Optimisation of organic compound removal in artificial recharge systems by redox control and enhanced oxidation

OXIRED – Phase 1

by

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Extended Summary

Subsurface passage as utilized during river bank filtration and artificial groundwater recharge has shown to be an effective barrier for multiple substances present in surface waters during drinking water production. Additionally it is widely used as polishing step after wastewater treatment. However, there are limitations concerning the removal of DOC and specific trace organics. The project "OXIRED" aims at assessing possibilities to overcome these limitations by combining subsurface passage with pre-oxidation by ozone.

In the first phase of the project, laboratory-scale column experiments were conducted in order to quantify removal for different settings under varying conditions. In a previous study different combinations of advanced oxidation and subsurface passage were evaluated concerning their potential removal efficiency and practical implementation on the basis of existing, published experiences and theoretical considerations. Two different scenarios were identified as promising for experiments in laboratory-scale columns with surface water and sewage treatment plant effluent:

A) surface water – oxidation – groundwater recharge

B) surface water – short bankfiltration – oxidation – groundwater recharge

The investigations were designed to lead to recommendations for the implementation of a combined system of subsurface passage and advanced oxidation in pilot scale experiments that will be carried out in the second phase of the project.

Prior to column experiments, batch tests following the R_{CT}-concept by Elovitz and von Gunten (1999) were carried out to characterize the reaction of ozone with the investigated water qualities [1]. Additional batch ozonation tests with subsequent analysis of biodegradable dissolved organic carbon (BDOC) were conducted in order to determine optimal ozone doses for DOC removal in column experiments. For laboratory-scale experiments a set of 8 soil columns (length: 1 m; diameter: 0.3 m) was operated at TUB to evaluate the effects of pre-ozonation of different source waters (secondary effluent, surface water, bank filtrate). Ozonation was conducted with gaseous ozone in a 13-L stirred tank reactor. Specific ozone doses of 0.7 $mg\ O_3/mg\ DOC_0$ and 0.9 $mg\ O_3/mg\ DOC_0$ were investigated. Trace organic compounds to be targeted were identified in a prior literature study on existing data on subsurface removal.

Results from laboratory-scale soil column experiments led to recommend specific ozone doses (z) of 0.7 $mg\ O_3/mg\ DOC_0$ for the following technical- and pilot-scale applications.

Removal of surface water DOC in the soil columns was increased from 22% without ozonation to 40% ($z = 0.7$) and 45% ($z = 0.9$) with preozonation and the DOC in the column effluent reached the level of tap water in Berlin within less than one week of retention time. At bank filtration and artificial recharge sites in Berlin similar removal rates were only observed within $3 - 6$ months of retention [2].

The transformation of many trace compounds was efficient with specific ozone doses of $0.6-0.7\ mg\ O_3/mg\ DOC_0$. Realistic surface water concentrations of carbamazepine,

sulfamethoxazole, diclofenac and bentazone were reduced below the limits of quantification (LOQ). The pesticides diuron and linuron were reduced close to LOQ. The substances MTBE, ETBE and atrazine were only partly transformed during ozonation. For efficient transformation of these substances, higher ozone doses or an optimisation of the oxidation process, for example as advanced oxidation process (AOP), should be considered.

Operating a preceding bank filtration (scenario B) will enhance the transformation efficiency of MTBE and ETBE. With similar ozone consumption the transformation of MTBE and ETBE was increased by $27-31\%$ and $28-33\%$ of the original removal, respectively. Other investigated compounds were efficiently transformed during ozonation of surface water independently of the preceding bank filtration step.
For the removal of bulk organic carbon only little improvement was observed for scenario B. Overall DOC removal increased from 45% with direct ozonation of surface water to up to 50% with a preceding soil column.

Despite the presence of relevant bromide concentrations ($\approx 100\ \mu g/L$) formation of the oxidation by-product bromate was not observed ($< 5\ \mu g/L$). However, this could also be a result of analytical problems, as later spiking tests showed. Formation of brominated organic compounds was also not observed. Adsorbable organic bromide (AOBr) even decreased by $50-60\%$ for secondary effluent and $80-90\%$ for surface water. The reduction of AOBr concentrations was accompanied by an increase of inorganic bromide by up to 40 $\mu g/L$ during ozonation of surface water.

In the two conducted in vitro genotoxicity tests (Ames test, micronucleus assay) no genotoxicity caused by ozonation of water samples was observed. Testing for cytotoxicity (glucose consumption rate, ROS generation) showed positive results in several samples. However, a systematic attribution of toxic effects to ozonation or subsequent soil passage was not possible. Reasons for cytotoxic effects were not evaluated within the scope of this project but it is assumed that they were caused by unknown cofactors.

These results show that the objectives of enhanced removal of trace organics and DOC by combining ozonation and subsurface passage are well met. Further investigations need to confirm this for the pilot scale, especially taking into account the formation, retention and toxicity of oxidation by-products.

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Optimisation of organic compound removal in artificial recharge systems by redox control and enhanced oxidation (OXIRED-1) – Final Report

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Thank you!

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1 Introduction

Underground passage as utilized during river bank filtration and artificial groundwater recharge has been proven to be an effective barrier for multiple substances present in surface waters during drinking water production. Additionally it is widely used as polishing step after wastewater treatment. However, there are limitations concerning the removal of dissolved organic carbon (DOC) and specific trace organics. The project " OXIRED – Phase 1" aims at assessing possibilities to overcome these limitations by combining underground passage and advanced oxidation (e.g. ozonation) as pre- or post-treatment.

In work package 1 of the project, existing data on underground removal were evaluated in order to identify substances that should be targeted in the investigations [3]. Additionally, different combinations of advanced oxidation and underground passage were evaluated concerning potential removal efficiency and practical implementation on the basis of existing, published experiences and theoretical considerations [4].

In work package 2, laboratory experiments with water from different sources in Berlin were carried out with the aim to limit larger scale experiments to scenarios that will most likely be successful. In the first phase, ozonation kinetics were evaluated and formation of biodegradable dissolved organic carbon (BDOC) by ozonation was analysed to optimize following column experiments. Subsequently, biodegradation of DOC and selected trace organics (from WP1) with pre-oxidation was observed in parallel columns with different source waters in order to assess the efficiency of different simulated treatment. In particular, two different systems for drinking water treatment from surface water were simulated, which are illustrated in figure 1.1. Additional columns were operated with secondary effluent to simulate tertiary treatment of wastewater. The investigations were carried out at the Technical University of Berlin and lead to recommendations for the design of the technical scale experiments and the pilot.

For work package 3, a 30 m column system at the UBA was used for a first test of the optimal scenario developed within WP 1 and 2 under conditions similar to

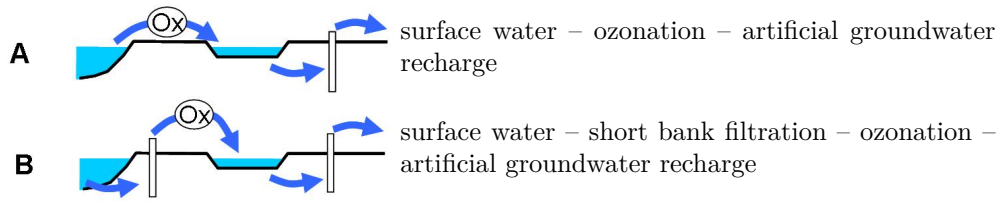

Figure 1.1: Investigated systems of combined treatment with ozonation and artificial groundwater recharge

those met in the field. The column are arranged with ozonation as pre-treatment and fed with surface water from Lake Tegel in Berlin. The investigations will lead to recommendations for the design of a pilot.

Work package 4 comprised the analytics on trace organics, conducted by the BWB laboratory on the samples obtained during the laboratory and technical scale experiments as well as a toxicological screening (cytotoxicity and genotoxicity) of selected samples by the UBA in Bad Elster.

In this final report, a detailed presentation of the results from laboratory experiments (WP 2) is given. Technical-scale column experiments have been started in September and will be ongoing in the following project (OXIRED-2).

2 Experimental

2.1 Batch experiments

2.1.1 Ozonation kinetics in different waters

Kinetics of ozonation strongly depends on the quality of the ozonated water. Batch experiments were conducted to characterize ozonation kinetics of different waters. The experimental set-up followed the R_{CT}-concept described by [1] measuring ozone decomposition and degradation of para-chlorobenzoic acid (pCBA) as indicator for radical exposure in a batch reactor. Therefore, 400 mL sample were mixed with 100 mL ozone enriched ultra pure water. Decomposition of ozone was measured using the indigo method [5], pCBA measurement was conducted using HPLC. All experiments were performed as duplicates.

The decomposition of ozone can be described as first order reaction:

$$ln\frac{[O_3]_t}{[O_3]_{t=0}} = k_{O3} \cdot t \tag{2.1}$$

Reaction of pCBA follows second order kinetics:

$$ln\frac{[pCBA]_t}{[pCBA]_{t=0}} = k_{pCBA,OH\bullet} \int [OH\bullet]dt \tag{2.2}$$

The R_{CT}-value is defined as the ratio between OH-radical and ozone exposition:

$$R_{CT} = \frac{\int [OH\bullet]dt}{\int [O_3]dt} \tag{2.3}$$

R_{CT} can be calculated from the slope of $ln([pCBA]_t/[pCBA]_{t=0})$ over the ozone exposition as long as the ratio of ozone radical and ozone exposure is constant over time.

Temperature, pH and total inorganic carbon were measured in samples before and after experiments. Temperature did not change during experiments and varied between 14 $^\circ C$ and 17 $^\circ C$. Results from pH and total inorganic carbon measurement are presented in table 2.1. Inorganic carbon was slightly reduced during experiments by $0.3 - 1.5$ mg/L. Initial pH was between 8 and 9 and decreased after ozonation.

2.1.2 Formation of BDOC by ozonation

In order to optimise the biodegradation after ozonation, batch tests were conducted analysing BDOC of ozonated water. Therefore, water was ozonated with varying

Table 2.1: pH and total inorganic carbon (TIC) during R_{CT} experiments (n.a.: not analysed)

	start	exp. 1	exp. 2
total inorganic carbon $[mg/L]$			
tap water	39.6	39.3	39.3
bank filtrate	39.4	38.5	38.1
surface water	32.5	31.9	31.1
secondary effluent	40.3	38.8	38.8
pH			
tap water	n.a.	n.a.	n.a.
bank filtrate	n.a.	8.3	8.3
surface water	8.4	7.9	8.0
secondary effluent	7.9	7.7	7.7

specific ozone doses. The ozonation unit is described in chapter 2.3. Bulk organic parameters were analysed from raw and ozonated water.

For the analysis of BDOC, a batch test described by Joret et al. (1990) [6] was modified. As inoculum, sand from Lake Tegel bank was sieved to a size of $0.2-1.0\ mm$ and rinsed ($V : w = 5 : 1$) eight times with tap water and two times with organic free water, respectively. The sand was stored over night in bank filtrate and used the day after sampling for experiments.
400 mL of ozonated water were incubated with 130 g of sand in 1 L Duran bottles at$20\,^{\circ}C$ in the dark. Incubation was conducted on a 150 rpm shaker and samples were taken and analysed for DOC after 1 hour (to measure effects of sorption) and on days 1, 3, 4, 5, 6, 7, 10 and 14. All BDOC tests were performed as duplicates.

2.1.3 Stripping test

Since some of the investigated compounds were expected to be volatile, additional stripping tests were conducted. Therefore, two different waters (surface water from Lake Tegel and column effluent from TUB) were spiked with trace compounds at 1 $\mu g/L$. Both solutions were then aerated with pure oxygen for approximately ten minutes (similar to ozonation time for lab-scale column experiments) using the ozonation unit at TUB. Samples were taken before and after aeration and analysed at BWB laboratories.

2.2 Laboratory-scale column experiments

For the laboratory-scale experiments, a set of eight soil columns was set up in the basement at TU Berlin (average room temperature of $22\,^{\circ}C$). The columns were filled with technical sand (particle size: $0.7 - 1.2\ mm$) and have been adapting to surface

water from Lake Tegel for four months. Columns were fed with a flow rate of 1.5 L/d from 13-L storage tanks, which were refilled weekly. Hydraulic retention time in the columns was determined in tracer tests to be 5-6 days. In order to test different treatment systems columns were operated with different feed waters (surface water; effluents from columns at TUB and UBA and secondary effluent). Surface water was taken from Lake Tegel, secondary effluent from the WWTP in Ruhleben. To simulate a short bank filtrate, effluent from the first column of the technical-scale column system was collected for approximately one month. Qualities of the operated waters are described in chapter 2.5. The detailed set-up is shown in figure 2.1. Reference columns without pretreatment were operated in order to evaluate benefits of ozonation prior to infiltration. During experiments, flow rate in columns 3 and 6 was adjusted to approximately 1 L/d since available feed water was short.

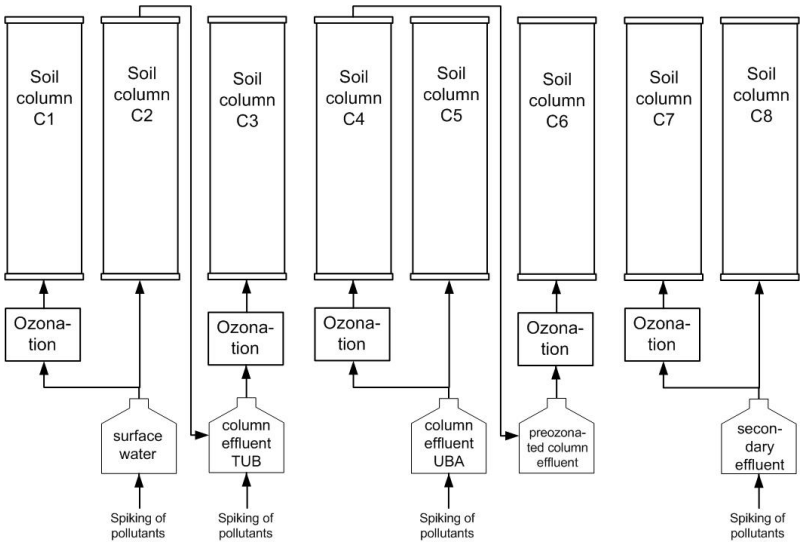

Figure 2.1: Experimental set-up of lab-scale column experiments

Two experiments with different ozone doses were conducted. The schedule of the experiments is presented in figure 2.2. Feed waters and column effluents were sampled weekly for analysis of bulk organic parameters DOC and UVA_{254}. LC-OCD and Nitrate-N were measured sporadically. Dissolved oxygen was monitored by optical fiber measurement in flow through cells. Analytics for trace organic compounds at BWB laboratories were conducted every three weeks in the first and biweekly in the second experiment.

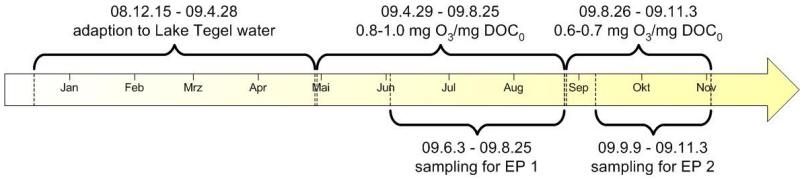

Figure 2.2: Schedule for laboratory scale column experiments

2.3 Ozonation for laboratory-scale column experiments

Ozonation for laboratory-scale column experiments as well as biodegradation tests was conducted using an available ozonation unit at TUB. Gaseous ozone was produced from pure oxygen and directly introduced into the sample in a 4-L-semi-batch stirred tank reactor. In-gas and off-gas ozone concentration, dissolved ozone and gas flow rate were measured continuously and an ozone mass balance was set up automatically by a computer. Additionally, pH was analysed in a by-pass. An example from ozonation for laboratory-scale column experiments is presented in figure 2.3. In order to set up a complete mass balance, all off-gas ozone was stripped with pure oxygen.

For the weekly ozonation of column influents a 13-L-reactor was purchased. Smaller sample volumes (column effluent TUB, preozonated column effluent) were still ozonated in the 4-L-reactor in two seperate batches. Ozonation was conducted without temperature adjustment at $20 - 25\,^{\circ}C$.

Ozone consumption, pH and total inorganic carbon (TIC) from ozonation for lab-scale column experiments are presented in table 2.2. Target ozone consumption was set to $0.8 - 1.0\ mg\ O_3/mg\ DOC_0$ and $0.7\ mg\ O_3/mg\ DOC_0$ in experiment 1 and 2, respectively. Since the consumption of ozone in the water is affected by many factors, applied ozone doses were variable and an exact adjustment was not feasible. The content of total inorganic carbon slightly decreased during ozonation whereas pH values slightly increased. Both effects are likely due to stripping of inorganic carbon.

Table 2.2: Ozone consumption, pH and total inorganic carbon during ozonation for laboratory-scale column experiments (averaged values)

experiment 1						
	ozone consumption		pH		total inorganic carbon	
	mg/L	$mgO_3/mgDOC_0$	before ozonation	after ozonation	before ozonation	after ozonation
secondary effluent	9.8 ± 0.6	0.88 ± 0.09	7.7 ± 0.1	8.1 ± 0.1	54.3 ± 7	51.7 ± 6.5
surface water	6.6 ± 0.2	0.94 ± 0.05	8.2 ± 0.1	8.4 ± 0.1	39 ± 1.5	38.3 ± 1.4
column effluent TUB	5.2 ± 0.4	0.85 ± 0.08	8.4 ± 0.1	8.6 ± 0.0	39.6 ± 1.8	38.8 ± 1.9
column effluent UBA	4.7 ± 0.3	0.95 ± 0.06	8.3 ± 0.1	8.4 ± 0.1	42.3 ± 0.7	41.7 ± 0.6
preozonated column effluent	2.9 ± 0.3	0.77 ± 0.1	8.5 ± 0.1	8.6 ± 0.1	41.9 ± 0.8	41.2 ± 0.9
experiment 2						
	ozone consumption		pH		total inorganic carbon	
	mg/L	$mgO_3/mgDOC_0$	before ozonation	after ozonation	before ozonation	after ozonation
secondary effluent	7.5 ± 0.4	0.71 ± 0.05	7.6 ± 0.2	8.0 ± 0.3	49.5 ± 6.7	47.6 ± 6.6
surface water	4.4 ± 0.3	0.69 ± 0.05	8.2 ± 0.2	8.4 ± 0.2	37.7 ± 0.3	37.1 ± 0.3
column effluent TUB	3.6 ± 0.3	0.69 ± 0.06	8.4 ± 0.2	8.6 ± 0.2	37.6 ± 0.4	37.1 ± 0.3
column effluent UBA	3.6 ± 0.2	0.73 ± 0.07	8.5 ± 0.1	8.5 ± 0.1	41.3 ± 0.4	41 ± 0.4
preozonated column effluent	2.1 ± 0.2	0.57 ± 0.05	8.5 ± 0.1	8.7 ± 0.1	41.3 ± 0.4	40.8 ± 0.3

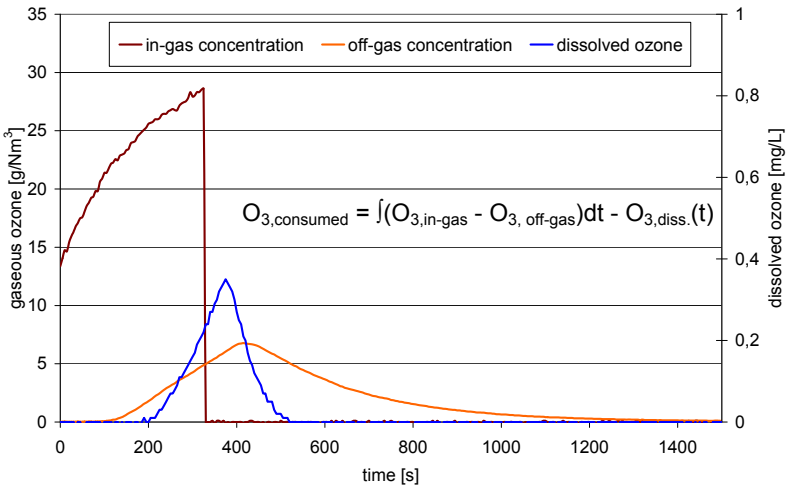

Figure 2.3: Ozonation of surface water for laboratory-scale column experiments (2009-08-26)

2.4 Technical scale column experiments

The schedule for the technical-scale columns is presented in figure 2.4. Columns have been operated with water from Lake Tegel since December 2008. Ozonation and spiking of trace substances started in July 2009. However, in September the ozone generator stopped working for five days and surface water without pre-ozonation was introduced into the columns. Therefore, it was decided to restart experiments in September.
Since retention times are around 30-45 days, experiments will be ongoing in OXIRED-2. Additionally, a second experiment with ozonation after the first column will be conducted in OXIRED-2 to simulate scheme B with ozonation of bank filtrate. For this reason, results from technical-scale experiments will be discussed in OXIRED-2.

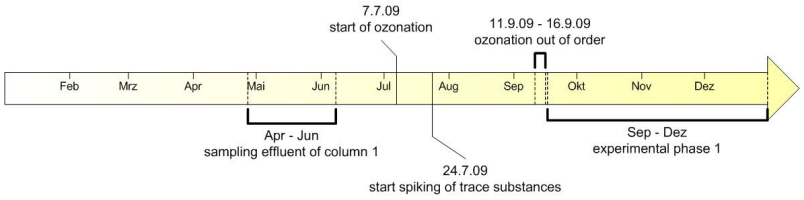

Figure 2.4: Schedule for technical scale column experiments

Table 2.3: Qualities of operated waters(n.a.: not analysed; 1) data from lab-scale column ($n > 20$) and R_{CT} experiments ($n = 1$); 2) data from 3 sampling campaigns in September and October ($n = 3$); 3) data from lab-scale column experiments ($n = 8$)

	DOC^1 mg/L	TIC^1 mg/L	UVA_{254}^1 m^{-1}	pH	$Bromide^2$ $\mu g/L$	$NO_3 - N^3$ mg/L
secondary effluent						
WWTP Ruhleben	11.1 ± 1.1	53.3 ± 7.5	27.5 ± 2.6	7.6 ± 0.2	89 ± 26	11.3 ± 2.6
surface water						
Lake Tegel	6.8 ± 0.5	39.2 ± 1.9	15.3 ± 1.0	8.2 ± 0.1	74 ± 12	1.9 ± 0.3
bank filtrate						
column effluent TUB	5.7 ± 0.5	39.5 ± 2.2	13.0 ± 1.2	8.4 ± 0.1	109 ± 27	2.0 ± 0.3
column effluent UBA	5.2 ± 0.4	41.9 ± 0.7	12.1 ± 0.4	8.4 ± 0.1	82 ± 5	2.6 ± 0.2
Well 20	5.8	49.1	13.9	7.8	n.a.	n.a.
preozonated column effluent	3.7 ± 0.3	41.7 ± 0.7		8.5 ± 0.1	103 ± 8	2.7 ± 0.2
tap water	5.0	49.5	10.2	8.4	n.a.	n.a.

2.5 Qualities of operated waters

The major quality parameters of the different waters used for experiments are presented in table 2.3.
Surface water was taken from Lake Tegel, Berlin, at the groundwater recharge facility. For the laboratory scale column experiments it was filtered through a microsieve (taken from the groundwater recharge facility in Tegel) in order to remove particles before ozonation and infiltration. Secondary effluent was taken from WWTP in Berlin Ruhleben and also filtered through a microsieve.
Bank filtrate was taken from extraction well 20 at the groundwater recharge facility in Berlin Tegel. Hydraulic retention time from the infiltration pond to the well is approximately 50 days [2]. Since water from the well was anaerobic and coagulation of iron was observed during batch experiments, it was not used for column experiments.

As simulated short bank filtrate for laboratory-scale column experiments, effluent from the first column from technical-scale experiments (column effluent UBA, appr. 7 days retention time) was collected prior to ozonation experiments. The water was stored at approximately $15\,^{\circ}C$.
Additionally, effluents from the columns 2 (column effluent TUB) and 4 (preozonated column effluent) were operated in laboratory scale experiments. Tap water was used as a reference in R_{CT}-experiments.

2.6 Analytics

2.6.1 Analytics of bulk organics and redox conditions

Samples for bulk organic analysis were filtered through a 0.45 μm cellulose nitrate filter. DOC and TIC were determined with an Elementar HighTOC analyser (Hanau, Germany). UV absorbance at 254 nm was measured using a Perkin-Elmer photometer Lambda 12 (Berlin, Germany). For the LC-OCD, a liquid chromatography method that quantitatively distinguishes between different fractions of DOC, a system of the DOC-Laboratory Dr. Huber (Karlsruhe, Germany) was used.
The anions nitrate, sulfate and bromide were detected by ion chromatography. Dissolved oxygen was analysed online by optical fiber measurement using the OXY-4 mini from PreSens GmbH (Regensburg, Germany).

2.6.2 Analytics of trace organic compounds (BWB)

Substances to be targeted within experiments were determined in WP 1. Analytics are available at BWB for the compounds presented in table 2.4. All targeted compounds for technical-scale and lab-scale experiments were spiked at 1 $\mu g/L$. EDTA was not spiked and analysed due to its high limit of quantification. The compound desethylatrazine was analysed as a metabolite of atrazine transformation and therefore not spiked.

Additionally, adsorbable organic bromide, chloride and iodide were analysed at BWB laboratories using the method DIN EN ISO 10304-1/2.

Table 2.4: Available measurement at BWB laboratories and spiking concentration

name	LOQ (limit of quantification	spiking concentration
MTBE	0.03 $\mu g/L$	1 $\mu g/L$
sulfamethoxazole	0.03 $\mu g/L$	1 $\mu g/L$
EDTA	2 $\mu g/L$	–
ETBE	0.03 $\mu g/L$	1 $\mu g/L$
carbamazepine	0.02 $\mu g/L$	1 $\mu g/L$
bentazone	0.01 $\mu g/L$	1 $\mu g/L$
atrazine	0.05 $\mu g/L$	1 $\mu g/L$
desethylatrazine	0.05 $\mu g/L$	–
linuron	0.05 $\mu g/L$	1 $\mu g/L$
diuron	0.05 $\mu g/L$	1 $\mu g/L$
diclofenac	0.01 $\mu g/L$	1 $\mu g/L$

2.6.3 Data evaluation

The determination of statistical significance was conducted using the Student T-test in Microsoft Excel. The level of significance was set to $\alpha = 0.05$. Removal rates (r) were

calculated using the equation $r = 1 - c/c_0$, with $c = LOQ$ for compounds removed below the limit of quantification (LOQ).

2.7 Biological test systems

There is scientific evidence that ozone has a great potential for degrading water pollutants, but at the same time ozone generates by-products. Some of them are known to be of environmental and health concern. The objectives of the biological part of the project were i) to assess possible toxic effects of chemical reactions that occur during ozonation and ii) to identify a suitable assay to monitor adverse effects of oxidation by-products.

Test batteries of bioassays can be used as screening tools to provide toxicity profiles. Especially in vitro assays with specific toxicological endpoints are promising tools for hazard assessment. Time and budgetary limitations exclude the use of a large battery of toxicity tests for routine screening of environmental samples, therefore a limited number of tests that are technically simple, standardized, fast, ecologically representative and reproducible are necessary. Genotoxicity and cytotoxicity tests are certainly among the recommended tests because they meet most of the aforementioned requirements.

2.7.1 Genotoxicity

The indentified risks and consequential reaction in environmental and healthcare policy make the handling of the topic "genotoxicity" a priority research area, and demonstrate an immediate need for regulatory measures with the aim of minimizing exposure to genotoxic compounds or, when technically possible, avoiding exposure altogether.

The first significant step is the identification of genotoxic impact. The aim of the first stage must be the positive detection of genotoxic potential in the water sample by using two or three short-term procedures. The objective of the basic test is achieving qualitative conclusions on the genotoxicity of a sample in a sense of YES or NO.

The most widely recommended initial genetic toxicology battery includes the bacterial reverse gene mutation assay (*Salmonella typhimurium* reverse mutation assay, Ames test), and an in vitro mammalian cell cytogenetic analysis (micronucleus assay).

Ames/ Salmonella Microsome Assay

One of the few tests that is recommended for routine screening is the Ames test.

Test principle: Bacterial assays belong to the basic set of tools for genotoxicity testing of chemicals and environmental samples. In the following we take a detailed look at the Ames/Salmonella microsome assay.

The *Salmonella typhimurium* reverse mutation assay is a true mutation test. Because of mutagenetic changes, the Salmonella strains used in this test require the amino acid histidine for growth (his^-). In addition, these strains contain mutations that increase the bacteria's sensitivity to some genotoxic agents. The rfa-mutation

increases the cell membranes‘ permeability against larger, particularly hydrophobic molecules. The uvrB-mutation causes the failure of an enzyme system that resects certain DNA damages. In the presence of genotoxic agents, reverse mutations to the wild-type (his^+-phenotype) frequently occur. These reverse mutations grow on agar plates containing mere traces of histidine, and can grow up to colonies, which can then be counted. The number of reverse mutations serves as a measure of genotoxicity. Different Salmonella strains were used to identify different types of mutagens or mutation types. In the standardized version of the Salmonella typhimurium reverse mutation test, strain TA 98 is used to detect frameshift mutations, while strain TA 100 is used to detect base substitution mutations.

Test protocol: Ames test was following the standard protocol of Maron and Ames (1983). The test procedure is shown in figure 2.5. Briefly, the standard plate incorporation assay was performed with Salmonella typhimurium TA 98 and TA 100 with (+S9) and without (-S9) in vitro extracellular microsomal activation (by S9 rat liver enzyme homogenate).
0.1 ml of a stationary overnight culture (ca. $1 - 2^8$ viable cells) was incubated with different volumes (0.25 ml with S9-mix and 0.5 ml without S9-mix) of the test samples, 0.5 ml S9-mix and 2 ml molten top agar and poured onto each selective agar plate. In experiments without metabolic activation, the S9-mix was replaced by phosphate buffered saline (PBS). Two plates were prepared in parallel for each experimental point and each experiment was repeated twice. For each tester strain a specific positive control was used: TA 98 without S9-mix: 4,6-dinitro-o-cresol (DNOC), 100 $\mu g/plate$; TA 98 with S9-mix: 2-acetamidofluorene (2-AAF), $50\mu g/plate$; TA 100 without S9-mix: bis(2-chlorethyl)-ammoniumchloride, 100 $\mu g/plate$ and TA 100 with S9-mix: 2-aminofluorene (2-AF), 100 $\mu g/plate$. Following a 48 hours incubation at 37 ° C in the dark for 2 days genotoxic activities were expressed as induction factors. The induction factor was calculated by using the following equation:

$$Induction\ factor\ (IF) = \frac{number\ of\ revertants\ of\ the\ test\ sample}{number\ of\ revertants\ of\ the\ negative\ control}$$

A genotoxic potential is assumed if the induction factor is higher than 1.2.

Micronucleus Assay

The micronucleus test will become the third internationally standardised test method for genotoxicity in water samples and will be a eukaryotic complement to the bacterial umu- and Ames test that had been published as standards already.

Test principle: The micronucleus assay has emerged as one of the preferred methods for assessing chromosome damage. A micronucleus is formed when, during cell division, a chromosome or chromosome fragment becomes separated from the spindle and therefore is not incorporated into one of the daughter nuclei. It remains in the cytoplasm and is encapsulated to form a small nucleus (figure 2.6).

Test procedure: Human HepG2 cells were kindly provided by S. Knasmüller (University Vienna, Austria). The cells were cultivated in MEM supplemented with 10%

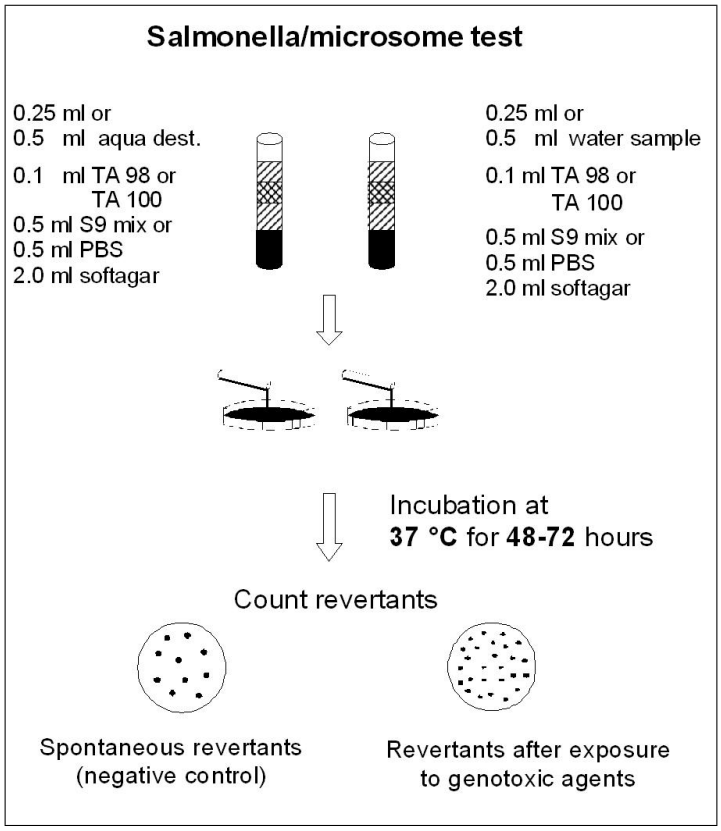

Figure 2.5: Flow chart of the Salmonella/microsome test (Ames test)

FCS. Five millilitres of the cell suspension – adjusted to a cell density of 30.000-80.000 cells per culture – were spread on microscope slides that were kept in chambers of QuadriPERM-dishes (QP, Greiner GmbH, Germany), so that each single chamber represented a separate culture. Two cultures were prepared for each test group (water samples or the control items). After seeding, the QP dishes were kept for 6 h in an incubator in order to ensure cell attachment. Then the culture medium was replaced by 4 ml of fresh MEM with 10% FCS (test without S9-mix). Water samples, negative (distilled water) and positive control (demecolcine 0.075 $\mu g/ml$) items were added at a volume of 1 mL, so that the resulting end volume was 5 mL per culture. The cultures were incubated for 24 h with the test and control items. Cells were then rinsed twice with Hank's balanced salt solution (with Ca^{2+} and Mg^{2+}; Biochrom AG, Germany), supplied with fresh medium with 10% FCS, and incubated for another 20 h. Incubation was followed by hypotonic treatment of the cells with 5 mL of 1.5% trisodium citrate solution per culture. Subsequently, the cells were fixed twice with a fixing liquid (150 mL ethanol, 50 mL glacial acid, and 2.5 mL of 37% formaldehyde) on the slides

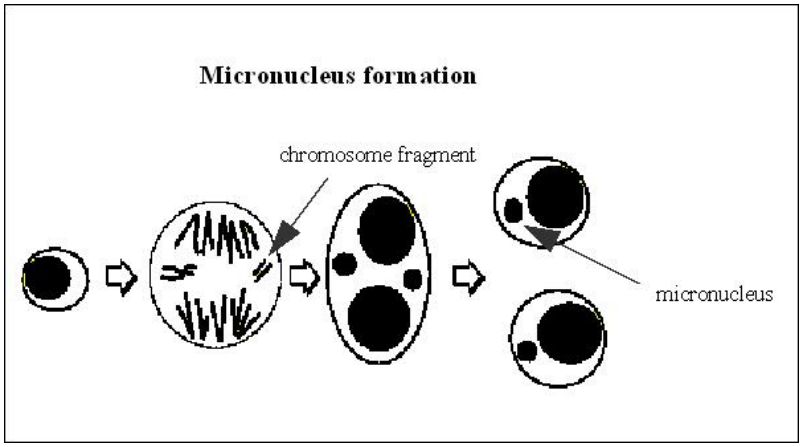

Figure 2.6: Mechanism of micronucleus formation

and stained with DAPI (Roth, Germany). For longer archiving, after evalution the slides were covered with Entellan (Merck, Germany). Each water sample was analysed once, without repetition.

At least 1000 cells of each culture had to be evaluated. The following criteria were applied for the classification as a micronucleus: the dimension of the micronucleus should not exceed 30% of the dimension of the normal cell nucleus, the micronucleus and the cell nucleus should show the same appearance in terms of colour, and the micronucleus had to be separated distinctly from the main nucleus. Only cells with a good cytoplasmatic contour were included in the evaluation. Taking into account variability of the micronuclei frequency the observed range of the negative controls in all series of experiments (historical control) was used as an aid when judging the relevance of effects. A sample was called positive if the mean limit of the observed range of the negative controls had been exceeded. The number of nuclear buds (NB) was also recorded providing additional important mechanistic information. The use of this additional information improves the predictive capacity of the assay. Cells with nuclear buds have nuclei with an apparent sharp constriction at one end of the nucleus suggestive of a budding process, i.e. elimination of

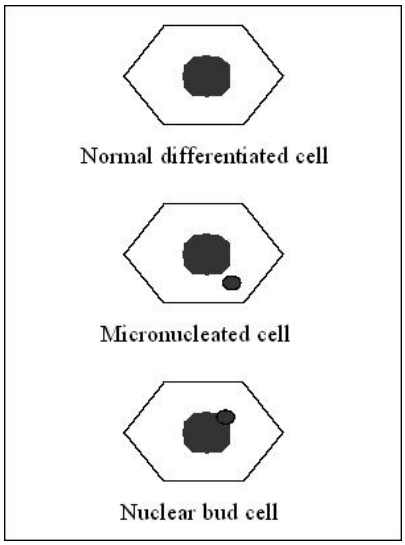

Figure 2.7: Typical example of micronuclei and nuclear buds

nuclear material by budding (figure 2.7). The nuclear bud and the nucleus are usually close proximity and are apparently attached to each other. The nuclear bud has the same morphology and straining properties as the nucleus, however its diameter may range from a half to quarter of that of the main nucleus. The mechanism leading to this morphology is not known but it may due to elimination of amplified DNA or DNA repair complexes.

2.7.2 Cytotoxicity

Early warning monitoring of environmental samples for genotoxicity as a parameter of first priority typically includes an analysis for mutagenicity in bacteria (Ames test) and for clasto- genicity in cultured mammalian cells (micronucleus assay). In addition an early assessment of cytotoxicity has become increasingly important. It is well established that cytotoxicity induced adverse effects, including secondary genotoxicity.

For cytotoxicity testing two procedures were applied, first the research of the glucose consumption rate, second the generation of reactive oxygen species. Research of glucose consumption rate produced results with regard alterations of proliferating and viability of cultured cells during long-term exposure to the test samples. Alterations of the generation of reactive oxygen species allowed an assessment with regard to the development of oxidative stress. Indirect mechanisms (e.g. oxidative damage) may be involved.

Glucose consumption rate

Test principle: Many cultured cells continually consume glucose from their culture medium because glucose is necessary for metabolic processes. By knowing initial glucose concentration in the media this method measures the overall metabolic activity of cells and reflects changes in viability and proliferation. This test is particularly suitable for cells with high metabolic activity and for extended sample exposure times. Cells are not exposed to any assay reagents and remain fully viable. Further more the test is used for kinetic and endpoint studies.

Test procedure: A suspension of HepG2 cells (5×10^5 $cells/ml$) in RPMI medium was seeded in a 96-well plate (200 μL per well). After 3 h incubation for adherence 100 μL of the test water samples or 100 μL of the water control (entionized water) are added per well. As a positive control cells were exposed to 1 μM Staurosporin (final concentration). Cells were treated with the individual samples for 72 h in the CO_2 incubator. The amount of remaining glucose in the cell culture medium was measured spectrophotometrically with the ELISA reader "Rainbow", Tecan using a testkit (GOD / POD method, Xenometrix). 100% glucose were measured in wells with cell culture medium and without cells. The reagents of the testkit utilize the coupled activities of glucose oxidase (GOD) und peroxidase (POD).

$$\text{D-glucose} + O_2 + H_20 \xrightarrow{GOD} \text{D-glucono-1,4-lactone} + H_2O_2$$

$$H_2O_2 + \text{dye-}H_2 \text{ (colorless)} \xrightarrow{POD} \text{dye (colored)} + H_20$$

50 μL of the supernatant of the 96 well plate were mixed with the GOD/POD reagent from the kit and incubated for 30 minutes. The reaction was stopped by adding 100 $\mu L/well$ 12 N HCl. The amount of oxidized dye in the supernatant was measured spectrophotometrically at 540 nm.

Measurement of Reactive Oxygen Species (ROS) generation

Test principle: Under normal conditions in cells one source of reactive oxygen species is the leakage of activated oxygen from mitochondria during oxidative phosphorylation. In mitochondria of cells O_2 is reduced by cytochrome c oxidase to form water. But this enzyme can release partly reduced species results in the production of free radicals (figure 2.8).

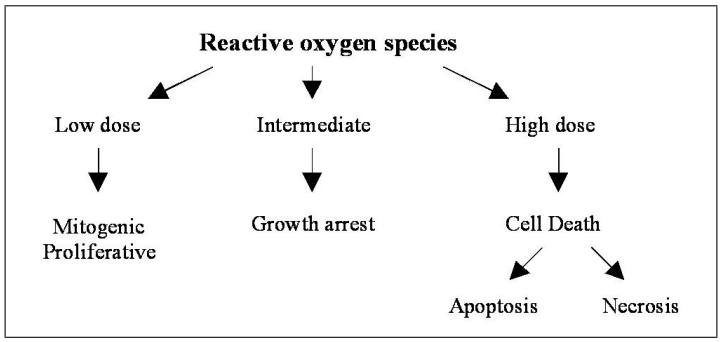

Figure 2.8: Variation of effects of reactive oxygen species on biological structures in dependence of their concentrations

Other respiratory chain enzymes can also produce partly reduced oxygen species including superoxide. These reactive oxygen species can also react with nitric oxide to produce reactive nitrogen species including peroxynitrite. Some of the important reactive oxygen species are shown in the following overview:

Table 2.5: Reactive oxygen species

Reactive Oxygen Species	structure
Hydrogen peroxide	H_2O_2
Hydroxyl radical	$HO\cdot$
Hypochlorous acid	$HOCl$
Nitric oxide	NO
Peroxyl radical including both alkylperoxyl and hydroperoxyl	$ROO\cdot$
Peroxynitrite anion	$ONOO^-$
Singlet oxygen	$^1O_2$
Superoxide anion	$\dot{O}_2{}^-$

For measurement of reactive oxygen species Dihydroethidium was chose, which is commonly used to analyze respiratory burst in phagocytes. Oxidation of dihydroethidium by reactive oxygen species results in ethidium. This oxidized ethidium intercalates within DNA, staining the cell nucleus to fluorescere red.
A significant proportion of the reactive oxygen and nitrogen species diffuse with controlled rate into the cytosol, where they react with various molecules, lipids, proteins, sugars and nucleotides. But a major portion remains in the mitochondria where they cause oxidative damage. By an inbalance between the production of reactive oxygen species and ability of biological systems to detoxify the reactive intermediates or easily repair the resulting damage oxidative stress is caused. Enhanced oxidative stress occurs in number degenerative diseases in human and wildlife.

Test procedure: A suspension of JURKAT cells (1×10^6 $cells/ml$) in RPMI medium was seeded in a 24-well plate (0.5 mL per well). 500 μL of the test water samples or 500 μL of the water control (entionized water or tap water, Bad Elster) were added per well. Positive control were cells exposed to various concentrations of the antibioticum staurosporin (final concentrations 0.5 μM and 0.3 μM). Cells were treated with the individual samples for 24 hours in a CO_2 incubator. After incubation cells were centrifuged, washed one time with phosphate buffered saline and resuspended with 1 $mL/well$ RPMI medium without phenolred. Intracellular generation of reactive oxygen species was measured using Dihydroethidium, Calbiochem. Cells were stained with 5 μM Dihydroethidium (final concentration) for 30 minutes at 37 °C. Ethidium fluorescence intensity resulting from dihydroethidium oxidation by ROS was measured using a FACS Calibur flow cytometer (BD Biosciences, Heidelberg). FL2 fluorescence of 10,000 cells was collected in each experiment. Experiments were repeated three times.

2.7.3 Sampling for biological tests

From laboratory-scale column experiments, samples for biological tests were collected twice during the first and once during the second experiment. Additionally, column effluents were sampled before starting the ozonation experiments during operation with surface water.
Biological tests comprised the analysis of all column influents and effluents. Column effluents were sampled after one week in order to analyse the same slug of water. In addition, ozonation experiments with surface water, column effluent UBA and secondary effluent were conducted without spiking of trace compounds to evaluate the effects of spiked compounds. Finally, the stock solution for spiking was tested.

3 Results

3.1 Characterisation of ozonation in different waters

To characterize kinetics of ozone decomposition and radical formation, R_{CT}-experiments were performed with surface water from Lake Tegel, bank filtrate from well 20, secondary effluent from Berlin, Ruhleben and tap water from TUB. The R_{CT}-concept described by Elovitz and von Gunten (1999) [1] is a standardized method to evaluate ozone depletion and the formation of radicals in a selected water. Presented results in this chapter are averaged values from duplicates.

Normalized ozone decomposition in different waters is presented in figure 3.1. Decomposition is significantly affected by DOC_0 of the water. Highest k_{O3} values were observed in experiments with secondary effluent, whereas ozone decomposition in tap water was comparatively low. Decomposition of surface water and bank filtrate was

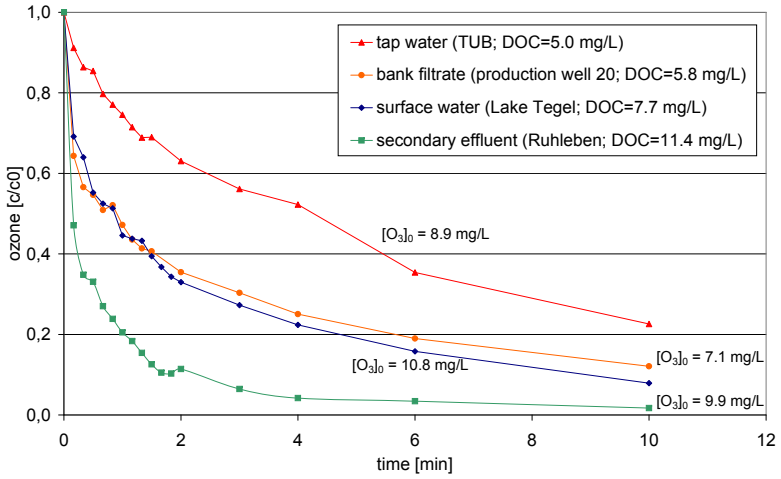

Figure 3.1: Normalized ozone decomposition in different waters

rather similar. In the beginning, k_{O3} was even higher in experiments with bank filtrate. This is likely due to reduced inorganic components in water from well 20, which instantaneously consume ozone. In a pilot-scale ozonation plant these components would be removed prior to ozonation by aeration and rapid sand filtration. Another explanation for the high reduction rate of ozone in bank filtrate can possibly be the

Table 3.1: Calculated k_{O3} of different source waters

	$0-10\ s$	$10-120\ s$		$120-600\ s$	
	k_{O3}	k_{O3}	R^2	k_{O3}	R^2
tap water	9.3×10^{-3}	3.4×10^{-3}	0.975	2.2×10^{-3}	0.988
bank filtrate	4.4×10^{-2}	3.9×10^{-3}	0.971	2.2×10^{-3}	0.99
surface water	3.7×10^{-2}	6.5×10^{-3}	0.979	3.0×10^{-3}	0.999
secondary effluent	7.5×10^{-2}	1.4×10^{-2}	0.966	3.6×10^{-3}	0.911

low initial O_3-concentration within those experiments. Results from k_{O3}-calculation are presented in table 3.1.

Figure 3.2 shows the results from pCBA-measurements. Similar to ozone decomposition removal rate of pCBA increases towards higher DOC_0 in the water. However, no significant difference between surface water and secondary effluent was observed. This is likely due to a shortage of ozone in experiments with secondary effluent.

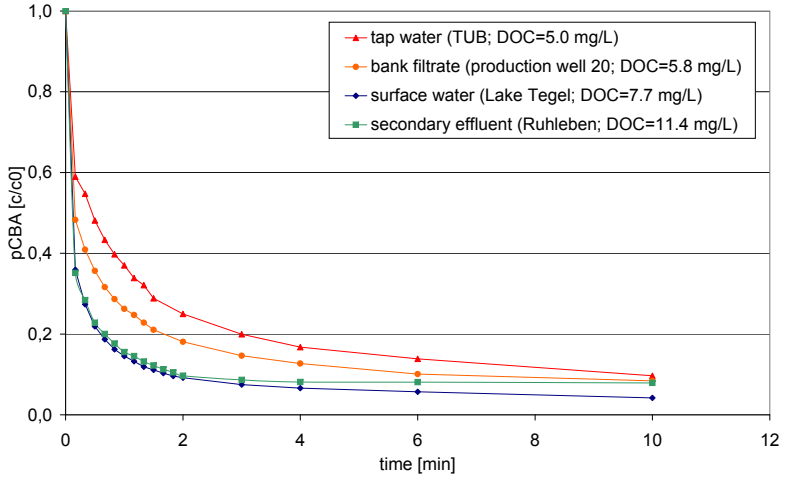

Figure 3.2: Removal of pCBA by ozonation of different waters ($pCBA_0 = 0.5 mg/L$)

Calculated R_{CT}-values are presented in table 3.2. Results for bank filtrate, surface water and secondary effluent are within the same order of magnitude. Kinetics of radical formation in tap water significantly differs from other waters. DOC in secondary effluent from Ruhleben, Lake Tegel water and bank filtrate is of similar origin. It is assumed that formation and degradation of DOC within the surface water and during bank filtration does not affect its ability to promote radical formation. However, since ozone concentration differs significantly between experiments and kinetics for secondary effluent seem to be limited by ozone exposure, results need to be evaluated

Table 3.2: Calculated R_{CT} for different source waters

	$0-10\ s$	$10-120\ s$		$120-600\ s$	
	R_{CT}	R_{CT}	R^2	R_{CT}	R^2
tap water	5.8×10^{-8}	1.1×10^{-8}	0.993	5.0×10^{-9}	0.993
bank filtrate	1.2×10^{-7}	2.5×10^{-8}	0.986	9.9×10^{-9}	0.976
surface water	1.0×10^{-7}	2.2×10^{-8}	0.978	7.8×10^{-9}	0.991
secondary effluent	1.3×10^{-7}	5.0×10^{-8}	0.998	9.3×10^{-9}	0.81

carefully. R_{CT}-values can be used for modelling of investigated trace substances with known k_{O3} and k_{OH}.

In summary, the experiments have shown that ozonation kinetics is highly affected by the water source. Towards higher DOC concentration in the water, an increase of ozone consumption was observed. Therefore, reduction of DOC prior to ozonation, for example by bank filtration, might be efficient to minimize ozone consumption.

Since some of the targeted substances are not efficiently removed by direct ozonation, the formation of radicals is essential for their removal during ozonation. Efficiency of pCBA removal, which was used as indicator for radical exposition indicates a better removal of trace substances during ozonation of water with high DOC. However, transferring results from batch experiments to ozonation with gaseous ozone is rather problematic and experiments are essential to revise the results.

Characterisation of ozonation

- All investigated waters can be characterized as highly reactive towards ozone with high potential to promote OH-radical formation.
- Consumption of ozone and promotion of radical formation depends on the DOC in the water.
  - Reaction rates with ozone ($t = 10 - 120\ sec.$) ranged from $k_{O3} = 3.4 \times 10^{-3}$ to $k_{O3} = 1.4 \times 10^{-2}$ in tap water and secondary effluent, respectively.
  - R_{CT}-values ($t = 10 - 120\ sec.$) ranged from $R_{CT} = 1.1 \times 10^{-8}$ to $R_{CT} = 5 \times 10^{-8}$ in tap water and secondary effluent, respectively.

3.2 Redox conditions in soil columns

To characterize redox conditions in laboratory-scale soil columns, dissolved oxygen (DO) was measured directly at the inlet and outlet of the columns and Nitrate-N was analysed in influent and effluent samples.

Evaluation of DO measurements was problematic because 1) hydraulic problems led to introduction of oxygen in column effluent flowthrough cells, 2) some results showed higher concentration of DO in column effluents compared to influents and 3) ozonation caused strong weekly variations of DO in column influents. As example for weekly variations, figure 3.3 illustrates analysis of DO in influent and effluent of column 3. Freshly ozonated water is highly oversaturated with oxygen concentrations up to $40 mg/L$. Within a week, concentration of DO in the container levels with ambient air saturation. In column effluents, a breakthrough of oxygen can be observed approximately after six days of retention time. Concentration in column effluents is significantly lower than in column influents, which is probably due to consumption in the column. However, a quantification of DO consumption is not feasible since results in column influents are out of the calibration range.

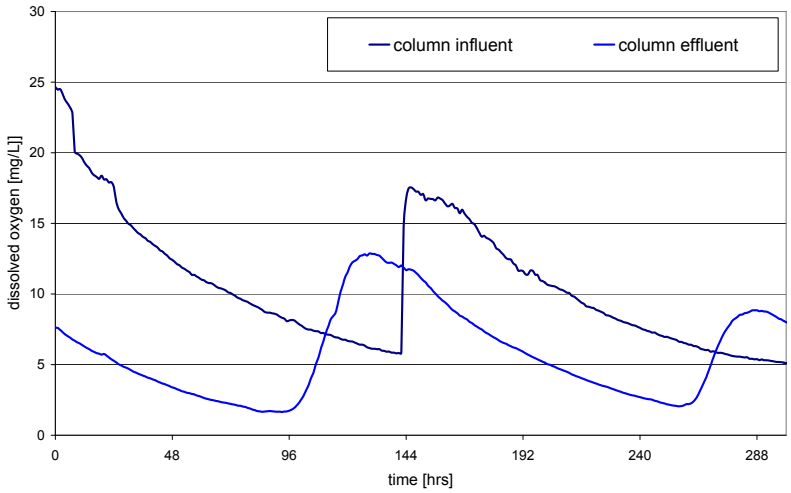

Figure 3.3: Results from continuous measurement fo dissolved oxygen in influent and effluent of column 3 (operated with column effluent TUB after ozonation)

Since DO in ozonated column influents was highly variable, effluent concentration underlied strong weekly variations. In column effluents operated with pre-ozonated surface water and secondary effluent, DO partly decreased to 1.0 mg/L. In all other columns, aerobic conditions were maintained with concentrations above 2.0 mg/L. DO concentrations in influents of the reference columns without pre-ozonation ranged from 3.5 to 8 mg/L.

Concentration of Nitrate-N in column influents ranged from 1.7 mg/L to above 10 mg/L in surface water and secondary effluent, respectively. During laboratory-scale experiments, Nitrate-N was analysed three times in the first and three times in the second experiment. No consumption of Nitrate-N was observed within the soil columns. As a summary it can be stated that laboratory-scale column experiments were conducted under aerobic conditions.

Redox conditions

- Dissolved oxygen in ozonated column influents was highly variable due to weekly ozonation.
- No reduction of Nitrate-N observed in soil columns
- Aerobic conditions in all soil columns during experiments

3.3 Bulk organic carbon

3.3.1 Transformation during ozonation

During ozonation of water with high DOC, ozone rapidly reacts with organic matter. In figure 3.4, results from batch ozonation of surface water with different ozone doses are presented. Whereas UV absorbance is quickly reduced, DOC is not significantly changed in the beginning of the reaction. A significant reduction of DOC by ozonation could only be observed with specific ozone doses higher than 1.0 $mg\ O_3/mg\ DOC_0$. This confirms the assumption that DOC is only transformed but not mineralised during ozonation. Additionally, it can be stated that ozone reacts very selective towards organic double bonds and aromatic structures.

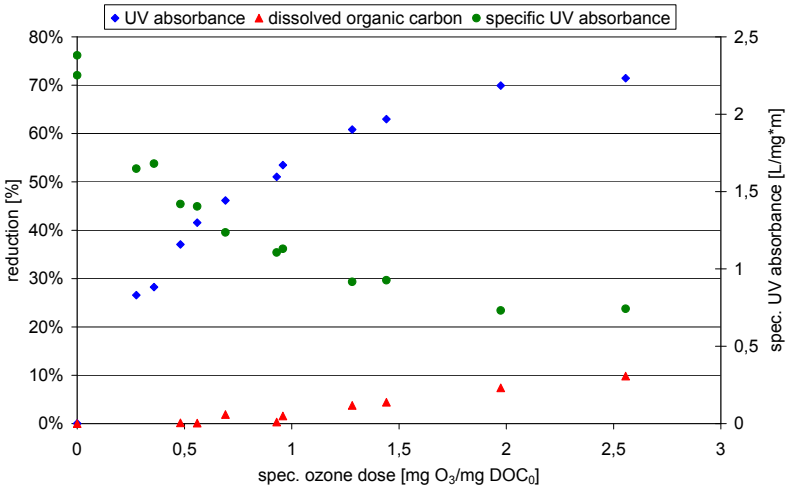

Figure 3.4: Reduction of DOC and UVA during ozonation of surface water (results from batch experiments)

Results from organic carbon detection (OCD) and UV detection (UVD) of LC-OCD measurements are presented in figures 3.5 and 3.6, respectively. Chromatograms

show the ozonation of different waters in the first experiment. Characteristic for the ozonation of dissolved organic matter is a formation of building blocks from humic substances, which can be observed after ozonation of all different waters. The fraction of biopolymers is not significantly affected by ozonation since biopolymers are predominantly aliphatic and not selectively attacked by ozone. Response of the UV signal was strongly reduced by ozonation confirming the high affinity of ozone to aromatic and unsaturated organics.

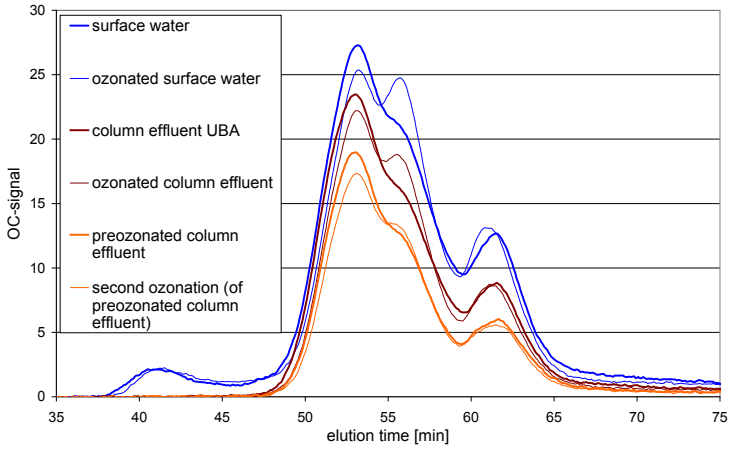

Figure 3.5: LC-OCD chromatograms from lab-scale ozonation (organic carbon detection; spec. ozone dose: appr. 0.9 $mg\ O_3/mg\ DOC_0$; data from 09-07-22)

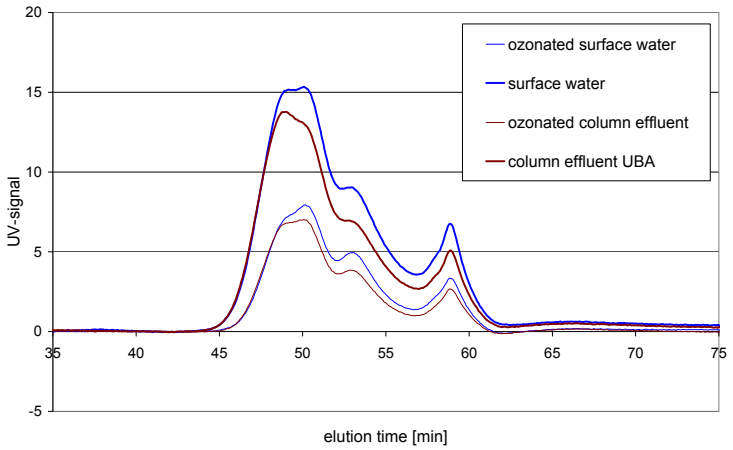

Figure 3.6: LC-OCD chromatograms from lab-scale ozonation (UV detection; spec. ozone dose: appr. 0.9 $mg\ O_3/mg\ DOC_0$; data from 09-07-22)

3.3.2 Enhancement of biodegradation by pre-ozonation

Prior to column experiments, the impact of ozone dose on biodegradability was investigated in batch experiments. Therefore, surface water and bank filtrate were ozonated with different ozone doses and biodegradable dissolved organic carbon (BDOC) was analysed. Experiments were designed i) to identify a maximum BDOC formed by ozonation and ii) to derive an optimal ozone dose for ozonation in laboratory and technical-scale column experiments.

Results are presented in figure 3.7. In both waters, BDOC was significantly increased by pre-ozonation. The DOC in surface water from Lake Tegel was reduced by more than 40% during experiments. Formation of BDOC was most efficient during ozonation with low doses. With increasing ozone dose direct mineralization of DOC during ozonation increased resulting in less efficient formation of BDOC. Formation of BDOC in bank filtrate was significantly lower in comparison to surface water. The bank filtrate for batch experiments was sampled from well 20 of the groundwater recharge facility in Berlin Tegel. The water was anaerobic and during experiments, ozone was consumed for the oxidation of reduced inorganic compounds. It can be assumed that reduction of DOC in aerobic or anoxic bank filtrate would already be efficient with lower ozone doses.

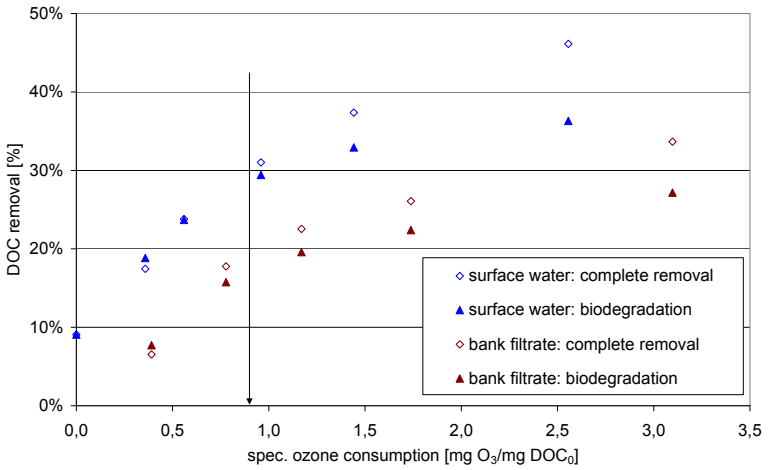

Figure 3.7: Enhancement of biodegradation by ozonation (batch experiments)

From both experiments, no ozone dose with maximum formation of BDOC could be identified. However, from results with surface water from Lake Tegel a specific ozone dose of 0.8 to 1.0 $mg\ O_3/mg\ DOC_0$ to efficiently increase formation of BDOC can be proposed. At higher ozone doses efficiency of BDOC formation significantly decreases. A similar conclusion for ozonation of bank filtrate from well 20 is not feasible since ozone was consumed by inorganic compounds. Schumacher (2005) [7] investigated BDOC formation with ozonation of effluent from a membrane bioreactor

($DOC_0 = 11.8 mg/L$) and secondary effluent from Ruhleben ($DOC_0 = 13,9\ mg/L$) and observed a maximum generation of BDOC with specific ozone consumption of 2.5 and 1.5 $mg\ O_3/mg\ DOC_0$, respectively. However, increasing consumption to values higher than 1.0 $mg\ O_3/mg\ DOC_0$ only leads to little enhancement of BDOC formation. From these results and in order to maintain comparability between different approaches, a specific ozone consumption of 0.8 to 1.0 $mg\ O_3/mg\ DOC_0$ was recommended for ozonation of all waters in laboratory-scale column experiments.

Ozonation for laboratory-scale column experiments was conducted weekly. Feed solutions were stored in 13-L containers at room temperature. To evaluate the removal in the feed container, solutions were sampled after 7 days for analysis of bulk organic parameters. Results are presented in figure 3.8 as averaged values from two measurements. DOC in surface water and column effluent UBA was stable within one week. In ozonated waters a fraction of DOC is already degraded in the feed container. However, it can be assumed that this fraction is easy biodegradable and would be removed in columns anyway. In this report, results for biodegradation in soil include preceding biodegradation in feed containers and tubing.

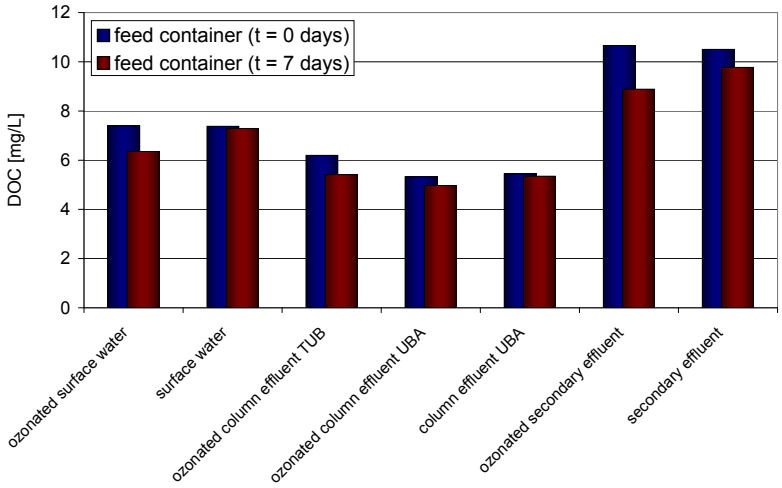

Figure 3.8: DOC removal in feed containers for column experiments

Figure 3.9 presents DOC results from columns operated with surface water over both experiment. LC-OCD chromatograms from these columns are presented in figure 3.10. In order to analyse the same slug of water column effluents were always sampled seven days after feed waters.

Both figures show a significant increase of DOC removal in the column operated with pre-ozonated water. The decrease of DOC by time is due to seasonal variations in surface water from Lake Tegel. During experiments, effluent DOC concentration decreased simultaneously to surface water DOC indicating no significant adaptation

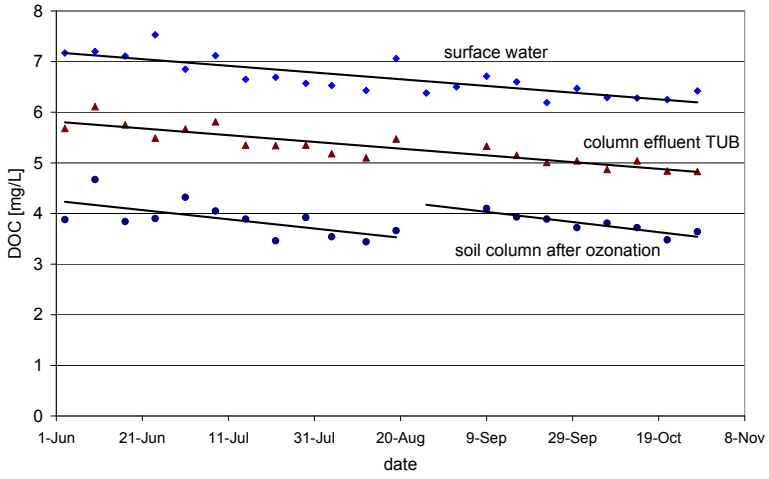

Figure 3.9: DOC results from laboratory scale columns with surface water over the entire experimental time

over time. The change of removal efficiency in the pre-treated column is caused by the start of the second experiment on Aug-26 with lower ozone doses. These results will be discussed later.

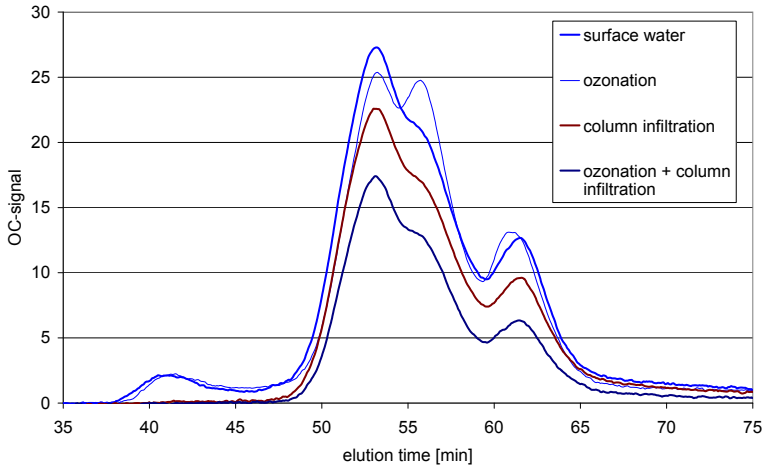

Figure 3.10: LC-OCD chromatograms from experiments with surface water (samples from 09-07-22 and 09-07-29)

According to size exclusion chromatograms ozonation does not selectively enhance removal of a specific molecular size. Biodegradation of all fractions is improved by

pre-ozonation. Results also show that the building blocks formed by ozonation are completely removed after soil passage. This confirms the assumption that many products formed by ozonation are easy biodegradable.

The biopolymers are biodegradable substances. They are completely degraded in soil columns without any pre-treatment. Thus, pre-ozonation does not improve their removal. However, most biopolymers are predominantly aliphatic. Since ozone selectively reacts with unsaturated and aromatic structures, it is assumed that consumption of ozone by biopolymers is negligible. Biological pre-treatment to remove biopolymeres, for example by slow sand filtration, is therefore not necessarily reducing ozone demand for oxidation.

In figure 3.11, DOC results from laboratory-scale column experiments with secondary effluent, surface water and bank filtrate (column effluent UBA) from both experiments are presented. In the first and second experiment specific ozone doses of 0.9 and 0.7 $mg\ O_3/mg\ DOC_0$ were applied, respectively. The results from the reference column without ozonation are averaged over the entire experiment.

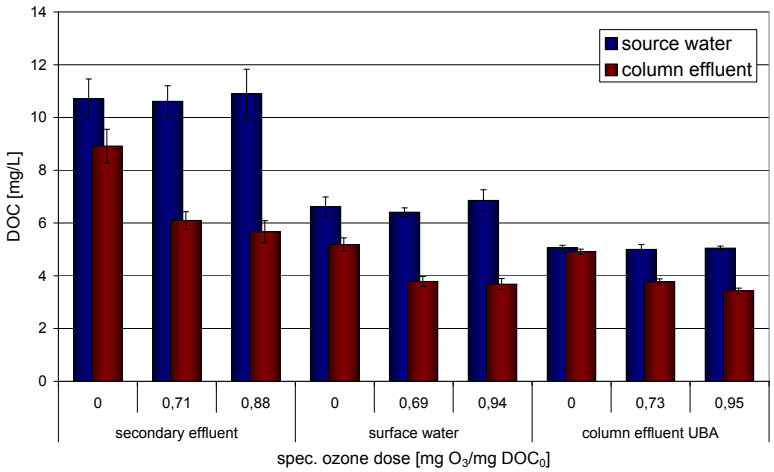

Figure 3.11: DOC results from laboratory-scale column experiments

The combination of ozonation and infiltration resulted in DOC reduction by more than 40% in surface water and secondary effluent. DOC in bank filtrate was reduced by 30%. In comparison, only about 20% of DOC from surface water and secondary effluent was removed in columns without pre-ozonation. The overall reduction of bulk organic carbon was more efficient in soil columns compared to batch experiments. This is probably due to longer adaptation and higher bacterial population in the columns.

Experiments with laboratory-scale columns confirmed the dependency of biodegradation efficiency on the specific ozone dose. Increasing the specific ozone dose for oxidation of secondary effluent and column effluent UBA from 0.7 to 0.9 $mg\ O_3/mg\ DOC_0$ resulted in significant decrease of effluent DOC concentration. In experiments with column effluent TUB (data not shown) and surface water no significant changes of

DOC concentration in column effluents were observed. However, this is likely due to the higher level of surface water DOC during experiments with specific ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$.

In summary, effects of different specific ozone doses on DOC removal were rather small compared to DOC level in the source water. It is therefore concluded that specific ozone doses higher than 0.7 $mg\ O_3/mg\ DOC_0$ are not beneficial concerning biodegradation of dissolved organic carbon. Lower doses have not been tested within OXIRED-1.

3.3.3 Benefits of a short bank filtration prior to ozonation

During laboratory-scale column experiments, two different variations for the combination of ozonation and underground passage were tested:

A) direct ozonation of surface water with subsequent groundwater recharge

B) ozonation of a short bank filtrate (column effluent) with subsequent groundwater recharge

In figure 3.12, the DOC reduction within both systems is illustrated. Two experiments were conducted with targeted specific ozone doses of 0.7 and 0.9 $mg\ O_3/mg\ DOC_0$. Numbers in the bottom of columns refer to the total ozone consumption during experiments. Presented results from the reference columns without ozonation are averaged values from both experiments.

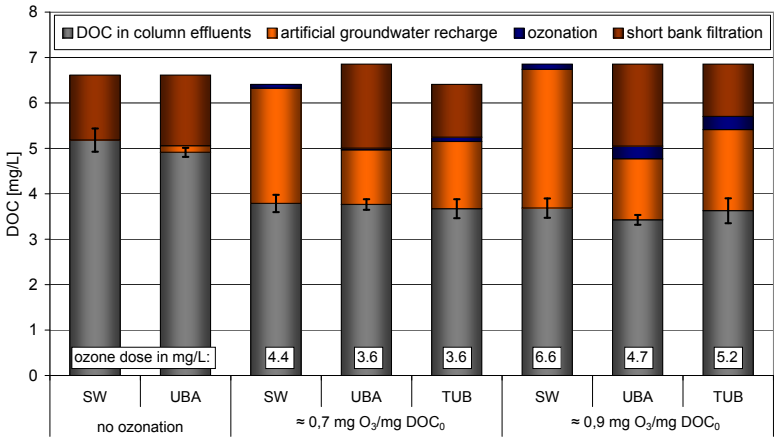

Figure 3.12: Comparing DOC reduction with different combinations of ozonation and subsurface passage (SW: surface water, UBA: column effluent UBA, TUB: column effluent TUB)

Comparing effluent concentration of the columns operated with surface water and column effluent TUB (short bank filtrate simulated at TUB) no significant differences

could be observed when similar specific ozone doses were applied. Ozonation and groundwater recharge of simulated bank filtrate from technical-scale columns (column effluent UBA) resulted in most efficient DOC removal. In the first experiment with specific ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$ effluent DOC was significantly lower compared to columns operated with surface water and column effluent TUB. Similar effluent concentration in the second experiment with specific ozone doses of 0.7 $mg\ O_3/mg\ DOC_0$ is probably due to different source water quality (column effluent UBA was collected prior to experiments when surface water DOC was relatively high). The better removal in columns at UBA is likely due to better adaptation of microorganisms since columns have been operating for several years.
The evaluation of data from both experiments with similar total ozone doses (surface water: 4.4 $mg\ O_3/L$; column effluent UBA: 4.7 $mg\ O_3/L$) demonstrates that the removal of surface water DOC can be improved by a preceding bank filtration. Surface water DOC could be reduced by $3.4 mg/L$ (50%) with simulated short bank filtration in technical-scale columns, ozonation (4.7 $mg\ O_3/L$) and subsequent soil passage. Without preceding soil passage only 2.6 mg/L (41%) were removed. However, it needs to be considered that these results are derived from two experiments with slightly diffferent source water quality and DOC concentration.

In summary, results demonstrate that ozone demand for oxidation can be significantly reduced in a preceding short bank filtration. Nevertheless, improvement of DOC removal was rather small and the relevance for drinking water treatment is arguable. Averaged DOC concentration in treated effluents ranged from 3.4 to 3.8 mg/L. Therefore it is concluded that a preceding bank filtration is no suitable treatment to efficiently improve DOC removal by ozonation and subsequent groundwater recharge. Figure 3.13 illustrates size exclusion chromatograms from experiments simulating both

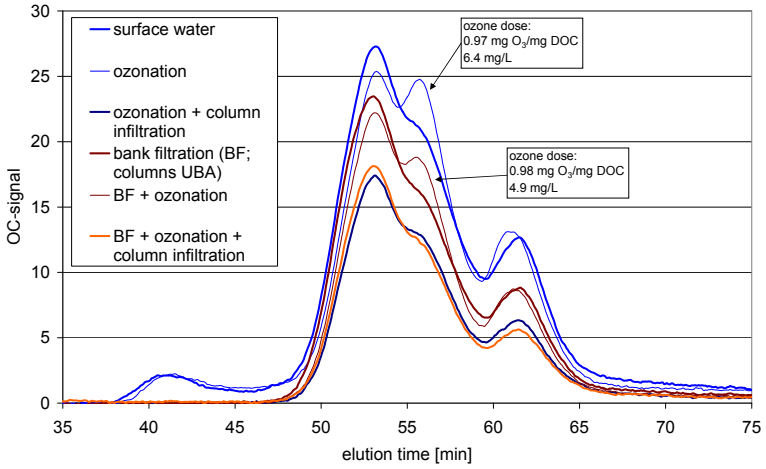

Figure 3.13: LC-OCD results from experiments with and without preceding short bank filtration (data from 09-07-22 and 09-07-29)

different variations. Results are taken from the first experiment with specific ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$. Chromatograms confirm that final DOC in both treatment systems is rather similar.

Bulk organic carbon

- DOC in the water is hardly mineralized by ozonation.
- UV absorbance at 254 nm is quickly reduced by selective reaction with ozone.
- The removal of DOC achieved with a combination of ozone and subsurface passage exceeds that of pure subsurface passage (after 6 d of travel time) by far.
  - DOC in ozonated secondary effluent was reduced by 42% ($z = 0.7$) and 47% ($z = 0.9$) compared to 17% removal during infiltration without preozonation.
  - DOC removal from surface water was increased from 22% (without ozonation) to 40% ($z = 0.7$) and 45% ($z = 0.9$).
  - In ozonated bank filtrate, 26% and 28% of DOC was degraded (3% biodegradation without ozonation).
- Recommended ozone dose for efficient DOC removal: 0.7 $mg\ O_3/mg\ DOC_0$
- With a preceding bank filtration efficiency of DOC removal could only slightly be improved (up to 50% overall removal in comparison to 45% with direct ozonation of surface water)

3.4 Trace organic compounds

3.4.1 Stripping test

Since some trace organic substances, namely MTBE and ETBE, were expected to be volatile, stripping tests were conducted prior to ozonation experiments. Therefore, surface water and column effluent were spiked with trace substances and aerated under conditions similar to ozonation. No significant reduction of trace substances was observed during stripping of both waters. As example, results from stripping test with surface water are presented in figure 3.14.

3.4.2 Transformation efficiency during ozonation

Reduction of trace substances during ozonation for laboratory-scale column experiments is presented in figure 3.15. Only results from the first experiment with specific

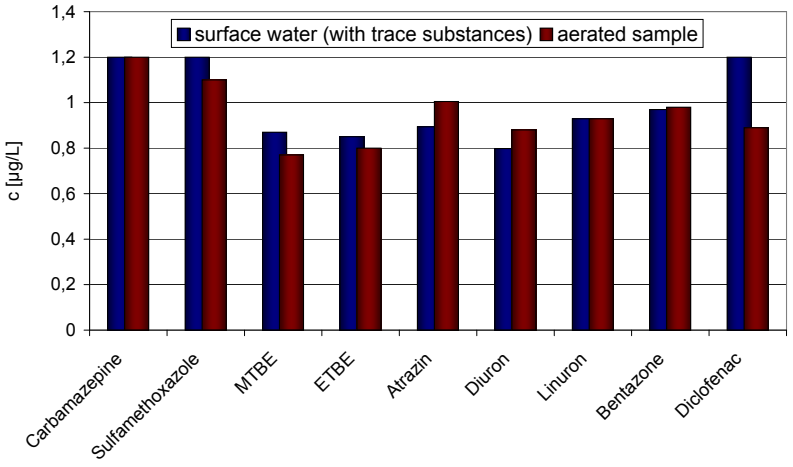

Figure 3.14: Concentration of trace substances before and after 10 min stripping with 55 L/h pure oxygen

ozone dose of 0.9 $mg\ O_3/mg\ DOC_0$ are presented. Substances were spiked at 1 $\mu g/L$ prior to ozonation.

Carbamazepine, sulfamethoxazole, diclofenac and bentazone were removed below detection limits in all ozonation experiments. For these substances good efficiency of transformation is also reported in literature. Second order rate constants for direct reaction with ozone are $k_{O3} \approx 3 \times 10^5\ M^{-1}s^{-1}, k_{O3} \approx 2.5 \times 10^6\ M^{-1}s^{-1} and k_{O3} \approx 1 \times 10^6\ M^{-1}s^{-1}$ for carbamazepine, sulfamethoxazole and diclofenac, respectively [8]. In the project PILOTOX, carbamazepine and diclofenac were reduced to concentrations below the detection limits with specific ozone doses above 0.4 $mg\ O_3/mg\ DOC_0$ [9]. Ozone doses for lab-scale experiments were significantly higher and complete transformation of these compounds was expected. High reactivity towards ozone has also been reported for bentazone [10, 11]. In respect to low sample volumes from column experiments, these four compounds were not analysed in further experiments.

Good removal was also observed for the ozonation of the pesticides linuron and diuron with specific ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$. However, linuron could sporadically be detected in ozonated samples. During ozonation with 0.7 $mg\ O_3/mg\ DOC_0$ transformation of linuron was incomplete and diuron was sporadically detected in ozonated samples (data not presented). The higher reactivity of diuron during ozonation (compared to linuron) is confirmed in literature [12, 10]. Second order rate constants of both pesticides (linuron: $k_{O3} = 1.9 \pm 0.2\ M^{-1}s^{-1}, k_{OH} = (5.9 \pm 0.1) \times 10^9\ M^{-1}s^{-1}$; diuron: $k_{O3} = 16.5 \pm 0.6\ M^{-1}s^{-1}, k_{OH} = (6.6 \pm 0.1) \times 10^9\ M^{-1}s^{-1}$) are significantly lower compared to those of carbamazepine, diclofenac and sulfamethoxazole [12].

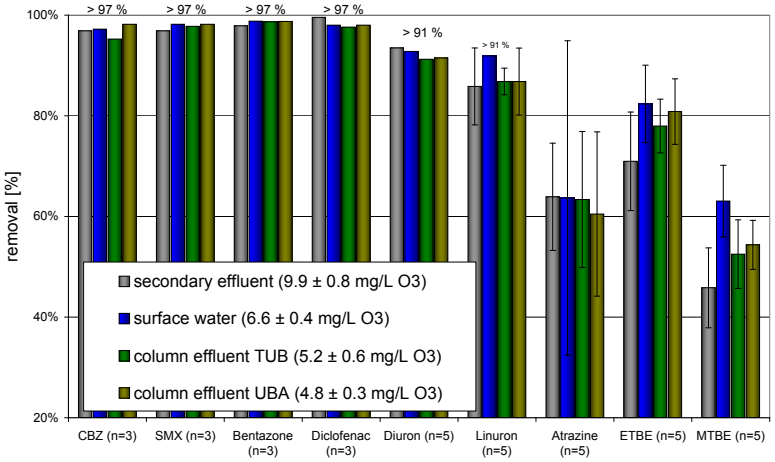

Figure 3.15: Averaged trace compound removal during ozonation in the first experiment ($Z \approx 0.9\ mg\ O_3/mg\ DOC_0, c_0 \approx 1 \mu g/L$)

Transformation of the pesticide atrazine and the fuel additives MTBE and ETBE was incomplete during ozonation in both experiments. MTBE was the most stable compound during ozonation, with ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$ its averaged reduction was approximately 50%. Low reactivity of MTBE towards ozone is confirmed in literature, second order rate constant for MTBE is $k_{O3} = 0.14\ M^{-1}s^{-1}$ [13]. It is assumed that MTBE transformation during ozonation is mainly driven by radical reactions.
Atrazine and ETBE are reduced by approximately 60% and 80%, respectively. According to rate constants from literature ($k_{O3} = 6.0\ M^{-1}s^{-1}, k_{OH} = 3 \times 10^9\ M^{-1}s^{-1}$) transformation of atrazine is surprisingly low in comparison to linuron [14]. However,reduction rate for atrazine was very variable during experiments and results need to be validated in further ozonation tests. To the author's knowledge, no rate constants for the ozonation of ETBE are available in literature.

3.4.3 Biodegradation in soil columns

Column influents and effluents were analysed for trace organic compounds three times in experiment one and three times in experiment two. However, analysed trace substances were chosen due to their high breakthrough potential in bank filtration and artificial recharge systems. Therefore, no significant removal in soil columns was expected for most investigated compounds.

Biodegradation of diclofenac was not analysed since diclofenac is photodegradable [15] and experiments were not conducted with full protection from light. The substances carbamazepine, sulfamethoxazole and bentazone were only analysed in the first experiment. Results from analysis of these compounds in the reference columns

without ozonation are presented in figure 3.16. Effluents from columns operated with ozonated water were not analysed since all three compounds were completely transformed during ozonation.

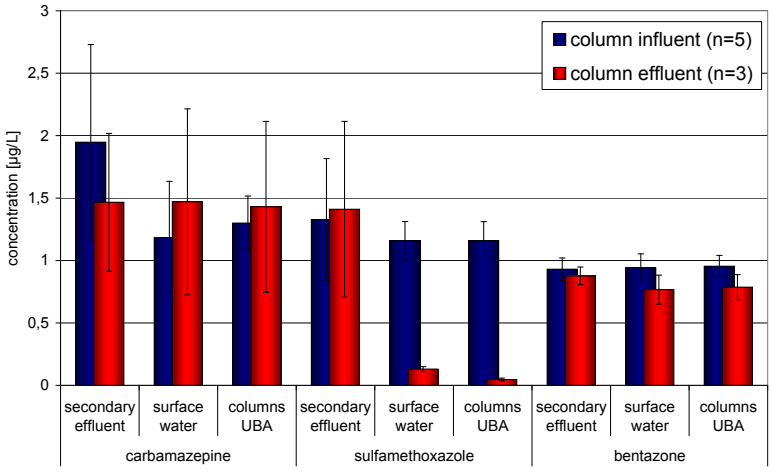

Figure 3.16: Concentration of carbamazepine, sulfamethoxazole and bentazone in soil columns (averaged values from reference columns without ozonation)

The antiepileptic drug carbamazepine is known as being very persistent during soil passage [16] and has recently been discussed as anthropogenic marker in the aquatic environment [17]. Influent concentrations of carbamazepine in all feed waters were highly variable by time. It is assumed that varying background concentration in the source waters are responsible for the variations. However, despite varying feed concentration the persistence of carbamazepine could be confirmed in soil column studies. A breakthrough was observed in all reference columns.

The antibiotic drug sulfamethoxazole was reduced by more than 90% in soil columns operating with surface water and short bank filtrate. By contrary, no significant removal was observed in the soil column operated with secondary effluent. Data on biodegradation of sulfamethoxazole in literature is contradictory. Its good removal during bank filtration was observed in several column studies at TU Berlin [18, 19]. Also for activated sludge systems aerobic degradation of sulfamethoxazole was reported [20]. However, field studies indicated less efficient removal during aerobic artificial recharge [2]. Whether different removal rates are caused by feed water quality or due to different bacterial population in the soil columns needs to be validated in complementary experiments.

Results from bentazone analytics indicate little removal of bentazone during soil columns. However, statistical tests show no significant differences between influent and effluent concentration. These results are confirmed by experiments with aquifer material by Tuxen et al., where bentazone neither was adsorbed to soil nor biodegraded [21].

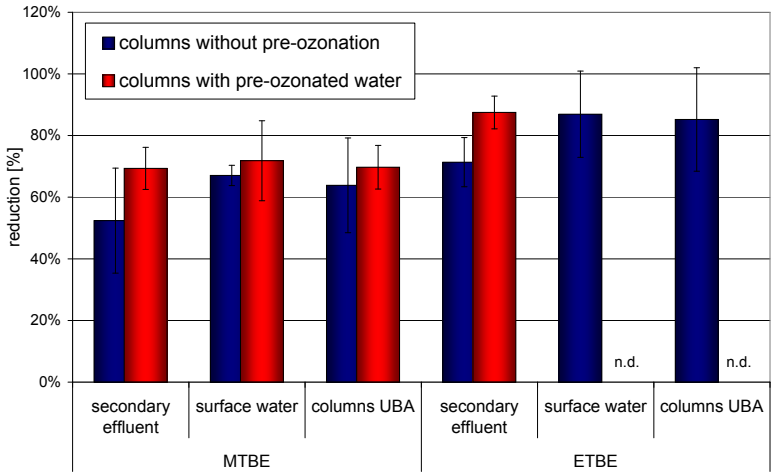

Figure 3.17: Reduction of MTBE and ETBE in laboratory scale soil columns ($n = 5$)

Reduction of MTBE and ETBE is presented in figure 3.17. Feed concentration of MTBE varied from 0.2 mg/L to 1.1 mg/L depending on ozonation and spiking efficiency. Reduction of ETBE in surface water and bank filtrate could not be calculated since it was reduced to concentration close to the limit of quantification by ozonation. Concentration of MTBE was reduced by approximately 50-70% in all soil columns. Removal efficiency for ETBE was even higher with 70-90%. The biodegradability of MTBE is confirmed in literature [22]. However, since both compounds are known as volatile and feed waters were stored in open containers, reduction of MTBE and ETBE might be also due to degassing prior to infiltration.

Biodegradation efficiencies of the analysed pesticides atrazine, diuron and linuron could not be evaluated. Results from analytics were highly variable and partly inconclusive. However, since the analysed pesticides could be detected in column effluents at concentration up to spiking level it is assumed that they were relatively stable in soil columns.

3.4.4 Benefits of a short bank filtration prior to ozonation

Ozonation of the fuel additives with different ozone doses in surface water and short bank filtrate is illustrated in figure 3.18. Applied ozone doses for $Z \approx 0.7\ mg\ O_3/mg\ DOC_0$ were $4.4 \pm 0.3\ mg/L$, $3.6 \pm 0.3\ mg/L$ and $3.6 \pm 0.2\ mg/L$ for ozonation of surface water, column effluent TUB and column effluent UBA, respectively. In experiments with $Z \approx 0.9\ mg\ O_3/mg\ DOC_0$ applied ozone doses were $6.6 \pm 0.2\ mg/L$, $5.2 \pm 0.4\ mg/L$ and $4.7 \pm 0.3\ mg/L$.

Experiments showed a good correlation between specific ozone doses and MTBE/ETBE removal efficiency. Reduction of both compounds was significantly better with higher ozone doses of 0.9 $mg\ O_3/mg\ DOC_0$.

Results also demonstrate that removal rates in surface water and bank filtrate are similar when identical specific ozone doses are applied. Statistic tests indicate no significant differences of reduction in different waters. Thus, ozone demand for oxidation of trace compounds is higher in surface water. This confirms the important role of DOC as a radical scavenger during ozonation. Hence, operating a short bank filtration is a suitable method to reduce ozone demand in the subsequent oxidation step and to optimise trace organic removal.

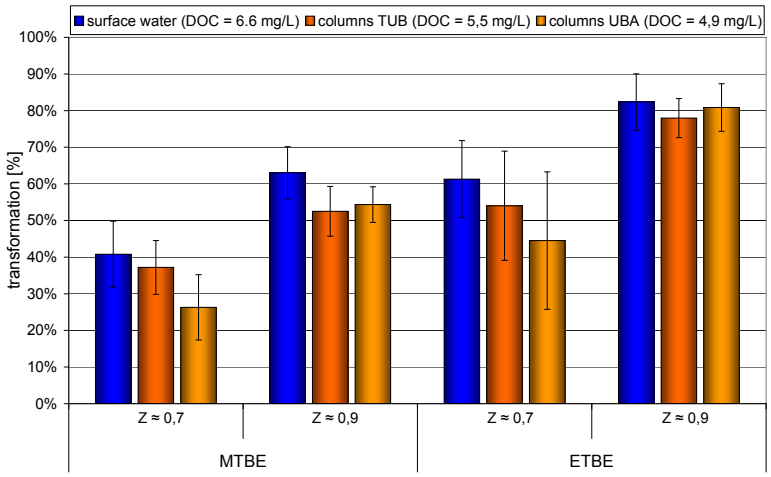

Figure 3.18: Reduction of MTBE and ETBE during ozonation of surface water and bank filtrate (columns TUB, columns UBA) with different specific ozone doses Z

Trace organic compounds

- No elimination of compounds by stripping with pure oxygen.
- Trace compounds carbamazepine, sulfamethoxazole, diclofenac and bentazone (1 $\mu g/L$) are efficiently reduced by ozonation below the limits of quantification (CMZ: 0.02 $\mu g/L$; SMX: 0.03 $\mu g/L$; bentazone, diclofenac: 0.01 $\mu g/L$)
- In most ozonation experiments, pesticides Diuron und Linuron were reduced by >90% and >80%, respectively (spiking concentration: 1 $\mu g/L$, LOQ: 0.05 $\mu g/L$)
- MTBE, ETBE and atrazine are only partly transformed. Based on available literature transformation is attributed to indirect reaction via OH radicals – thus transformation can possibly be enhanced by using advanced oxidation processes (AOP).
- Efficiency of transformation increases significantly with rising ozone doses.
- Preceding BF-step improves the transformation efficiency of MTBE (41% to 52-54%) and ETBE (61% to 78-81%) with similar ozone consumption.
- No or low biodegradation of most compounds in soil columns.
- Reduction of MTBE and ETBE can possibly be attributed to degassing from the feed container.
- Sulfamethoxazole is well removed in columns operated with bank filtrate and surface water but not significantly reduced in column with secondary effluent.

3.5 Formation and biodegradation of oxidation by-products and metabolites

The oxidation by-product bromate was analysed in ozonated column influents. Additionally, AOBr (adsorbable organic bromine) was measured as indicator for the formation of brominated organic compounds.

Desethylatrazine has been identified in recent studies as metabolite from transformation of atrazine [14]. Its formation during ozonation and its degradability in soil columns was investigated in laboratory-scale experiments.

3.5.1 Bromate

Bromate is a well known disinfection by-product from ozonation of bromide containing waters [23]. The International Agency for Research on Cancer classified bromate as a possible human carcinogen. Therefore maximum contamination levels are set by many authorities. In the EU, maximum bromate concentration for drinking water is regulated in the Drinking Water Directive to 10 $\mu g/L$. The limit of quantification for bromate in BWB laboratories is 5 $\mu g/L$.

During laboratory scale experiments the five different ozonated column influents were analysed for bromate. Samples were taken twice in the first and three times in the second experiment. No bromate was detected in any of the samples ($LOQ = 0.5\ \mu g/L$).

Table 3.3: Important water parameter for bromate formation in laboratory scale experiments

	bromide	pH	DOC_0	residual O_3, max. $[(mg/L)]$	
	$[\mu g/L]$		$[mg/L]$	exp. 1	exp. 2
secondary effluent	89 ± 26	7.6	11.4	0.4	0.3
surface water	74 ± 12	8.2	6.6	0.7	0.3
columns TUB	119 ± 18	8.4	5.5	0.7	0.5
columns UBA	82 ± 5	8.4	4.9	1.6	0.8
preozonated column effluent	103 ± 8	8.5	3.8	2.1	0.8

In table 3.3 relevant ozonation parameters for bromate formation are summarized. Formation of bromate during ozonation strongly depends on ozone exposure (Legube, Parinet et al. 2004). Due to the instantaneous reaction of ozone with the water matrix, dissolution of ozone is very low in most experiments. Only during ozonation of column effluent UBA and preozonated column effluent, ozone exposure reached higher level with concentration of dissolved ozone up to $2mg/L$. However, also in these experiments no formation of bromate was observed. This might be due to competitive reactions of organic matter with hypobromic acid [24]. It is also possible that bromide concentration was not sufficient since bromide was only analysed in the second experiment. Since bromate was not formed during ozonation removal efficiency in the soil columns could not be evaluated in laboratory and technical scale column experiments.

3.5.2 Brominated organic compounds

During ozonation brominated organic compounds can be formed by a reaction of organic compounds and hypobromic acid, an intermediate product from reaction with bromide and ozone [23]. AOBr was measured during laboratory scale experiments in the second phase. Results are presented in figure 3.19. Formation of organic bromine was not observed. Instead, AOBr decreased during ozonation by 60-80% in all analysed waters. These findings were confirmed by IC-analyses showing an increase of

inorganic bromide during ozonation.
In contrast to adsorbable organic bromide, adsorbable organic iodide was neither reduced during ozonation nor in aerobic soil columns.

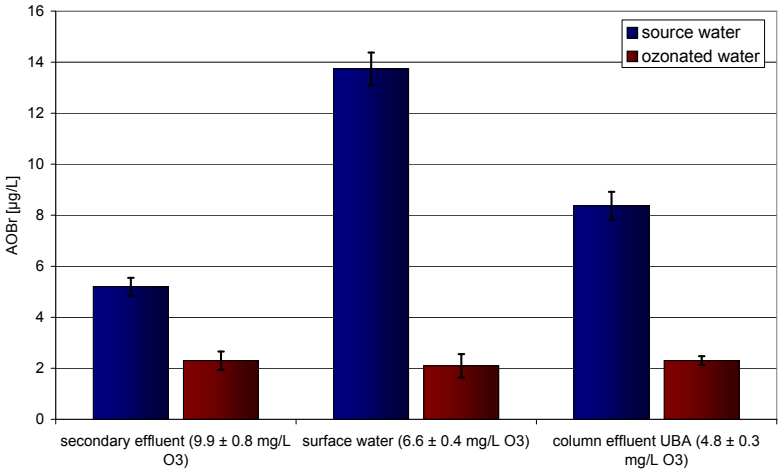

Figure 3.19: Results from AOBr monitoring in column influents ($n = 3$)

3.5.3 Desethylatrazine

Desethylatrazine is one of the major metabolites from ozonation of atrazine [14]. It was not spiked during experiments in order to monitor its formation during oxidation.

Results from atrazine and desethylatrazine analysis during experiments with bank filtrate (column effluent TUB) are presented in figure 3.20. Data from other columns are not shown. A statistical evaluation of the data was not feasible since results were not always conclusive (see, for example, data from jun-24). Additionally, desethylatrazine was measured unexpectedly in all source waters during two sampling campaigns (may-05 and july-15). The source of this apparent contamination could not be identified. However, two major conclusions can be taken from results despite analytical problems:

1. Desethylatrazine is formed as a major metabolite from ozonation of atrazine.
2. Atrazine in source water can not completely be detected as cumulated atrazine and desethylatrazine in ozonated water.

These results indicate that desethylatrazine is not the only metabolite from atrazine oxidation confirming results presented by Gottschalk [25], who also detected desisopropyl atrazine as metabolite. Additionally, Gottschalk showed further oxidation of desethylatrazine during ozonation [25].

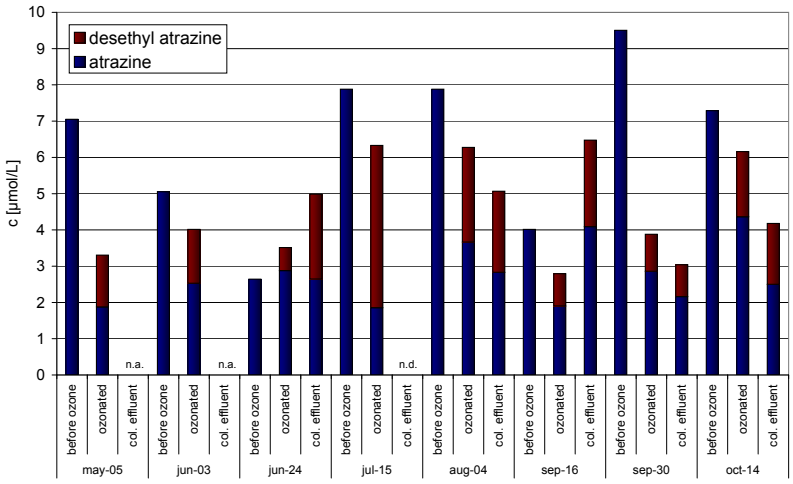

Figure 3.20: Monitoring of atrazine and desethylatrazine in laboratory-scale column experiments with bank filtrate (column effluent TUB, n.d.: not detected, n.a.: not analysed)

During soil passage no significant changes of desethylatrazine were observed indicating that this metabolite of atrazine is biologically stable.

During ozonation of secondary effluent no significant formation of desethyl atrazine was observed despite atrazine removal was similar to other waters. These findings are not conclusive and might be due to analytical inaccuracy. However, despite desethylatrazine was not detected in ozonated secondary effluent it was detected in column effluent.

Oxidation by-products and metabolites

- Despite relevant bromide concentrations (around 100 $\mu g/L$) bromate formation was not observed (LOQ: 5 $\mu g/L$) due to high reactivity of DOC (free ozone up to 1.6 mg/L during ozonation of column effluent UBA)
- Formation of brominated organic compounds as possible oxidation by-products was not observed. Instead AOBr was reduced during ozonation.
- Desethylatrazine is formed as a major metabolite from ozonation of atrazine.

3.6 Biological test systems

3.6.1 Genotoxicity

Ames/Salmonella microsome assay

All test samples were negative for all test variants in both tester strains in the presence and absence of S9 metabolic activation mix. Only two reference samples were slightly positive in TA 98 without metabolic activation in one of the two independent experiments (IF 1.3). This slightly positive result can be overruled by negative results in the other test variants. Summarized results obtained in the Ames assay are presented in the supporting information.

Micronucleus assay

The results obtained in the assay are shown in the supporting information. In general rates from our historical negative control data ranged from 0 to 6. There was no clearly increase of the number of micronuclei over the negative control frequency for all test variants. Only two test samples, namely column influents operated with ozonated bank filtrate (column effluent TUB) and ozonated secondary effluent (experiment 1, sampling campaign 2, ozone consumption: 0.9 $mg\ O_3/mg\ DOC_0$) showed an increased number of micronuclei (14 respectively 10) compared to the negative control (6). However, these results were not confirmed in the other two sampling campaigns.

The frequency of nuclear buds in the negative control ranged from 0 to 1.0%. A doubling of the frequency of nuclear buds were observed in the following samples:

1. C5-effluent, C6-effluent and C8-effluent (reference samples prior to ozonation experiments; all columns operated with surface water without ozonation).

2. ozonated secondary effluent (unspiked) (experiment 1; sampling campaign 2; ozone consumption: 0.9 $mg\ O_3/mg\ DOC_0$).

Also the positive results from nuclear buds were not confirmed in additional measurements. It is assumed that sporadical positive results in the micornucleus assay are caused by unknown cofactors. Thus, systematic effects of ozonation on genotoxicity were not confirmed.

3.6.2 Cytotoxicity

Reference samples were taken from all laboratory-scale column effluents prior to ozonation experiments. In comparison to cells exposed to the same volume of entionized water (negative control) glucose consumption rate of HepG2 cells was not diminished in these samples after 72 hours exposure time.
Generation of reactive oxygen species in JURKAT cells was slightly increased after 24 h expositon time to reference samples from column effluents. Percentage of reactive oxygen species generation was on average 5% to 10% higher in comparison to negative control. This increase in common for environmental samples and is probably caused by the natural background of humic substances. In environmental samples this increase of 5% to 10%

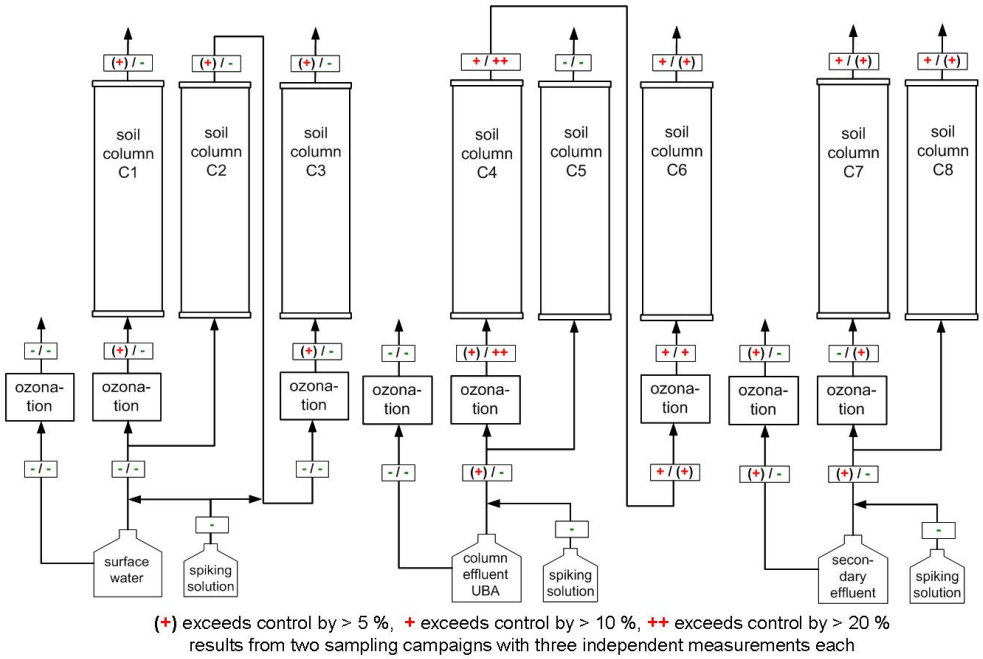

Figure 3.21: ROS-formation in laboratory-scale experiments (results from experiment 1 with 0.9 $mg\ O_3/mg\ DOC_0$)

Results from reactive oxygen species generation in experiment 1 are presented in figure 3.21. Highest formation of reactive oxygen species was observed in experiments with simulated bank filtrate from column effluent UBA. Negative control in ozonated column influent (column 4) was exceeded by 7% and 27% in sampling campaigns 1 and 2, respectively. In the subsequent treatment by column infiltration, second ozonation

and additional infiltration (column 6), generation of ROS was only slightly reduced. Elevated cytotoxicity in these samples is strenthened by results from glucose consumption rates. From all samples only the influent and effluent of column 4 operated with ozonated column effluent (columns UBA) caused a distinct diminution of glucose consumption rate of HepG2 cells which indicated cytotoxicity effects after 72 h exposure time.
The elevated cytotoxicity in these samples could not be completely explained. Parallel experiments (column 3) were conducted with effluent from columns at TUB. Due to similar retention time in the column, water was expected to be similar to column effluent UBA. However, percentage of reactive oxygen species not or only slightly increased (5% to 10% higher in comparison to negative control) in experiments with column effluent TUB. In addition, ozonation of the unspiked column effluent did not cause formation of ROS. Thus, ROS generation was caused by the combination of column effluent UBA with spiking of trace substances and ozonation. However, in experiments with lower ozone dose (0.7 $mg\ O_3/mg\ DOC_0$) generation of ROS was only confirmed in the influent of column 6 (14% higher compared to negative control, data not presented).
Objective of this study was to evaluate the possible effects of ozonation on toxicity. The causes for positive tests from experiments with column effluent UBA were not further investigated.

Results from experiments with secondary effluent were partly inconclusive. In the first experiments mainly the effluents from columns operated with secondary effluent caused elevated ROS generation. In the second experiment the highest percentages of reactive oxygen species were measured after exposure to the unspiked secondary effluent before and after ozonation with 24% and 25% higher values in comparison to the negative control, whereas secondary effluent spiked with trace compounds did not cause elevated ROS formation. It is assumed that highly positive results in these samples are caused by contamination.

In experiments with surface water no negative effects of ozonation and artificial recharge were observed. Negative control with entionized water was only sporadically exceeded by more than 5%.

In all experiments the antibioticum staurosporine was engaged as a positive control. At a final concentration of 1 μM and after 72 h hours exposure time staurosporine diminished the glucose consumption rate of HepG2 cells in the mean to a value of 38% of the negative control.
At final concentrations of 0.5 and 0.3 μM and after 24 h exposure time to staurosporine generation of reactive oxygen species was raised dose dependent, in the mean to a value of 60% and 44%.

Biological test systems

- No genotoxic effects were observed during ozonation and subsurface passage.
- Systematic cytotoxic effects of ozonation were also not observed. The cause for toxic effects in some samples was not identified and is attributed to unknown co-factors.
- ROS production test was identified as an easy and sensitive test to identify and monitor cytotoxic effects in water samples.

4 Conclusions

Experiments in OXIRED-1 have demonstrated the combination of groundwater recharge/ soil aquifer treatment with pre-ozonation being a suitable solution to enhance the removal of DOC and many relevant trace organics from wastewater impacted waters. Although the toxicity of the resulting waters was investigated and not seen as critical, it needs to be stated that apart from bromate and desethylatrazine no other transformation products were analysed. Further investigations need to take these into account.

4.1 Trace organic compounds

Transformation of the investigated trace organic substances during laboratory-scale experiments is summarized in table 4.1. Most substances were not efficiently biodegraded in soil columns under the aerobic conditions encountered. This was expected since the selected compounds were chosen due to their high or medium breakthrough potential in groundwater recharge systems.

Realistic surface water concentrations of carbamazepine, sulfamethoxazole, diclofenac and bentazone are efficiently reduced by ozonation below the limits of quantification (LOQ). Concentrations of diuron and linuron are reduced close to LOQ. For these compounds specific ozone doses of $0.6 - 0.7\ mg\ O_3/mg\ DOC_0$ were sufficient for efficient transformation.
The substances MTBE, ETBE and atrazine are only partly transformed during ozonation. Based on available literature transformation is attributed to indirect reaction via OH radicals. For these compounds an optimisation of the ozonation, for example by applying advanced oxidation processes (AOP), should be considered.

The results of the laboratory experiments show that transformation of trace organic substances during artificial groundwater recharge is not affected by pre-ozonation. Therefore, trace compound removal of the combined system can be estimated regarding both treatment processes separately. The removal efficiency of many trace organic substances during artificial recharge systems is well documented in literature [26]. Also the transformation during ozonation is well investigated [8, 10, 27]. However, characteristics of ozonation are highly affected by the water matrix and trace organic

Table 4.1: Summary of trace organic removal results from laboratory-scale experiments for substances with high relevance to water suppliers (++: complete removal (below LOQ); +: good removal ($> 70\%$); +/-: partial removal($30 - 70\%$); -: poor removal ($< 30\%$); n.a.: not analysed)

	RBF/AR	ozonation
High potential for breakthrough in RBF/AR systems		
MTBE	(+/−)	+/−
sulfamethoxazole	+	++
ETBE	(+)	+/−
carbamazepine	−	++
Medium / uncertain potential for breakthrough in RBF/AR systems		
bentazone	−	++
atrazine	−	+/−
linuron	−	+
diuron	−	+
diclofenac	n.a.	++

removal needs to be estimated with respect to the ozonated water. A method to characterize the consumption of ozone and the formation of OH-radicals was proposed by Elovitz and von Gunten (1999) [1]. Batch experiments with water from Berlin have demonstrated a fast consumption of ozone and a high formation of OH-Radicals. However, since ozone doses and the type of reactor for laboratory-scale ozonation were different to the test system, modeling of trace compound removal was not conducted. The modeling of trace compound removal in wastewater application with high DOC is currently being investigated [28].

One major concern regarding the ozonation of trace organic compounds is the formation and possible persistence of unknown metabolites. Several metabolites have been identified in recent studies [13, 14, 29]. Biodegradability of metabolites in artificial recharge systems is mostly unknown.
During the laboratory-scale experiments, desethylatrazine (DEA), a major metabolite from transformation of atrazine [14], was detected in ozonated samples. During subsequent soil passage, DEA was not significantly removed. These results indicate that i) many trace organic compounds are not completely mineralised by ozonation and soil aquifer treatment and ii) metabolites from ozonation are not necessarily better biodegrable than the parent substances. However, degradation of other metabolites needs to be investigated in further experiments in combination with toxicological tests.

4.2 Bulk organic carbon

The removal of bulk organic carbon during groundwater recharge was significantly improved by pre-treatment with ozone. Removal of surface water DOC in the soil columns was increased by preozonation from 22% to 40% ($z = 0.7$) and 45% ($z = 0.9$) and DOC in column effluent reached the level of tap water in Berlin within less than one week of retention time. At bank filtration and artificial recharge sites in Berlin similar removal rates were only observed within $3 - 6$ months of retention [2].

DOC in the finished water after ozonation and short infiltration was analysed using LC-OCD. Chromatograms did not indicate relevant differences in size contribution in comparison to bank filtrate. However, due to the selective reaction of ozone with unsaturated and aromatic structures, UV absorbance at 254 nm decreased during oxidation by more than 50%. The specific UV absorbance after biodegradation in soil columns ($1.3-1.7\ L/mg{\cdot}m$) is still very low compared to $2.3-2.7\ L/mg{\cdot}m$ in columns without preozonation indicating a high amount of aliphatic structures in the column effluents. Whether these compounds are biologically stable after six days of retention time in the column or can further be biodegraded during longer soil passage is currently being investigated in technical-scale experiments with travel times of $30 - 45\ d$.

From batch test specific ozone doses of $0.8 - 1.0\ mg\ O_3/mg\ DOC_0$ were recommended to optimise removal of DOC. Experiments with laboratory-scale soil columns showed, that specific ozone doses of $0.7\ mg\ O_3/mg\ DOC_0$ were already sufficient to efficiently enhance biodegradation. Increasing of ozone doses only slightly improved DOC removal during infiltration. It is assumed that the humic background DOC in the area of Berlin is very inert towards biodegradation or oxidation by ozone. Column experiments with lower ozone dose were not conducted in OXIRED-1.

Since bacterial regrowth is a serious concern in drinking water treatment and distribution the biological stability of the treated water after ozonation and artificial recharge is of great interest for water companies. It is therefore proposed to integrate the analysis of biological stability by measuring assimilable organic carbon content (AOC) in the treated water after soil column tests.

4.3 Advanced wastewater treatment or drinking water treatment

Parallel experiments with surface water and secondary effluent have demonstrated that ozonation with subsequent soil aquifer treatment may have benefit for both, drinking water treatment systems as well as for tertiary treatment of wastewater. Biodegradation of DOC in the soil columns was similar during experiments with secondary effluent

and surface water (42% to 47% and 40% to 45%, respectively). Most investigated trace organic substances were efficiently removed by ozonation in both waters. However, results also showed higher ozone consumption during treatment of secondary effluent due to the higher level of DOC resulting in increased ozone demand and energy costs. Nevertheless, ozonation and infiltration as tertiary wastewater treatment is beneficial to reduce concentrations of anthropogenic pollutants in the aquatic environment.

4.4 Benefits of a preceding short bank filtration step

The benefits of a short bank filtration prior to ozonation were evaluated in comparison to direct ozonation of surface water by operating parallel columns with surface water and simulated bank filtrate. All investigated trace substances except for MTBE, ETBE and atrazine were removed below or close to the LOQ during ozonation of both, surface water and bank filtrate. Results from MTBE and ETBE analysis indicate more efficient oxidation after a preceding bank filtration. With similar ozone consumption the transformation efficiency of MTBE and ETBE was improved by $27 - 31\%$ and $28 - 33\%$ of the original removal, respectively. It is concluded that a preceding bank filtration step is suitable to reduce ozone demand for the selective oxidation of most poorly reactive compounds such as the analysed fuel additives, but possibly also some pesticides and iodinated contrast media.

For the removal of bulk organic carbon only little improvement was observed by applying a preceding bank filtration step. Overall DOC removal was increased from 45% with direct ozonation of surface water to up to 50% with a preceding soil column. However, in all column effluents specific ozone doses of 0.7 $mg\ O_3/mg\ DOC_0$ were sufficient to reduce DOC to a level of $3.5 - 4.0\ mg/L$ after 6 days of retention time independent from the treatment system. Whether DOC is further degraded after longer retention times is currently being investigated in technical-scale experiments.

Apart from the investigated parameters, local factors, such as surface water quality and bank characteristics, need to be considered for the design of an ozonation and infiltration unit. On the one hand, particles and algae in surface water may increase ozone consumption rendering pre-treatment necessary, which can be spared using prior bank filtration (during experiments, particles were removed with a microsieve). On the other hand, a preceding bank filtration step with anaerobic passages can result in solubilisation of reduced iron and manganese. These substances increase the ozone demand and potentially cause clogging in the infiltration ponds.

4.5 Oxidation by-products

Formation of the oxidation by-product bromate was not detectable ($< 5\ \mu g/L$) despite the presence of its precursor bromide ($\approx 100\ \mu g/L$). Bromate formation depends on ozonation conditions and it is assumed that ozone exposure was not sufficient for significant formation of bromate due to the presence of highly reactive DOC. Formation of brominated organic compounds was also not observed during laboratory-scale experiments. Adsorbable organic bromide (AOBr) even decreased during ozonation by $50 - 60\%$ during ozonation of secondary effluent to $80 - 90\%$ in surface water. The reduction of AOBr concentrations was accompanied by an increase of inorganic bromide by up to 40 $\mu g/L$ during ozonation of surface water.

Since the formation of oxidation by-products during treatment with ozone is a serious concern it should be addressed in further experiments. In addition to bromate, N-Nitrosodimethylamine was identified as a relevant oxidation by-product from ozonation [30]. One major chance for the combination of ozonation and soil passage is the possible reduction of potentially toxic by-products from ozonation in the subsequent artificial recharge system. It is therefore recommended to carry out experiments to evaluate the degradation of bromate and N- Nitrosodimethylamine in the second phase of the project.

4.6 Toxicology

In the two conducted in vitro genotoxicity tests (Ames test, micronucleus assay) no genotoxicity caused by ozonation of water samples was observed. Testing for cytotoxicity (glucose consumption rate, ROS generation) showed positive results in several samples. However, a systematic attribution of toxic effects to ozonation was not possible. Reasons for cytotoxic effects were not evaluated within the scope of this project but it is assumed that they were caused by unknown cofactors.

Comparison of the two procedures applied for cytotoxicity testing showed that the assessment of reactive oxygen species generation was more sensitive. Although the glucose consumption rate measurement was a long-term assay with 72 h exposure time only two samples caused distinctly diminished glucose consumption rates as parameter for cell damage. These two samples also showed distinctly increased reactive oxygen species generation after 24 h exposure time demonstrating the good reproducibility of results.
Experiments showed that the analysis of ROS generation is a suitable test to monitor water treatment systems as indicator for possible adverse effects for human health and ecological systems. It is a fast and sensitive assay which can be easily standardized and is recommended for further investigations.

4.7 Transferability to other sites

All experiments in this project were conducted with water from the area of Berlin, which is characterised by a relatively high background DOC. For the treatment of these waters, specific ozone doses of 0.7 $mg\ O_3/mg\ DOC_0$ are sufficient to reduce the concentrations of many trace organic substances below or close to LOQ and to enhance DOC removal during groundwater recharge. For compounds which are basically transformed by radical reactions (e.g. Atrazine, MTBE), optimisation of ozonation, for example by preceding bank filtration or advanced oxidation processes (AOP) needs to be considered.

Since experiments were conducted using industrial sand, transferability to other sites is basically a question of source water quality. To estimate ozone decomposition and radical formation during ozonation, simple batch tests following the R_{CT}-concept [1] can be conducted. In order to evaluate the potential and the optimal ozone dose for removal of bulk organics, a biological test system such as soil columns needs to be established. In order to assess transferability of results to other sites, it is recommended to carry out experiments with a different source water.

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