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

Final Report of OXIRED – Phase 2

by

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for

KompetenzZentrum Wasser Berlin gGmbH

Preparation of this report was financed in part through funds provided by BWB and Veolia

Berlin, October 25, 2011

Extended Summary

Subsurface passage as utilized during 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 oxidation by ozone.

Results from the first phase of the project have demonstrated that oxidation with ozone is a suitable method to reduce the concentrations of several relevant trace organic compounds (e.g. carbamazepine, sulfamethoxazole) and to significantly enhance biodegradation of DOC during subsequent soil passage. For efficient removal of DOC in the soil columns, specific ozone consumptions of 0.6 to 0.7 mgO_3/DOC_0 were sufficient.

Project objectives in OXIRED-2 were to i) verify results from laboratory scale experiments at a larger scale with longer retention times, ii) study feasibility under field conditions with seasonal variations by operating a pilot unit, iii) evaluate the formation of oxidation by-products and their persistence during subsurface passage and iv) propose a standardized test protocol to analyse benefits of ozonation and artificial groundwater recharge at different sites.

To investigate effects of ozonation on groundwater recharge with longer retention times, a technical scale column system with a length of 30 m and a hydraulic retention time of approximately six weeks was operated at the UBA's experimental site in Berlin Marienfelde. Pilot studies were conducted at Lake Tegel using an ozone unit from ITT-Wedeco with a 4 g/h generator and subsequent slow sand filtration. Reduction of bromate was assessed in laboratory scale soil columns under different redox conditions. In addition, anoxic reduction of bromate was evaluated in a diploma thesis at TU Berlin. To analyse effects of DOC removal after ozonation, a standardized test protocol using recirculating columns was proposed and tested.

Results from the different experiments confirmed the conclusions of the first phase of the project. Removal of surface water DOC during infiltration significantly increased with preozonation. In pilot studies, effluent DOC of approximately 4.7 mg/L after 1 d of retention time was measured, which is comparable to residual DOC from artificial groundwater recharge in Berlin Tegel after 30 days retention time [1]. In addition, strong effects of temperature on DOC removal were observed. During experiments with ozonation, overall DOC reduction decreased from approximately 40% in October to about 30% in the end of November. Biological testing of slow sand filter effluent revealed no genotoxic or cytotoxic effects in the water prior to further infiltration into the aquifer.

Many persistent trace compounds were efficiently transformed during ozonation with specific ozone doses of 0.8 $mg\ O_3/mg\ DOC_0$. For example, realistic surface water concentrations of carbamazepine, sulfamethoxazole, phenazone and bentazone were reduced below the limits of quantification (LOQ).

Primidone was only partly transformed during ozonation (70%). Since primidone is persistent during infiltration, a breakthrough in combined ozonation and artificial recharge can be expected. Also the substances MTBE and ETBE, the pesticide atrazine and some metabolites detected in Lake Tegel persist partially during treatment with ozone and subsequent groundwater recharge. 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.

Efficient reduction of the concentration of adsorbable organic iodine (AOI), an indicator for x-ray contrast media, during ozonation or infiltration was not observed. In contrast, adsorbable organic bromine decreased by $70 - 80$ % during ozonation.

Formation of the oxidation by-product bromate during ozonation of Lake Tegel water with a specific ozone consumption of up to 1.0 $mg\ O_3/mg\ DOC_0$ was below the limit of the German drinking water directive. Removal during subsurface passage was observed under anoxic conditions in presence of biodegradable organic carbon. Since artificial recharge after ozonation is likely aerobic, no significant reduction of bromate can be expected. Thus, formation of bromate needs to be controlled during surface water ozonation.

Formation of nitrosamines was monitored in batch experiments with a specific ozone consumption of up to 1.15 $mg\ O_3/mg\ DOC_0$. No formation of nitrosamines including NDMA (LOQ: 5 ng/L) was observed.

Operating a preceding bank filtration step will reduce ozone demand for efficient DOC removal. In addition, problems with particles from source water can be minimised. However, additional energy consumption for operation of extraction wells has to be taken into account.

Overall, the presented results confirm that the objectives of enhanced removal of trace organics and DOC by combining ozonation and subsurface passage are well met. Further investigations need to focus on seasonal variations in long-term pilot studies and the formation, retention and toxicity of transformation products.

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Deliverable number

D 1.1

Acknowledgements

The project team is grateful to BWB and Veolia for sponsoring and supporting the OXIRED-project. We thank all involved persons at the technical divisions and research and development departments as well as the technical committee for the valuable discussions and provided information.

Thank you!

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Abbreviations

AAA	Acetylaminoantipyrin
AMDOPH	1-Acetyl-1methyl-2-dimethyl-oxamoyl1-2-phenylhydrazid
AMPH	1-Acetyl-1-methyl-2-phenylhydrazid
AOBr	adsorbable organic bromine
AOCl	adsorbable organic chloride
AOI	adsorbable organic iodine
AOP	advanced oxidation processes
AR	artificial groundwater recharge
BF	bank filtration
BWB	Berliner Wasser Betriebe
CBZ	Carbamazepin
DEA	Desethylatrazine
DO	Dissolved oxygen
EDTA	Ethylendiamintetraessigsäure
ETBE	Ethyl-tert-butylether
HPLC	high performance liquid chromatography
MS	mass spectroscopy
MTBE	Methyl-tert-butylether
NDMA	N-Nitrosodimethylamin
NOM	natural organic matter
OBP	oxidation by-products
o-TSA	ortho-Toluensulfonamid
p-TSA	para-Toluensulfonamid
ROS	reactive oxygen species
SAT	soil-aquifer-treatment
SMX	Sulfamethoxazol
STP	Standardized Test Protocol
TUB	Technical University of Berlin
UBA	Federal Environment Agency (Umweltbundesamt)

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 within a multi-barrier concept. Additionally it is widely used as polishing step after wastewater treatment. Especially particulate and particle-bound substances (e.g. algae and bacteria) are efficiently removed by physical straining. Multiple bio- and geochemical reactions in the subsurface lead to a reduction of many dissolved substances as well (e.g. pharmaceuticals and industrial chemicals). However, there are limitations concerning the removal of dissolved organic carbon (DOC) and specific trace organics.

The project "OXIRED" aims at assessing possibilities to overcome these limitations by combining underground passage and advanced oxidation (e.g. ozonation) as pre- or post-treatment for an optimized removal of DOC and selected trace organics.

In the first phase of the project, literature studies as well as batch and laboratory scale experiments were carried out in order to evaluate the theoretical benefit and limitations of such a system and identify knowledge gaps. Results from laboratory-scale column experiments have demonstrated that oxidation with ozone is a suitable method to reduce the concentrations of several relevant trace organic compounds and to significantly enhance biodegradation during subsequent soil passage. For efficient removal of DOC in the soil columns, specific ozone consumptions of 0.6 to 0.7 mgO_3/DOC_0 were sufficient.

Objectives of work package 1 in the project OXIRED-2 were to i) verify results from laboratory scale experiments at a larger scale with longer retention times, ii) operate a pilot unit to study feasibility under field conditions with seasonal variations, iii) evaluate the formation of oxidation by-products and their persistence during subsurface passage and iv) propose a standardized test protocol to analyse benefits of ozonation and artificial groundwater recharge at different sites.

To verify results from laboratory-scale experiments, a technical scale column system consisting of six stainless steel columns with a total length of 30 m was operated in Berlin, Marienfelde. In a first experiment, direct ozonation of surface water was tested with subsequent infiltration into the soil columns. In the second experiment, surface water was directly infiltrated into the first column of the system to simulate a short bank filtration step before ozonation. The ozonated water was then infiltrated into the second column of the system.

Pilot studies were carried out at Lake Tegel in Berlin from summer to late autumn 2010. The combination of an ozonation unit with a slow sand filter was set up in order to assess the effects of ozonation on the processes in the infiltration pond prior to infiltration into the aquifer.

The formation of oxidation by-products during ozonation was investigated in batch tests with various ozone dosages. The degradation of bromate during subsurface passage was monitored in laboratory-scale columns under different redox conditions. In addition, a diploma thesis on the reduction of bromate under anoxic conditions was compiled at TU Berlin within OXIRED-2.

A standardized test protocol was proposed to evaluate biodegradation of DOC from surface water after ozonation at different ozone dosages. Therefore, a set of small columns was tested, which were operated in recirculating mode to analyse BDOC.

In addition, the laboratory of the Federal Environment Agency (UBA) in Bad Elster analysed samples from different experiments for adverse effects on human and ecology. Tests on genotoxicity (Ames assay, micronucleus assay) and cytotoxicity (necrosis, ROS-induction) were carried out.

In this final report, a detailed presentation of the results from work package 1 is given.

2 Experimental

2.1 Batch experiments

2.1.1 Batch ozonation at TU Berlin

In order to investigate the formation of nitrosamines and bromate during ozonation, batch experiments were conducted with surface water from Lake Tegel applying different specific ozone dosages. The ozonation unit is given in figure 2.1. Gaseous ozone was produced from pure oxygen and directly introduced into the sample in a 4-L-semi-batch stirred tank reactor. The ozone concentration in the in-gas and off-gas, dissolved ozone and gas flow rate were measured continuously and an ozone mass balance was set up automatically by a computer. An example of ozonation for laboratory scale column experiments is presented in figure 2.2. In order to set up a complete mass balance, all off-gas ozone was stripped with pure oxygen.

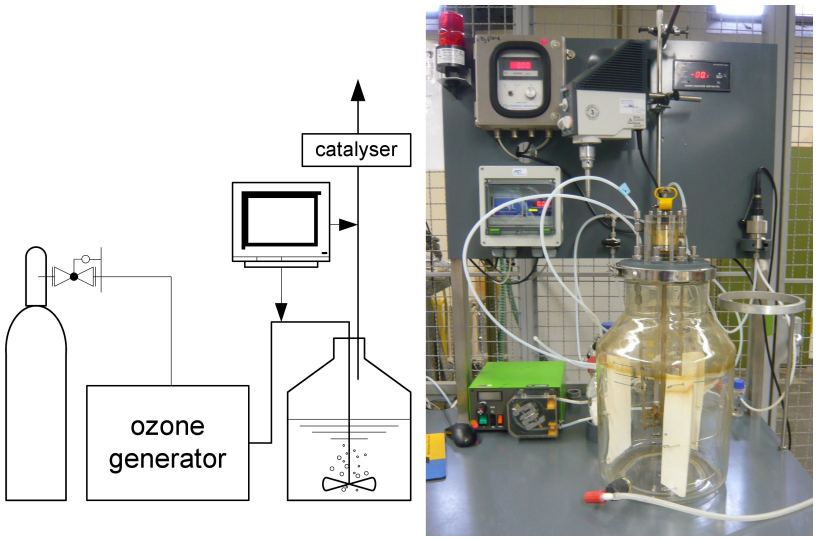

Figure 2.1: Setup of ozonation arrangement

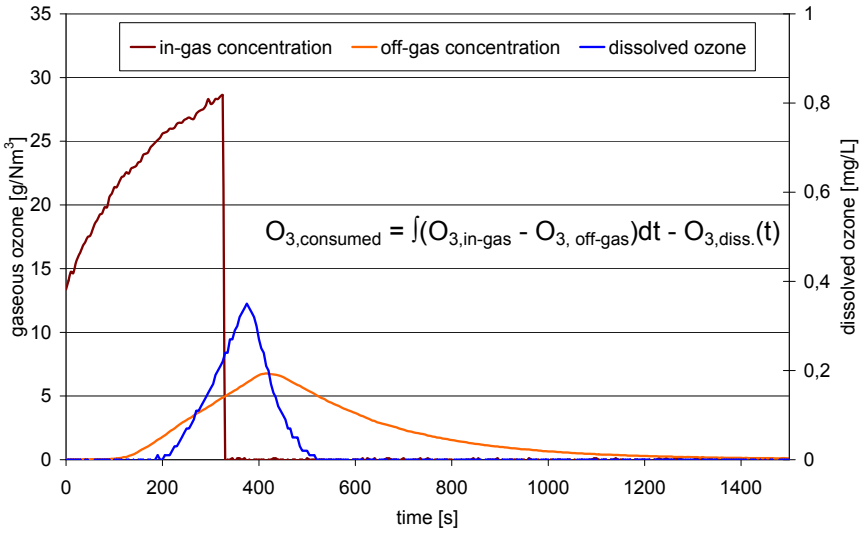

Figure 2.2: Example for a mass balance of ozone during ozonation of a sample in a batch reactor: in- and off-gas.

2.1.2 Diploma thesis on biodegradation of bromate

To investigate the possible reduction of the oxidation by-product bromate, a diploma thesis was conducted at the TU Berlin by Simon Kuhnt. In this study, six small recirculating columns with a length of 30 cm and a diameter of 5 cm were set up at a constant temperature of 10 $^{\circ}C$. The columns were filled with aquifer sand from 26 meter depth sampled during a well construction by BWB close to Lake Tegel and operated under anoxic to anaerobic conditions, since bromate was expected to serve as electron acceptor for bacteria. Feed water was provided from 2 L bottles with a flow rate of 0.26 L/h, anoxic conditions were established by daily purging of the reservoir with nitrogen gas. Dissolved oxygen was measured by optical fiber measurement. The schematic set-up of one column is shown in figure 2.3. In three different experiments the influence of biodegradable DOC, redox conditions and initial bromate concentration on the removal of bromate was investigated.

2.1.3 Standardized test protocol

Since the effect of ozonation on the biodegradability of DOC depends on the characteristics of the ozonated water, it was proposed to develop a simple, standardized test protocol (STP) to evaluate the potential for DOC removal by ozonation and subsequent groundwater recharge at different sites.
The recirculating columns from experiments with bromate (see 2.1.2) were tested for the evaluation of BDOC formation. The columns were adapted to surface water from Lake Tegel for three weeks under aerobic conditions. Parallel biodegradation experiments were conducted as duplicates using surface water i) without ozonation, ii) with a specific ozone dose of 0.5 $mg\ O_3/mg\ DOC$ and iii) with a specific ozone dose of 1.0 $mg\ O_3/mg\ DOC$. In addition, laboratory scale columns (see chapter 2.2) were operated with similar ozone dosage under aerobic conditions to verify the results from standardized test protocol.

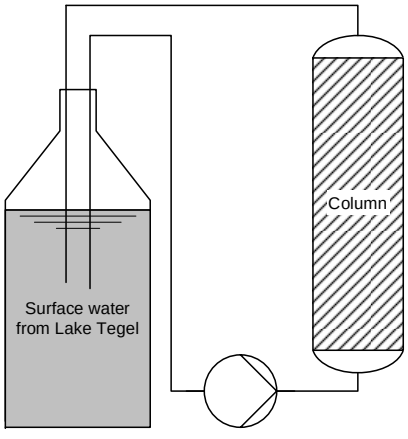

Figure 2.3: Schematic setup of one of the six circulating columns for investigations concerning the standardized test protocol

2.2 Laboratory scale column experiment

For the laboratory scale column experiments four soil columns were set up in the basement at TU Berlin (average room temperature of 22 $^{\circ}C$). The columns have a length of 100 cm and a diameter of 12 cm. They were filled with

technical sand (particle size: $0.7 - 1.2\ mm$). The soil columns were adapted to surface water from Lake Tegel during OXIRED-1. The columns were fed with a flow rate of 1.5 L/d from storage tanks of 13 L, which were refilled weekly with surface water from Lake Tegel. The hydraulic retention time in the columns was determined in tracer tests to be 5-6 days. During the experiments, the flow rate in column C 3 was adjusted to approximately 1 L/d since available feed water was short.
The laboratory scale columns were operated under different redox conditions to investigate the reduction of bromate. The setup is shown in figure 2.4. Column C 1 was operated as a reference without preozonation. Columns C 2 and C 3 were fed with ozonated surface water under aerobic and anoxic conditions, respectively. Column C 4 was operated anoxically to simulate an anoxic soil passage after aerobic infiltration.
Anoxic conditions were established by degassing the inflow with nitrogen, ozonation was conducted with a specific ozone consumption of approximately 0.7 $mg\ O_3/mg\ DOC_0$ and bromate was spiked at 100 $\mu g/L$.

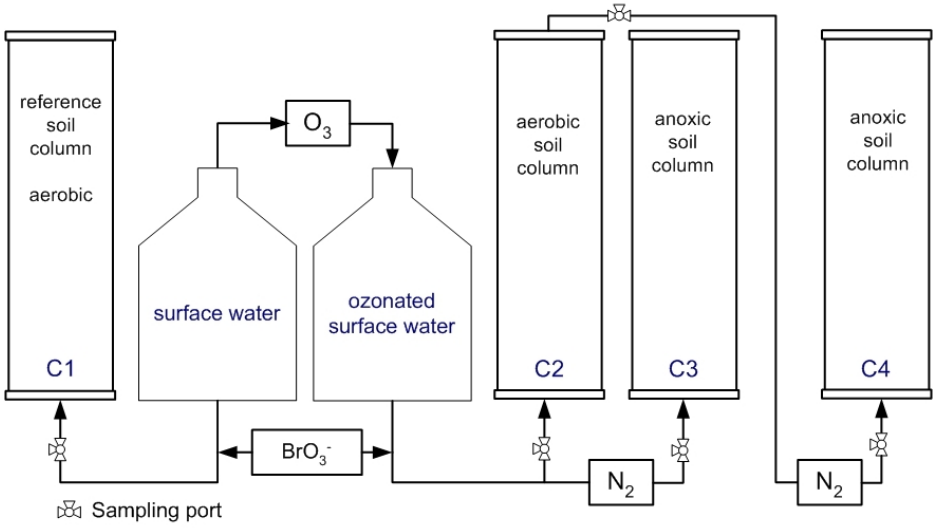

Figure 2.4: Setup of the laboratory scale column experiment with spiking of bromate

Feed waters and column influents and effluents were sampled weekly from 21 October 2010 to 6 January 2011 for analysis of DOC, UVA_{254}, bromate and NO_3. Dissolved oxygen in influents and effluents was monitored by optical fiber measurement in flow through cells.

2.3 Technical scale column experiments

The technical scale columns were set up during the project NASRI on the site of the Federal Environment Agency (UBA) in Berlin-Marienfelde. The system consists of 6 stainless steel columns (l=5 m, d=0.4 m) in series. The columns are filled with technical sand and were adapted to surface water from Lake Tegel for 6 months prior to experiments. The water flow rate was set to 1.3 L/h resulting in a hydraulic retention time of approximately 42 days (7 days per column).

Two long-term experiments were conducted using the technical scale column system (experiment 1: July 09 - April 10; experiment 2: April - November 10). The setup for the first experiment investigating direct ozonation of surface water is described in figure 2.5. To simulate ozonation after a short bank filtration step, the set-up was changed by ozonating the effluent of the first column. Ozonated water was then infiltrated into the second column. Sampling for bulk and trace organic compounds was conducted weekly, dissolved oxygen was measured in column effluents by optical fiber measurement in flow through cells.

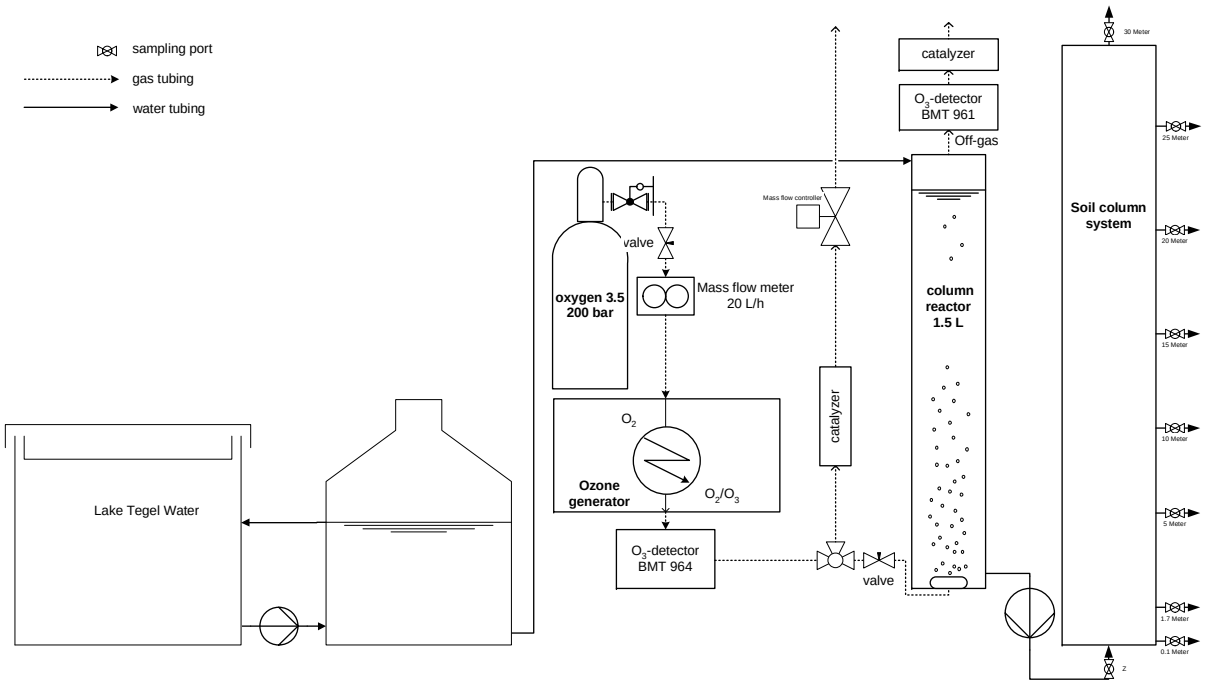

Figure 2.5: Setup of the technical scale column experiment at UBA Marienfelde. The soil column system consists of six columns with a total length of 30 meters.

Continuous ozonation for technical scale experiments was complicated due to the comparably low flow rates in soil columns. Ozonation was set up using a Certizon C 100 Ozonizer (Sander, Uetze/Eltze), which is designed for application in aquaria. After several problems with the ozone generation, the generator was replaced by a 5 g/h generator (Modell 500, Fischer Labor- und Verfahrenstechnik). Operation of this generator at very low capacity resulted in very unstable ozone production and strong variability of ozone dosage. The averaged ozone consumption was approximately 5.8 mg/L (specific ozone consumption $Z = 0.83 \pm 0.27\ mg\ O_3/mg\ DOC_0$) and 4.6 mg/L ($Z = 0.81 \pm 0.34\ mg\ O_3/mg\ DOC_0$) for direct ozonation of surface water and ozonation of column effluent, respectively.

2.4 Pilot plant

Co-authors of this chapter: G. Grützmacher, U. Miehe and D. Orlikowski

The objective of operating a pilot plant was to test the effects of ozonation on the first meter of infiltration during artificial groundwater recharge at larger scale. To simulate processes in the infiltration basin a slow sand filter from the TUB was set up at Lake Tegel. The pilot plant was installed and operated in cooperation between TUB and KWB.

The set-up of the slow sand filter is shown in figure 2.6. The filter with a height of 1 m and a base area of about 1 m^2 was filled with sand from the infiltration pond of the groundwater recharge facility in Berlin, Tegel (size of grains: 0.2 to 2 mm). The hydraulic retention time (HTR) was 12 to 48 hours.

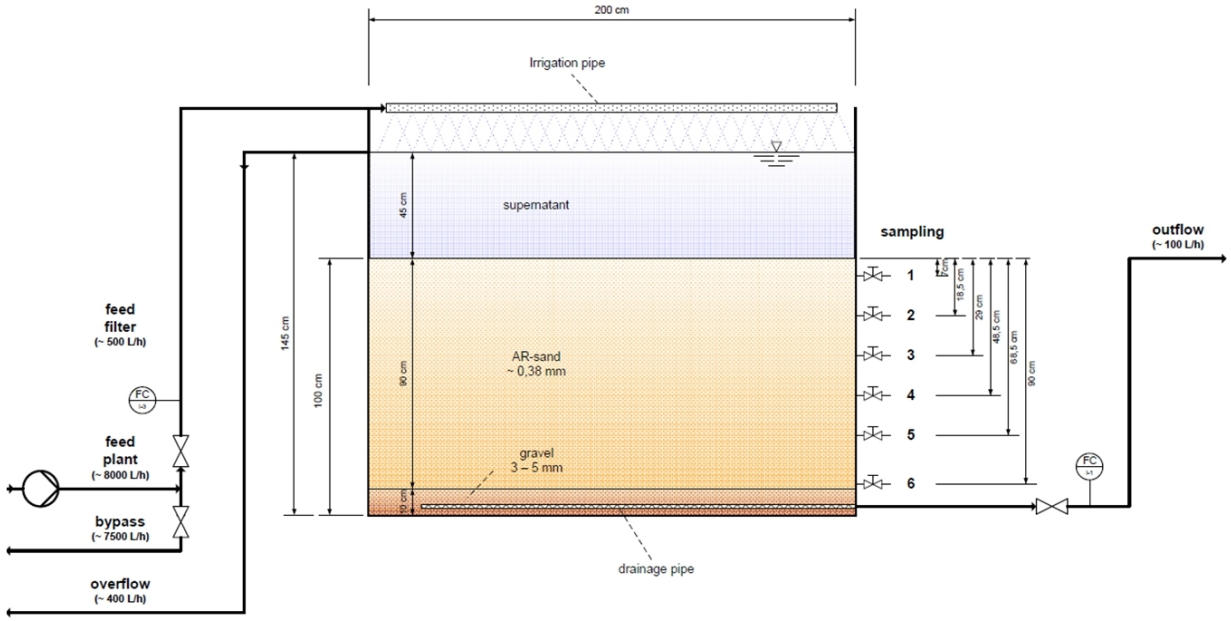

Figure 2.6: Schematic setup of the slow sand filter

The slow sand filter was set up on the 21^{st} of June 2010 and operated for three month without preozonation (phase 1).

For the preozonation of filter influent in the second phase an ozonation unit was leased from ITT-Wedeco (Herford, Germany). Ozone was produced from pure oxygen with an 8 g/h ozone generator. The ozone was injected via a Venturi valve. The ozone reactor was a 50 m PVC hose. Ozonation commenced on 20^{th} of September. After approximately ten days the ozonation unit operated stable with an ozone consumption of about 6.1 mg/L. Dissolved ozone in the effluent of the reactor was below LOQ. Water from Lake Tegel was filtered with a microsieve (28 μm) before ozonation. The setup for the second phase of pilot scale study (ozonation unit and slow sand filter) is demonstrated in figure 2.7.

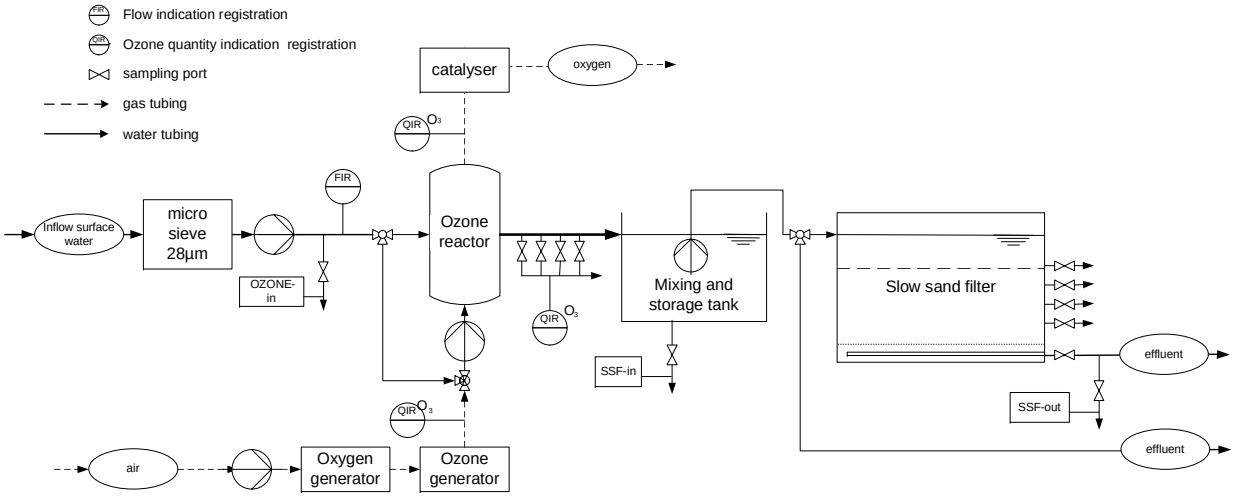

Figure 2.7: Schematic overview of the setup of the pilot plant at Lake Tegel. The sampling points are indicated as "Ozone-in", "SSF-in" and "SSF-out".

The flow rates for the ozonation unit and slow sand filter were in the range of 0.4 to 0.8 m^3/h and 0.05 to 0.2 m^3/h, respectively. Excess water from ozonation was discarded. The water flow corresponded to a filter velocity in the range of 0.3 and 2 m/d (see figure 2.8), which is comparable to the velocity in the ground water recharge basins at Lake Tegel. When the filter velocity dropped below 0.05 m/d, the top layer of the filter media was cleaned.

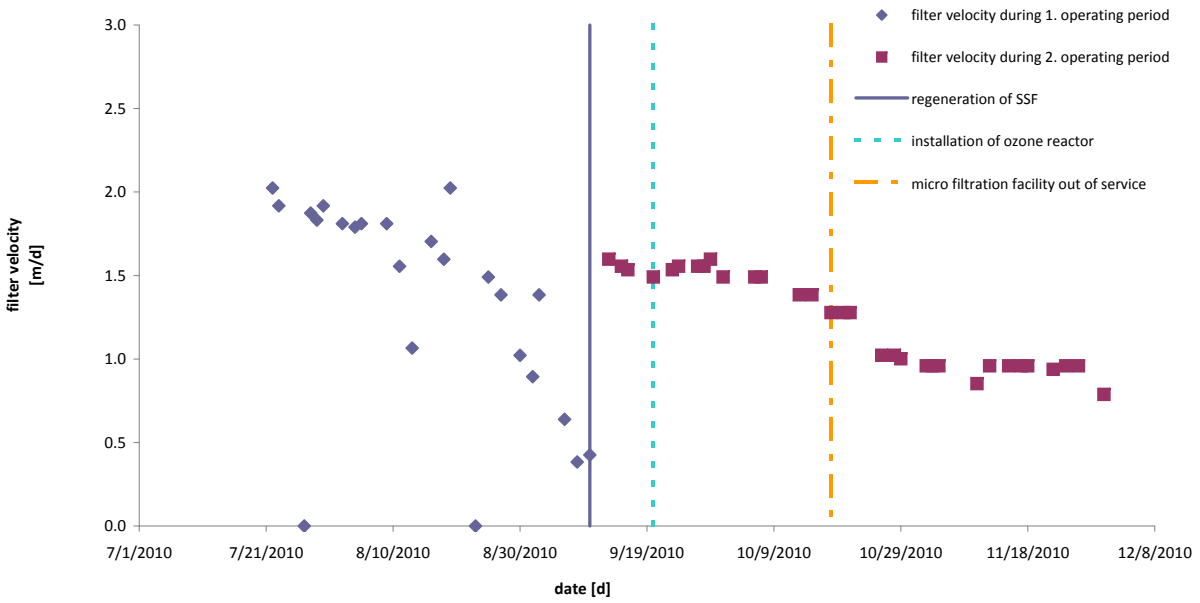

Figure 2.8: Filter velocity of the slow sand filter at pilot plant during phase 1 and 2 of the experiment.

Samples were taken three times a week for bulk analysis and weekly for analysis of trace organic compounds including adsorbable organic halogens. Toxicological testing was conducted twice during operation without preozonation and three times in the second phase with ozonation and slow sand filtration.

2.5 Analytics

All samples for bulk and trace analysis as well as for toxicological testing were collected in glass flasks. Samples from lab scale and technical scale experiments were evaluated with respect to hydraulic retention times.

2.5.1 Bulk organics and redox parameters

Samples for bulk organic analysis were filtered through a 0.45 μm cellulose nitrate filter. DOC was determined with an Elementar varioTOC analyser (Hanau, Germany).
UV absorbance at 254 nm was measured using a Perkin-Elmer photometer Lambda 12 (Berlin, Germany). The anions nitrate, nitrite, sulfate and bromide were detected in unfiltered samples by ion chromatography. Dissolved oxygen was analyzed online by optical fiber measurement using the OXY-4 mini from PreSens GmbH (Regensburg, Germany).

2.5.2 Trace organic compounds (BWB)

All analysis for trace organic substances was conducted at BWB laboratories. For technical scale experiments, analysed substances except desethylatrazine (DEA) were spiked at a target concentration of 1 $\mu g/L$. DEA was monitored as transformation product from ozonation of atrazine. For LOQ see table 3.2.
Additionally, adsorbable organic bromide, chloride and iodide were analyzed at BWB laboratories using the method DIN EN ISO 10304-1/2.

2.5.3 Nitrosamines

The analysis of N-Nitrosodimethylamin (NDMA) and other nitrosamines was carried out at Rheinisch-Westfälisches Institut Für Wasser (IWW). The limit of quantification for these substances was 0.005 $\mu g/L$.

2.5.4 Bromate

The oxidation by-product bromate was analysed using the HPLC-MS/MS method described by [3]. All samples were measured after filtration through a 0.45 μm cellulose nitrate filter. No additional cleanup

was applied before analysis for bromate. Samples from spiking experiments were analysed using a HP 1100 from Hewlett Packard and a Quattro LC of Micromass. External standards were prepared in matrix (surface water, tap water) and bromate was identified using the isotopic ratio of bromine. Analysis at trace concentrations was conducted using a TSQ-Vantage from Thermo Fisher Scientific. As internal standard $Br^{18}O_3$ was used. Identification and quantification was accomplished using m/z of 110.9 and 112.9 (loss of one oxygen atom). The limit of quantification was 0.5 $\mu g/L$.

2.6 Biological test systems

The experiments on toxic effects are carried out by Dr. Grummt from UBA Bad Elster. 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.

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.

2.6.1 Genotoxicity

The identified 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 2.9. 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). 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.

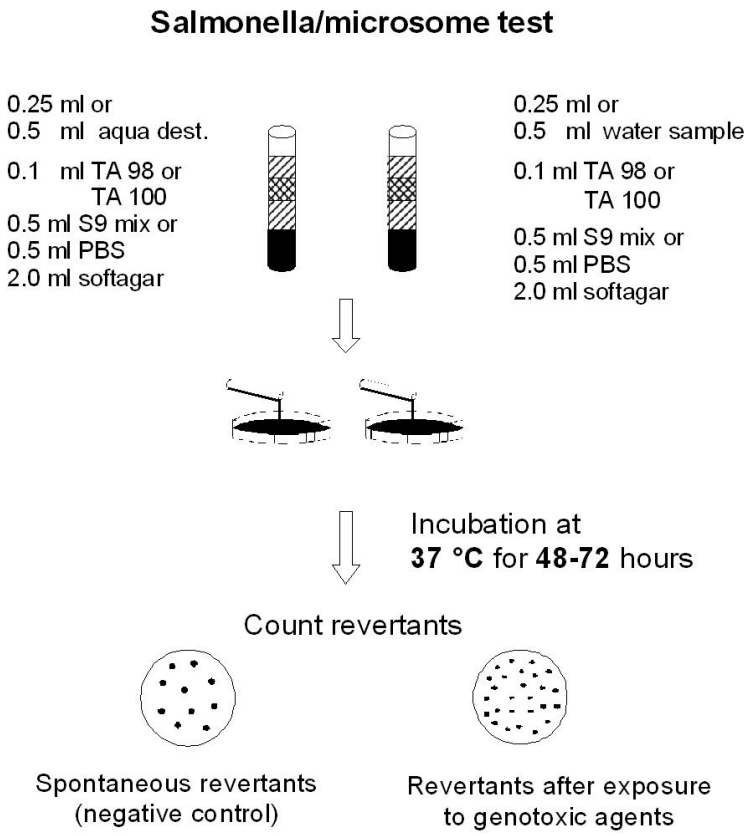

Figure 2.9: Flow chart of the Salmonella/microsome test (Ames test)

Micronucleus Assay

The micronucleus test will become the third internationally standardized 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 2.10).

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

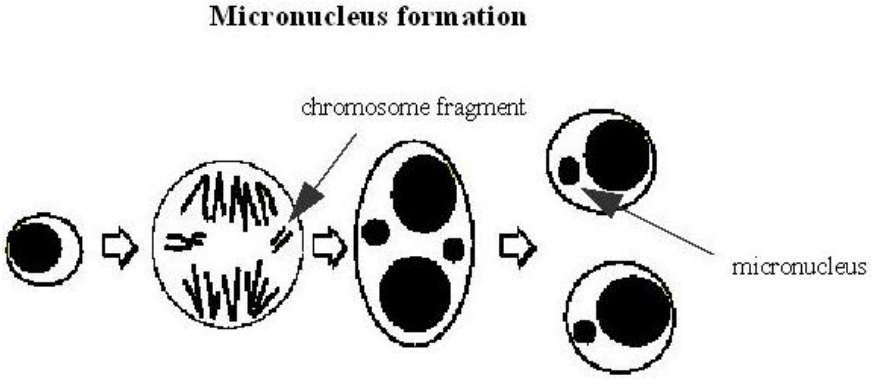

Figure 2.10: Mechanism of micronucleus formation

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 and stained with DAPI (Roth, Germany). For longer archiving, after evalution the slides were covered with Entellan (Merck, Germany). Each water sample was analyzed 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 color, 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.

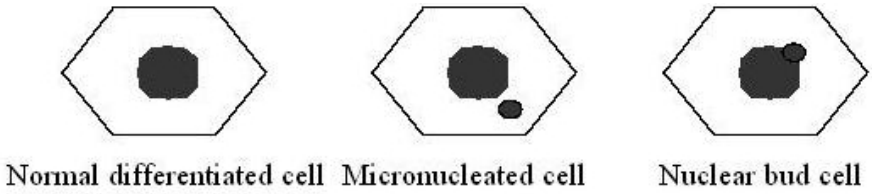

Figure 2.11: Typical example of micronuclei and nuclear buds

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 nuclear material by budding (figure 2.11). 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.

2.6.2 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 induces adverse effects, including secondary genotoxicity.

For cytotoxicity testing two procedures were applied, firstly the determination of the glucose consumption rate, secondly the generation of reactive oxygen species. Determination of glucose consumption rate produced results with regard to 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.

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 resulting in the production of free radicals (figure 2.12).

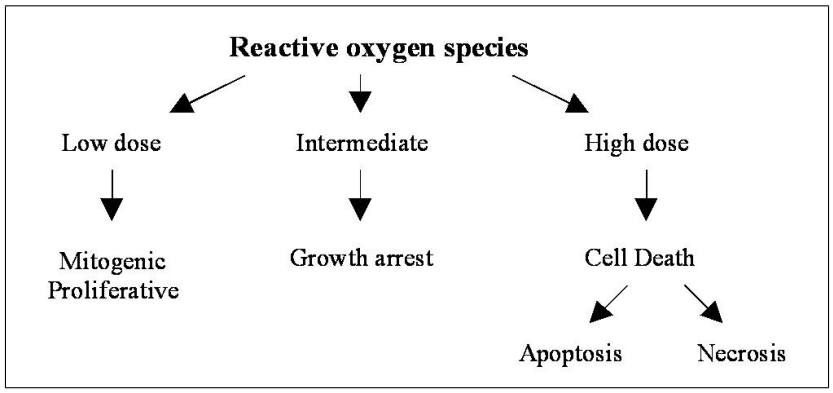

Figure 2.12: Variation of effects of reactive oxygen species on biological structures in dependence of their concentrations

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 2.1: 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 chosen, which is commonly used to analyze respiratory burst in phagocytes. Oxidation of dihydroethidium by reactive oxygen species results in ethidium production. 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 $^\circ$ 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 determined in each experiment. Experiments were repeated three times.

3 Results

3.1 Redox Conditions

3.1.1 Technical-scale soil columns

Results from dissolved oxygen (DO) measurements in different column effluents during the beginning of ozonation are shown in figure 3.1. During ozonation of surface water, pure oxygen is introduced into the water resulting in high oversaturation with dissolved oxygen. This high concentration of dissolved oxygen was then observed in all column effluents. However, an accurate measurement was not feasible since DO was measured by optical fiber measurement calibrated from 0-100%. Differences in concentrations between different columns may therefore be caused by analytical inaccuracy.

With the assumption of unretarded oxygen breakthrough a hydraulic retention time of approximately 7 days in each column could be estimated. This results in an overall retention time of 42 days in technical-scale columns.

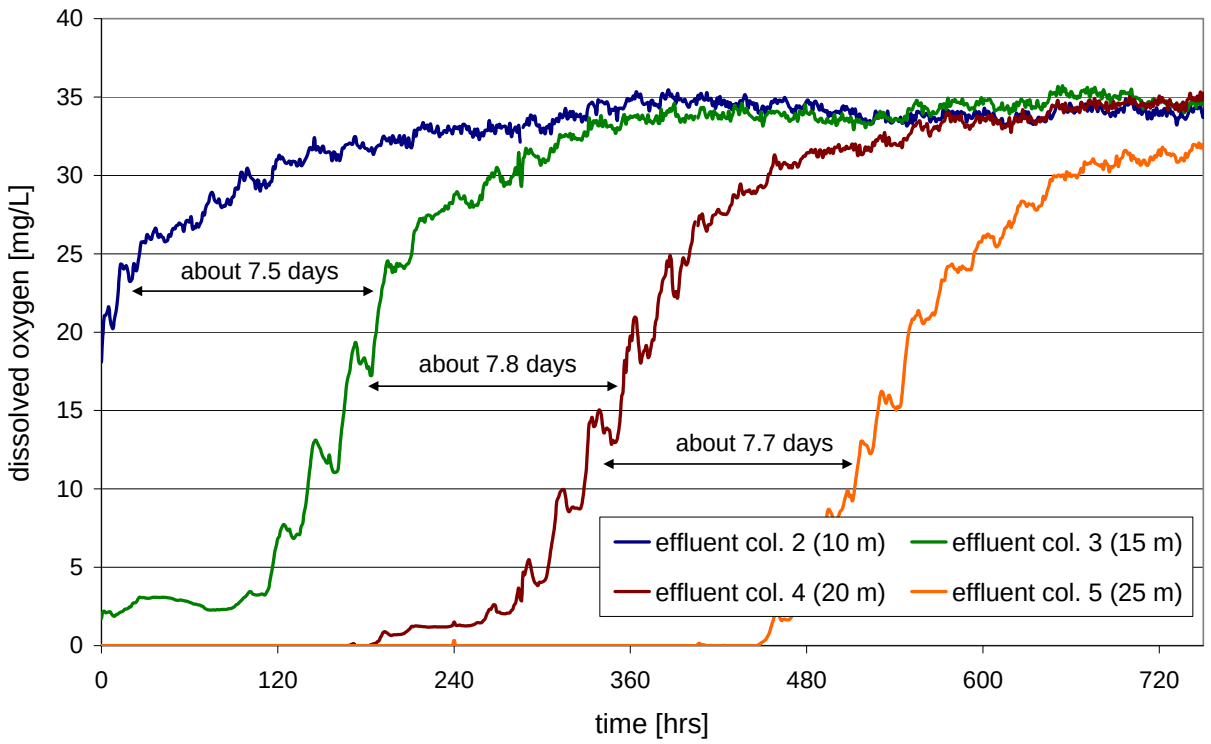

Figure 3.1: Dissolved oxygen in column effluents at technical-scale experiments: results from measurement during the beginning of ozonation in the first phase

3.1.2 Pilot plant

The oxygen concentration in the in- and outflow of the slow sand filter as well as in the influent to the ozonation unit is shown in figure 3.2 over the entire experimental time. Dissolved oxygen in the influent to the pilot plant (Lake Tegel Water) was about 8 mg/L for most of the time. The brief drop in oxygen levels at the start of ozonation in the inlet was due to a lack of mixing of an intermediate container.

During experiments without preozonation, the oxygen was reduced in the slow sand filter to a concentration below 1 mg/L indicating partially anoxic conditions in the filter. With ozonation, the inflow of the filter was highly oversaturated with dissolved oxygen at levels of about 20 mg/L. However, DO probes were only calibrated for a range from 0 to 100% saturation and measured concentration after ozonation were likely not

accurate. Despite this analytical inaccuracy it can be stated that differences to results from technical-scale experiments are significant, where measured DO was significantly higher, which is likely due to differences in experimental set-up. Whereas technical-scale experiments were conducted in a closed system, evaporation of DO from intermediate tanks and the filter can be expected in the pilot plant. The dissolved oxygen in the effluent increased during ozonation to concentration about 11 mg/L. Thus, the introduction of dissolved oxygen during ozonation can be seen as a beneficial side effect to maintain oxic conditions during subsurface passage.

A mass balance of oxygen consumption was not feasible due to inaccurate measurement at high concentrations. In order to balance the consumption during infiltration after ozonation more accurate measurement such as the Winkler method is necessary.

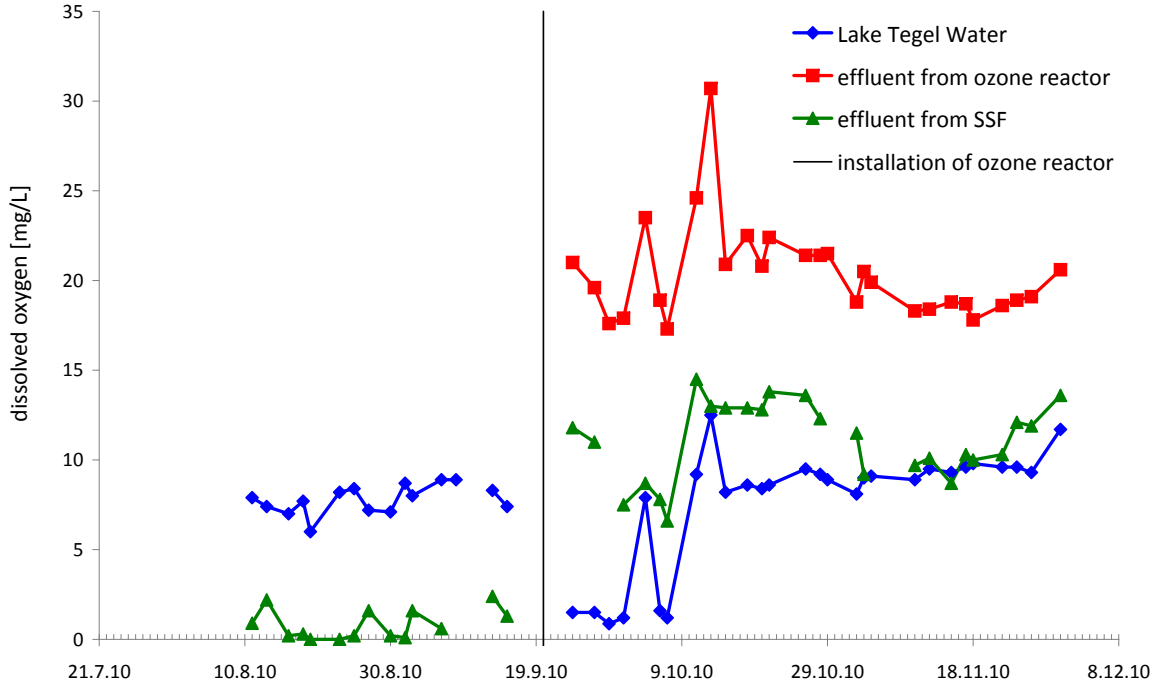

Figure 3.2: Results from dissolved oxygen measurement at the pilot plant

3.2 Removal of dissolved organic carbon (DOC)

3.2.1 Technical scale column experiments

Figure 3.3 shows the ozone consumption as well as the DOC concentration in the influent and effluent of the technical scale column system in both experiments. The ozone consumption is depicted as daily averages with standard deviation. Results show high fluctuation of ozone dosage over time due to the experimental set-up. Continuous ozonation was carried out with a generator for aquaria which did not work reliably. Therefore, ozone was generated using a laboratory generator producing unstable ozone dosages at rather low capacity. The average ozone consumptions in the first and second experiment were approximately 5.8 mg/L (specific ozone consumption $Z = 0.83 \pm 0.27\ mg\ O_3/mg\ DOC_0$) and 4.6 mg/L ($Z = 0.81 \pm 0.34\ mg\ O_3/mg\ DOC_0$), respectively. Results from DOC measurements were flow time corrected for 6 weeks. Despite the strong fluctuation of ozone dosage, DOC removal in the column system was rather constant at about 42%.

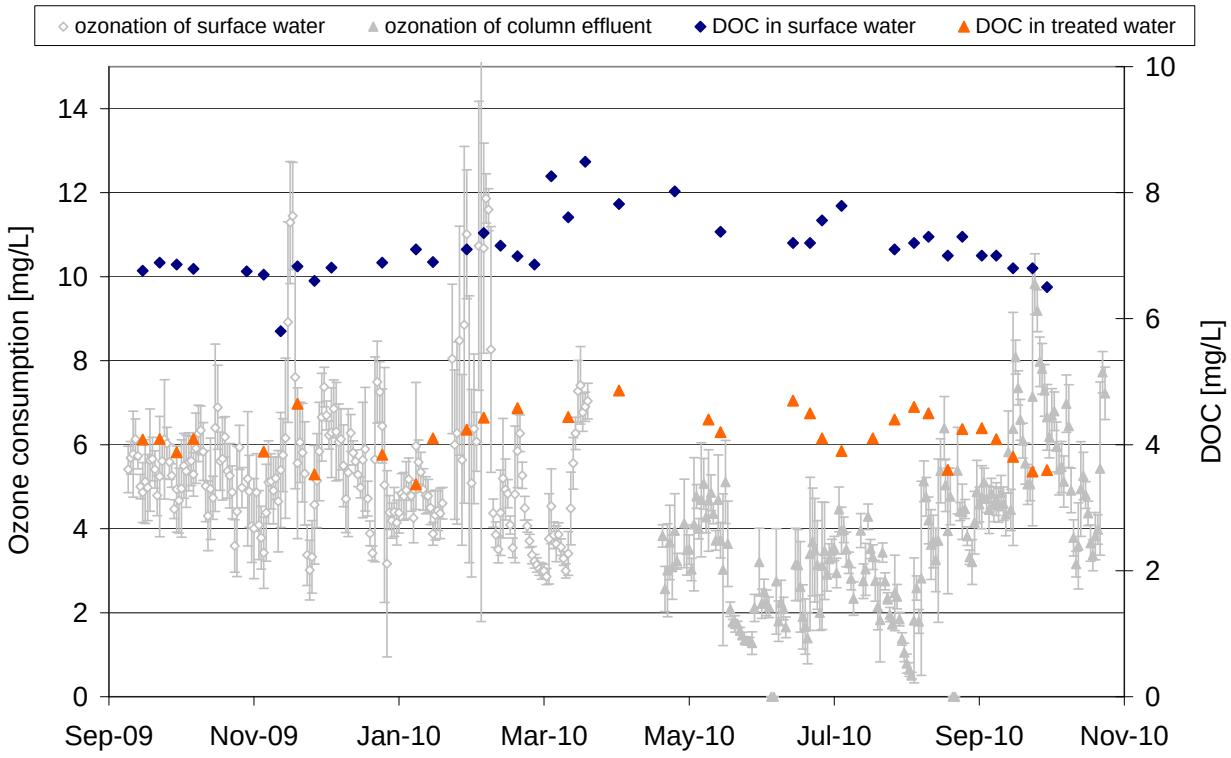

Figure 3.3: DOC concentration of inflow to the soil column system and after the passage of 30 m.

The objective of experiments in technical scale columns was to verify results from laboratory scale experiments concerning the benefits of a preceding bank filtration before ozonation. Therefore, two experiments were conducted with i) direct ozonation of surface water and ii) ozonation of the effluent of the first column to simulate a short bank filtration. DOC results from both experiments are presented in figure 3.4 as box plot diagrams.
Both experiments show good removal of DOC in the first column after ozonation. After 10 m of travel distance, similar DOC concentrations of approximately $4.5 - 4.7\ mg/L$ were measured in both experiments. This confirms results from laboratory scale experiments, where fast reduction of DOC was observed after ozonation (OXIRED-1).
During further infiltration, DOC reduction was relatively low in both experiments. Measured DOC in the effluent of technical scale columns was 4.1 mg/L after direct ozonation of surface water and 4.2 mg/L after ozonation of column effluent. Due to different applied ozone consumption it can be concluded from technical scale experiments that a preceding bank filtration step can reduce ozone demand by approximately 20%. This confirms results from laboratory scale columns conducted in OXIRED-1.

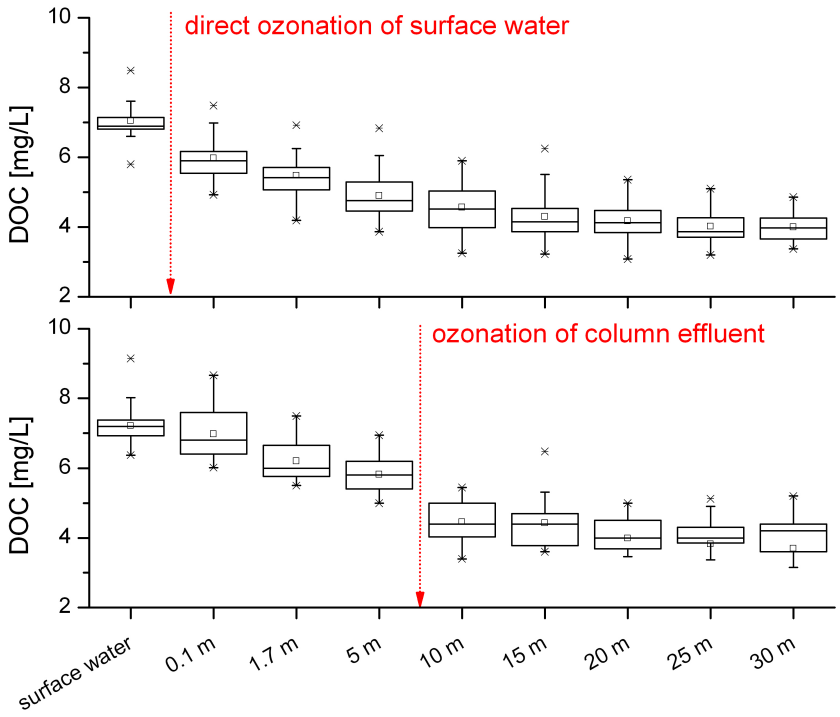

Figure 3.4: Removal of DOC at technical scale column experiment; comparison of the two experimental phases: direct ozonation and ozonation of the column effluent.

3.2.2 Pilot plant

Results from DOC measurement in the pilot plant experiment are shown in figure 3.5 as normalized concentration. Concentration in the source water from Lake Tegel was 7.2 +/- 0.4 mg/L. The removal of DOC in the slow sand filter without ozonation (phase 1) was about 23 % with retention times of 12 to 24 hours. After installation of ozonation the reduction of DOC significantly increased with efficiencies of up to 40%. Later, the efficiency of DOC removal decreased which was likely due to decreasing temperatures in late autumn. On average, total DOC removal by ozonation and slow sand filtration was 34%.

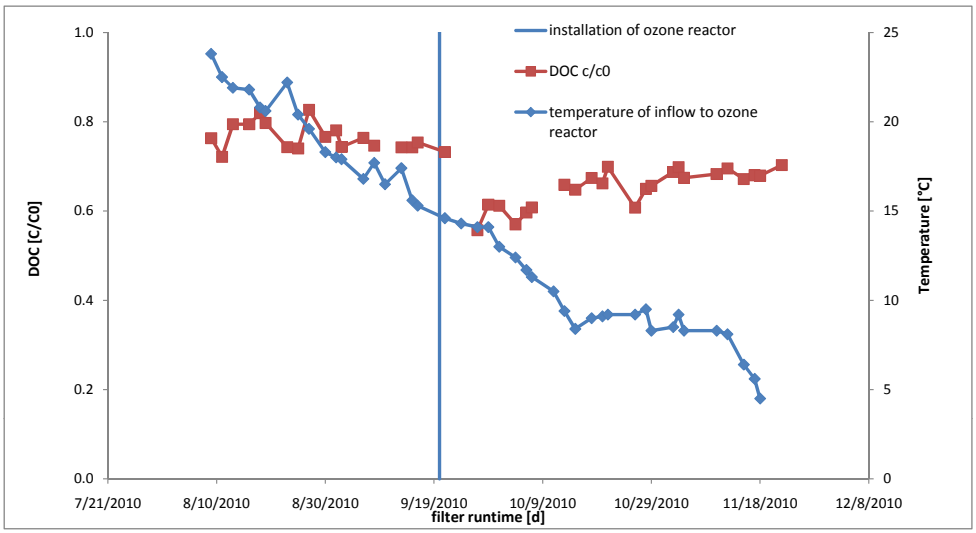

Figure 3.5: DOC removal at pilot plant and water temperature of Lake Tegel before and during ozonation (experimental phase 1 and 2 respectively)

3.2.3 Comparison of DOC degradation with results from OXIRED-1

The results for DOC removal in the different experiments (technical scale, laboratory scale column experiments and pilot plant) are compared in table 3.1.
DOC reduction without ozonation in the first column within 5 to 7 days hydraulic retention time is similar in laboratory and technical scale experiments 22% and 26%, respectively) indicating similar adaptation to surface water from Lake Tegel.
During further infiltration, DOC removal in the technical-scale columns increased to 37%. Earlier experiments with these columns during the NASRI-project showed significantly better DOC reduction of approximately 47% [4]. A reason for the better performance of soil columns during the NASRI-project can be seen in the longer adaptation period. Whereas the columns were operated for several years during the NASRI-project, they were adapted for only 6 to 7 months to Lake Tegel water prior to ozonation in OXIRED. In the time between both projects, columns were fed with groundwater from Berlin Marienfelde providing only a limited amount of bioavailable substrate for bacteria.

In the slow sand filter of the pilot plant, removal degrees from laboratory scale columns were already reached within retention time of one day. In technical scale columns only 19% of DOC were removed after approximately 2 days of retention time. The faster biodegradation in the slow sand filter is likely due to good adaptation, since pilot plant experiments were conducted with adapted sand from the infiltration pond at Lake Tegel. Grünheid et al. (2005) reported DOC removal of around 30% after three days of travel time from monitoring of groundwater recharge in Berlin Tegel [1]. It can therefore be concluded that results from slow sand filter are a good indication for processes in the upper layer of the infiltration basin.

Table 3.1: Comparison of DOC results from laboratory and technical scale columns with pilot plant

	laboratory scale experiments exp. 1	laboratory scale experiments exp. 2	technical scale experiments	pilot plant
surface water DOC $[mg/L]$	6.85	6.41	7.06	7.19
O_3 consumption $[mg\ O_3/mg\ DOC_0]$	0.94±0.05	0.69±0.05	0.83±0.27	0.8
DOC removal without preozonation [%]				
temperature	22°C	22°C	12°C	15 – 23°C
0.5 - appr. 2 days retention time			19	23
5 to 7 days retention time	22	22	26	
42 days retention time			37	
DOC removal after preozonation [%]				
temperature	22°C	22°C	12°C	5 – 15°C
0.5 - appr. 2 days retention time			21	34
5 to 7 days retention time	45	40	28	
42 days retention time			42	

Effects of ozonation on the removal of DOC varied significantly between the experiments. Whereas biodegradation in laboratory scale experiments was strongly improved by preozonation (from 22% to > 40%) only insignificant changes were observed in the first column of the technical-scale system. The high fluctuations of ozone dosage might be an explanation for the bad performance of technical-scale columns, since water with different quality and availability of substrates was infiltrated into the columns causing stress for the bacteria. In addition, higher temperature during laboratory scale experiments probably resulted in better biodegradation.

Results from pilot experiments confirmed the improvement of DOC removal during initial infiltration by preozonation. However, efficiency of biodegradation was significantly lower compared to laboratory scale columns. This can be explained by rapidly decreasing water temperatures during field experiments, since the strong impact of temperature on biodegradation during groundwater recharge is known [5].

- Preceding bank filtration step can reduce ozone demand by approximately 20%
- Results from pilot plant confirmed improvement of DOC removal by preozonation
- DOC reduction in SSF improved by ozonation from 23% to 34%
- Temperature confirmed as important parameter for DOC removal during infiltration

3.3 Transformation of trace organic compounds

3.3.1 Technical scale column experiments

The removal of trace organic compounds at technical scale column experiments was investigated to confirm the results gained in laboratory scale column experiments during OXIRED phase 1. Therefore, the relevant trace compounds Carbamazepine (CBZ), Sulfamethoxazole (SMX), Bentazone, MTBE, ETBE, Atrazine, Linuron and Diuron were spiked during experiments with direct ozonation at target concentrations of 1 $\mu g/L$. In addition to the spiked compounds the metabolite Desethylatrazine as well as adsorbable organic chlorine (AOCl), bromine (AOBr) and iodine (AOI) were measured by BWB laboratories.

Spiking and analysis of CBZ, SMX and bentazone was stopped after two sampling campaigns since these substances were rapidly transformed during ozonation. Removal of $>$ 99 % for bentazone and SMX and $>$ 95 % for CBZ were calculated (concentrations below LOQ set to $\frac{1}{2}$ LOQ).

The concentrations of the other parameters are shown in figure 3.6 as box plots. Data from Diuron and Linuron are not shown since the concentrations in ozonated samples were close to the detection limit. However, both compounds were detected in several samples after soil passage of 25 m indicating a high persistence to removal in the columns.

AOBr was reduced by ozonation from concentrations of around 15 $\mu g/L$ to below 5 $\mu g/L$. Significant reduction of AOBr by ozonation was also observed in laboratory scale experiments during OXIRED-1. In those experiments, concurrent IC-analyses showed an increase of inorganic bromide by ozonation. During soil passage no further reduction of AOBr was observed. Adsorbable organic iodine was measured as indicator for iodinated contrast media. The relatively low reactivity of AOI during ozonation observed in OXIRED-1 was confirmed in technical scale experiments. During subsurface passage no significant deiodination was observed, which has also been reported in literature for aerobic groundwater recharge (e.g. [1]). Concentrations of AOCl in surface water were below or close to LOQ in all samples.

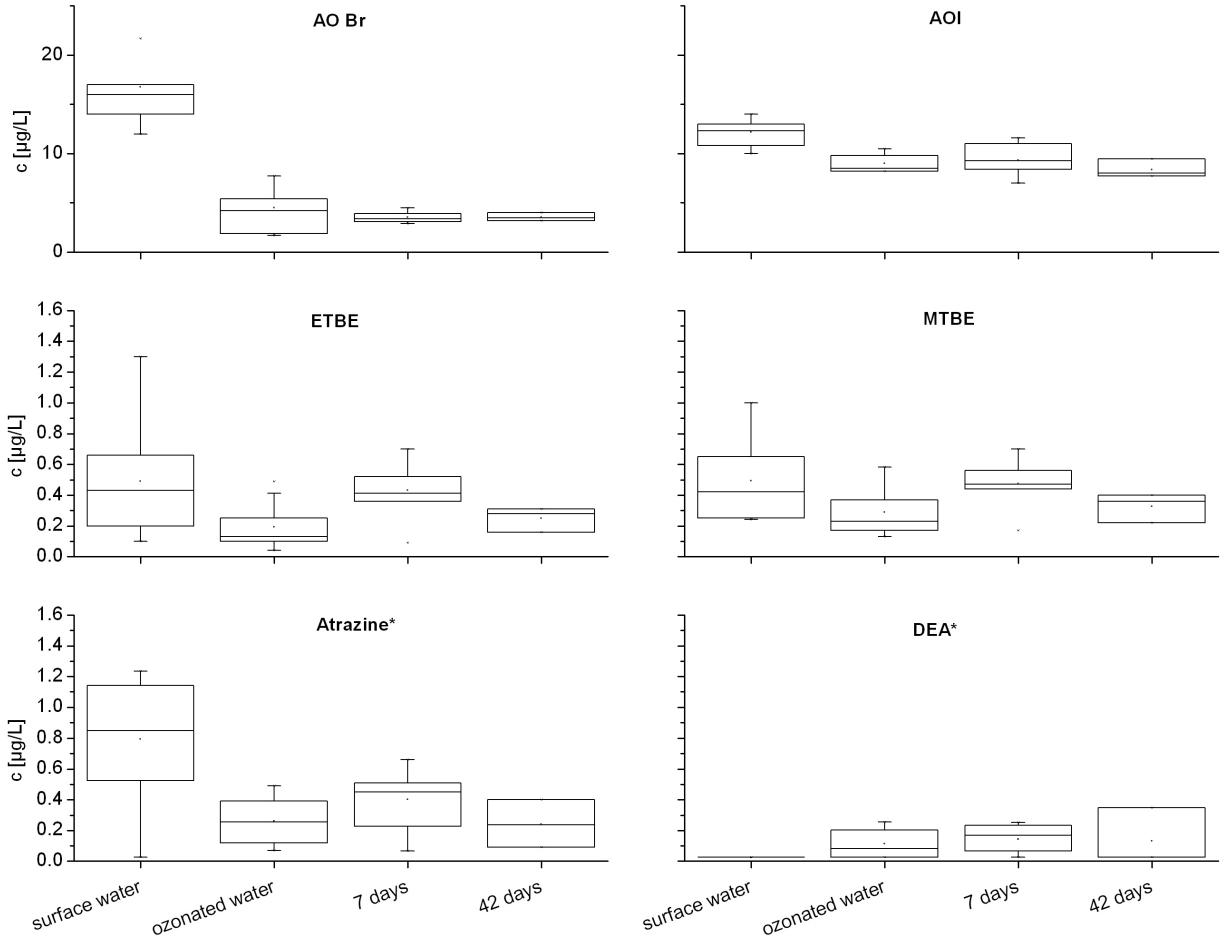

Figure 3.6: Concentration of AOBr, AOI, ETBE, MTBE, Atrazine and Desethylatrazine in technical scale column experiments (* some samples are not quantified, samples below LOQ are calculated as LOQ)

The fuel additives MTBE and ETBE were only partly transformed during ozonation which is confirmed by results from OXIRED-1 as well as other literature for MTBE [6]. The increase of MTBE and ETBE in the first soil column can not be explained. It is assumed that inacurracy is caused by unstable spiking, which was likely due to volatisation from spiking solution. In addition, sampling could not always be conducted with respect to hydraulic retention time.

Desethylatrazine was reported as transformation product from ozonation of atrazine [7] and its formation was also observed in laboratory scale experiments. Technical scale experiments confirmed the formation of DEA from atrazine. Both compounds, atrazine and its transformation product DEA were persistent in soil columns.

3.3.2 Pilot plant

During pilot scale investigations several trace organic compounds were analysed weekly at BWB laboratories. Results from monitoring of trace compounds in the source water of the pilot plant (Lake Tegel) are summarized in table 3.2.

Table 3.2: Summary of analysed substances in Lake Tegel (LOQ: Limit of quantification; n. c.: not calculated; n. q.: not quantified)

substance	*LOQ* $[\mu g/L]$	n	$n \leq LOQ$	c_{max} $[\mu g/L]$	$\bar{c_0}$ $[\mu g/L]$	*standard deviation*
Substances quantified in all surface water samples from Lake Tegel						
ETBE	0.03	8	0	0.59	0.33	0.18
AMDOPH	0.02	7	0	0.15	0.13	0.02
Carbamazepine	0.02	7	0	1.30	1.12	0.25
Phenazon	0.05	7	0	0.21	0.12	0.04
AAA	0.05	7	0	0.41	0.35	0.06
FAA	0.05	7	0	0.59	0.52	0.08
Primidone	0.02	7	0	0.16	0.13	0.03
p-TSA	0.05	8	0	0.22	0.13	0.04
BSA	0.03	8	0	0.11	0.08	0.02
Sulfamethoxazole	0.03	8	0	0.25	0.17	0.05
Metoprolol	0.03	8	0	0.38	0.28	0.09
Benzotriazol	0.02	7	0	2.50	2.26	0.24
Tolyltriazole	0.02	7	0	1.40	0.99	0.21
Substances sporadically detected in Lake Tegel						
MTBE	0.03	8	2	0.10	n. c.	n. c.
AMPH	0.05	7	1	0.07	n. c.	n. c.
o-TSA	0.05	8	5	0.06	n. c.	n. c.
Phenobarbital	0.05	8	5	0.08	n. c.	n. c.
NBBSA	0.02	7	3	0.23	n. c.	n. c.
Substances below LOQ in surface water from Lake Tegel						
Dimethylaminophenazon	0.05	7	7	n. q.	n. q.	n. c.
DP	0.05	7	7	n. q.	n. q.	n. c.
Propyphenazon	0.05	7	7	n. q.	n. q.	n. c.
Koffein	0.10	7	7	n. q.	n. q.	n. c.
Bisphenol A	0.03	8	8	n. q.	n. q.	n. c.

Removal efficiencies for compounds, which were continuously quantified in Lake Tegel water, are summarized in table 3.3. During operation of slow sand filtration without preozonation many of the investigated compounds showed a high persistence. Only the compounds ETBE and Phenazone as well as the metabolite AAA were efficiently removed in the filter. Moderate removal was observed for Metoprolol and the metabolite FAA, the concentration of Sulfamethoxazole was reduced by approximately 30%.

During ozonation, concentrations of many compounds were efficiently reduced. Especially the pharmaceuticals CBZ and SMX, which are of interest for artificial recharge systems, are highly reactive to ozone. However, some trace contaminants, like ETBE, the metabolite AMDOPH and the antiepileptic drug Primidone were only partly transformed during ozonation.

Removal in the slow sand filter after ozonation was not calculated for most compounds, since concentrations were reduced below or close to LOQ by ozonation not allowing adequate calculation of further degradation.

Finally, it can be stated that the combination of ozonation and artificial recharge is suitable for the removal of many trace organic contaminants. Only the compounds AMDOPH, Benzotriazol and Primidone were detected regularly after treatment with ozonation and slow sand filtration.

Table 3.3: Removal of trace organics at pilot plant (n. c.: not calculated, because c_0 was too low for calculation of removal efficiency)

substance	removal in SSF without ozonation	removal during ozonation	removal in SSF after ozonation	total removal
ETBE	$> 96\%^1$	$\sim 50\%$	n. c.	$> 90\%^1$
AMDOPH	$< 10\%$	$\sim 50\%$	$< 10\%$	$\sim 50\%$
Carbamazepine	$< 10\%$	$> 98\%^1$	n. c.	$> 98\%^1$
Phenazon	$> 70\%^1$	$> 70\%^1$	n. c.	$> 70\%^1$
AAA	$> 70\%^1$	$> 90\%^1$	n. c.	$> 90\%^1$
FAA	$\sim 70\%$	$> 90\%^1$	n. c.	$> 90\%^1$
Primidone	$< 10\%$	$\sim 70\%$	n. c.	$\sim 70\%^2$
p-TSA	$< 10\%$	$> 50\%^2$	n. c.	$> 50\%^2$
BSA	$< 10\%$	$> 50\%^2$	n. c.	$> 50\%^2$
Sulfamethoxazole	$\sim 30\%$	$> 80\%^1$	n. c.	$> 80\%^1$
Metoprolol	$\sim 60\%$	$> 90\%^1$	n. c.	$> 90\%^1$
Benzotriazol	$< 10\%$	$\sim 85\%$	$< 10\%$	$\sim 85\%$
Tolyltriazole	$< 10\%$	$> 94\%^2$	n. c.	$> 94\%^2$

[1] removal below LOQ in all samples, samples below LOQ calculated as $\frac{1}{2}$LOQ

[2] several samples below LOQ, data shown as minimum reduction, samples below LOQ calculated as $\frac{1}{2}$LOQ

Results from analysis of AOI and AOBr are presented in figure 3.7. AOBr decreased significantly during ozonation of surface water. However, this decrease can also be due to stripping of volatile THMs. During infiltration into the sand filter, an increase of AOBr was observed. This increase probably results from AOBr production by algae in the infiltrating water since the slow sand filter was exposed to sunlight and similar effects were not observed in dark soil column experiments. However, the production of AOBr in the slow sand filter influent was also not observed in experiments without preozonation.
The AOI was only slightly reduced during ozonation and stable during slow sand filtration.

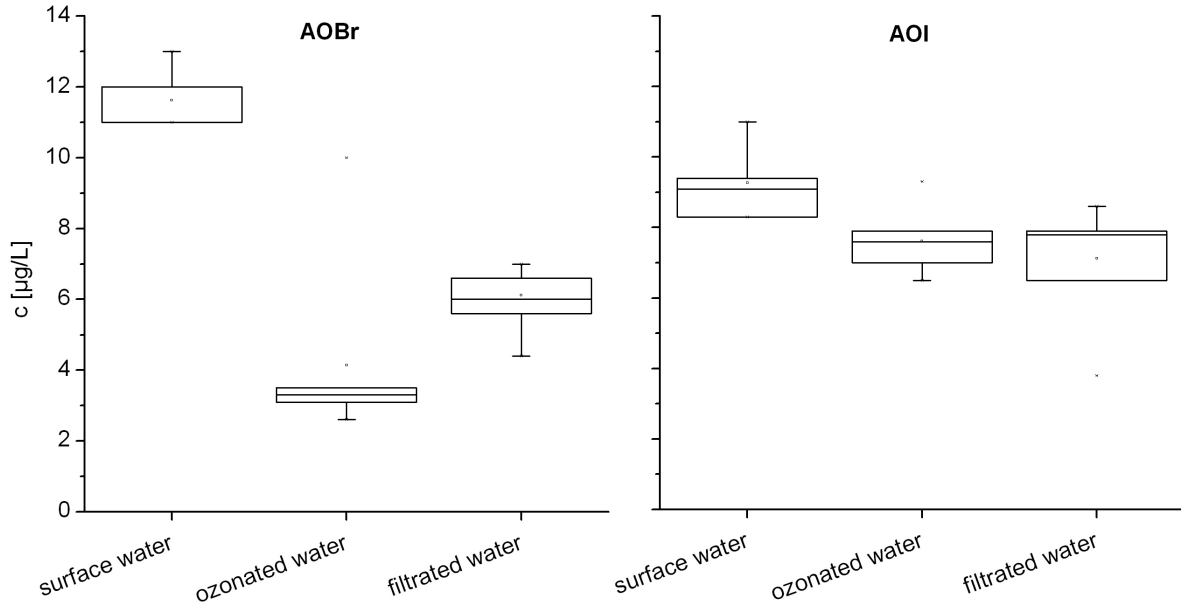

Figure 3.7: Removal of AOBr and AOI at pilot plant; Box-plot diagram of the measured values in the surface water, after the ozonation unit and after the filtration in the slow sand filter.

3.3.3 Comparison of trace organic removal from different experiments

In tables 3.4 and 3.5 results from removal of trace organics in different experiments are compared.

The AOBr from surface water was efficiently reduced during ozonation in all experiments. During infiltration in soil columns, no further degradation of AOBr was observed. In the pilot plant a significant increase of AOBr was measured in the slow sand filter which is probably caused by production from algae in the infiltrating water. Results from AOI measurements were comparable in all experiments. During ozonation, AOI was reduced by less than 30%. During infiltration and soil passage no reduction of AOI was observed, since all experiments were conducted under aerobic conditions.

The antibiotic SMX and the antiepileptic drug CBZ were rapidly transformed by ozone in all experiments. This is confirmed by high reaction rates with these compounds to ozone in literature [8]. Results from experiments without ozonation were not consistent. Whereas almost complete degradation of SMX was observed in laboratory scale columns, the concentration was only partly reduced in the slow sand filter. This difference can be explained by different initial concentration since SMX was spiked at 1 $\mu g/L$ during laboratory scale experiments. Baumgarten et al. (2011) reported initial concentration of SMX being a driving factor for its biodegradation [9]. In addition, it has to be stated that the retention time in the slow sand filter was significantly lower compared to laboratory scale soil columns. CBZ was persistent during soil passage in all experiments. This was expected since CBZ is discussed as a possible anthropogenic marker in the environment [10]. However, under anoxic/anaerobic conditions, removal of CBZ has been reported [11].

Reduction of the pesticides atrazine, diuron and linuron was investigated in laboratory and technical scale experiments. During ozonation, the concentration of atrazine was reduced by 60-70%. The reactions of atrazine during ozonation are well documented in literature [7]. The known transformation product desethylatrazine was detected in both experiments after ozonation. Both, atrazine and its transformation product were persistent in soil columns.

Transformation of Linuron and Diuron during ozonation was approximately 90%. Results from soil column experiments indicate high persistence to biodegradation of these substances.

The fuel additives MTBE and ETBE were spiked during laboratory scale and technical scale experiments and monitored unspiked during pilot studies. Both compounds were only partly transformed during ozonation (40-80%) with ETBE showing slightly higher reactivity. The low reactivity of MTBE with ozone is well documented in literature [6].
Concentration of ETBE was reduced below LOQ during infiltration in laboratory and pilot scale studies. However, during 42 days of soil passage in technical scale experiments, ETBE was not reduced. Whether removal in other experiments is due to biodegradation or volatilisation from feed water needs to be clarified in further experiments.

Table 3.4: Reduction of trace organic compounds by ozonation: Summary of results from different experiments (n. a.: not analyzed in this experiment; n. c.: not calculated, because c_0 was too low for calculation of removal efficiency)

	pilot scale	technical scale	lab scale exp. 1	lab scale exp. 2
ozone consumption $[mg/L]$	5.70 ± 0.29	5.79 ± 1.87	6.60 ± 0.36	4.40 ± 0.33
spec. O_3 consumption $[mg/mg\ DOC_0]$	0.81 ± 0.05	0.83 ± 0.27	0.95 ± 0.06	0.69 ± 0.05
AOBr	∼ 70%	∼ 70%	n.a.	∼ 80%
AOI	< 30%	∼ 30%	n.a.	< 30%
MTBE	∼ 40%	∼ 40%	∼ 60%	∼ 40%
ETBE	∼ 50%	∼ 60%	∼ 80%	∼ 60%
Carbamazepine (CBZ)	> 98%	> 95%	> 97%	n.a.
Sulfamethoxazole (SMX)	> 82%	> 99%	> 98%	n.a.
Atrazine	n.a.	∼ 70%	∼ 70%	∼ 60%
Linuron	n.a.	∼ 80%	> 90%	∼ 80%
Diuron	n.a.	> 90%	> 90%	> 90%

Table 3.5: Reduction of trace organic compounds during infiltration, pilot plant: results from first experiment without ozonation, technical scale experiments: results from experiments with direct ozonation of surface water, lab scale: results from reference column without ozonation (n.a.: not analyzed; n.c.: not calculated, because c_0 was too low for calculation of removal efficiency)

	pilot scale	technical scale	lab scale
retention time [d]	0.5 - 1	42	5 - 6
AOBr	$< 10\%$	$< 10\%$	$< 20\%$
AOI	$< 10\%$	$< 10\%$	$< 10\%$
MTBE	$\sim 30\%$	$< 10\%$	70%
ETBE	$> 96\%$	$< 10\%$	$\sim 80\%$
Carbamazepine	$< 10\%$	n.a.	$< 10\%$
Sulfamethoxazole	30%	n.a.	90%
Atrazine	n.a.	$< 10\%$	$< 10\%$[1]
Linuron	n.a.	$> 90\%$	$< 10\%$[1]
Diuron	n.a.	n.c.	$< 10\%$[1]

[1] strong variation in effluent concentration

- Efficient removal of several trace organic compounds by ozone (e.g. CBZ, AAA, FAA, SMX, Metoprolol and Tolyltriazole) - otherwise persistent during subsurface passage
- Efficient reduction of AOBr during ozonation of surface water
- No significant elimination of AOI during treatment with ozonation and subsequent groundwater recharge

3.4 Formation and removal of oxidation by-products (OBP)

A major drawback of applications with ozone is the formation of oxidation by-products. The most discussed by-products from ozonation are bromate and N-nitroso-dimethylamin (NDMA). Besides these a variety of organic by-products can be formed from the oxidation of natural organic matter (NOM) [12]: aldehydes, ketones, keto aldehydes, carboxylic acids, keto acids, hydroxy acids, alcohols and esters.

During Oxired-2, experiments focussed on the by-product bromate and a group of different nitrosamines including NDMA.

3.4.1 Bromate

Bromate is a "genotoxic carcinogen inducing, for example, renal cell tumors in rats" [12]. It is declared a potential human carcinogen. For bromate, a limit value of 10 $\mu g/L$ is set in the German drinking water directive.

Formation of bromate during ozonation

Bromide concentrations of 100 $\mu g/L$ in Lake Tegel Water were reported in Oxired-1 [13], resulting in high potential for bromate formation. The formation of bromate is influenced by many parameters, such as natural organic matter or ammonium [14, 15]. Strategies for bromate minimization include addition of H_2O_2 or ammonium and lowering the pH [15].

In this study the formation of bromate was investigated in batch experiments and in the pilot plant. Results

are shown in figures 3.8 and 3.9.

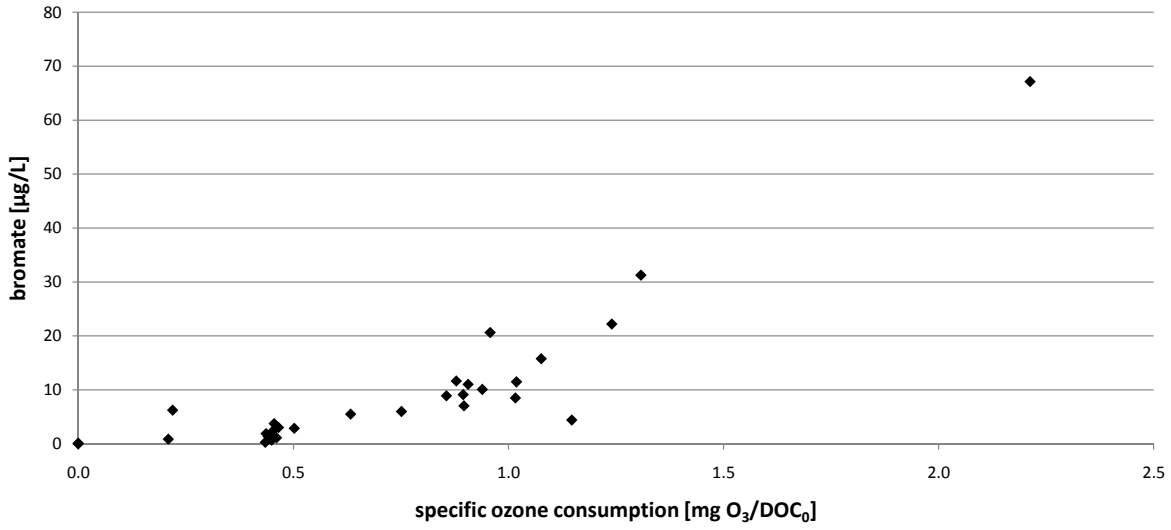

Figure 3.8: Formation of bromate during ozonation in batch experiments

According to figure 3.8 the concentration of bromate increases with the ozone consumption. In samples that were not ozonated no bromate was found. To avoid the formation of bromate in concentrations above the limit value of 10 $\mu g/L$ water should be ozonated with specific ozone consumption below 0.9 $mg\ O_3/mg\ DOC$.

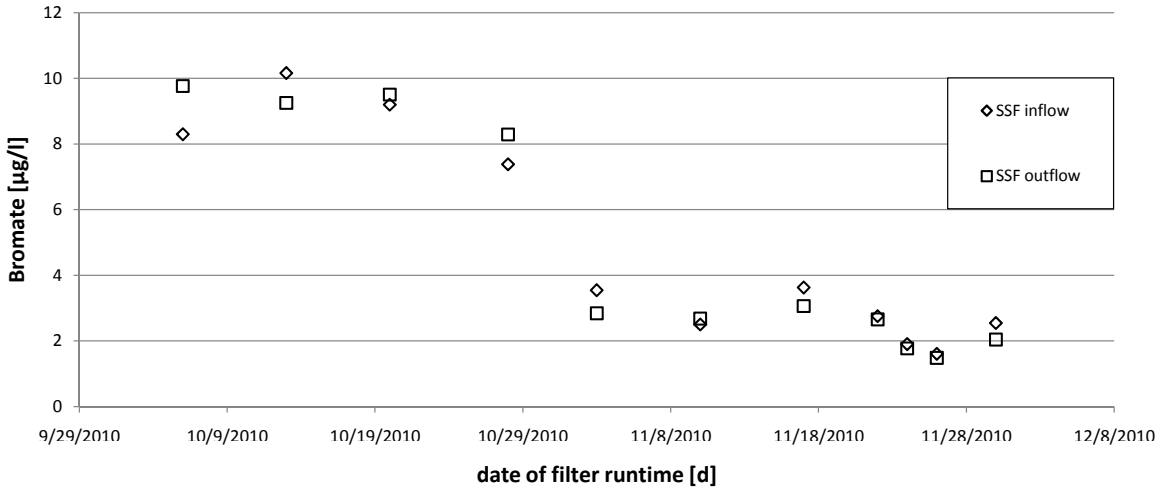

Figure 3.9: Formation of bromate at ozone reactor of pilot plant, no degradation observed

At the pilot plant bromate was formed during ozonation (see figure 3.9) but at different concentrations. The samples investigated during the first 30 days show a concentration of bromate of about 9 $\mu g/L$ while later samples reached only a concentration of about 3 $\mu g/L$. So far, this gap can not be explained conclusively. However, it is possible that changes of process parameters after early November caused this change. Another possible reason is a seasonal change in ammonium concentration in the surface water of Lake Tegel. In addition, decreasing temperature can be an explanation for lower formation of bromate (see [16]).

Removal of bromate during sub surface passage

The removal of bromate during subsurface passage was investigated in laboratory scale column experiments under different redox conditions. Bromate was spiked at 100 $\mu g/L$. Ozonation for the anoxic and the

aerobic soil column were conducted with specific ozone consumption of 0.7 $mg\ O_3/mg\ DOC$. Operational conditions in the columns are summarized in table 3.6. Aerobic DOC removal (23% without ozonation and 39% with preozonation) was comparable to previous results presented in OXIRED-1.

The set-up for the anoxic experiments included purging with nitrogen between the feed storage and the soil column. Since ozonation produced easy biodegradable DOC and biodegradation could be expected in the partly aerobic feed storage (compare [17]), additional sampling of the anoxic column influent was conducted in order to balance removal under anoxic conditions. Approximately 0.8 mg/L of DOC were biodegraded in the feed storage and tubing prior to infiltration into the columns. During anoxic soil passage only about 10% of DOC were removed.

Table 3.6: Operational conditions in laboratory scale columns (average values of 8-12 measurements)

	reference col.		anoxic col.		aerobic col.		anoxic after aerobic	
	feed tank	effluent	influent	effluent	feed tank	effluent	influent	effluent
DOC $[mg/L]$	7.3	5.6	6.2	5.6	7.1	4.3	4.3	4.3
oxygen [mg/L]	5.8	1.8	0	0.1	13.3	4.9	0.3	0.1
nitrate [mg/L]	7.6	7.7	7.3	6.3	7.6	7.7	7.7	7.2
prevealing redox conditions	mostly aerobic		anoxic		aerobic		anoxic	

Data from bromate analyses are shown in figure 3.10 as box plot diagrams. Results confirm the assumption that bromate is only removed under anoxic conditions. Whereas bromate reduction by approximately 20% was observed in the anoxic column after ozonation, no removal occurred during aerobic infiltration. These results are confirmed by monitoring of bromate during pilot scale studies (see figure 3.9). In the anoxic column after aerobic infiltration, bromate degradation was probably limited by the availability of BDOC.

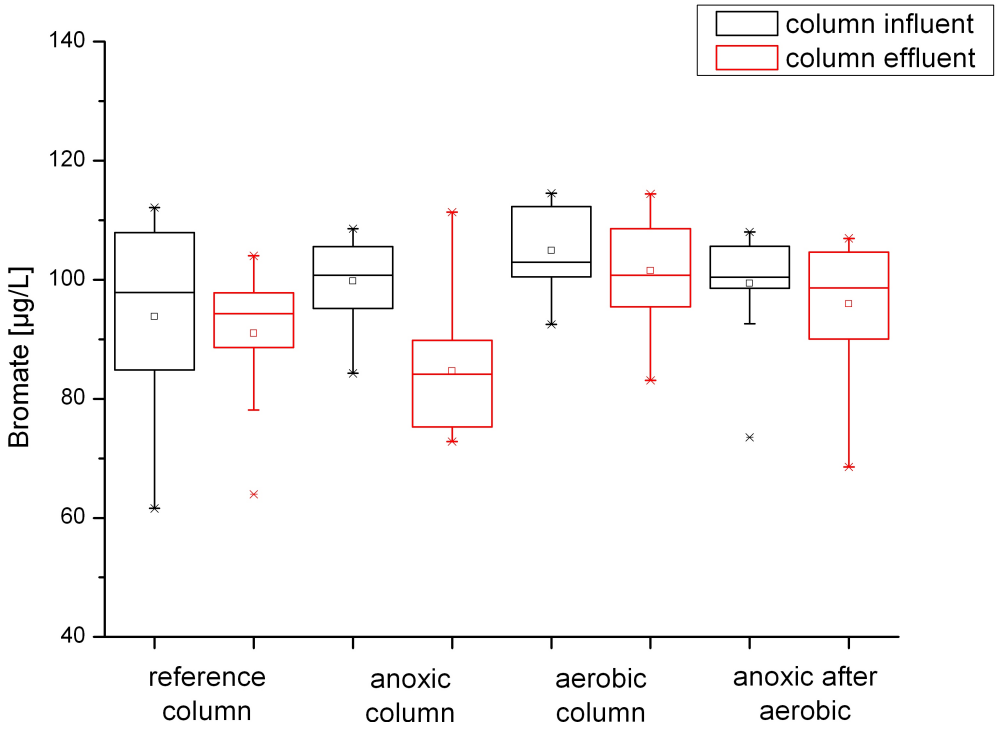

Figure 3.10: Concentrations of bromate in laboratory scale columns under different redox conditions

Results from laboratory scale experiments were confirmed in the diploma thesis of Simon Kuhnt [2]. In the third experiment of his thesis he ozonated surface water from Lake Tegel, added bromate at different

concentrations (see table 3.7) and operated recirculating columns under anoxic conditions over a time period of 13 days. Due to ozonation of the water, sufficient amount of BDOC was available for bacteria. Normalized bromate concentrations are shown in figure 3.11. Within ten days of retention time, bromate concentrations were reduced by approximately 60%. Experiments also demonstrated that bromate can be used as alternative electron acceptor in presence and absence of nitrate.

Table 3.7: Operational parameters for recirculating column experiments for the investigation of bromate removal under anoxic conditions [2]

		CC1	CC2	CC3	CC4	CC5	CC6
Ozone consumption	$[mgO_3/mgDOC_0]$	0.9	0.9	0.9	0.9	0.9	0.9
Bromate dosage	$[\mu g/L]$	0	0	50	50	1000	1000

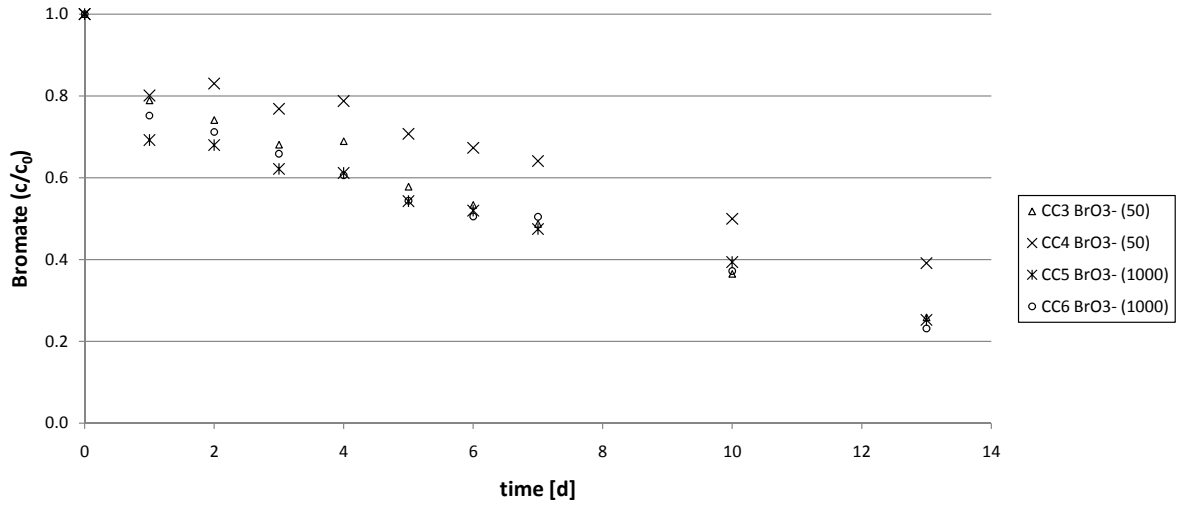

Figure 3.11: Results from bromate measurements in anoxic recirculating column experiments [2]

The removal of bromate under aerobic conditions in the slow sand filter at the pilot plant is shown in figure 3.9. There is no degradation of bromate observed. This confirms the results from laboratory scale experiments.

3.4.2 Nitrosamines

The formation of nitrosamines during ozonation was investigated in batch experiments. The results are listed in table 3.8. The analysed nitrosamines were under the limit of quantification in samples of all investigated ozone consumptions.

Table 3.8: Results of measurement of nitrosamines in ozonated surface water from Lake Tegel

Z $[mgO_3/mgDOC_0]$	0	0.21	0.45	0.94	0.96	1.08	1.15	1.31	2.21
N-Nitrosodimethylamin (NDMA)									
N-Nitrosoethylmethylamin									
N-Nitrosodi-n-propylamin									
N-Nitrosodi-n-butylamin				$< 0.005\mu g/L$					
N-Nitrosodiethylamin									
N-Nitrosomorpholin									
N-Nitrosopiperidin									
N-Nitrosodipyrrolidin									

The removal of nitrosamines especially NDMA was not investigated in column experiments as no nitrosamines were detected in ozonated samples. The analysis of NDMA is difficult and not developed at laboratories at TU Berlin or BWB yet. However, von Gunten [12] observed a low risk of a break-through of nitrosamines after an oxidation and subsequent subsurface passage. Especially the carcinogic by-product NDMA is expected to be easily degradable in subsurface passage.

- Formation of bromate during ozonation of Lake Tegel water below $10 \mu g/L$ with spec. ozone consumption of less than 0.9 $mg\ O_3/mg\ DOC_0$
- No degradation of bromate in subsurface under aerobic conditions
- 60% removal of bromate in subsurface under anoxic conditions within 10 days
- BDOC necessary for degradation of bromate during subsurface passage
- No formation of nitrosamines observed during ozonation from Lake Tegel (sp. ozone consumptions between 0.21 and 2.21 $mgO_3/mgDOC_0$; LOQ: 5 ng/L) of surface water observed

3.5 Standardized test protocol

The standardized test protocol is part of the decision tree. Its objective is to predict the DOC removal at new or existing artificial recharge sites where the combination with ozonation is an option. The test protocol should answer the question whether a site is suitable for ozonation of surface water and a subsequent subsurface passage. Therefore it is necessary to investigate the amount of the easily degradable BDOC. A simple setup allows the transportation to the specific site. For this reason, short recirculating columns were set up and tested. The results of the test over a runtime of 25 days is shown in figure 3.12.

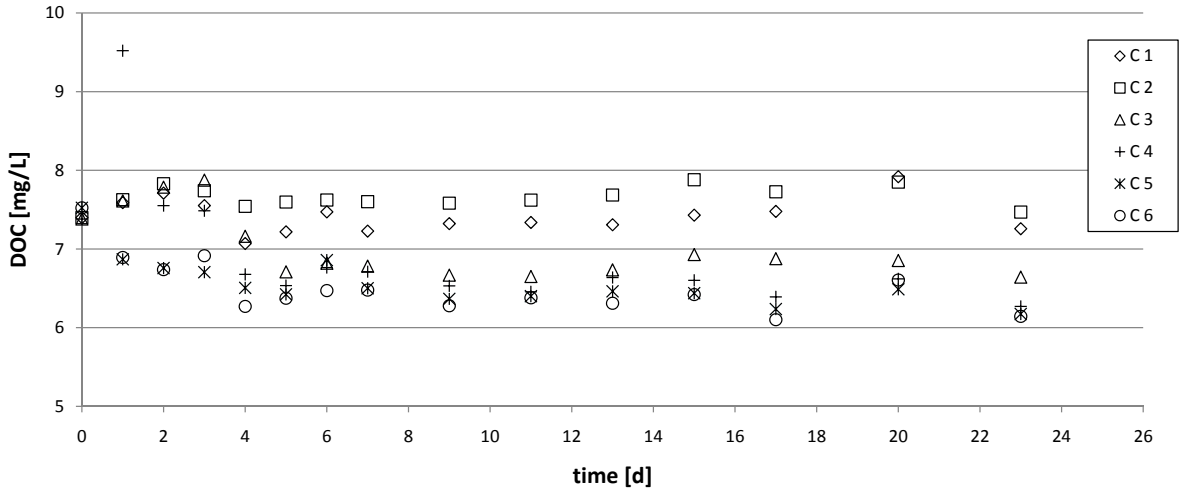

Figure 3.12: Development of DOC in circulating columns over a runtime of 25 days

In columns C 1 to C 4 the values of DOC increased during the first 4 days. This effect is likely attributed to leaching/ desorption of DOC from the column sand. BDOC determined in columns was calcuated as difference from DOC after 23 days to DOC_0.
The reduction of DOC in recirculating columns is shown in figure 3.13 in comparison to parallel measurement in laboratory scale columns and results from batch experiments in Oxired-1.

A strong correlation of ozone consumption and DOC removal was observed in all experiments. As expected, results from laboratory scale columns showed most efficient removal of DOC due to their good adaptation to surface water and relatively long retention times. The comparably low biodegradation in the column with a specific ozone dose of 0.5 $mg\ O_3/mg\ DOC_0$ is likely to less adaptation of bacteria, since this column

was operated under anoxic conditions in the preceding experiment.

Calculated BDOC from standardized test protocol is comparably low due to leaching of DOC from column sand. However, also results from batch experiment in Oxired-1 underestimated biodegradation in soil columns. Therefore, additional tests to optimize the standardized test protocol need to be conducted. It is proposed to operate the recirculating columns with organic-free technical sand in the project OXIMAR.

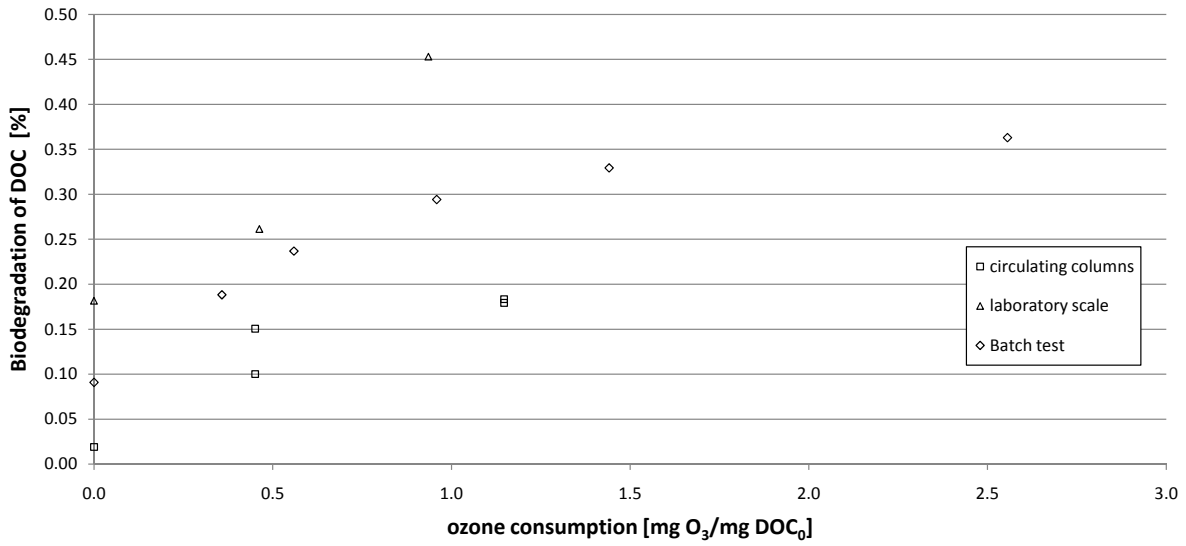

Figure 3.13: Comparison of the biodegradation of DOC in circulating columns with the results in laboratory scale columns and in batch tests (carried out in OXIRED phase 1)

- Results from standardized test protocol not fully satisfactory due to leaching effects from column sand (anoxic aquifer material)
- DOC removal in soil columns higher than in simple batch tests
- Further tests with recirculating columns filled with organic-free technical sand are recommended

3.6 Toxicity testing

One of the objectives of this study was to evaluate the possible toxicological effects of ozonation. The results concerning toxicity at the cell level can lead to statements of effects on the level of the organism. Concerning genotoxicity the results can be translated directly. The difficulty concerning the results of the cytotoxicological tests is the threshold, from which a toxic effect can be detected in the organism.

3.6.1 Genotoxicity

Ames/Salmonella microsome assay

All tested samples of technical scale columns were negative for all test variants in both tester strains in the presence and absence of S9 metabolic activation mix.

Micronucleus assay

The results obtained in the micronucleus assay were negative in all samples.

Table 3.9: Results of investigations on genotoxicity (Ames assay: 1^{st} testing immediately after sample receipt; 2^{nd} testing after 2 weeks)

Sample	Ames assay		Micronucleus assay
	1^{st} testing	2^{nd} testing	
*Samples from **technical scale experiments** on 2010/03/23*			
source water	-	-	-
Ozonated surface water	-	-	-
after 0.1 m column passage	-	-	-
after 1.7 m column passage	-	-	-
after 5 m column passage	-	-	-
after 10 m column passage	-	-	-
after 15 m column passage	-	-	-
after 20 m column passage	-	-	-
after 25 m column passage	-	-	-
after 30 m column passage	-	-	-
*Samples from **pilot plant** on 2010/08/27*			
inflow to slow sand filter	-	-	-
outflow of slow sand filter	-	-	-
*Samples from **pilot plant** on 2010/09/08*			
inflow to slow sand filter	-	-	-
outflow of slow sand filter	-	-	-
*Samples from **pilot plant** on 2010/11/12*			
inflow to ozonation unit	-	-	-
inflow to slow sand filter	-	-	-
outflow of slow sand filter	-	-	-
*Samples from **pilot plant** on 2010/11/26*			
inflow to ozonation unit	-	-	-
inflow to slow sand filter	-	-	-
outflow of slow sand filter	-	-	-
*Samples from **pilot plant** on 2010/11/29*			
inflow to ozonation unit	-	-	-
inflow to slow sand filter	-	-	-
outflow of slow sand filter	-	-	-

3.6.2 Cytotoxicity

Results from cytotoxicity testing are shown in 3.10. Three samples from the pilot plant induce the formation of reactive oxygen species (ROS). One sample (outflow of the ozonation unit) shows a ROS-induction of more than 20% over control level. Two of them (the outflow of slow sand filter at two different dates of sampling) show a ROS-induction of more than 5% over control level - however, both without prior ozonation. Several samples from the technical scale column experiment show a ROS-induction of more than 5% over control level. Surprisingly, it is not the sample of the ozonated surface water that is part of this marked samples, but the samples after 0.1, 1.7, 15 and 25 meters.

Table 3.10: Results of investigations on cytotoxicity, (ROS-Induction: (+) >5% over control; + >10% over control; ++ >20% over control)

Sample	Necrosis	ROS-Induction
*Samples from **technical scale experiments** on 2010/03/23*		
source water	-	-
Ozonated surface water	-	-
after 0.1 m column passage	-	(+)
after 1.7 m column passage	-	(+)
after 5 m column passage	-	-
after 10 m column passage	-	-
after 15 m column passage	-	(+)
after 20 m column passage	-	-
after 25 m column passage	-	(+)
after 30 m column passage	-	-
*Samples from **pilot plant** on 2010/08/27*		
inflow to slow sand filter	-	-
outflow of slow sand filter	-	(+)
*Samples from **pilot plant** on 2010/09/08*		
inflow to slow sand filter	-	-
outflow of slow sand filter	-	(+)
*Samples from **pilot plant** on 2010/11/12*		
inflow to ozonation unit	-	-
inflow to slow sand filter	-	-
outflow of slow sand filter	-	-
*Samples from **pilot plant** on 2010/11/26*		
inflow to ozonation unit	-	-
inflow to slow sand filter	-	++
outflow of slow sand filter	-	-
*Samples from **pilot plant** on 2010/11/29*		
inflow to ozonation unit	-	-
inflow to slow sand filter	-	-
outflow of slow sand filter	-	-

- No systematic genotoxic effects of ozonation or subsurface passage were observed.
- No systematic cytotoxic effects of ozonation. The cause for ROS-induction of more than 5% over control in some samples is not identified and is attributed to unknown co-factors.
- ROS-induction test was identified as a robust, cost-effective and sensitive test to identify and monitor cytotoxic effects in water samples.

4 Conclusions

Results from OXIRED-1 have demonstrated that the combination of ozonation and a subsequent subsurface passage is a feaible solution to enhance the removal of DOC and many relevant trace organics from wastewater impacted waters. Experiments in Oxired-2 confirm these conclusions at a larger scale.

4.1 Degradation of DOC

Results from pilot plant confirm the improvement of DOC removal during infiltration at short retention times. DOC in the effluent of the slow sand filter reached a level of 4.7 mg/L. From monitoring the artificial recharge site in Berlin Tegel, similar values were reported as residual DOC after one month of travel time [1]. In addition, results from the pilot plant indicate a strong impact of seasonal variations, since effluent DOC increased significantly with decreasing temperature.

Technical scale experiments were set up to verify results from Oxired-1 at a larger scale. DOC removal in technical scale columns increased by preozonation from 37% to 42% . However, especially results from the first column showed significantly less efficient biodegradation compared to laboratory scale experiments, which is likely due to unstable operation of online ozonation. In order to assess effects of ozonation on biodegradation during longer subsurface passage, field scale experiments need to be conducted. Long-term field studies are also necessary to investigate the effects of seasonal variations on DOC removal.

The formation of biodegradable DOC strongly depends on the characteristics of the ozonated water. In order to assess the biodegradability of DOC after ozonation, a test protocol for analysis of BDOC after ozonation needs to be established. Batch tests operated in Oxired-1 gave a good indication for effects of ozonation but significantly underestimated DOC removal reported from soil columns. Therefore, a test protocol using recirculating columns was proposed and tested in work package 1. Columns were filled with aquifer material from Berlin Tegel. Results from proposed standardized test protocol were not fully satisfactory due to leaching of DOC from column sand even after several weeks of adaptation.

Further experiments to optimize the standardized test protocol are therefore recommended. In order to eliminate DOC leaching from sand, columns need to be filled with organic-free technical sand. Necessary times for adaptation of sand to surface water also need to be assessed in experiments. In addition, other options, such as the change of filter media have to be taken into account.

4.2 Trace organic compounds

The transformation of trace organic substances during ozonation and subsurface passage in different experiments is summarized in table 4.1. Brackets indicate uncertainties, e.g. due to contradictory results. As expected, most substances with high and medium potential for breakthrough in bank filtration and artificial recharge were not efficiently degraded in the slow sand filter and the soil columns. For the antibiotic sulfamethoxazole, removal of 30% was observed during slow sand filtration with a hydraulic retention time of 12-24 h whereas removal rates of 90% were observed in soil column experiments. Monitoring of sulfamethoxazole during artificial recharge in Berlin confirmed moderate removal of approximately 50% [1]. The more efficient reduction in column experiments is likely due to elevated concentration from spiking.

Ozonation at technical and pilot scale experiments confirmed a good reactivity of carbamazepine and sulfamethoxazole, as observed in Oxired-1. Also concentrations of the pesticides linuron and diuron were efficiently reduced by ozonation with specific ozone consumption of about 0.8 $mg\ O_3/mg\ DOC_0$. In ad-

Table 4.1: Trace organic removal during ozonation and artificial recharge: Summary of results from laboratory, technical and pilot scale experiments (++: complete removal (below LOQ in all samples); +: good removal (> 70%); +/-: partial removal(30 − 70%); -: poor removal (< 30%); n.a.: not analysed

	SSF/AR	ozonation	ozonation + SSF/AR
High potential for breakthrough in RBF/AR systems			
MTBE	(+/-)	+/-	(+/-)
ETBE	(+)	+/-	(+)
Sulfamethoxazole	(+/-)	++	++
Carbamazepine	-	++	++
Primidone	-	+/-	+/-
Medium / uncertain potential for breakthrough in RBF/AR systems			
Bentazone	-	++	++
Atrazine	-	+/-	+/-
Linuron	(-)	+	+
Diuron	(-)	++	++
Diclofenac	n.a.	++	n.a.
Other substances detected in Lake Tegel water			
Phenazon	++	++	++
AMDOPH	-	+/-	+/-
AAA	++	++	++
FAA	+/-	++	++
p-TSA	-	+/-	+/-
BSA	-	+/-	+/-
Metoprolol	+/-	++	++
Benzotriazol	-	+	+
Tolyltriazole	-	+	+

dition, results from monitoring at the pilot plant demonstrated that ozonation is a suitable method for transformation of several other compounds and metabolites.

Literature on the oxidation of primidone with ozone is scarce. Ternes et al. (2002) reported moderate removal during ozonation of drinking water [18]. Moderate removal was also observed in pilot scale investigations. Since primidone is persistent during subsurface passage, a breakthrough during combined ozonation and artificial recharge treatment is likely. Besides primidone, the fuel additives MTBE and ETBE, the pesticide atrazine and some metabolites, which were detected in surface water, may potentially persist.

For efficient transformation of these substances, the optimisation of oxidation processes is necessary. Further experiments should therefore adress the combination of ozone and H_2O_2 as advanced oxidation process (AOP).

Adsorbable organic iodine (AOI) was monitored in all experiments as indicator for iodinated x-ray contrast media. Reduction during ozonation was rather low, AOI decreased by up to 30%. From monitoring of aerobic groundwater recharge, Grünheid et al. (2005) reported no efficient removal of AOI [1]. This was confirmed in column and slow sand filter experiments.

Adsorbable organic bromine (AOBr) was efficiently reduced during ozonation in Oxired-1. Results from Oxired-2 proved again an efficient removal, as AOBr decreased by $70 - 80\%$. No significant reduction was observed during infiltration. However, results from the pilot plant indicate, that new production by algae might occur in the exposed supernatant water in infiltration ponds after ozonation.

One major concern regarding the ozonation of trace organic compounds is the formation of unknown transformation products. During experiments, desethylatrazine was detected as a major transformation product

from ozonation of atrazine. Its persistence in soil columns demonstrates that transformation products from ozonation are not necessarily better biodegradable than the parent compound.

However, further experiments are necessary to assess the formation of transformation products from ozonation as well as their biodegradability in artificial recharge systems. Therefore, new analytical methods for non target analysis, such as high resolution mass spectrometry (HRMS), need to be employed.

4.3 Benefits of a preceding bank filtration

Results from technical scale experiments simulating a preceding bank filtration basically support conclusions from Oxired-1. The preceding bank filtration step reduced ozone demand for similar efficiency of DOC reduction by approximately 20%.

On the other hand, additional investment costs and increasing energy costs for additional pumping in extraction wells need to be considered. Therefore, the favored treatment system has to be selected depending on local factors like surface water quality and bank characteristics rather than ozone demand for DOC removal.

4.4 Redox conditions

In addition to the improvement of DOC removal, the experiments have also demonstrated that ozonation is a suitable method to increase the redox potential during artificial groundwater recharge. Dissolved oxygen in the influent of the slow sand filter was highly oversaturated after ozonation. In the filter effluent, dissolved oxygen was constantly around 11 mg/L. Constant aerobic conditions are likely for artificial recharge with preceding ozonation in Berlin. However, these assumptions need to be confirmed in field studies.

4.5 Oxidation by-products

Formation of bromate was monitored in batch experiments and during pilot studies. Results from batch experiments showed bromate formation being strongly related to specific ozone consumption. Concentrations of bromate in surface water from Lake Tegel exceed the limit of 10 $\mu g/L$ in the German drinking water directive only at specific ozone consumption of more than 0.9 $mg\ O_3/mg\ DOC_0$. Formation of bromate in pilot studies was below 10 $\mu g/L$. In the beginning of November, bromate concentration in ozonated samples spontaneously decreased from around 8 $\mu g/L$ to 3 $\mu g/L$. A possible explanation for this drop is an increase of ammonia concentration in the surface water. However, this assumption could not be supported due to a lack of analytical data.

Under anoxic conditions, bacteria can utilize bromate as electron acceptor [19]. Experiments with soil columns confirmed a significant reduction of bromate under anoxic conditions in the presence of biodegradable DOC. Artificial recharge after ozonation is expected to be mostly aerobic. Thus, reduction of bromate is probably negligible and the formation of bromate needs to be controlled during ozonation. However, since recommended specific ozone dosages are in the range of $0.6 - 0.8\ mg\ O_3/mg\ DOC_0$, no conflict with the limit value of German drinking water directive (10 $\mu g/L$) is expected and additional measures to control bromate formation are not necessary.

A formation of nitrosamines including NDMA from ozonation of Lake Tegel water with specific ozone consumptions of up to 1.15 $mgO_3/mgDOC_0$ was not observed. The limit of quantification for analysed nitrosamines was 5 ng/L. Since nitrosamimes are reported as highly carcinogenic and there is evidence on their formation from other studies (e.g. [20]), formation of nitrosamines should be also addressed in further studies.

4.6 Toxicity

Neither systematic genotoxic nor cytotoxic effects of ozonation or subsurface passage are observed. Except for few samples no positive test results were found. The ROS-induction test was identified as a robust, cost-effective and sensitive test to identify and monitor cytotoxic effects in water samples.

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