uncharged molecules), obtained from MS fragmentation patterns (MS/MS spectra), as well as metabolic logic. Two main fragmentation characteristics observed in MS/MS spectra were used to determine the positions of transformation within the molecule: i) presence of one or several fragments indicating the cleavage of a C₃H₆ group which is characteristic for the isopropyl moiety present in bentazone; and ii) cleavage of CO₂ which is indicative for the presence of a carboxylic acid moiety in negative ESI MS. Based on this, the isopropyl-moiety remained intact for all transformation products except TP270 (carboxy-bentazone) and TP192. In addition, the presence of one (TP234, TP276, TP284 and carboxy-bentazone) or two (TP304, TP278) carboxylic acid moieties was identified in most transformation products (Figure 1).

Figure 1 Suggested degradation pathways of bentazone in rapid sand filters. The three boxes represent degradation in different environments with different colors. Bentazone degradation pathways in soil (grey), 10, 12 in contact with methanotrophic culture enriched from a rapid sand filter at Sjølsø waterworks plant II (green) 20 and in filter sand from Sjølsø waterworks plant II (blue). Three biodegradation pathways were identified in filter sand, different colors show different pathways (also marked by numbers). At environmentally relevant bentazone concentrations ($<10\mu\text{g L}^{-1}$) only carboxy-bentazone (TP270) was detected from degradation in filter sand. *Mark transformation products with several structural isomers. All the suggested structures are tentative.

Based on the MS/MS spectra of carboxy-bentazone revealing the cleavage of a $\text{C}_3\text{H}_4\text{O}_2$ group instead of C_3H_6 , it is clear that this transformation product was formed by oxidation of one of the isopropyl CH_3 groups to the corresponding carboxylic acid. This is further supported by the loss of a CO_2 group as well as by results showing the hydroxylation of the isopropyl-moiety by methanotrophs as one main biotransformation pathway for bentazone (Figure 1) (20). For TP246 the obtained exact mass indicated the cleavage of two carbon atoms and the incorporation of two oxygen atoms. Even though information for TP246 obtained from MS/MS spectra was

limited, one plausible explanation is the formation of a dialdehyde compound, following a similar mechanism as postulated for TP276 (see next section) (Figure 1). In addition, TP284 was formed, containing an additional carboxy group. MS/MS spectra revealed that this carboxy group can be cleaved (loss of CO₂ in MS/MS spectrum), yielding a fragment with the same exact mass as the parent compound bentazone. Similar results have been observed for the biodegradation of benzotriazole (25) and is most likely attributable to *N*-methylation followed by oxidation to the carboxylic acid. For TP259, TP244 and TP192 information obtained from HRMS analysis was insufficient to postulate a chemical structure. It is noteworthy that a transformation product with the same exact mass as TP192 has recently been reported for the photocatalytic degradation of bentazone by TiO₂/UV-A, even though the authors were also unable to postulate a chemical structure (26).

3.2 Degradation of 6- and 8-OH-bentazone in filter sand (BM1 and BM2)

The presence of carboxylic acid moieties in TP304, TP278, TP276, and TP234 clearly indicates the enzymatic cleavage of the aromatic ring in bentazone. This biodegradation reaction, which is common for aromatic compounds (27,28), involves hydroxylation reactions as primary biodegradation step (10,20). This is supported by previous studies showing the formation of 6-OH-, 8-OH-, isopropyl-OH- and di-OH-bentazone during bentazone degradation by a methanotrophic enrichment culture enriched from the same rapid sand filter at Sjælsø waterworks Plant II (20). However, none of the hydroxylated transformation products could be detected during degradation of bentazone (neither at 5 mg L⁻¹ nor at 10 µg L⁻¹) in filter sand. Probably the initial hydroxylation reaction is the rate-limiting step and the hydroxylated transformation products are too transient to accumulate to detectable levels in filter sand. To test this hypothesis, we investigated the removal rate and formation of transformation products during degradation of 6-OH- and 8-OH-bentazone in filter sand and this was compared to bentazone degradation (Figure 2).

Both 6-OH- and 8-OH-bentazone were removed rapidly with a decrease from an initial concentration of >0.3 mg L⁻¹ to below detection limit (0.01 mg L⁻¹) within the first 30 minutes in all biologically active microcosms (experiment BM2; Figure 2b and c). The removal rate of 6-OH-bentazone was 15 nmoles g_{sand}⁻¹ h⁻¹, and 30 nmoles g_{sand}⁻¹ h⁻¹ for 8-OH-bentazone (based on linear regression of the first 10 minutes) (Figure 2b and c). Hence, the removal rate of 6-OH- and 8-OH-bentazone in the filter sand was two orders of magnitude faster than of bentazone (which was 170×10⁻³ nmoles g_{sand}⁻¹ h⁻¹ based on linear regression of the removal over the first two hours, at 0.8 mg L⁻¹) (Figure 2a). The rapid removal of 6-OH- and 8-OH-bentazone was confirmed by experiment BM1 (data not shown). Biodegradation of 6-OH- and 8-OH-bentazone in filter sand resulted in the formation of TP304, TP278, TP244 and TP192 (Table S3) which also were observed during bentazone degradation (see section above). These results thus support the high biodegradability of 6-OH- and 8-OH-bentazone and explain their absence in biodegradation experiments of bentazone in filter sand.

Neither 6-OH- nor 8-OH-bentazone was removed in controls without sand filter material (water only) (Figure 2b and c). In autoclaved controls with filter sand 61% of 6-OH-bentazone and 35% of 8-OH-bentazone was removed after four hours (experiment BM2; Figure 2b and c). Removal was substantially slower in the autoclaved controls than in biologically active microcosms, emphasizing that

biological processes governed the rapid removal in filter sand. To investigate whether 6-OH- or 8-OH-bentazone adsorbed to the filter material, filter sand taken at the end of experiment BM2 was subjected to solvent (methanol) extraction. However, neither 6-OH- nor 8-OH-bentazone could be extracted from the filter sand. The removal of both compounds in autoclaved controls can thus be attributed either to remaining microbial activity or more likely to an abiotic reaction of the compounds with the sand filter material, as has been shown in soils (10,11).

Figure 2 Removal of bentazone, 6-OH- and 8-OH-bentazone in filtersand. Removal investigated in microcosms with 50 g filter sand and 150 mL water (triplicates) and in water only and in autoclaved controls (single microcosms). Mean concentration and standard deviation of A) bentazone, B) 6-OH-bentazone and C) 8-OH-bentazone. Notice: x-axis varies from 4 h to 168 h.

3.3 Formation of transformation products at environmentally relevant concentrations (low concentrations in FTP1 and FTP2)

In order to investigate whether bentazone is transformed similarly at environmentally relevant concentrations and whether this leads to accumulation of any transformation products, additional experiments were performed using LC/MS/MS (initial concentration: $10 \mu\text{g L}^{-1}$) and liquid scintillation counting (initial concentration: $1 \mu\text{g L}^{-1}$ ^{14}C -bentazone) (experiment FTP2). Under these conditions the filter sand removed 74% of the initial bentazone (LC/MS/MS) during 7 days in experiment FTP2, while only $33.3 \pm 6.5\%$ ^{14}C -ring-bentazone and $39.7 \pm 1.3\%$ ^{14}C -carbonyl-bentazone was removed in the same period (Figure 3a). The relative removal of ^{14}C -carbonyl-bentazone was similar in experiment FTP1, where $25.4 \pm 1.0\%$ was removed during 7 days (Figure 3a). Thus, the removed fraction of ^{14}C -bentazone was similar between experiments (FTP1 and FTP2) and unaffected by the initial concentration (1 to $8.3 \mu\text{g L}^{-1}$).

The difference in the removal of bentazone and ^{14}C -bentazone suggested an accumulation of one or more transformation products that remained in the water phase after bentazone degradation (Figure 3a). The equal removal of ^{14}C -carbonyl-bentazone and ^{14}C -ring-bentazone further indicated that the structures of the accumulated transformation product(s) contained both the benzene-ring and the carbonyl-group (Figure 3a). In accordance with this, carboxy-bentazone was the only transformation product detected by LC/MS/MS in microcosm with bentazone at environmentally relevant concentrations (day 14). Assuming a similar response of the transformation products in the mass spectrometer compared to bentazone and hydroxy-bentazone (i.e. similar ionization efficiencies), the limit of detection should be in the 10-100 ng/L range. If any other transformation products are formed during bentazone degradation in filter sand (at $10 \mu\text{g L}^{-1}$), they would as such constitute less than 1% of the initial bentazone.

Figure 3 Removal of bentazone and ^{14}C -bentazone and $^{14}\text{CO}_2$ -formation at low concentrations. Microcosms with 100 g filter sand and 100 mL water. Mean concentration and standard deviation given as percentage of the initial concentration during experiment FTP2 for bentazone (10 $\mu\text{g/L}$ –1, duplicates), ^{14}C -carbonyl- (1 $\mu\text{g/L}$ –1, four replicates), ^{14}C -ring-bentazone (1 $\mu\text{g/L}$ –1, triplicates) and autoclaved controls (1 $\mu\text{g/L}$ –1, single microcosms) with ^{14}C -carbonyl- and ^{14}C -ring-bentazone. Removal of ^{14}C -carbonyl-bentazone (8.3 $\mu\text{g/L}$ –1, duplicates) in experiment FTP1 is also included. A) Removal of bentazone and ^{14}C -bentazone from water phase and B) $^{14}\text{CO}_2$ -formation from mineralization of ^{14}C -carbonyl- and ^{14}C -ring-bentazone.

The $^{14}\text{CO}_2$ -formation revealed a mineralization of $7.8 \pm 0.5\%$ of the initial ^{14}C -carbonyl-bentazone during 13 days, while $21.3 \pm 1.1\%$ of the ^{14}C -ring-bentazone was mineralised in the same period (Figure 3b). The mineralization of ^{14}C -ring-BTZ was larger than observed previously (20), probably due to increased oxygen availability in the present study.

3.4 Degradation pathway

Recently, a methanotrophic culture enriched from a rapid sand filters transformed bentazone to 6-OH-, 8-OH-, isopropyl-OH- and di-OH-bentazone (Figure 1), but did

not degrade these transformation products further (20). Co-metabolic degradation of bentazone by methanotrophs is a plausible explanation for the degradation in filter sand, since methanotrophs are a part of the core taxa of the filters (29,30). However, initial hydroxylation reactions by methanotrophs could lead to dead-end products in filter sand which could hamper full mineralization. In soils hydroxylation reactions are also important for the fate of bentazone, where 6-OH- and 8-OH-bentazone were identified as main transformation products (Figure 1) (10–12). Generally, it is assumed that bentazone is hydroxylated by several fungal species in soils, and that these transformation products are rapidly incorporated in the organic soil matter (10).

The 10 transformation products formed during the degradation of bentazone in filter sand (Table S3), have, to the authors knowledge, not previously been identified or ascribed to biodegradation, although some have also been observed during photocatalytic degradation by TiO₂/UV-A (26). Degradation in filter sand might deviate from degradation in soils, partly because fungi are widely present in soils (31,32), while preliminary studies of sand filters have focused on prokaryotes (29,30,33), but also because sand filters do not have the same humic matrix. Additionally, aeration of anaerobic groundwater dramatically changes the redox potential, which induces a change in the microbial community from the aquifer to the rapid sand filters. Hence, the rapid sand filters constitutes a diverse microbial community depending on the groundwater chemistry (29,30,33). None of the hydroxylated bentazone transformation products previously identified in soils and methanotrophic cultures were detected in filter sand (Table S3 in SI). However, the presence of carboxylic acid moieties in several of the transformation products (TP304, TP278, TP276, TP234) identified in filter sand indicated the relevance of oxidation and subsequent cleavage of the aromatic ring in bentazone (Table S3 in SI). This was confirmed by degradation experiments with 6-OH- and 8-OH-bentazone, showing that these were very transient in filter sand (Figure 2), and that their degradation led to the formation of transformation products (TP304, TP278, TP244 and TP192) that were also identified during bentazone degradation (Table S3 in SI). This highlights that OH-bentazone transformation products were essential intermediates during bentazone degradation in filter sand.

Overall, the results suggest the relevance of three main biotransformation pathways (Figure 1) for bentazone in rapid sand filter: 1) oxidation of the isopropyl-moiety via oxidation of a CH₃ group to the corresponding carboxylic acid, 2) oxidation of the aromatic ring leading to ring cleavage and subsequent decarboxylation reactions, and 3) *N*-methylation followed by oxidation to a carboxylic acid.

Two of the biotransformation pathways clearly depend on initial hydroxylation reactions (degradation pathway 1 and 2) and might be associated with methanotrophic activity in the sand filters. While oxidation of the isopropyl-moiety (pathway 1), has only been reported for methanotrophs previously (20), oxidation of the aromatic ring (pathway 2) is an omnipresent biodegradation reaction (10,11,20,34). However, in contrast to methanotrophic bentazone degradation, the results highlight the relevance of other microbial processes in filter sand that led to further degradation of bentazone through *both* pathway 1 and 2. The *N*-methylation (pathway 3) was not observed in the methanotrophic culture, but has been observed during biodegradation of bentazone in soil (Figure 1) (12). Hence, the diverse microbial community in filter sand

(29,30,33) constituted an environment where several different microbial processes interacted and contributed to a rapid microbial degradation of bentazone.

At environmentally relevant low concentrations, LC/MS/MS-analysis confirmed that carboxy-bentazone was formed during the degradation, and thus pathway 1 was of importance at these concentrations and it was not possible to determine whether carboxy-bentazone was a dead-end product (Table S3 in SI). The different mineralization of ^{14}C -carbonyl-bentazone versus ^{14}C -ring-bentazone strongly indicated that at least 13.5% of the initial bentazone (equal to 30% of the removed fraction of ^{14}C -bentazone) (Figure 3b) was metabolised through pathway 2 and that transformation products in this pathway were very transient, leading to full mineralization. The *N*-methylation (pathway 3) was the only pathway, which was not confirmed at these concentrations, which could be due to insufficient sensitivity of the analytical method. Thus, at environmentally relevant concentrations, bentazone degradation in filter sand followed at least two of the same degradation pathways as at elevated concentrations. This showed that the same mechanism, the initial hydroxylation, governed biodegradation of bentazone at both elevated and environmentally relevant concentrations.

It would be interesting to be able to steer the microbial community towards the most favourable degradation pathway, avoiding accumulation of dead-end products and enhancing full mineralization. In this regard, pathway 2 is likely the most favourable degradation pathway, since our results showed that pathway 2 resulted in full mineralization (Figure 1 and Figure 3).

3.5 Hazard screening of transformation products – Human endpoints

Only five (TP304, TP278, TP276, TP234 and carboxy-bentazone) of the 10 transformation products identified at elevated bentazone concentrations (5 mg L^{-1}) accumulated to detectable levels during 14 days (see Table S3 in SI). At environmentally relevant concentrations ($<10 \text{ } \mu\text{g L}^{-1}$) the only detectable transformation product was carboxy-bentazone. Therefore, we assumed that these five transformation products would be most relevant in a waterworks context and we performed a QSAR hazard screening to investigate their potential hazards. Additionally, we included five transformation products identified in previous studies (10,12,20). Since we investigated drinking water systems, human endpoints were of particular interest, including oral availability, human intestinal adsorption (HIA), carcinogenicity, genotoxicity, endocrine disrupting effects, heart effects and reproduction and developmental toxicity.

Table 2 Predicted physio-chemical properties, aquatic and human toxicology of produced bentazone (BTZ) transformation products by EPI Suite, Leadscape and the Danish QSAR-database. Bentazone and the TPs were outside the applicability domain of the carcinogenicity models (7 models) and the Human Adverse Cardiotoxic Effects Suite (13 models). TP270 was the only transformation product detected in filter sand at environmentally relevant concentrations ($<10 \mu\text{g L}^{-1}$).

	Log K_{ow}	Water solubility	Henry law's constant	BCF	Aquatic toxicology			Lipinsky oral availability	HIA**	Heart effects	Endocrine disrupting	Genotox	Reprod Develop tox
					Microalgae*	Daphnia magna**	Fathead minnow#						
		(mg L^{-1})	($\text{atm m}^3/\text{mole}$)	(L/kg)	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})		%	hERG	(14 models)	(16 models)	(37 models)
Soil	BTZ	2.34	$2.7 \cdot 10^2$	$1.9 \cdot 10^{-10}$	16	$5.3 \cdot 10^1$	5.2	19	Passed	90	OD	Negative	Negative
	AIBA	1.82	$1.6 \cdot 10^3$	$3.0 \cdot 10^{-12}$	7.3	ND	ND	98	Passed	96	Negative	Negative	Negative
	N-methyl-BTZ	2.22	$2.9 \cdot 10^2$	$4.1 \cdot 10^{-9}$	13	ND	ND	10	Passed	94	ND	Positive (7)	Negative
MOB culture	6OH-BTZ	1.19	$8.0 \cdot 10^3$	$2.1 \cdot 10^{-14}$	2.8	5.0	6.2	13	Passed	85	Negative	Positive (3)	Negative
	8OH-BTZ	1.19	$8.0 \cdot 10^3$	$2.1 \cdot 10^{-14}$	2.8	5.0	6.2	13	Passed	85	Negative	Positive (3)	Negative
	TP256A	0.20	$7.2 \cdot 10^2$	$7.3 \cdot 10^{-15}$	3.2	$6.8 \cdot 10^1$	8.7	ND	Passed	84	Negative	Positive (1)	Positive (1)
Filter sand	TP304	-0.76	$2.6 \cdot 10^3$	$8.9 \cdot 10^{-21}$	3.2	$7.9 \cdot 10^4$	$1.8 \cdot 10^1$	ND	Passed	70	Negative	Positive (1)	Negative
	TP276	-0.88	$4.7 \cdot 10^3$	$3.5 \cdot 10^{-18}$	3.2	$7.7 \cdot 10^4$	$4.9 \cdot 10^2$	ND	Passed	78	OD	Negative	Positive (1)
	TP278	-2.60	$3.3 \cdot 10^2$	$4.3 \cdot 10^{-19}$	3.2	$6.7 \cdot 10^2$	$4.3 \cdot 10^1$	ND	Passed	68	OD	Negative	Positive (1)
	TP234	-1.19	$1.4 \cdot 10^4$	$5.8 \cdot 10^{-14}$	3.2	$5.3 \cdot 10^2$	$5.1 \cdot 10^1$	ND	Passed	79	OD	Negative	Positive (1)
$<10 \mu\text{g L}^{-1}$	TP270	0.43	$7.8 \cdot 10^3$	$2.2 \cdot 10^{-14}$	3.2	$4.3 \cdot 10^2$	$2.5 \cdot 10^1$	ND	Passed	81	OD	Negative	Negative

OD = Out of Domain

ND = Not determined

*72h growth inhibition, EC50

**48h immobilisation, EC50

#96h mortality, LC50

##HIA = Human Intestinal Adsorption

479 The molecular properties of all transformation products showed that they were likely
 480 to be orally active in humans, since they passed 'Lipinski's rule for oral availability'
 481 and they could partially be absorbed in the human body according to the Human
 482 Intestinal adsorption (HIA) model (Table 2). It was not possible to determine the
 483 carcinogenicity or heart effects of bentazone and the transformation products, since
 484 they were outside the applicability domain of the models. However, the hERG
 485 blocking model (DTU-model) indicated no cardio toxic effects of the transformation
 486 products (Table 2).

487 Several of the Leadscape models indicated that some of the transformation products
 488 could have endocrine disrupting and/or genotoxic effects (Table 2). In the
 489 Reproductive and Developmental Toxicity Suite, four structures gave positive outputs,
 490 however, these outputs were based on two analogs which showed a weak similarity
 491 with the transformation products (the two closest determining analogs were close to
 492 the cut-off of 0.7). Carboxy-bentazone did not result in any positive outputs for human
 493 endpoints (Table 2).

494 The QSAR-models for 6-OH-, 8OH-, Isopropyl-OH-, di-OH- and N-methyl-
 495 bentazone, gave some positive outputs, e.g. for genotoxicity (Table 2). However,
 496 hazards of 6-OH- and 8-OH-bentazone have been investigated by the World Health
 497 Organization, and both compounds are less toxic than bentazone and show no
 498 genotoxicity or subchronic toxicity (34).

499 In conclusion, the statistical predictions from the applied QSAR models generally
 500 indicated a detoxification during biodegradation of bentazone, with respect to the
 501 investigated human endpoints. For some of the transformation products (TP304,
 502 TP276, TP278, and TP234) positive results indicate adverse effects on reproduction or
 503 development. However, these transformation products were not detected at
 504 environmentally relevant concentrations. Only carboxy-bentazone was detected
 505 during degradation of bentazone and since the QSAR-models did not give any positive
 506 outputs for carboxy-bentazone, degradation of bentazone in real filter sand did not
 507 indicate a risk for humans.

3.6 *Hazard screening of formed transformation products – Environmental endpoints*

The hazard screening also included potential environmental effects. The investigations included aquatic toxicity, phase distribution and bioaccumulation potential of the compounds as environmental endpoints.

Based on estimated log K_{ow} and the Henry's law constants, all the transformation products are expected to behave similarly in the environment (Table 2). The bioconcentration factors (BCF) were lower for all transformation products than for bentazone, and the transformations products were therefore less prone to bioaccumulate. Predictions of the aquatic toxicity by a DTU-developed model in the Leadscope software, suggested that the toxicity of the investigated transformation products was reduced compared to bentazone. Thus, biodegradation of bentazone in filter sand reduced the toxicity towards aquatic organisms (Table 2).

The QSAR-models for 6-OH-, 8OH-, Isopropyl-OH-, di-OH- and *N*-methyl-bentazone predicted that these compounds were more or equally toxic to aquatic organisms compared to bentazone (Table 2). Similarly, the European Food Safety Authority (EFSA) concluded that *N*-methyl-bentazone is more toxic to aquatic organisms than bentazone, while 6-OH- and 8-OH-bentazone both pose a low risk to aquatic organisms – equal to bentazone (12).

Thus, the QSAR models predicted that a biodegradation of bentazone to *N*-methyl-bentazone, AIBA (10, 12) or 6-OH-, 8OH- and Isopropyl-OH-bentazone, as we detected in methanotrophic enrichment cultures (20), would induce a similar or increased risk compared to bentazone. Even though these transformation products are most likely formed during degradation of bentazone in filter sand, they are very transient and never detected during degradation at environmentally relevant bentazone concentrations. The further biodegradation of bentazone in real filter sand reduced the bioaccumulation potential and the toxicity towards aquatic organisms (Table 2).

Carboxy-bentazone was the only detectable transformation product at environmentally relevant bentazone concentrations, and QSAR-models investigating both human and environmentally relevant endpoints did not identify any potential hazards of carboxy-bentazone. Hence, degradation of bentazone in filter sand at environmentally relevant concentrations indicated no risk for humans or the environment, and the hazards decreased compared to bentazone.

In the European Union (EU) the guideline value for pesticides in drinking water is based on a zero tolerance policy, but transformation products, such as carboxy-bentazone, that do not pose a risk for humans or the environment can be approved even when occurring at concentrations higher than $10 \mu\text{g L}^{-1}$ (6).

4. Conclusion

The results obtained in this study led to the following conclusions:

- 10 transformation products were identified during biological bentazone degradation at elevated concentrations (5 mg L^{-1}).

- Hydroxylation reactions are the most important initial biodegradation steps in filter sand but hydroxylated transformation products including 6-OH- and 8-OH-bentazone are very transient and are rapidly transformed.
- Transformation of bentazone can be attributed to three transformation pathways; 1) oxidation of the isopropyl-moiety to the corresponding carboxylic acid, 2) oxidation of the aromatic ring leading to ring cleavage and subsequent decarboxylation reactions, and 3) *N*-methylation followed by oxidation to a carboxylic acid.
- Biodegradation of bentazone in filter sand differs from other environments. In contrast to methanotrophic enrichment cultures which only transformed bentazone to various hydroxylated transformation products (20), the diverse microbial community in filter sand constituted an environment where several microbial processes interacted and degraded bentazone further, leading to mineralization.
- At environmentally relevant concentrations the only detectable transformation product was carboxy-bentazone. QSAR-models evaluating both human and environmentally relevant endpoints showed that the hazard of carboxy-bentazone decreased substantially compared to bentazone.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was partly financed by the Grundfos Prize. The authors thank the staff at Sjølsø waterworks, Nordvand A/S for help with filter sand collection and Hélène Deliniere and Mikael Emil Olsson for technical assistance. Thanks to Eva Bay Wedebye and Nikolai Georgiev Nikolov for performing the hazard screening of the detected transformation products and assistance with interpretation of the data. We confirm that there are no known conflicts of interest associated with this publication.

5. References

1. IWA. International statistics for water services [Internet]. 2014. Available from: <http://www.iwa-network.org/wp-content/uploads/2016/06/International-Statistics-for-Water-Services-2014.pdf>
2. Mouchet P. From conventional to biological removal of iron and manganese in France. *J / Am Water Work Assoc.* 1992;84(4):158–67.
3. Benner J, Helbling DE, Kohler HE, Wittebol J, Kaiser E, Prasse C, et al. Is biological treatment a viable alternative for micropollutant removal in drinking water treatment processes? *Water Res* [Internet]. 2013;47(16):5955–76. Available from: <http://dx.doi.org/10.1016/j.watres.2013.07.015>
4. GEUS & Danish Ministry of Energy Utilities and Climate. Groundwater monitoring 1989 – 2015. 2016.
5. European Commission. Council Directive 98/83/EC of 3 November 1998 on

- 587 the quality of water intended for human consumption. Off J Eur Communities.
588 1998;L330:32–54.
- 589 6. European Commission. Guidance document on the assessment of the relevance
590 of metabolites in groundwater of substances regulated under council directive
591 91/414/EEC [Internet]. HEALTH & CONSUMER PROTECTION
592 DIRECTORATE-GENERAL. 2003. p. 1–14. Available from:
593 [https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-](https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_fate_metabolites-groundwtr.pdf)
594 [proc_guide_fate_metabolites-groundwtr.pdf](https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_fate_metabolites-groundwtr.pdf)
- 595 7. European Commission. EU pesticides database [Internet]. 2017. Available
596 from: [http://ec.europa.eu/food/plant/pesticides/eu-pesticides-](http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN)
597 [database/public/?event=homepage&language=EN](http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN)
- 598 8. Heijman SGJ, Siegers W, Sterk R, Hopman R. Prediction of breakthrough of
599 pesticides in GAC-filters and breakthrough of colour in ion-exchange-filters.
600 In: Water Science and Technology: Water Supply. 2002. p. 103–8.
- 601 9. Suty H, De Traversay C, Cost M. Applications of advanced oxidation processes:
602 present and future. Water Sci Technol. 2004;49(4):227–33.
- 603 10. Huber R, Otto S. Environmental behavior of bentazon.pdf. George W. Ware,
604 editor. Rev Environ Contam Toxicol. 1994;137:111–34.
- 605 11. Knauber WR, Krotzky AJ, Schink B. Microbial metabolism and further fate of
606 bentazon in soil. Environ Sci Technol. 2000;34(4):598–603.
- 607 12. European Food Safety Authority. Conclusion on the peer review of the pesticide
608 risk assessment of the active substance bentazone. EFSA J [Internet].
609 2015;13(4). Available from: <http://doi.wiley.com/10.2903/j.efsa.2015.4077>
- 610 13. Clausen L, Fabricius I, Madsen L. Adsorption of pesticides onto quartz, calcite,
611 kaolinite, and alpha-alumina. J Environ Qual. 2001;30(3):846–57.
- 612 14. Albrechtsen HJ, Mills MS, Aamand J, Bjerg PL. Degradation of herbicides in
613 shallow Danish aquifers: An integrated laboratory and field study. In: Pest
614 Management Science. 2001. p. 341–50.
- 615 15. Broholm MM, Rügge K, Tuxen N, Højberg AL, Mosbaek H, Bjerg PL. Fate of
616 herbicides in a shallow aerobic aquifer: A continuous field injection experiment
617 (Vejen, Denmark). Water Resour Res [Internet]. 2001 Dec [cited 2017 Apr
618 24];37(12):3163–76. Available from:
619 <http://doi.wiley.com/10.1029/2000WR000002>
- 620 16. Kaiser E, Prasse C, Wagner M, Bröder K, Ternes TA. Transformation of
621 oxcarbazepine and human metabolites of carbamazepine and oxcarbazepine in
622 wastewater treatment and sand filters. Environ Sci Technol.
623 2014;48(17):10208–16.
- 624 17. Hedegaard MJ, Arvin E, Corfitzen CB, Albrechtsen HJ. Mecoprop (MCP)P
625 removal in full-scale rapid sand filters at a groundwater-based waterworks. Sci

- 626 Total Environ. 2014;499:257–64.
- 627 18. Zearley TL, Summers RS. Removal of trace organic micropollutants by
628 drinking water biological filters. *Environ Sci Technol*. 2012;46(17):9412–9.
- 629 19. Hedegaard MJ, Albrechtsen HJ. Microbial pesticide removal in rapid sand
630 filters for drinking water treatment - Potential and kinetics. *Water Res*
631 [Internet]. 2014;48(1):71–81. Available from:
632 <http://dx.doi.org/10.1016/j.watres.2013.09.024>
- 633 20. Hedegaard MJ, Deliniere H, Prasse C, Dechesne A, Smets BF, Albrechtsen H-
634 J. Evidence of co-metabolic bentazone transformation by methanotrophic
635 enrichment from a groundwater-fed rapid sand filter. *Water Res*. 2018;129.
- 636 21. Gülay A, Musovic S, Albrechtsen H-J, Al-Soud WA, Sørensen SJ, Smets BF.
637 Ecological patterns, diversity and core taxa of microbial communities in
638 groundwater-fed rapid gravity filters. *ISME J* [Internet]. 2016;10(9):2209–22.
639 Available from: <http://www.nature.com/doi/10.1038/ismej.2016.16>
- 640 22. Helbling DE, Johnson DR, Honti M, Fenner K. Micropollutant
641 biotransformation kinetics associate with WWTP process parameters and
642 microbial community characteristics. *Environ Sci Technol*.
643 2012;46(19):10579–88.
- 644 23. Johnson DR, Helbling DE, Lee TK, Park J, Fenner K, Kohler HPE, et al.
645 Association of biodiversity with the rates of micropollutant biotransformations
646 among full-scale wastewater treatment plant communities. *Appl Environ*
647 *Microbiol*. 2015;
- 648 24. Janniche GS, Lindberg E, Mouvet C, Albrechtsen HJ. Mineralization of
649 isoproturon, mecoprop and acetochlor in a deep unsaturated limestone and
650 sandy aquifer. *Chemosphere*. 2010;81(7):823–31.
- 651 25. Huntscha S, Hofstetter TB, Schymanski EL, Spahr S, Hollender J.
652 Biotransformation of benzotriazoles: Insights from transformation product
653 identification and compound-specific isotope analysis. *Environ Sci Technol*.
654 2014;48(8):4435–43.
- 655 26. Berberidou C, Kitsiou V, Kazala E, Lambropoulou DA, Kouras A, Kosma CI,
656 et al. Study of the decomposition and detoxification of the herbicide bentazon
657 by heterogeneous photocatalysis: Kinetics, intermediates and transformation
658 pathways. *Appl Catal B Environ* [Internet]. 2017;200:150–63. Available from:
659 <http://dx.doi.org/10.1016/j.apcatb.2016.06.068>
- 660 27. Arora PK. Bacterial degradation of monocyclic aromatic amine. Vol. 6,
661 *Frontiers in Microbiology*. 2015.
- 662 28. Kolomytseva MP, Baskunov BP, Golovleva LA. Intradiol pathway of para-
663 cresol conversion by *Rhodococcus opacus* 1CP. *Biotechnol J*. 2007;2(7):886–
664 93.

29. Gulay A, Musovic S, Albrechtsen H-J, Al-Soud WA, Sorensen SJ, Smets BF. Ecological patterns, diversity and core taxa of microbial communities in groundwater-fed rapid gravity filters [Internet]. Vol. 10, The ISME Journal. 2016. p. 2209–22. Available from: <http://dx.doi.org/10.1038/ismej.2016.16>
30. Albers CN, Ellegaard-Jensen L, Harder CB, Rosendahl S, Knudsen BE, Ekelund F, et al. Groundwater chemistry determines the prokaryotic community structure of waterworks sand filters. *Environ Sci Technol*. 2015;49(2):839–46.
31. Strickland MS, Rousk J. Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*. 2010.
32. Van Der Heijden MGA, Bardgett RD, Van Straalen NM. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*. 2008.
33. Palomo A, Jane Fowler S, Gülay A, Rasmussen S, Sicheritz-Ponten T, Smets BF. Metagenomic analysis of rapid gravity sand filter microbial communities suggests novel physiology of *Nitrospira* spp. *ISME J* [Internet]. 2016;10(11):2569–81. Available from: <http://www.nature.com/doi/10.1038/ismej.2016.63>
34. Kanungo D, Dellarco V, Davies L. Bentazone. *World Heal Organ*. 2012;4(25057):31–98.

Supplementary information for 'Degradation pathways of the herbicide bentazone with filter sand used for drinking water treatment'

Mathilde J. Hedegaard, Carsten Prasse and Hans-Jørgen Albrechtsen

S1 Introduction

Table S1 Chemical names, abbreviations and IUPAC definitions.

Name	Abbreviation	IUPAC definition
Bentazone	BTZ	3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
6-OH-bentazone	6-OH-BTZ	6-Hydroxy-3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
8-OH-bentazone	8-OH-BTZ	8-Hydroxy-3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
AIBA	-	2-amino-N-propan-2-ylbenzamide
N-methyl-bentazone	N-methyl-BTZ	3-isopropyl-1-methyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
Isopropyl-OH-bentazone	Isopropyl-OH-BTZ	3-(1-hydroxypropan-2-yl)-1H-benzo[c][2,1,3]thiadiazin-4(3H)-one-2,2-dioxide
Di-OH-bentazone	Di-OH-BTZ	-

S2 Materials and methods

S2.5.3 Bentazone and OH-bentazone measurements by HPLC-DAD

Chromatographic separation was achieved on a 150 mm x 4.6 mm x 5 µm BDS Hypersil C18 column (Thermo Scientific), held at 30 degrees, using 5mM H₂SO₄/acetonitrile (55/45) isocratic for 5 minutes at 1.25 ml min⁻¹. Detection was achieved at 230 nm with the absorbance spectrum collected between 190 and 400 nm.

S2.5.4 Identification of bentazone transformation products by high-resolution MS

The ion source parameters were: source temperature 550 °C; capillary voltage -4.5 kV; curtain gas 40 psi; ion source gas 1 35 psi, ion source gas 2 45 psi. Exact mass of parent compounds and main fragments were obtained using full scan TOF-MS and MS/MS mode with information dependent acquisition (IDA) experiments (MS²). The resolution of measurements was 35,000 at m/z = 400 and the mass accuracy below 5 ppm. A scan range of m/z 100-500 was used.

Chromatographic separation was carried out on a 4 µm Synergi Hydro-RP column (150 x 3 mm i.d.) equipped with a SecurityGuard column (4 x 3 mm i.d.; Phenomenex, Aschaffenburg, Germany). Flow rate was set to 400 mL min⁻¹ using 0.1% formic acid (A) and acetonitrile (B) as mobile phases. The percentage of (A) was changed linearly as follows: 0-10 min, 100%; 25 min, 10%; 28 min, 10%; 29 min, 100%; 35 min, 100%.

S2.5.5 LC/MS/MS analysis

A Hydro-RP column (150x3 mm, 4 µm; Phenomenex, Aschaffenburg, Germany) was used for chromatographic separation using 0.1% acetic acid (A) and methanol (B) as mobile phases. Separation of analytes was achieved applying the following gradient: 0-4 min, 100% A; 7 min, 30% A; 17 min, 10% A; 18 min, 100% A. The run time was 22 min, flow rate was 0.4 mL min⁻¹, and column oven temperature was set to 25 °C. Sample volume was set to 100 µL. Multi-reaction monitoring (MRM) was used for detection of all compounds (Table S2) using the following parameters: gas temp.: 350°C; gas flow: 9 L min⁻¹; nebulizer: 45 psi; sheath gas heater: 400°C; sheath gas flow: 9 L min⁻¹; capillary voltage: -3600 V.

Table S2 LC/MS/MS multi-reaction monitoring (MRM) parameters used for detection of TPs identified in biodegradation experiments with bentazone, 6-OH-bentazone and 8-OH-bentazone.

Name	Parent ion mass [M-H] ⁻	Fragment ion mass	Fragmentor voltage [V]	Collision energy [V]	Cell exit potential [V]
Bentazone	239	197	130	24	6
		175	130	22	4
		132	130	22	4
6-OH-bentazone	255	213	130	14	6
		191	130	12	4
		148	130	18	4
8-OH-bentazone	255	213	130	14	6
		191	130	12	4
		148	130	18	4
TP304	303	259	130	10	7
		215	130	10	7
		173	130	20	7
TP284	283	239	130	10	7
		197	130	20	7
		132	130	30	7
TP278	277	233	130	10	7
		189	130	10	7
		125	130	10	7
TP270	269	225	130	10	7
		197	130	20	7
		189	130	10	7
TP244	243	201	130	20	7
		121	130	20	7
		78	130	30	7
TP235	234	191	130	10	7
		173	130	20	7
		147	130	20	7
TP192	191	163	130	10	7
		122	130	20	7
		78	130	40	7

731 **S3 Results and discussion**

Table S3 Identified transformation products (TPs) from degradation of bentazone (5 mg L⁻¹) in contact with filter sand from a rapid sand filter in FTP1 (28 days) using high-resolution mass spectrometry in negative ionization mode. * Mark the TPs which presence were confirmed after 14 days in FTP2. # Mark the TPs which were detected during degradation of 6-OH- and/or 8-OH-bentazone. ⌘ Mark the TPs which were detected during degradation of bentazone at low concentrations (10 µg L⁻¹).

ESI ⁻				
	Exact mass	Sum formula	Δppm	
BTZ	239.0494	C₁₀H₁₁N₂O₃S	1.5	
	197.0026	C ₇ H ₅ N ₂ O ₃ S	2.7	-C ₃ H ₆
	175.0877	C ₁₀ H ₁₁ N ₂ O	3.5	-SO ₂
	147.0816	C ₁₀ H ₁₁ O	4.6	
	133.0409	C ₇ H ₅ N ₂ O	5.5	
	132.0331	C ₇ H ₄ N ₂ O	5.6	
TP304*#	303.0291	C ₁₀ H ₁₁ N ₂ O ₇ S	1.3	
	259.0374	C ₉ H ₁₁ N ₂ O ₅ S	5.3	-CO ₂
	215.0477	C ₈ H ₁₁ N ₂ O ₃ S	5.9	-CO ₂ , -CO ₂
	173.0019	C ₅ H ₅ N ₂ O ₃ S	1.0	-CO ₂ , -CO ₂ , -C ₃ H ₆
	110.0244	C ₅ H ₄ NO ₂	1.8	
	94.02964	C ₅ H ₄ NO	3.8	
	77.9641	NO ₂ S	9.9	
TP284	283.0395	C ₁₁ H ₁₁ N ₂ O ₅ S	2.2	
	240.9915	C ₈ H ₅ N ₂ O ₅ S	1.3	-C ₃ H ₆
	239.0502	C ₁₀ H ₁₁ N ₂ O ₃ S	4.8	-CO ₂
	197.0028	C ₇ H ₅ N ₂ O ₃ S	3.9	-CO ₂ , -C ₃ H ₆
	175.0870	C ₁₀ H ₁₁ N ₂ O	0.3	-CO ₂ , -SO ₂
	133.0397	C ₇ H ₅ N ₂ O	3.0	-CO ₂ , -SO ₂ , -C ₃ H ₆
	132.0325	C ₇ H ₄ N ₂ O	1.1	
	117.0455	C ₇ H ₅ N ₂	2.8	
TP278*#	277.0131	C ₈ H ₉ N ₂ O ₇ S	0.1	
	233.0228	C ₇ H ₉ N ₂ O ₅ S	1.5	-CO ₂
	189.0334	C ₆ H ₉ N ₂ O ₃ S	0.1	-CO ₂ , -CO ₂
	146.9861	C ₃ H ₃ N ₂ O ₃ S	1.6	-CO ₂ , -CO ₂ , -C ₃ H ₆
	125.0714	C ₆ H ₉ N ₂ O	0.4	-CO ₂ , -CO ₂ , -SO ₂
	68.01405	C ₃ H ₂ NO	6.0	
TP276*	275.0341	C ₉ H ₁₁ N ₂ O ₆ S	1.1	
	231.0431	C ₈ H ₁₁ N ₂ O ₄ S	3.3	-CO ₂
	188.9985	C ₅ H ₅ N ₂ O ₄ S	7.9	-CO ₂ , -C ₃ H ₆
	162.0210	C ₅ H ₈ NO ₃ S	9.0	
	145.9921	C ₄ H ₄ NO ₃ S	6.5	
	110.0242	C ₅ H ₄ NO ₂	0.4	
TP270*⌘	269.0238	C ₁₀ H ₉ N ₂ O ₅ S	2.1	
	225.0341	C ₉ H ₉ N ₂ O ₃ S	3.1	-CO ₂
	197.0027	C ₇ H ₅ N ₂ O ₃ S	3.1	-C ₃ H ₄ O ₂
	189.0665	C ₁₀ H ₉ N ₂ O ₂	0.8	-SO ₃
	161.0723	C ₉ H ₉ N ₂ O	5.4	
	145.0407	C ₈ H ₅ N ₂ O	3.6	
	143.0591	C ₆ H ₉ NO ₃	6.2	
	133.0396	C ₇ H ₅ N ₂ O	3.9	-C ₃ H ₄ O ₂ , -SO ₂
	132.0324	C ₇ H ₄ N ₂ O	0.4	

	117.0454	C ₇ H ₅ N ₂	1.7	
	105.0333	C ₇ H ₅ O	6.4	
	92.05052	C ₆ H ₆ N	5.4	
	79.9579	SO ₃	13.9	
TP259	258.0171	C ₅ H ₁₀ N ₂ O ₈ S	5.5	
	215.9692	C ₂ H ₄ N ₂ O ₈ S	1.7	-C ₃ H ₆
	172.9662	C ₄ HN ₂ O ₄ S	3.1	
	136.0132	C ₂ H ₄ N ₂ O ₅	2.7	-C ₃ H ₆ , -SO ₃
	107.0587	C ₃ H ₉ NO ₃	4.8	
	77.9651	NO ₂ S	2.0	
TP246	245.0234	C ₈ H ₉ N ₂ O ₅ S	0.7	
	202.9773	C ₅ H ₃ N ₂ O ₅ S	5.1	-C ₃ H ₆
	123.0188	C ₅ H ₃ N ₂ O ₂	5.0	-C ₃ H ₆ , -SO ₃
	77.9653	NO ₂ S	4.4	
TP244[#]	243.0445	C ₉ H ₁₁ N ₂ O ₄ S	2.2	
	200.9960	C ₆ H ₅ N ₂ O ₄ S	4.8	-C ₃ H ₆
	121.0408	C ₆ H ₅ N ₂ O	5.6	-C ₃ H ₆ , -SO ₃
	77.96553	NO ₂ S	7.2	
TP234*	233.0241	C ₇ H ₉ N ₂ O ₅ S	3.7	
	190.9766	C ₄ H ₃ N ₂ O ₅ S	1.9	-C ₃ H ₆
	189.0334	C ₆ H ₉ N ₂ O ₃ S	0.1	-CO ₂
	172.9664	C ₄ HN ₂ O ₄ S	4.3	-C ₃ H ₆ , -H ₂ O
	146.9861	C ₃ H ₃ N ₂ O ₃ S	1.7	-CO ₂ , -C ₃ H ₆
	125.0714	C ₆ H ₉ N ₂ O	0.6	-CO ₂ , -SO ₂
	83.0251	C ₃ H ₃ N ₂ O	7.8	-CO ₂ , -SO ₂ , -C ₃ H ₆
	79.9578	SO ₃	12.4	
TP192[#]	191.0138	C ₅ H ₇ N ₂ O ₄ S	5.6	
	163.0174	C ₄ H ₇ N ₂ O ₃ S	1.4	-CO
	121.9550	CNO ₄ S	2.2	
	77.9651	NO ₂ S	2.4	

Table S4 Structure of substances in the hazard screening.	
BTZ
AIBA
N-methyl-BTZ
6OH-BTZ
8OH-BTZ
Isopropyl-OH-BTZ
TP304
TP276
TP278
TP234
TP270

733

734