Impact of the water matrix on the effect and the side effect of MP UV/H_2O_2 treatment for the removal of organic micropollutants in drinking water production

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Abbreviations

General abbreviations

AOC	Assimilable Organic Carbon
AOP	Advanced Oxidation Process
a.u.	Arbitrary unit
BAC(F)	Biological Activated Carbon (Filtration)
BAT	Best Available Technology
BDOC	Biodegradable Organic Carbon
BMD	Benchmark Dose
$BMDL_{10}$	Benchmark Dose giving 10% extra cancer incidence
BMDS	Benchmark Dose Software
cb	Collimated Beam
cf	Concentration Factor
CFD	Computational Fluid Dynamics
CSF	Coagulation, Sedimentation Filtration
DBP	Disinfection Byproduct
DOC	Dissolved Organic Carbon
E	Einstein
EBCT	Empty Bed Contact Time
EC	European Community
EDI	Estimated Daily Intake
EED	Electrical Energy Dose
EEO	Electrical Energy per Order
EFSA	European Food Safety Authority
EU	European Union
GAC	Granular Activated Carbon
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
H	UV fluence
HAA	Haloacetic Acid
HAN	Haloaceto nitrile
HPLC	High Pressure Liquid Chromatography
HWL	Het Water Laboratorium
IHSS	International Humic Substances Society
IX	Ion Exchange

kW	Kilo Watt
LC-OCD-OND	Liquid Chromatography-Organic Carbon Detection-Organic Nitrogen Detection
M(s)	Mole(s)
LP	Low Pressure
MP	Medium Pressure
MOE	Margin of Exposure
N-DBP	Nitrogenous Disinfection Byproducts
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
NOM	Natural Organic Matter
OCD	Organic Carbon Detection
OND	Organic Nitrogen Detection
PAC	Powdered Activated Carbon
RIWA	International Association of River Water Works
RO	Reverse Osmosis
SCE	Sister Chromatid Exchange
SPE	Solid Phase Extraction
SUVA	Specific Ultra Violet Absorption
TEF	Toxic Equivalency Factor
THM	Trihalomethanes
TOC	Total Organic Carbon
TTC	Threshold of Toxicological Concern
TTHM	Total Trihalomethanes
UF	Ultra Filtration
UK	United Kingdom
UPLC	Ultra Performance Liquid Chromatography
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
UVT	Ultra Violet Transmittance
WHO	World Health Organisation
WTP	Water Treatment Plant
ε	Molar absorption coefficient
ϕ	Quantum yield

Chemical abbreviations

AMPA	Aminomethylphosphonic acid
As(III)	Arsenic
ClO_2	Chlorine dioxide
CN^-	Cyanide
CO_2	Carbon dioxide
2,4D	2,4-Dichlorophenoxyacetic acid
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethene
DMSO	Dimethylsulfoxide
EDTA	Ethenediaminetetra acetic acid
Fe(II)	Iron
Hg	Mercury
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
$KBrO_3$	Potassium bromate
MIB	Methylisoborneol
MTBE	Methyl-t-butylether
NDMA	Nitrosodimethylamine
2-NF	2-Nitrofluorene
NO_2^-	Nitrite
NO_3^-	Nitrate
4-NQO	4-nitroquinoline-1-oxide
NTA	Nitriloacetic acid
OH	Hydroxyl
O_3	Ozone
pCBA	Parachlorobenzoic acid
PCE	Perchloroethene
PFOA	Perfluorooctanioc acid
PFOS	Perfluorooctanesulfonic acid
SO_3^{2-}	Sulfite
TCA	Trichloroacetic acid
TCE	Trichloroethene
TiO_2	Titanium dioxide

Chapter 1 Introduction

1.1 General

Nowadays thousands low molecular weight organic chemicals are used in daily life, improving the quality of life and health. Because of this abundant use, these compounds are found everywhere including in sources for drinking water (Houtman, 2010). In view of the low concentration and low molecular weight, these compounds are defined as organic micropollutants, referring to both anthropogenic chemicals such as pesticides and pharmaceuticals and natural compounds such as algae toxins. Organic contaminant control is a major issue in advanced drinking water treatment. In the introduction of this thesis the developments in the approach for organic contaminant control in drinking water treatment are presented.

1.2 Sources for drinking water

1.2.1 Groundwater

In The Netherlands about two third of the drinking water is produced from ground water (Geudens, 2012). The residence time of groundwater in the subsurface guarantees a good microbiological quality, so chemical disinfection can be avoided. After extraction from local wells, conventional groundwater treatment by aeration and rapid sand filtration to remove iron, manganese, ammonia and methane achieves a good drinking water quality (De Moel et al., 2004). Since the sixties of the last century pollution with organic micropollutants has been observed locally. A few examples of micropollutants that are encountered are: dry cleaning agents such as trichloroethene (TCE) and tetra(per)chloroethene (PCE), lead replacement agents such as methyl-t-butylether (MTBE) and pesticides such as the weed killer bromacil. To remove these pollutants, treatment processes such as air stripping and/or granular activated carbon (GAC) filtration have been added to the conventional groundwater treatment (Van Paassen et al., 1985).

1.2.2 Surface water

After the available quantity of groundwater became insufficient, surface water has been used as an alternative source for drinking water production as well. Protected catchment areas and reservoirs fed by pristine rivers served as drinking water sources, requiring no treatment prior to distribution. Due to upstream activities, eutrophication and natural erosion, treatment became necessary. Disinfection to remove human pathogens to a negligible infection level, and colour and turbidity removal have become basic surface water treatment requirements. In conventional surface water treatment disinfection is achieved

by chlorination, while colour and turbidity are lowered by coagulation, sedimentation and filtration. Industrial activities, run off from agriculture and livestock and discharges from domestic waste water treatment plants have affected the surface water quality (Kolpin et al., 2002). Taste and odour causing compounds and numerous other organic micropollutants have become a threat for the drinking water quality. With increasing pollution, conventional surface water treatment required extension with advanced treatment processes such as oxidative treatment by for example ozonation or adsorptive treatment by for example GAC filtration.

1.3 Organic matter

1.3.1 Natural organic matter

Natural organic matter (NOM) is defined as the withering material from plants, algae, animals and invertebrates and their degradation products, categorized as biopolymers (polysaccharides and polypeptides), geopolymers (humic substances) and random polymers of a variety of biological monomers. The NOM content is generally expressed by the total organic carbon (TOC) or dissolved organic matter (DOC) content, measured as the carbon content respectively without or with prefiltration of the sample (Kördel et al., 1997).

Humic substances are coloured, high molecular weight organic substances, that can be subdivided in fulvic acids, humic acids and humins. The difference between these three groups is determined by their ability to dissolve in both base and acid solutions (fulvic acids), only in base solutions (humic acids) and neither base nor acid solution (humins). There is a wide variety in carbon and nitrogen content, colour and molecular size between the different types of humic substances, mainly caused by their origin: terrestrial or aquatic. Terrestrial humic substances originate from lignin and contain more aromatic rings while aquatic humic substances have more protein and carbohydrate structures.

Generally aquatic humic substances have a lower molecular weight than terrestrial humic substances. The molecular structure of aquatic humic acids may consist of: monoaromatic rings with three to six substituents such as alkyl, carboxylic, keto or hydroxyl groups, short aliphatic carbon chains, polynuclear aromatic, aliphatic rings and fused rings including furan and pyridine rings. These structural features are not exclusive. For example the aromatic segments may include a variety of isomers as substituents and the polycyclic structures may not be limited to two or three ring systems (Kördel et al., 1997).

Analytical methods have been developed to characterize the behaviour of NOM in drinking water treatment. As already indicated above, the NOM content can be expressed by the DOC and TOC content. The biodegradability of NOM can be expressed by the assimilable organic carbon (AOC) (Van der Kooij, 1992) or biodegradable organic carbon (BDOC) content (Servais et al., 1989). To characterize the structure, nitrogen content and molecular size of NOM are determined. To characterize molecular size, Huber et al.(Huber et al., 2011) developed a liquid chromatography method, combined with UV detection.

1.3.2 Organic micropollutants in surface water

Anthropogenic micropollutants in surface water originate mainly from organic chemistry. Although their chemical structure is similar to the structure of humic substances, their specific mode of action makes them a treatment target for drinking water production. The first organic micropollutants were not anthropogenic but natural compounds. Taste and odour complaints about distributed drinking water produced from surface water were related to seasonal blue-green algae blooms, releasing taste and odour causing compounds such as geosmin and methylisoborneol (MIB). The human taste and odour threshold for geosmin and MIB is 10 ng/L, a level that can be exceeded during algae blooms. Geosmin and MIB are relevant from an esthetic rather than a health perspective. However blue-green algae may also release neurotoxins such as microcystin during their bloom (WHO, 1999).

The presence of anthropogenic micropollutants is not an issue from recent date. Surface water has been under the influence of human activities for many decades or even centuries. For a long time the presence of anthropogenic micropollutants was not established because analytical tools and monitoring programs were lacking. After analytical tools such as chromatography and mass spectrometry became available the problem was still not recognized, since all compounds were present below the detection limit. Only after isolation and concentration methods were combined with advanced analytical tools the presence of organic micropollutants was established (Meijers, 1970). In the fifties and sixties of the last century phenolic wastes became a major concern, followed by the group of classic pesticides (lindane, endrin, dieldrin, DDE, DDT and endosulfan) (Jones and De Voogt, 1999). Also the presence of organic solvents such as TCE and PCE was a threat for both ground and surface water. A major concern was the presence of trihalomethanes (THMs) and other halogenated compounds, produced by the reaction of chlorine with NOM (Rook, 1974).

In the seventies and eighties of the last century hundreds of organic micropollutants were found in water sources used for the production of drinking water (Meijers, 1970), and their presence was considered a potential concern for the environment and human health. In

1987, for the first time an anthropogenic compound was considered to be a direct threat for the drinking water quality in The Netherlands. The pesticide bentazone was found in surface water in concentrations above the EU standard for pesticides in drinking water of 0.1 µg/L (Smeenk et al., 1988). It was shown that by conventional surface water treatment (coagulation, sedimentation, filtration) bentazone was not removed. In 1990, an inventory showed that in The Netherlands besides bentazone about 320 other pesticides were applied. Although for most pesticides no analytical methods were available at that time, it was expected they would reach the drinking water when no treatment for organic contaminant control was present (Hopman et al., 1990). In the past 25 years the character of the applied pesticides gradually changed from strongly apolar to rather polar, water soluble, because the type of compounds should be environmentally more friendly (Pieters et al., 2004). An improved and more sophisticated mode of action of the applied pesticides reduced the required dosage, resulting in lower concentrations in surface water. Around 1990 commonly applied pesticides such as bentazone and atrazine were found in concentrations higher than the EU standard of 0.1 µg/L (Bannink et al., 2010).

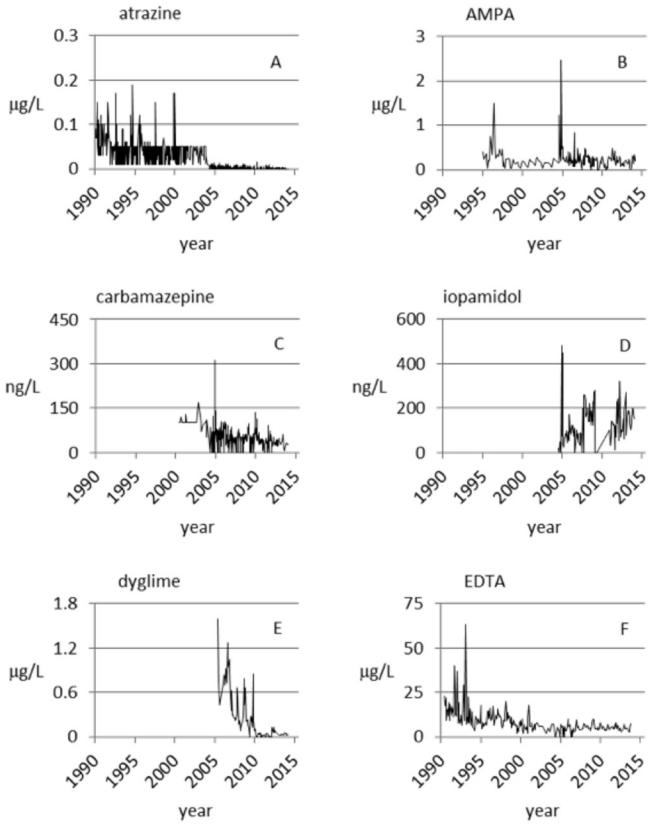


Figure 1.1: Occurence of atrazine (A), AMPA (B), carbamazepine (C), iopamidol (D), diglyme (E) and EDTA (F) in IJssel Lake water, period 1990 – 2013

In the IJssel Lake atrazine was found in concentrations up to 0.2 µg/L. In a later phase atrazine was blacklisted and not found any more since 2000 (Figure 1.1A). Since early 2000 pesticides such as diuron, glyphosate and its active compound AMPA were found. For AMPA the average and highest concentration were 0.3 µg/L and 2.5 µg/L respectively (Figure 1.1B). More recent monitoring programs were focused on the presence of other micropollutants as well, such as endocrine disrupting compounds, pharmaceuticals, X-ray contrast media, personal care products, solvents and complexing agents. Since 2004 the pharmaceutical carbamazepine was found in an average and highest concentration of 50 ng/L and 300 ng/L respectively (Figure 1.1C). Contrast media such as iopamidol were found in concentrations around 150 ng/L (Figure 1.1D). Since 2005 the solvent diglyme was found in concentrations up to 1.6 µg/L, but after 2010 this solvent was not found anymore (Figure 1.1E). Finally the complexing agent EDTA was found since 1990 in concentrations up to 60 µg/L. Since 2005 the average concentration was around 5 µg/L (Figure 1.1F).

Although the effect on human health of such low concentrations is judged negligible (Schriks et al., 2010), these types of compounds should not be present in drinking water. In addition, the presence or absence of these types of compounds play an important part in the customer confidence in drinking water. The widespread production and use of organic chemicals will cause a contamination of drinking water sources now and in the future. Climate changes influencing algae blooms and water discharge of rivers may cause new contaminants to emerge. The first emerging compounds were taste and odour compounds, algae toxins, phenolics and pesticides followed by endocrine disruptors and pharmaceuticals (Snyder et al., 2007), while currently perfluorinated compounds (Eschauzier et al., 2013) and nanoparticles (Van Wezel et al., 2011) are emerging. Therefore a broad variety of organic chemicals with different characteristics (adsorbability, degradability, etc.) may be present, requiring a control strategy based on multiple barriers.

1.4 Organic contaminant control

1.4.1 Rationale for organic contaminant control in water treatment

Organic contaminant control in water treatment refers to the removal or degradation of organic matter, present in the source. Different drivers and reasons determine the required organic contaminant control. For instance for esthetical reasons, colour removal is pursued. Colour removal is achieved by coagulation with iron or aluminium salts, sedimentation and filtration. Additional removal can be achieved by oxidation processes such as ozonation, chlorination or potassium permanganate dosage, by adsorption on activated carbon or by removal with nanofiltration. A second reason for organic matter

removal is the improvement of the efficiency of water treatment steps for disinfection and organic micropollutant control. When ozonation or chlorination is applied for disinfection, a large fraction of the disinfectant reacts with the organic matter, reducing the disinfection capacity or increasing the required chemical dose. Furthermore, oxidation of organic matter causes the formation of biodegradable organic compounds, reducing the biostability of the produced drinking water. A third reason to remove organic matter is disinfection byproduct control. When chlorination is applied, reaction with organic matter causes formation of low molecular weight halogenated organic compounds (Kruithof, 1986). The health impact of disinfection byproducts is suggested by epidemiological studies relating the occurrence of bladder cancer to the presence of disinfection byproducts (Richardson et al., 2007).

1.4.2 Philosophy organic micropollutant control

A robust organic micropollutant control strategy consists of multiple barriers. In The Netherlands, the drinking water supply companies using the River Rhine (IJssel Lake) and River Meuse as their drinking water source aim for a surface water quality requiring simple treatment such as coagulation, sedimentation, filtration and disinfection only. To enable this, a long term vision was developed, focusing on water catchment area protection. This is the main task of RIWA Rhine and Meuse, representing the drinking water companies in the catchment areas of these rivers (Bannink et al., 2010).

1.4.3 Organic micropollutant control in water treatment practice

The preferred solution for organic micropollutant control is preventing these compounds from entering the drinking water sources. This may be achieved by: restricted pesticide use in agricultural areas and/or more controlled dosing realizing restricted leaching of pesticides into surface water, use of less and better biodegradable pharmaceuticals and personal care products, stricter collection of unused pharmaceuticals, treatment of hospital waste water and more advanced waste water treatment. On long term these measures may lead to lower emission levels. However negative developments may be anticipated as well such as: increased pharmaceutical use by an aging population and higher concentrations of all organic micropollutants in summer by less water discharge caused by climate changes. Therefore, although prevention is the preferred approach, preventive measures only do not meet the current water quality requirements, so in addition treatment is required.

An alternative approach for organic micropollutant control is removal. In general this can be achieved by membrane filtration or adsorption. These processes avoid the formation of potentially harmful reaction products (Verliefde et al., 2007). Membrane processes such as nanofiltration or reverse osmosis remove most dissolved organic compounds. However, even reverse osmosis membranes, the membranes with the smallest pores, do not achieve a complete removal of low molecular weight compounds (Verliefde et al., 2007). Other drawbacks of reverse osmosis are a high energy consumption and the production of a waste stream, which must be disposed of. Therefore membrane filtration, by in example reverse osmosis, is not considered the best available technologie for organic micropollutant control, making adsorption processes the preferred technology. For organic micropollutant control both powdered activated carbon (PAC) dosage and GAC filtration are applied. Adsorptive processes are feasible when hydrophobic micropollutants must be removed (De Ridder et al., 2013). In current practice with a tendency towards the presence of more polar organic micropollutants, use of activated carbon alone does not provide a robust barrier anymore. Therefore, because prevention and removal do not provide a robust solution, degradation of organic micropollutants is considered an attractive additional barrier (Glaze et al., 1987).

A third approach for organic contaminant control is the use of technologies to degrade organic micropollutants into harmless metabolites by oxidative treatment. Commonly applied oxidative technologies in drinking water treatment are ozonation and advanced oxidation by the combined use of O_3/H_2O_2, UV/H_2O_2 or O_3/UV (Glaze et al., 1987).

1.5 Oxidative treatment

1.5.1 General

Chlorination is applied for disinfection and oxidation based on the oxidative properties of chlorine. For organic contaminant control, besides chlorination, commonly applied oxidative processes in drinking water treatment include ozonation, based on an electrophilic attack of molecular ozone on the organics and advanced oxidation processes involving the formation of hydroxyl radicals.

1.5.2 Chlorination

Aqueous chlorine is an oxidizing agent, used in drinking water treatment for disinfection and chemical oxidation. Chlorination is applied to reduce taste and odour and to oxidize inorganic species such as ammonia, SO_3^{2-}, CN^-, NO_2^-, As(III) and Fe(II), by an electrophilic attack on the inorganic compounds. In the case of organic compounds, second-order rate constants for chlorination vary over 10 orders of magnitude (Deborde and Von Gunten, 2008). Oxidation, addition and electrophilic substitution reactions are possible pathways. However, from a kinetic point of view, usually only an electrophilic attack is significant. Comparing chlorine with ozone reactivity towards aromatic compounds (electrophilic attack) shows that reaction rate constants for chlorine are about four orders of magnitude

lower than those for ozone (Deborde and Von Gunten, 2008), making chlorination for oxidation of organic micropolutants attractive in specific cases only.

1.5.3 Ozonation

Ozone is a strong oxidizing agent. Ozone is a gas and is dispersed in the water phase by bubble diffusors or injection systems, followed by a residence time in a contact chamber. The most important applications of ozone in water treatment are disinfection and organic contaminant control (Buffle, 2005). Many applications of ozone have been developed including control of algae, removal of taste and odour, colour, iron and manganese, microflocculation, partial oxidation of dissolved organics and control of the formation of halogenated organic compounds by chlorination. Ozone, a strong selective oxidant, is very reactive against unsaturated and aromatic compounds but does not convert saturated aliphatic compounds. The reaction rate constants for the reaction of pesticides with molecular ozone vary between < 0.04 $M^{-1}s^{-1}$ for lindane and 4.4 x 10^4 $M^{-1}s^{-1}$ for aldicarb (Hoigné, 1982). This means that in practice lindane is not degraded at all, while aldicarb is converted within a few seconds.

Because of its selectivity ozone does not cause a complete mineralization of all organic compounds to CO_2 and H_2O. Therefore after ozonation, reaction products are present in the treated water. Extensive research has been carried out into the reaction product formation (Richardson et al., 1999). In general the reaction products are less harmful and more biodegradable than the parent compounds (Krasner et al., 1993; Paode et al., 1997). Biodegradable compounds are also produced by oxidation of the organic water matrix (Van der Kooij et al., 1989). By ozonation of bromide rich water, bromate, a suspect carcinogen for humans (Kurokawa et al., 1990), is formed. The bromate formation depends on the presence of NOM and the pH (Von Gunten et al., 1998). Because of the selective reaction by ozone and the bromate formation, a non selective degradation process without the formation of harmful compounds has been pursued. Therefore advanced oxidation processes have been selected.

1.5.4 Advanced oxidation processes

The effect of advanced oxidation processes is based on the formation and reaction of hydroxyl radicals. Hydroxyl radicals are rather non selective agents, reacting with most organic compounds by adding to aromatic or unsaturated sites, by abstracting hydrogen atoms or by reacting with S-, N-, P-atoms present in the molecule. Many hydroxyl radical producing processes have been described: Fenton (Fe^{2+}/H_2O_2), photo Fenton (Fe^{2}/H_2O_2/UV), photocatalytic oxidation (UV/TiO_2 or O_3/TiO_2), high pH O_3, peroxone process (O_3/H_2O_2), ultraviolet hydrogen peroxide process (UV/H_2O_2) and the hybrid combination of ozone, ultraviolet light and hydrogen peroxide (O_3/UV/H_2O_2).

For many years the focus of advanced oxidation was on the application of O_3/H_2O_2 for the degradation of organic micropollutants such as pesticides, pharmaceuticals, personal care products, endocrine disruptors, algae toxins, etc (Meijers et al., 1995; Chen et al., 2006). In general advanced oxidation is implemented after a conventional surface water treatment, coagulation, sedimentation and filtration (CSF) and prior to post biological activated carbon (BAC) filtration.

The reaction rate constants for hydroxyl radicals are very high, the rate constant for the reaction with atrazine is 2.7 x 10^9 $M^{-1}s^{-1}$ (Acero et al., 2000). Therefore after hydroxyl radicals are produced the reaction with atrazine takes place in microseconds. In principle a complete mineralization can be achieved by O_3/H_2O_2 advanced oxidation. However, under economically feasible conditions this does not take place and reaction products are formed. Just like for ozonation intensive research showed the reaction products in general to be more biodegradable and less harmful. Once again biodegradable compounds were also produced by the oxidation of the organic water matrix (Krasner et al., 1993). Although by O_3/H_2O_2 based advanced oxidation a non-selective degradation was achieved, bromate formation in bromide rich water could be restricted but not be avoided completely. Therefore application of UV/H_2O_2 based advanced oxidation has gained interest as a possible alternative advanced oxidation method (Kruithof et al., 2007).

1.5.5 UV/H_2O_2 based advanced oxidation

UV/H_2O_2 treatment is based on the photolysis by UV light. The photolysis of H_2O_2 causes the formation of hydroxyl radicals (table 1.1). UV photolysis can also cause degradation of organic compounds, depending on the molar absorption coefficient and the quantum yield of these compounds. For example the pesticide atrazine has a high molar absorption coefficient at 254 nm (3683 $M^{-1}cm^{-1}$) and a rather high quantum yield (0.033 M/Einstein) (Bolton et al., 2002) resulting in a feasible degradation. On the other hand the solvent 1,4-dioxane does not absorb UV light at all so this compound is not degraded by UV photolysis. Therefore this type of compound must be degraded by hydroxyl radical oxidation.

Table 1.1: OH radical formation by UV photolysis of H_2O_2

Reaction	molar absorption coefficient / quantum yield
1) $H_2O_2 + h\nu \rightarrow 2\ OH^{\cdot}$	$\varepsilon = 18.6\ M^{-1}cm^{-1}$;
	$\phi = 0.5$ mol/E
2) $HO_2^- + H_2O + h\nu \rightarrow 2\ OH^{\cdot} + OH^-$	$\varepsilon = 228\ M^{-1}cm^{-1}$;
	$\phi = 0.5$ mol/E

Just like O_3/H_2O_2 treatment, UV/H_2O_2 treatment can achieve non-selective degradation, but it does not cause bromate formation in bromide rich water (Kruithof et al., 1997). UV/ H_2O_2 based advanced oxidation has been introduced successfully in drinking water treatment as a non-selective barrier against organic micropollutants. One of the first full scale UV/H_2O_2 installations was installed by PWN at water treatment plant (WTP) Andijk (Martijn et al., 2007). For UV/ H_2O_2 treatment low pressure (LP) and medium pressure (MP) mercury lamps are applied. LP lamps have a dominant emission of UV light at 254 nm, MP lamps have an emission in the wavelength area 200 - 300 nm (Figure 1.2) (Bolton, 2010).

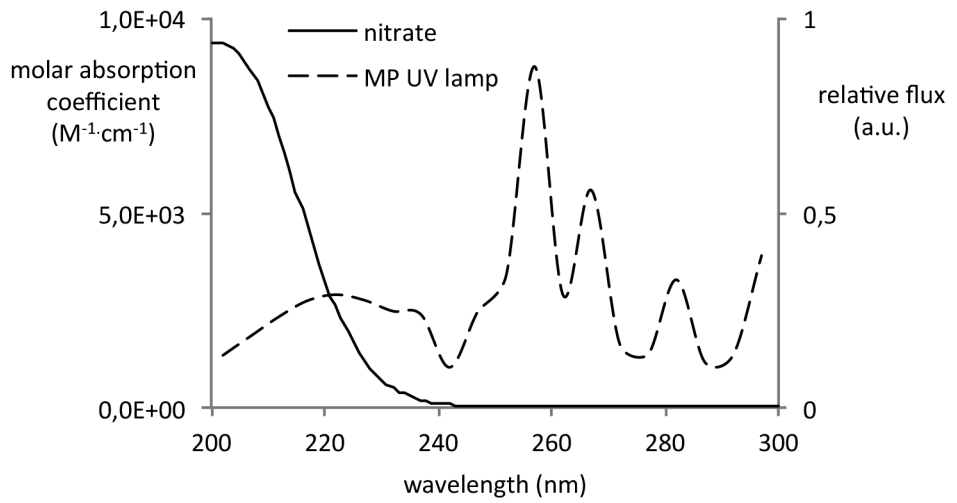


Figure 1.2: Emission spectrum of a medium pressure UV lamp and the molar absorption coefficient between 200 and 300 nm of nitrate in demineralized water

The research described in this thesis is focused on the application of MP UV lamps. It is generally accepted that the formed reaction products from NOM by oxidative treatment are biodegradable (Van der Kooij et al., 1989). It is anticipated that oxidation products from the organic water matrix do not have a significant health impact (Sarathy et al., 2011). Many authors present the formation of potentially harmful reaction products from organic priority compounds upon (MP) UV/ H_2O_2 treatment. However, it is anticipated that they do not contribute significantly to a harmful effect under drinking water concentrations and well defined treatment conditions (Snyder et al., 2003).

An important issue is the UV photolysis of nitrate. Nitrate has two absorption bands in the UV region, one in the near UV region from 260 nm to 350 nm with its maximum at 300 nm and a much more intensive band below 260 nm with its maximum at 200 nm (Krishnan and Guha, 1934). The molar absorption coefficient of nitrate between 200 and 300 nm (figure 1.2) illustrates the relevance of the absorption of UV light by nitrate for the application of MP UV technology in water treatment. The emission spectrum of MP UV lamps shows a substantial emission in the wavelengths (<240 nm) where nitrate has a high molar absorption coefficient (figure 1.2). Especially at these emitted lower wavelengths, UV photolysis causes a strong nitrite formation (Mack and Bolton, 1999). However, the impact of UV photolysis of nitrate on harmful organic reaction product formation and the possible health impact (Habermeyer et al., 2015) is not (well) documented yet, although the hazard of nitrate photolysis was predicted (Mack and Bolton, 1999; Reckhow et al. 2010).

1.6 Effect measurements and preliminary risk assessment

1.6.1 Ames test

Given the numerous potentially genotoxic reaction products and metabolites that are formed by UV photolysis or hydroxyl radical oxidation of water matrix constituents and targeted organic micropollutants, water samples were tested using a bioassay. To characterize the effect of chlorination byproduct formation, the application of Ames testing (Ames et al., 1972) was introduced. In a later phase Ames testing has also been applied to characterize the effect of other water treatment processes such as ozonation and GAC filtration (Kruithof, 1986). Recently, a high throughput alternative of this test has become available (Flückinger et al., 2004). In this research, the high throughput Ames fluctuation test is performed to screen water samples for MP UV induced genotoxicity without knowing the properties, concentrations and number of the compounds responsible for a response.

The Ames test is an in vitro test using a biological assay to assess the genotoxic potential of chemical compounds. Since the 1970's the test is used as a standard method for detecting potentially genotoxic and carcinogenic compounds. A bacterium is used in the assay as an indicator for DNA damage (McCann and Ames, 1976). The bacterium used in this test is genetically modified *Salmonella* Typhimurium, preventing the bacterium from synthesizing histidine (his) which is required for growth. The mutagenic potential of tested chemicals is assessed by exposing his- organisms to different concentrations of chemicals by which the genotoxic effect of the chemical compounds results in a mutation giving the bacteria the ability to grow without histidine, and form colonies (reverse mutation). To perform the Ames test on water samples, sample concentration is required. For this research, samples were concentrated up to a factor of 20,000, using OASIS HLB solid phase extraction (Heringa et al., 2011). The Ames II test, used in these experiments, applies a liquid culture instead of Agar plates, and *Salmonella* Typhimurium strains containing TAMix (TA7001, TA7002, TA7003, TA7004, TA7005 and TA7006) and TA98, and small sample quantities to obtain faster results. Some tested compounds are carcinogenic or genotoxic only when they are metabolized in the body; therefore a mixture of rat liver enzymes is used in the Ames test to mimic metabolic activation, referred to as +S9 (Flückinger et al., 2004).

1.6.2 Threshold of toxicological concern

The threshold of toxicological concern (TTC) approach is a concept to establish a human exposure threshold value below which no appreciable health risk occurs (Kroes et al., 2004). The TTC concept may be applied when the presence of a new contaminant in food is observed, for which no toxicological information is available. It could also be useful in setting priorities for testing large groups of functionally similar chemicals to which exposure is generally very low, such as flavourings (Barlow, 2005) or deriving target values for drinking water contaminants (Mons et al., 2013). These conditions also apply for genotoxic compounds formed by MP UV treatment of nitrate rich pretreated surface and groundwater. The formation of a large number of similar, genotoxic compounds at very low concentrations, for which toxicity data are lacking, requires a preliminary risk assessment. Application of the TTC concept can demonstrate whether or not the formation of genotoxic compounds by MP UV treatment poses an acceptable risk. When considering to apply the TTC approach it is important to realize that certain classes of genotoxic carcinogens have been excluded from application of the TTC concept, including for example high-potency carcinogens such as aflatoxins, azoxy compounds, N-nitroso compounds, benzidines, hydrazines and compounds with an unknown chemical structure (EFSA, 2012). This may hamper application of the TTC concept in the risk assessment of reaction products of UV/H_2O_2 treatment of NOM containing water samples, pointing at a need for another approach to perform a risk assessment. An alternative is the so-called Margin of Exposure (MOE) approach presented in the next section.

1.6.3 Margin of exposure

The Margin of Exposure (MOE) approach was suggested by the European Food Safety Authority (EFSA) as an approach which can be applied for risk assessment of substances or impurities, that are both genotoxic and carcinogenic. The MOE approach allows comparison between compounds to support prioritisation for risk management action (Barlow et al., 2005) (EFSA, 2005).

The MOE is calculated by dividing the lower confidence limit of the benchmark dose (BMD) giving 10% extra cancer incidence ($BMDL_{10}$), obtained from experimental data on tumour incidence, by the estimated daily intake (EDI) by humans; MOE = $BMDL_{10}$ / EDI (human).

The MOE can be used by risk managers to determine the priority of concern for public health, to prioritise the possible actions required. An MOE value gives an indication of the level of concern, but is not a precise risk quantification. Also, MOE values depend on the carcinogenicity data selected to be used in a BMD approach to determine the $BMDL_{10}$ and the estimation of human dietary exposure.
An MOE value of 10,000 has been proposed as the cut-off for deciding if a certain level of exposure is of concern. This value of 10,000 incorporates a factor 100, consisting of a factor 10 for possible inter-species differences, and another factor 10 for differences between human individuals. It also incorporates an additional factor of 10 to account for inter-individual human variability in cell cycle control and DNA repair and a factor 10 when the MOE is based on the $BMDL_{10}$ not being a no effect level (EFSA, 2005).
A MOE value of 10,000 means that the amount consumed is ten thousand times lower than the lower confidence bound of the dose that causes 10% extra tumour incidence above background levels in an animal bioassay. An MOE of 10,000 or higher, based on animal cancer bioassay data, is considered to be a low concern from a public health standpoint and a low priority for risk management actions. An MOE value, lower than 10,000 might raise a potential concern for human health, and indicates a high priority for risk management (EFSA, 2005).

1.7 This thesis

1.7.1 Problem definition

In surface water, an important source for drinking water, numerous organic micropollutants are present. Regulatory requirements, maintaining customer confidence and restricting a possible health impact require barriers for organic micropollutants in surface water treatment processes. Advanced oxidation, based on the in-situ formation of hydroxyl radicals, is a robust technology to oxidize organic micropollutants. However, advanced

oxidation processes may form harmful reaction products from NOM and inorganic water matrix constituents. In addition, NOM and inorganic water matrix constituents reduce the efficiency of the advanced oxidation process.

1.7.2 Scope and outline

The aim of this thesis is to characterize the effect and the side effect of micropollutant control of pretreated surface water for drinking water production with advanced oxidation. This research focuses on advanced oxidation by medium pressure UV/H_2O_2 treatment. The aim of this research is to determine the impact of the water matrix on the efficacy of MP UV/H_2O_2 treatment for the removal of organic micropollutants and on the formation of harmful reaction products from the water matrix. Regarding undesired side effects, this research aims to unravel the cause and to determine the possible health concern. Chapter 1 of the thesis presents an introduction with background information on micropollutants and methods for their control during drinking water production. The MP UV/H_2O_2 process is introduced in chapter 2. Degradation of pesticides, pharmaceuticals and a few industrial compounds, anthropogenic substances commonly detected in surface water, is shown. The contribution of the two degradation mechanisms of MP UV/H_2O_2 technology, UV photolysis and hydroxyl radical oxidation, is illustrated. Furthermore, the embedding of the MP UV/H_2O_2 process in a surface water treatment facility for drinking water production is described. The effect of the NOM content and the nitrate concentration in pretreated surface water for the production of drinking water on the required electrical energy dose for the formation of hydroxyl radicals, is presented in chapter 3. Competition for photons by the water matrix reduces the efficacy to form hydroxyl radicals from hydrogen peroxide while scavenging of formed hydroxyl radicals by the water matrix further reduces the efficacy of the process. Improved pretreatment to remove NOM and nitrate reduces the required electrical energy for the MP UV/H_2O_2 process. Water matrix related reaction product formation is a known effect of oxidation and advanced oxidation technologies in water treatment. Disinfection byproducts from chlorination and their health impact are extensively studied since the 1970's. Formation of the carcinogen bromate by ozonation of bromide containing water types for drinking water production is currently limiting the applicability of ozone based processes, both for disinfection and advanced oxidation. For medium pressure UV based processes, only formation of nitrite by nitrate photolysis in nitrate rich water types is reported. Although the hazard of nitrate photolysis is predicted, the effect is not measured until recently. Chapter 4 shows the effect of nitrate photolysis by MP UV/H_2O_2 treatment in the presence of organic matter, on a response in an effect derived bioassay: the Ames fluctuation test. The balance for the inorganic nitrogen content before and after MP UV treatment is compared to show a possible nitrogen incorporation in NOM by MP UV/H_2O_2 treatment which may be the cause of the formation of genotoxic compounds. In chapter 5 model compound studies using phenol, standard NOM from the International Humic Substances Society and nitrate are presented. Conversion of the concentrations showing positive responses in the Ames fluctuation test into 4-nitroquinoline 1-oxide (4-NQO) equivalent concentrations, allowing a comparison to 4-NQO a known and relevant genotoxic carcinogen, is pursued in chapter 6. Based on the 4-NQO equivalent concentration and available tumour data for this genotoxic carcinogen, a margin of exposure (MOE) based risk inventarisation is performed. The objective of this effort is to judge the urgency to act upon the observed effects in concentrated water samples without knowing the identity of the genotoxic compounds. The formation of genotoxic compounds by MP UV nitrate photolysis is studied, using practical water matrices with different types of NOM and nitrate content. Practical MP UV treatment regimes are applied such as MP UV disinfection conditions (40 mJ/cm^2), MP UV/H_2O_2 treatment conditions (600 mJ/cm^2 with 6 mg/L H_2O_2) and MP UV photolysis (600 mJ/cm^2). Responses in the Ames fluctuation test for concentrated water samples are converted into 4-NQO equivalent concentrations to apply a preliminary risk assessment. In addition, it is studied to what extent a representative selection of micropollutants contributes to the response in the Ames fluctuation test (chapter 7). Finally chapter 8 presents an overall discussion of the results obtained in the thesis and considerations on future perspectives. In the discussion, the need for the removal of water matrix constituents, both for improvement of the energy efficiency of MP UV/H_2O_2 treatment and for control of reaction product formation is evaluated. Furthermore, the desire and need to have a barrier for organic contaminant control based on advanced oxidation versus the formation of harmful reaction products is discussed. The structure of this thesis is shown in figure 1.3.

Problem analysis	Chapter 1: Introduction
Organic contaminant control	Chapter 2: MP UV/H_2O_2 treatment: an essential process in a multi barrier approach against trace chemical contaminants Chapter3: Impact of IX-MF pretreatment on the feasability of MP UV/H_2O_2 treatment for the degradation of NDMA and 1,4-dioxane
Genotoxicity & reaction products and mechanisms	Chapter 4: MP UV photolysis and MP UV/H_2O_2 treatment: the silver bullet for reaction product and genotoxicity formation in water production Chapter 5: Formation of genotoxic compounds by medium pressure ultraviolet treatment of nitrate-rich water
Risk analysis & health impact	Chapter 6: Development of a 4-NQQ toxic equivalency factor (TEF) approach to enable a preliminary risk assessment of unknown genotoxic compounds detected by the Ames II test in UV/H_2O_2 water treatment samples Chapter 7: Induced genotoxicity in nitrate rich water treated with medium pressure ultraviolet processes
Discussion	Chapter 8: Discussion

Figure 1.3: Structure of this thesis

Abstract

The presence of pesticides, endocrine disruptors and pharmaceuticals caused PWN Water Supply Company North-Holland to implement multiple barriers for organic contaminant control in their surface water treatment plants. A combination of advanced oxidation by medium pressure (MP) UV/H_2O_2 treatment and granular activated carbon (GAC) filtration is installed. MP UV experiments in a standard pilot reactor were carried out to investigate the degradation of a representative selection of pesticides found in the IJssel Lake, a source for drinking water production. It was observed that atrazine and diuron were more sensitive to MP UV photodegradation while bentazone and bromacil were primarily degraded by hydroxyl radical oxidation. Addition of H_2O_2 increased the decay rate of all selected herbicides. Using computational fluid dynamics, irradiance distribution and kinetic models, an optimized MP UV-reactor was designed. In tests with a pilot reactor, constructed according to this new design, the predicted performance was confirmed, both for photodegradation and hydroxyl radical oxidation. During the research period, the scope broadened from pesticides to pharmaceuticals, endocrine disrupting compounds, solvents and algae toxins. At process conditions 0.56 kWh/m^3 and 6 mg/L H_2O_2, 80–100% degradation was achieved for compounds such as mecoprop, clofibric acid and diclofenac. A somewhat lower degradation was found for dicamba, 2,4-D, bentazone, ibuprofen, carbamazepine and sulphametoxalol. The developed modelling was used to design a full scale MP UV/H_2O_2 system with an electric energy of 0.56 kWh/m^3 for treatment of 3,200 m^3/h. In a site acceptance test, degradation of atrazine was measured at two UV-doses at a fixed H_2O_2 dose of 6 mg/L. The installation performed as predicted by the design models and design criteria were met. At WTP Andijk, MP UV/H_2O_2 treatment was integrated in the existing process train, preceded by conventional surface water treatment (coagulation, sedimentation and filtration) and followed by GAC filtration, providing a robust barrier against reaction products.

This chapter is derived from: Kruithof, J.C., Martijn, A.J., 2013. UV/H_2O_2 treatment: an essential process in a multi barrier approach against trace chemical contaminants, *Water Science & Technology: Water Supply,* 13(1): 130-138

Chapter 2 MP UV/H_2O_2 treatment: an essential process in a multibarrier approach against trace chemical contaminants

2.1 Introduction

The presence of pesticides, endocrine disruptors and pharmaceuticals caused PWN to implement multiple barriers in their surface water treatment plants. In addition to reverse osmosis (RO), a combination of advanced oxidation by medium pressure (MP) UV/H_2O_2 treatment and granular activated carbon (GAC) filtration was implemented. Bench scale experiments focussed on degradation of a representative selection of pesticides observed in PWN's main raw water source, the IJssel Lake. Main objective of this phase of the study was to establish the feasibility of MP UV in the presence and absence of H_2O_2 for pesticide degradation. A database of quantum yields and hydroxyl radical reaction rate constants was developed. Applying newly developed CFD models, existing irradiance distribution and kinetic models using the UV quantum yield and hydroxyl radical reaction rate constants from the database, an optimized MP UV pilot reactor was designed and constructed. In tests with this pilot reactor, the predicted performance was investigated, both for photodegradation and hydroxyl radical oxidation. Based on the pilot scale experiments, a full scale installation has been constructed. To check the performance of this installation, a site acceptance test was carried out.

2.2 Treatment objectives for organic contaminant control

In the 1980 and 1990, at the early awareness of micropollutants being a threat for drinking water production, the focus was on pesticide degradation. In the Netherlands, around 350 pesticides were used with a large variety in persistence, degradability and toxicity. In IJssel Lake water many of these pesticides such as atrazine, pyrazon, diuron, bentazone, bromacil, methabenzthiaxuon, dicamba, 2,4-D, trichlorpyr and TCA have been found in concentrations up to 1 µg/L. After storage the concentration levelled off at 0.5 µg/L. For this type of compounds, the EC and Dutch drinking water standard of 0.1 µg/L must be satisfied (Drinkwaterregeling, 2011). In view of the concentration after storage the required removal/degradation by treatment is 80%. MP UV/H_2O_2 treatment was pursued to achieve this objective. Degradation by MP UV/H_2O_2 treatment is based on a combination of MP UV photolysis and hydroxyl radical oxidation. At the start of this research effort, hardly any data for quantum yield φ and hydroxyl radical oxidation rate constant k_{OH} of a number of pesticides were available in literature. Therefore a database was developed based on

collimated beam experiments. Some data are presented in Table 2.1 (Stefan et al., 2005; Stefan and Bolton, 2005).

Table 2.1: Data for ϕ and k_{OH} of a number of pesticides

Pesticide	ϕ (M^{-1})	k_{OH} x 10^9 (M^{-1} s^{-1})
atrazine	0.005 (254 nm)	2.4-3.0
2,4-D	0.0262 (254 nm)	2.3
diuron	0.22 (254 nm) 0.014 (296 nm)	4.6
isoproturon	0.045 (254 nm) 0.0045 (275 nm)	5.2
simazine	0.083 (254 nm)	2.9
TCA	-	0.06

Applying a model for MP UV photolysis, hydroxyl radical oxidation and scavenging, it was shown that 80% pesticide degradation can be achieved under realistic process conditions. More recently the focus was extended to the presence of endocrine disruptors, pharmaceuticals, algae toxins and solvents. In IJssel Lake water endocrine disruptors such as bisphenol A and diethylphtalate, pharmaceuticals such as diclofenac, ibuprofen, phenazone, carbamazepine, antibiotics and X-ray contrast media were found in concentrations up to several hundred ng/L. Also algae toxins such as geosmin, methyl-isoborneol (MIB) and microcystine and solvents such as diglyme were identified. Finally disinfection byproducts such as NDMA, complexing agents such as EDTA and NTA and fuel retardants such as trichloroethylphosphate, PFOA and PFOS were found. Partly in cooperation with UV manufacturers, the following research efforts were carried out into:

- degradation of pesticides with a standard pilot installation for disinfection purposes;
- development of pilot and full scale reactors for organic contaminant control and advanced models to predict the degradation by MP UV photolysis and hydroxyl radical oxidation and the impact of scavenging on degradation;
- application of MP UV/H_2O_2 treatment for the degradation of pesticides and pharmaceuticals in an advanced pilot installation;
- degradation of pesticides and complexing agents by full scale MP UV/H_2O_2 application.

2.3 Materials and methods

Standard UV equipment consisted of three Berson InLine250 reactors in series. Each reactor was equipped with two medium pressure UV lamps of 2 kW. The power level of the UV lamps could be set at 60, 80 and 100% of their maximum power output. Individual reactors could be switched off. The flow in the standard UV equipment was varied between 5 and 12 m^3/h. The hydrogen peroxide dosage was varied between 0 and 15 mg/L. For MP UV photolysis experiments, no hydrogen peroxide was dosed. The advanced UV pilot reactor, designed for AOP, was a Trojan SWIFT4L12 UV reactor. The reactor was equipped with four medium pressure UV lamps of 2.8 kW. The output of the UV lamps could be controlled continuously between 30 and 100% of the maximum output. The flow was varied between 10 and 40 m^3/h. The hydrogen peroxide dosage was varied between 0 and 15 mg/L. The full scale MP UV/H_2O_2 installation of WTP Andijk consists of 12 Trojan SWIFT16L30 UV reactors, arranged in three trains of four reactors each. Each UV reactor is equipped with 16 medium pressure UV lamps of 12 kW. The output of the UV lamps could be controlled continuously between 30 and 100% of the maximum output. The design capacity of the system is 0.56 kWh/m^3 to treat 3,200 m^3/h of CSF pretreated IJssel Lake water. The hydrogen peroxide dosage was varied between 0 and 15 mg/L. For the pilot experiments, pesticides and pharmaceuticals were added to the feed water of the UV reactor to such a level that the residual after treatment was well above the detection limit of the analytical method of 0.02 µg/L. Chemical analyses were performed using gas chromatography (GC) and high pressure liquid chromatography (HPLC). The performance of the full scale installation was monitored by using gas chromatography-mass spectrometry (GC-MS).

2.4 Results and discussion

2.4.1 MP UV/H_2O_2 research in standard pilot equipment

The degradation of 10 emerging pesticides: atrazine, pyrazon, diuron, bentazone, bromacil, methabenzthiaxuon, dicamba, 2,4-D, trichlorpyr and TCA were studied in three Berson Inline 250 reactors in series. In the first place, the degradation by MP UV photolysis was investigated for an electric energy ranging from 0.25 to 2.0 kWh/m^3. All investigated pesticides showed a significant degradation by MP UV photolysis. The conversion for an electric energy input of 1.0 kWh/m^3, (a UV dose of ~ 1,000 mJ/cm^2) is presented in figure 2.1.

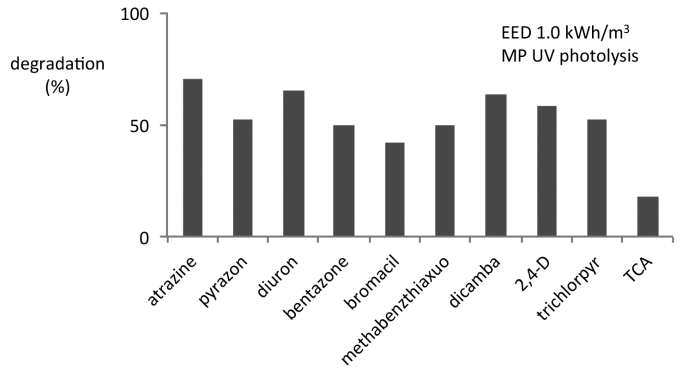


Figure 2.1: Pesticide degradation by MP UV photolysis for an electric energy dose (EED) of 1.0 kWh/m³

For an electric energy input of 1.0 kWh/m³, degradation by MP UV photolysis ranged from 18% for TCA to 70% for atrazine showing that TCA is least susceptible and atrazine most susceptible for MP UV photolysis. A higher conversion should be realized by adding H_2O_2 to initiate an additional oxidation by hydroxyl radicals. The degradation of the same 10 pesticides by combined MP UV photolysis and OH radical oxidation was studied for a range of electric energy consumptions (0.33–2.2 kWh/m³) and H_2O_2 dosages (0–15 mg/L). The degradation for a compound with a high susceptibility for MP UV photolysis (atrazine) and OH radical oxidation (pyrazon) in the same standard pilot reactor configuration is presented in figure 2.2.

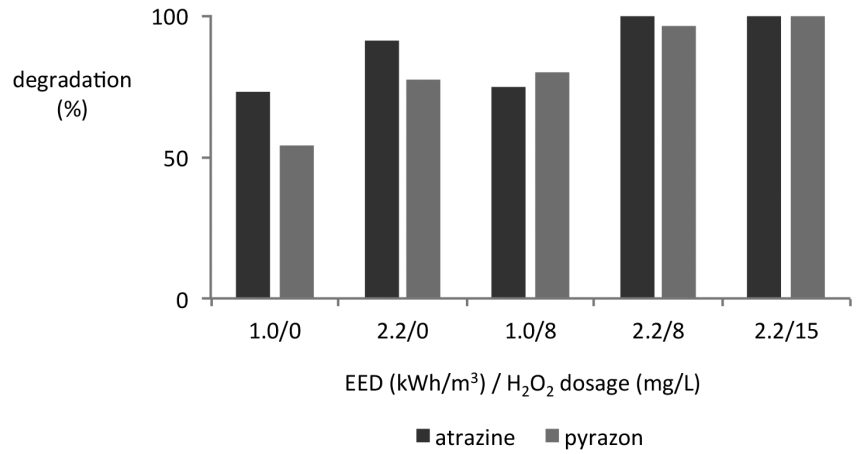


Figure 2.2: Pyrazon and atrazine degradation by MP UV/H_2O_2 treatment in a standard UV reactor for disinfection purposes

As already mentioned atrazine was photolized for 70% by an electric energy of 1 kWh/m³. To increase the degradation to the target conversion of 80% a dosage of 13 mg/L H_2O_2 was needed. Pyrazon was photolized for 54% by an electric energy of 1.0 kWh/m³. To increase this conversion to 80% a dosage of 8 mg/L H_2O_2 was needed. These results established the feasibility of MP UV/H_2O_2 treatment, although the energy consumption was rather high. Therefore a reduction of the required energy consumption was pursued by developing a new reactor.

2.4.2 Development of a reactor for organic contaminant control

To reduce the required energy consumption and H_2O_2 dosage for 80% atrazine degradation (1.0 kWh/m³ and 13 mg/L H_2O_2), an advanced pilot and full scale reactor were developed for organic contaminant control. The full scale SWIFT 30 reactor without baffling showed some short circuiting at the top and the bottom of the reactor. This short circuiting is shown by the yellow spots in the reactor area (see figure 2.3(a)). Therefore this reactor was equipped with baffles at the top and bottom to decrease short circuiting with a minimal impact on head loss. The baffled reactor showed an excellent residence time distribution as shown by a strong decrease of the yellow spots in the reactor area (see figure 2.3(b)).

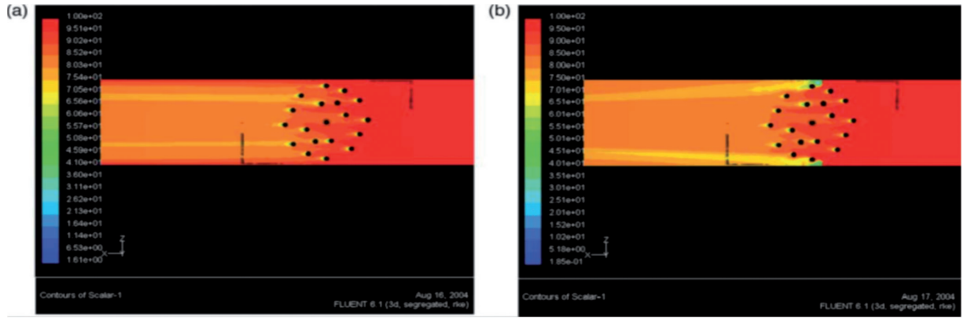


Figure 2.3: CFD modelling for a SWIFT 16L30 reactor without (a) and with baffling (b)

The baffled reactor had an excellent calculated efficiency and was implemented in the full scale WTP Andijk. For the pilot plant research, a SWIFT 4L12 reactor was developed with the same calculated efficiency. Advanced kinetic models were developed to predict the degradation of organic micropollutants by MP UV/H_2O_2 treatment (Stefan et al., 2005). In collimated beam research kinetic parameters (quantum yield, hydroxyl radical reaction rate constant) were determined. Combined CFD and kinetic modelling were used to show the efficiency of the developed reactors. For the newly designed 4L12 pilot reactor, a relationship between the realized degradation and the degradation predicted by the advanced modelling was established (see figure 2.4).

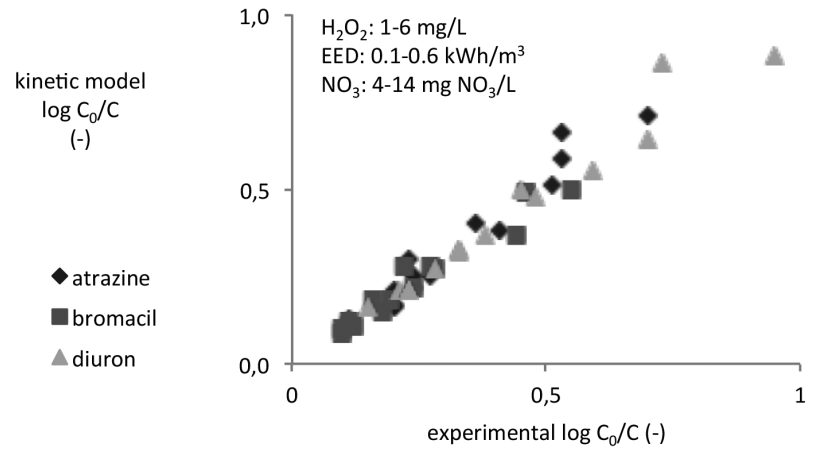


Figure 2.4: Predicted versus experimental log degradation for atrazine, bromacil and diuron

For several process conditions (0.10–0.6 kWh/m³ and 1–6 mg/L H_2O_2), an excellent relation was shown between the predicted and experimental degradation of atrazine, bromacil and diuron.

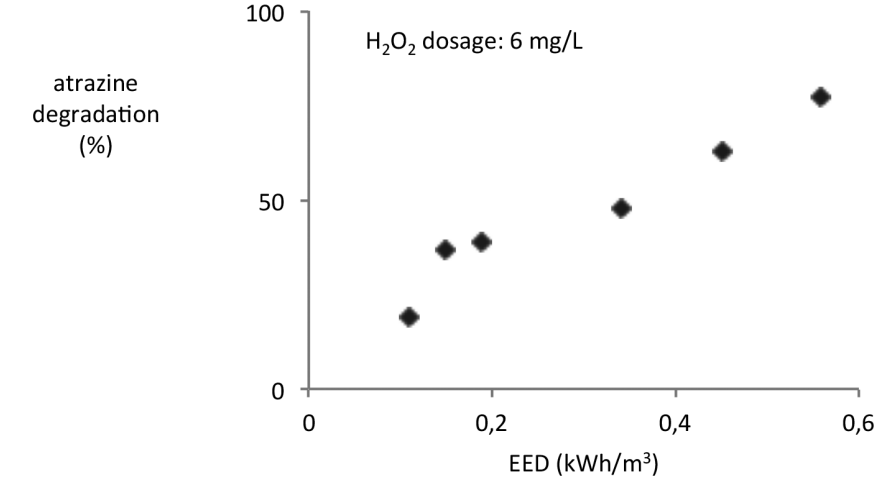


Figure 2.5: Atrazine degradation as a function of the electrical energy dose (EED) for a H_2O_2 dose of 6 mg/L in the newly designed SWIFT4L12 UV reactor for organic contaminant control purposes

Figure 2.5 shows the atrazine degradation as a function of the energy consumption. In the standard in line pilot reactors, to satisfy a degradation target for atrazine degradation of 80% an energy consumption of 1.0 kWh/m³ together with a H_2O_2 dose of 13 mg/L was needed. In the newly designed SWIFT 4L12 reactor only 0.56 kWh/m³ and 6 mg/L H_2O_2 were needed, lowering the electric energy consumption by 44% and the H_2O_2 dose by 54% respectively. So the new reactor design strongly increased the economic feasibility of MP UV/H_2O_2 treatment.

2.4.3 MP UV/H_2O_2 research in advanced pilot equipment

In this phase of the study, the focus was extended from pesticides to endocrine disruptors, pharmaceuticals, algae toxins and solvents. Degradation of endocrine disruptor bisphenol A, pharmaceutical carbamazepine, algae toxin microcystin and solvent diglyme was investigated in collimated beam experiments for a set of selected process conditions:

- UV dose of 600 mJ/cm²;
- UV dose of 600 mJ/cm² and a H_2O_2 dosage of 6 mg/L
- UV dose of 600 mJ/cm² and a H_2O_2 dosage of 15 mg/L
- UV dose of 1200 mJ/cm² and a H_2O_2 dosage of 6 mg/L
- UV dose of 1200 mJ/cm² and a H_2O_2 dosage of 15 mg/L

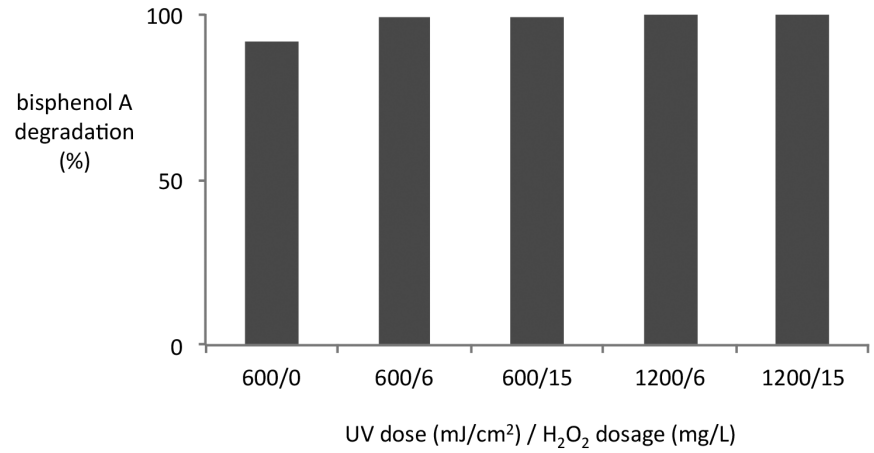


Figure 2.6: Bisphenol A degradation by MP UV/H_2O_2 treatment for selected process conditions in collimated beam experiments

For bisphenol A, by MP UV photolysis with 600 mJ/cm², a degradation of more than 90% was achieved. For the other process conditions, the conversion was close to 100% (figure 2.6). For carbamazepine with a conversion of 58%, the target degradation of 80% was not achieved by photolysis with 600 mJ/cm² only. The target degradation was exceeded for both 600 mJ/cm² with 15 mg/L H_2O_2 and 1200 mJ/cm² with 6 mg/L H_2O_2 (Figure 2.7).

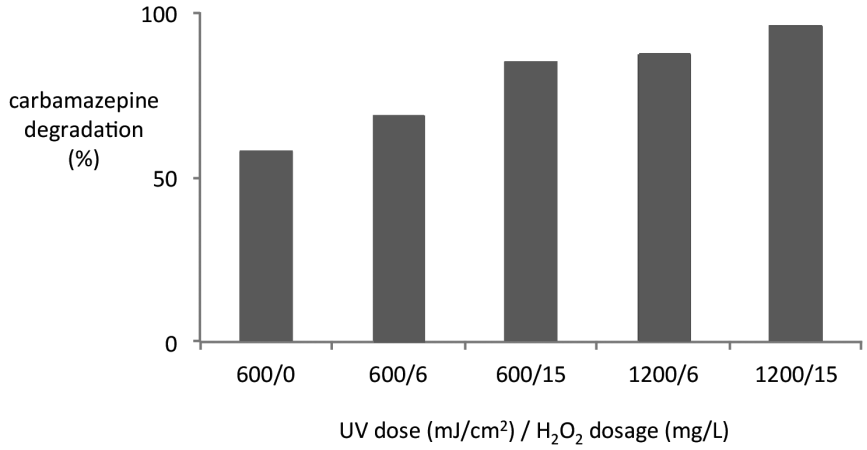


Figure 2.7: Carbamazepine degradation by MP UV/H_2O_2 treatment for selected process conditions in collimated beam experiments

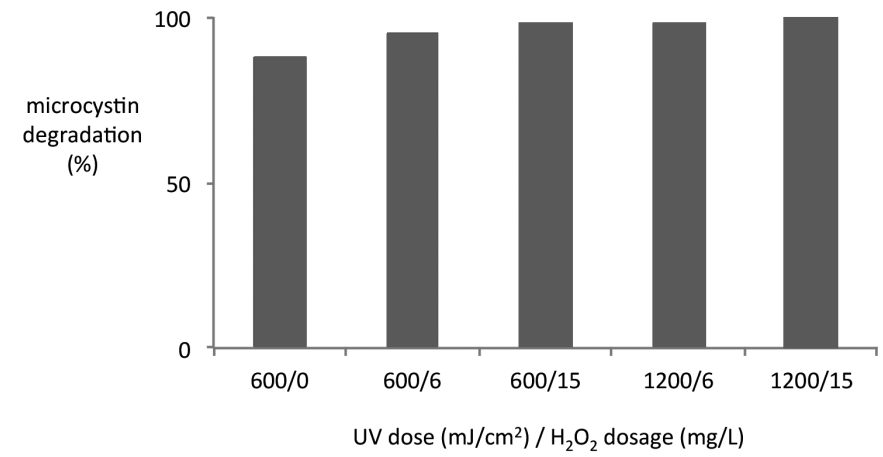


Figure 2.8: Microcystin degradation by MP UV/H_2O_2 treatment for selected process conditions in collimated beam experiments

Microcystin showed the same behaviour as bisphenol A. Using MP UV photolysis with 600 mJ/cm², the degradation target of 80% was realized. For the other conditions the conversion was close to 100% (figure 2.8). Of the selected compounds, diglyme proved to be most resistant against MP UV photolysis. A UV dose of 600 mJ/cm² achieved a degradation of about 20% only. For a UV dose of 600 mJ/cm² in combination with a H_2O_2 dosage of 5 mg/L, the degradation increased to 60% while 80% conversion could be achieved by both 600 mJ/cm² with 15 mg/L H_2O_2 or 1200 mJ/cm² with 6 mg/L H_2O_2 (figure 2.9).

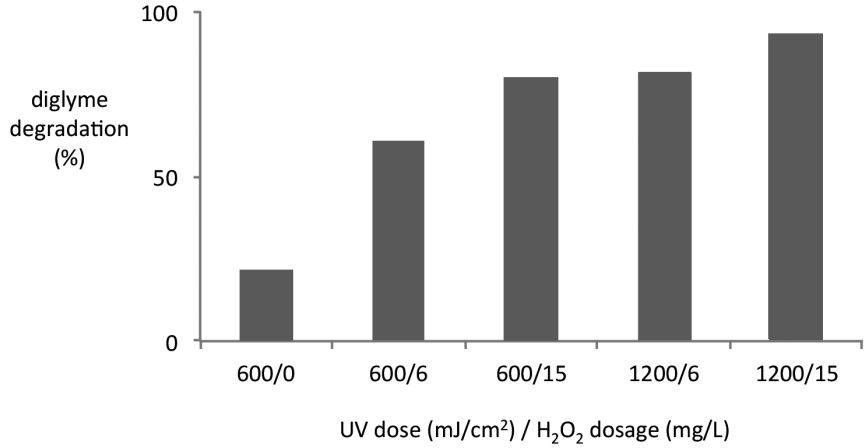


Figure 2.9: Diglyme degradation by MP UV/H_2O_2 treatment for selected process conditions in collimated beam experiments

Additional pilot scale experiments were carried out in the advanced pilot reactor. For standard MP UV/H_2O_2 conditions, electric energy demand (EED) 0.56 kWh/m³ (UV dose 540 mJ/cm²), H_2O_2 dose 6 mg/L the degradation in the advanced pilot reactor for a set of pesticides and pharmaceuticals are presented in Figures 2.10 and 2.11.

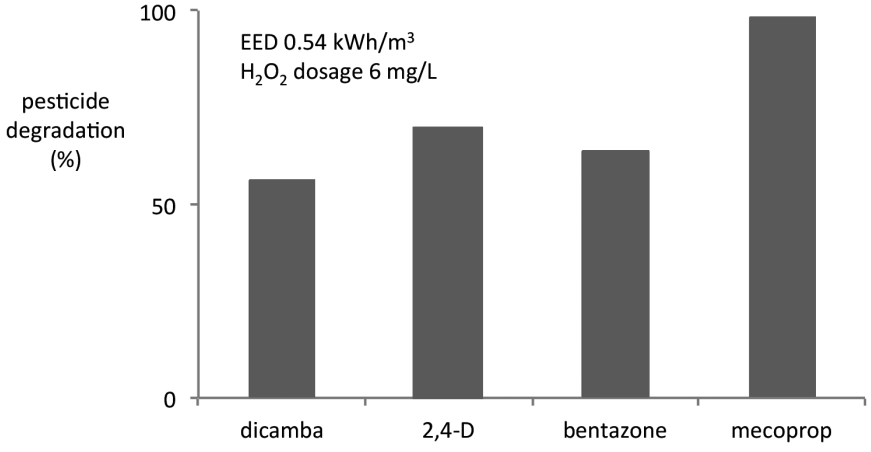


Figure 2.10: Pesticide degradation in pretreated IJssel Lake water in the SWIFT 4L12 reactor (EED 0.56 kWh/m³, H_2O_2 dose 6 mg/L)

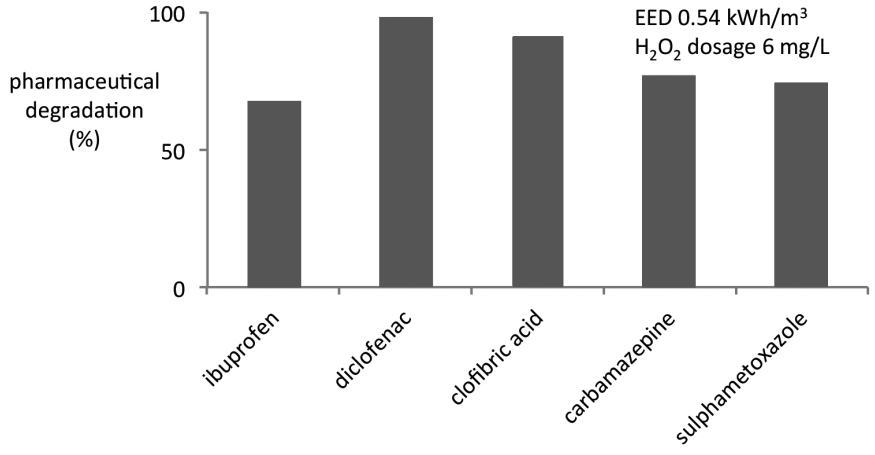


Figure 2.11: Pharmaceutical degradation in pretreated IJssel Lake water in the SWIFT 4L12 reactor (EED 0.56 kWh/m^3, H_2O_2 dose 6 mg/L)

With the SWIFT 4L12 reactor a degradation >60% was achieved for all pollutants under standard conditions.

2.4.4 Full scale MP UV/H_2O_2 application

At WTP Andijk MP UV/H_2O_2 treatment was implemented after CSF pretreatment prior to granular activated carbon (GAC) filtration. At the same time breakpoint chlorination was stopped (Figure 2.12).

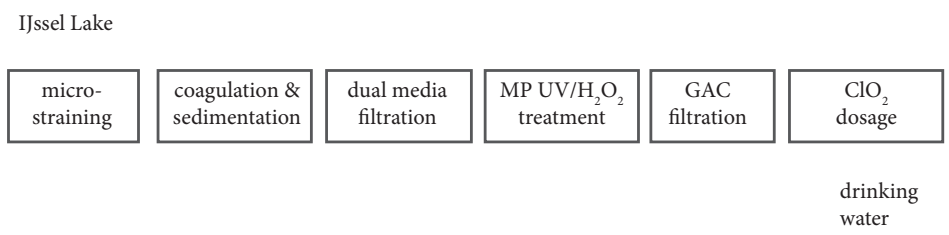


Figure 2.12: Treatment scheme WTP Andijk 2004-2015

A MP UV/H_2O_2 system consisting of 12 SWIFT 16L30 reactors in three rows of four reactors each was installed. The installation is equipped with a control unit calculating the atrazine degradation capacity under actual process conditions. Before start up of the retrofit plant, a site acceptance test was performed. The actual atrazine degradation, the degradation calculated by the control unit and the degradation predicted by the kinetic model is presented in figure 2.13.

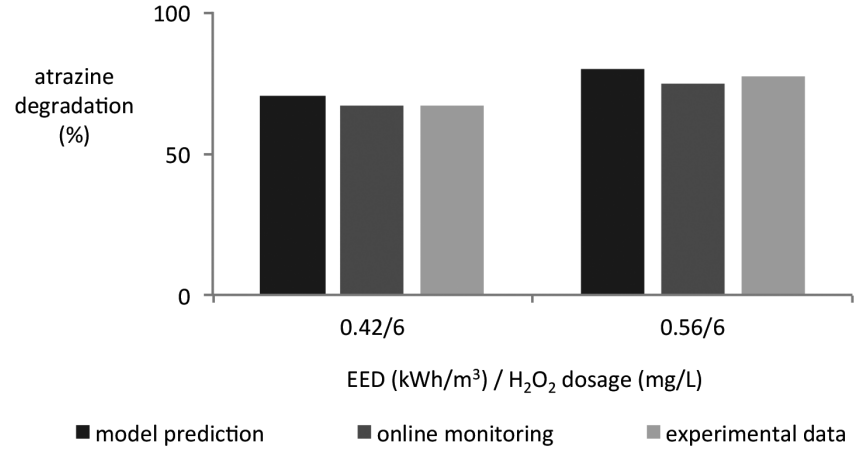


Figure 2.13: Model prediction, online monitoring and experimental data of atrazine degradation for an electric energy of 0.42 and 0.56 kWh/m^3 with 6 mg/L H_2O_2 (full scale data)

Good agreement was found between the measured degradation, the model calculation and the prediction by the installation software. At a UVT254 of 87%, the realized degradation of 77± 9% satisfied the target conversion of 80%. In IJssel Lake water 25 priority pollutants were identified in 2004. Most compounds were removed to a concentration below 0.1 µg/L by GAC filtration only. The removal of EDTA by GAC filtration only was poor. The removal of EDTA before and after the installation of MP UV/H_2O_2 treatment is presented in Figure 2.14.

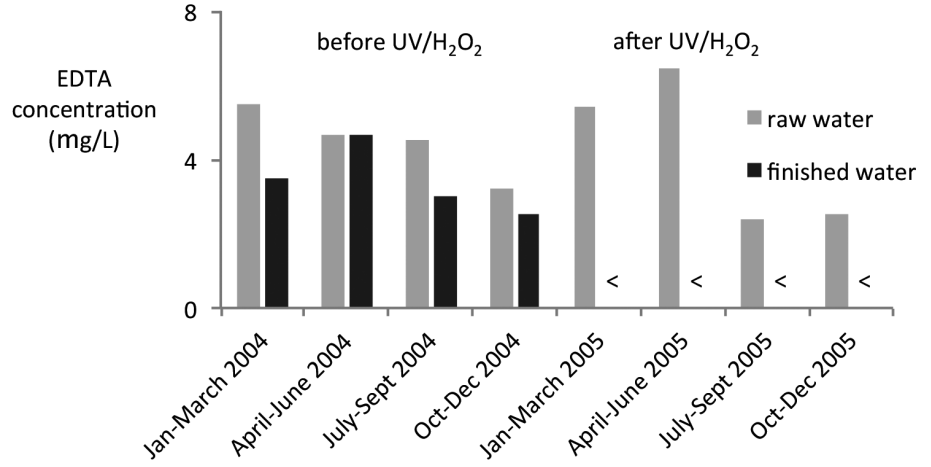


Figure 2.14: EDTA concentration in raw and finished water before and after installation of MP UV/H_2O_2 treatment (full scale data, 2004-2005)

After the installation of MP UV/H_2O_2 treatment, no EDTA was detected in the finished water, even when the raw water concentration was as high as 6 µg/L. Amongst the micropollutants found in raw IJssel Lake water were anti-epileptic carbamazepine, flame retardant trichloropropylphosphate, detergent Surfynol 104 and melanine. After installation of MP UV/H_2O_2 treatment, these compounds were removed to below the detection limit of 0.02 µg/L by combination of MP UV photolysis and hydroxyl radical oxidation. The solvent diglyme does not absorb UV light. Therefore hardly any degradation achieved by MP UV photolysis, while 50% degradation was achieved by hydroxyl radical oxidation. Additional collimated beam research showed that almost all priority pollutants were degraded to a certain extent by MP UV/H_2O_2 treatment. The contribution of MP UV photolysis was determined by the UV absorbance and quantum yield. The contribution of hydroxyl radical oxidation was determined by the hydroxyl radical reaction rate constant, depending on unsaturated sites and/or H-atoms present in the compound. Perfluorinated flame retardants perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) do neither absorb UV light nor contain unsaturated sites or H-atoms. Therefore these compounds were not degraded at all. Fortunately from a treatment perspective, these compounds were adsorbed by GAC. The full scale installation is in operation since October 2004. The process has proven to be a robust and reliable barrier against organic micropollutants. In addition it is a strong and robust barrier against pathogenic micro-organisms (Belosevic et al., 2001). Although improved significantly the electric energy consumption still deserves further attention.

2.5 Perspective

MP UV/H_2O_2 treatment has shown to be a robust, non-selective barrier against most organic micropollutants. Its application in standard UV equipment, developed for disinfection purposes, was economically feasible, although rather expensive. Development of an advanced reactor for organic contaminant control has improved the economics of the process by about 50%. With the currently available MP UV technology, a significant further cost reduction by reactor optimization may not be expected. Nevertheless a further cost reduction should be pursued to make MP UV/H_2O_2 treatment even more attractive. An option to decrease the operational costs of UV/H_2O_2 treatment even further is to increase the UV transmission (UVT) of the water. The UVT of the water is predominantly determined by the natural organic matter (NOM) and nitrate content, which should be lowered by pretreatment as much as possible.

Abstract

Increasingly, advanced oxidation with MP UV/H_2O_2 is considered as a best available technology for organic contaminant control. Although the required energy consumption of MP UV/H_2O_2 is still substantial, the process has proven to be economically feasible for organic contaminant control purposes such as NDMA degradation, taste and odour removal and as a non selective barrier against organic micropollutants. The economic feasibility would increase significantly with a reduced energy consumption, making this technology even more attractive to solve a wide range of water treatment problems. A significant reduction of the energy consumption can be achieved by a strong increase of the UV-transmittance by advanced pretreatment. The most important UV absorbing compounds in raw IJssel Lake water are NOM and nitrate. Both NOM and nitrate content can be lowered by pretreatment, for instance by coagulation or ion exchange. Compared to conventional pretreatment, ion exchange improves the water quality in terms of increased UV transmittance, extended DOC removal and nitrate removal, improving the conditions for MP UV/H_2O_2 treatment. The degradation of organic micropollutants was studied in collimated beam experiments. NDMA was selected as a reference compound sensitive for MP UV photolysis while 1,4-dioxane was selected as a reference compound sensitive for OH-radical oxidation only. Quantum yield for NDMA and reaction rate constant for 1,4-dioxane were determined in milliQ water. Both competition for MP UV radiation (NDMA) and competition for OH-radical scavenging (1,4-dioxane) were heavily impacted by the water matrix. The best results were achieved after ion exchange pretreatment, resulting in the lowest DOC and nitrate content. These results were confirmed in pilot research. Compared to coagulation pretreatment, ion exchange pretreatment reduced the required electrical energy per order for NDMA degradation and 1,4 dioxane degradation by MP UV/H_2O_2 treatment by about 50%.

This chapter is derived from: Martijn, A.J., Fuller, A.L., Malley, J.P., Kruithof, J.C., 2010. Impact of IX-UF pretreatment on the feasibility of UV/H_2O_2 treatment for degradation of NDMA and 1,4 dioxane. *Ozone Science and Engineering* 30 (6), 383-390

Chapter 3 Impact of IX-UF pretreatment on the feasibility of MP UV/H_2O_2 treatment for the degradation of NDMA and 1,4-dioxane

3.1 Introduction

Two degradation mechanisms play a role in MP UV/H_2O_2 treatment, MP UV photolysis and OH-radical oxidation, contributing to the non selectivity of this AOP. Some compounds are only degraded by OH-radical oxidation (1,4-dioxane), others mainly by MP UV photolysis (NDMA) but the majority of organic micropollutants is degraded by a combination of both MP UV photolysis and OH-radical oxidation (atrazine) (figure 3.1). The efficacy of the MP UV/H_2O_2 process is strongly impacted by the water matrix. UV-absorbing compounds (nitrate, natural organic matter (NOM)) cause competition for MP UV light, while water constituents (DOC, nitrite, carbonate/bicarbonate), other than the degradation targets and H_2O_2 act as OH-radical scavengers.

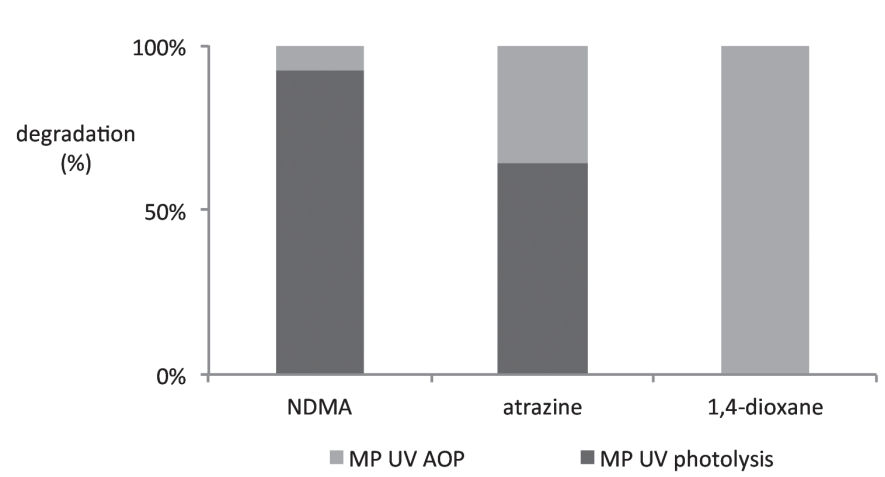


Figure 3.1: Contribution of MP UV photolysis and hydroxyl radical oxidation to the degradation by MP UV/H_2O_2 treatment for NDMA, 1,4 dioxane and atrazine degradation

Advanced oxidation by MP UV/H_2O_2 treatment is increasingly considered as a best available technology (BAT) for organic contaminant control. The energy consumption of the process is an important issue. Development of an advanced kinetic and CFD model enabled the design of a reactor that reduced the power consumption by more than 40% compared to a standard reactor design for disinfection purposes (chapter 2). Although

the required energy consumption by the MP UV/H_2O_2 process in this reactor type is still substantial, the process has proven to be economically feasible for organic contaminant control purposes such as NDMA degradation, taste and odor removal and as a non selective barrier against organic micropollutants. The economic feasibility would increase significantly with a reduced energy consumption and would make this technology even more attractive to solve a wide range of water treatment problems. Advanced pretreatment may play an important part to achieve this goal.

3.2 Key water quality parameters

A significant reduction of energy consumption by MP UV/H_2O_2 treatment can be achieved by the removal of UV absorbing constituents in the pretreatment, thereby reducing the competition for UV light. In addition the removal of these compounds reduces the scavenging of OH-radicals, produced by the photolysis of H_2O_2. Therefore the composition of the water matrix was analysed. The most important water constituents influencing the efficacy of the UV/H_2O_2 process were natural organic matter (NOM) and nitrate. Nitrate absorbs UV light in the same wave length range and has a higher molar absorption coefficient than H_2O_2, introducing competition for photons (especially when broad spectrum UV light is applied). NOM (measured as DOC) is also a strong UV absorber and is the most important OH-radical scavenger.

Pretreatment impacted the content and composition of the water matrix. CSF lowered the DOC content, reducing UV absorbance and OH-radical scavenging, but did not impact the nitrate concentration. Advanced pretreatment with ion exchange and ultrafiltration (IX-UF) removed both nitrate and DOC (Galjaard et al., 2005). The strong removal of DOC and nitrate by IX-UF relative to CSF pretreatment benefits the formation of OH-radicals while scavenging of OH-radicals is reduced by DOC removal. An additional advantage of IX-UF is that any desired DOC and nitrate removal can be achieved by setting process conditions. Therefore IX-UF pretreatment provides the opportunity to create favourable conditions for the application of the MP UV/H_2O_2 process, regardless of the raw water composition.

Table 3.1 presents the nitrate concentration in raw water, after CSF and IX-UF pretreatment. Raw IJssel Lake water showed seasonal variations in nitrate content between 1 and 12 mg/L. CSF treatment did not impact the nitrate concentration. IX-UF lowered the nitrate concentration with 65%, reducing both the fluctuation and the absolute concentration.

Table 3.1: Annual min, max and average nitrate concentration in raw water, after CSF and IX-UF treatment

Water type	min	max	average
	mg NO_3/L	mg NO_3/L	mg NO_3/L
Raw water	1.2	12.4	6.5
CSF treatment	1.7	9.4	5.8
IX-UF treatment	0.5	4.2	2.3

The DOC content (6.0 mg/L) in raw water was rather stable over the year. DOC was removed by CSF for approximately 30%. With IX-UF pretreatment, the removal of DOC was increased to 50% on average (table 3.2).

Table 3.2: Annual min, max and average DOC concentration in raw water, after CSF and IX-UF treatment

Water type	min	max	average
	mg C/L	mg C/L	mg C/L
Raw water	5.1	7.2	6.0
CSF treatment	2.9	5.1	4.0
IX-UF treatment	2.1	4.4	2.9

The impact of pretreatment on the most relevant water matrix parameters for MP UV/H_2O_2 treatment, nitrate and DOC, are summarized in figure 3.2.

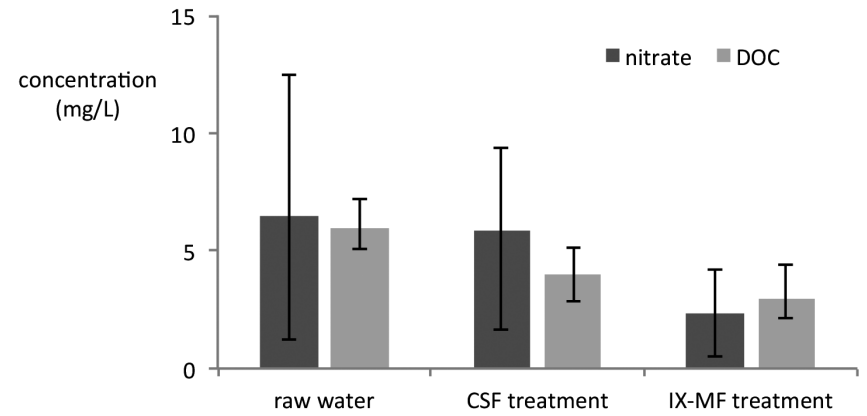


Figure 3.2: Annual average nitrate and DOC concentration in raw water, after CSF pretreatment and in IX-UF treated water

For MP UV/H_2O_2 purposes both DOC and nitrate content should be lowered by pretreatment as much as possible. CSF lowered the DOC content from 6.0 mg/L to 4.0 mg/L, while nitrate removal was absent. At the applied process conditions IX-UF lowered both DOC and nitrate concentration to 2.9mg/L and 2.3 mg/L respectively. The impact of the improved water quality on the degradation of organic micropollutants was studied in collimated beam experiments. Nitrosodimethylamine (NDMA) was selected as a reference compound sensitive for UV photolysis while 1,4-dioxane and para-chlorobenzoic acid (pCBA) were selected as reference compounds sensitive for OH-radical oxidation.

3.3 Effect pretreatment on efficacy MP UV/H_2O_2 treatment

The effect of pretreatment on the UV absorption spectrum of the water matrix (panel A, B and C) and the spectrum of H_2O_2 (panel D) are presented in figure 3.3. DOC and nitrate were measured in all water types. In addition the found contents were spiked in milliQ, simulating the raw water, CSF and IX-UF composition. A UV scan (200-300 nm) of raw water, CSF treated water and IX-UF treated water, was compared to a UV-scan of the same concentration of DOC (IHSS Nordic Lake NOM) and nitrate in milliQ. The absorption spectra confirmed that DOC and nitrate were the major UV absorbing constituents. Note that H_2O_2 absorbs in the same wavelength range as nitrate but with much lower absorbance.

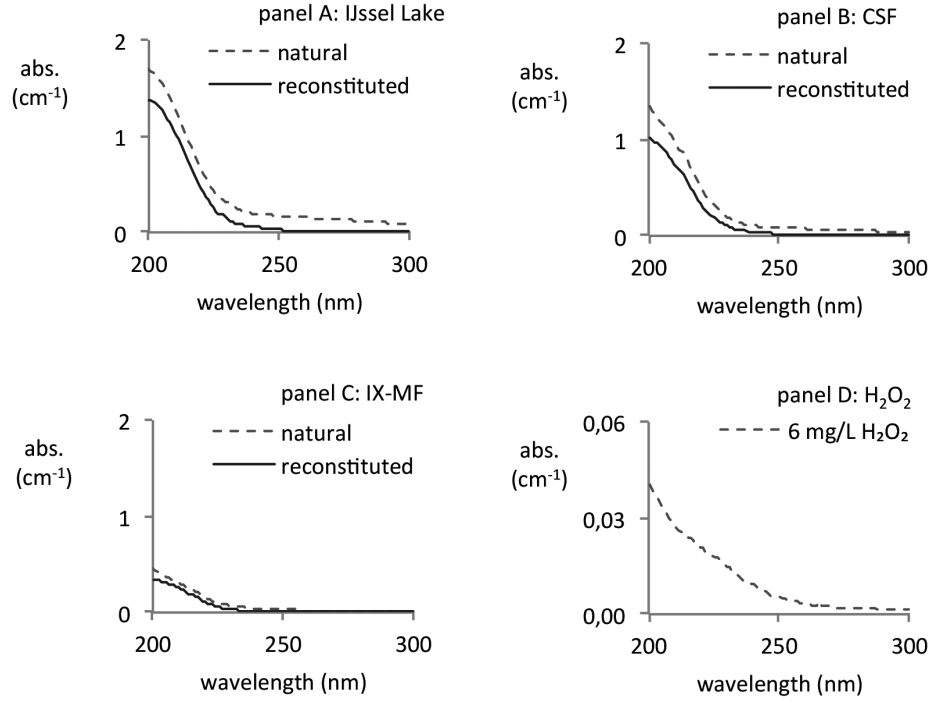


Figure 3.3: UV absorbance of natural and reconstituted raw water (panel A), CSF treated water (panel B) and IX-UF treated water (panel C) and the UV absorbance of a solution containing 6 mg/L H_2O_2 (panel D)

The available fraction of the UV light for the formation of OH-radicals by photolysis of H_2O_2 was calculated for two wavelengths, 240 nm and 254 nm, in raw, in CSF and in IX-UF treated water (table 3.3). All three water types showed a higher UV absorbance by H_2O_2 at the lowest wavelength, indicating that OH-radical formation at 240 nm was more efficient than at 254 nm (table 3.3). The effect of pretreatment on the UV-absorption is illustrated as well by the results presented in table 3.3. An increase of approximately 2-2.5 times in available UV light from CSF to IX-UF pretreatment was observed.

Table 3.3: Fraction UV light absorbed by H_2O_2 (6 mg/L) in raw, CSF and IX-UF treated water at a wave length of 240 nm and 254 nm

Water type	240 nm	254 nm
Raw water	4.5%	2.6%
CSF treated water	8.2%	5.3%
IX-UF treatment	19.4%	14.7%

Based on the emission spectrum of the used MP UV lamp, relative to the total photon flow, the photon flow absorbed by the water matrix constituents, the H_2O_2 dosage and the reference compounds (NDMA for MP UV photolysis and 1,4-dioxane for OH-radical oxidation) were calculated using a spreadsheet provided by Bolton (table 3.4) (Bolton, 2001). Due to experimental constraints, a high NDMA concentration was applied, resulting in a comparable absorbed photon flow for NDMA and H_2O_2.

Table 3.4: Relative photon flow absorbed by the water matrix, H_2O_2 (6 mg/L), NDMA (500 µg/L) and 1,4- dioxane (200 µg/L) in raw, CSF and IX-UF treated water

	raw	CSF treated water	IX-UF treatment
% photon flow absorbed by matrix	89%	85%	72%
% photon flow absorbed by H_2O_2	4%	6%	11%
% photon flow absorbed by NDMA	7%	9%	17%
% photon flow absorbed by 1,4-dioxane	0%	0%	0%

Compared to the results of table 3.3 where only the absorption spectrum of the water matrix and H_2O_2 were taken into account, the effect of pretreatment was less pronounced, but still substantial.

3.4 Effect of pretreatment on advanced oxidation

3.4.1 Effect of pretreatment on MP UV photolysis

NDMA absorbs within the UV-C spectral range 200-260nm and has a relatively high quantum yield. The compound was selected because degradation is almost entirely due to MP UV photolysis while degradation by OH-radical oxidation is insignificant (Stefan et al., 2002). In MP UV collimated beam experiments, the degradation of NDMA was studied in milliQ, CSF and IX-UF treated water for several H_2O_2 dosages. The irradiance time was calculated according to Bolton et al. (Bolton et al., 2002).

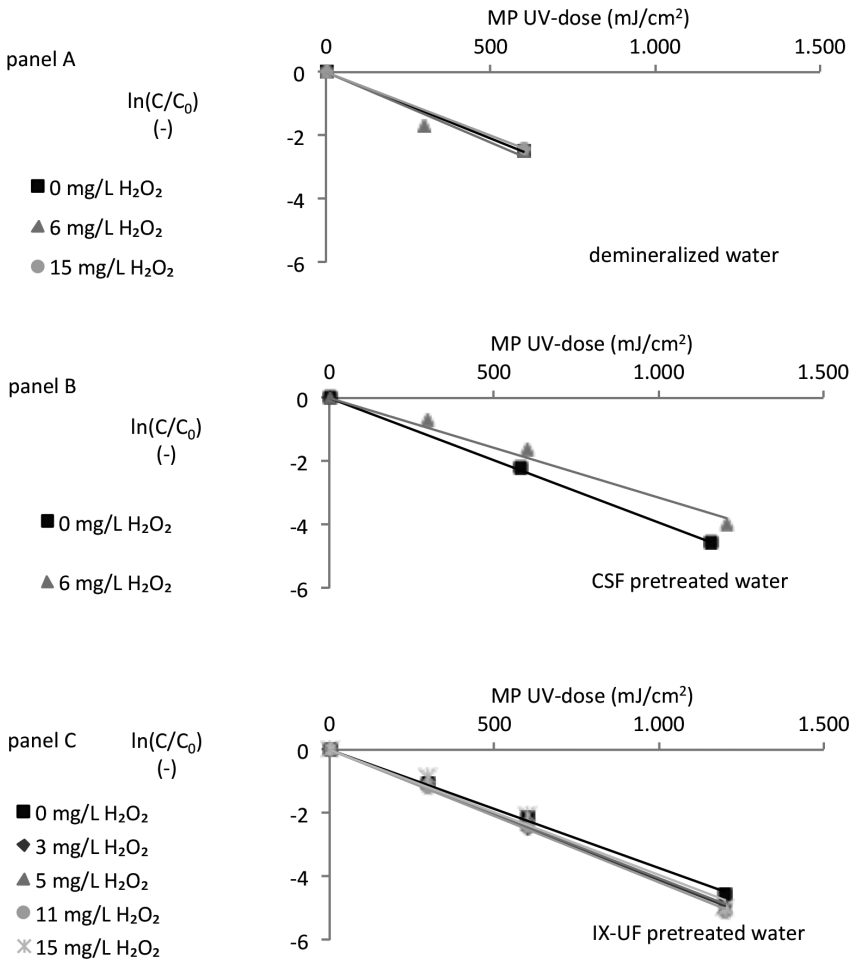


Figure 3.4: NDMA degradation in milliQ (panel A), in CSF (panel B) and IX-UF treated water (panel C) as a function of the MP UV-dose for a range of H_2O_2 concentrations (MP UV CB experiments)

The results from the collimated beam experiments show that the H_2O_2 concentration had a very small impact on the NDMA degradation, confirming that the predominant nature of the degradation mechanism of NDMA is MP UV photolysis. Matrix effects (competition for UV-light) were taken into account by adjusting the irradiation time to achieve the same UV dose for the three water types. The quantum yield was derived from the experimental results (figure 3.4). Literature values (Stefan et al., 2002) were consistently lower (approximately 15%) than quantum yields determined in this study (table 3.5) due to differences in the applied molar absorption coefficient.

Table 3.5: Experimentally obtained quantum yield for NDMA

	milliQ	CSF treatment	IX-UF treatment
ϕ ($M\ E^{-1}$)	0.431	0.394	0.392

For MP UV pilot experiments, at a range of H_2O_2 dosages, the electric energy per order (EEO) required for NDMA degradation in IX-UF pretreated water was determined. For a single H_2O_2 dosage, the EEO for NDMA degradation in CSF treated water was calculated as well (figure 3.5).

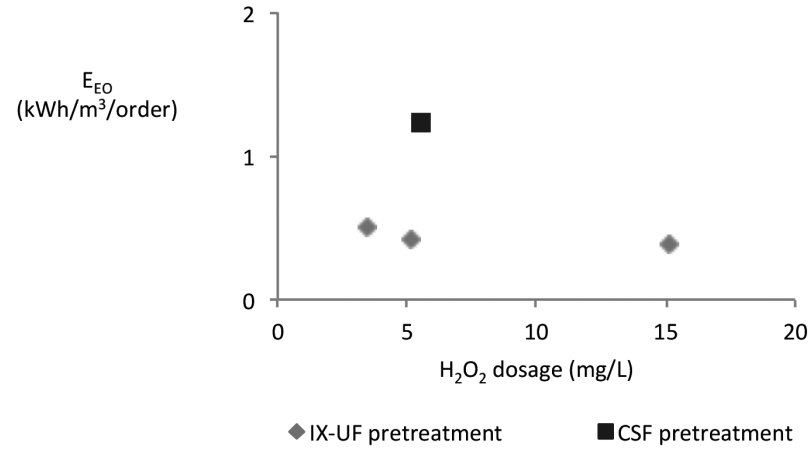


Figure 3.5: Electrical energy per order for NDMA degradation by MP UV/H_2O_2 treatment as function of H_2O_2 concentration for CSF and IX-UF treated water (pilot experiments)

In IX-UF treated water the EEO for NDMA degradation was independent of the H_2O_2 dosage confirming the photolytic character of NDMA degradation. The higher EEO in CSF treated water was due to the water matrix, creating more competition for UV light. The EEO for NDMA in IX-UF treated water (0.41 kWh/m³), compared to the EEO for NDMA in CSF treated water (1.24 kWh/m³) exceeded the estimation based upon the photon flow.

3.4.2 Effect of pretreatment on OH-radical oxidation

1,4-dioxane was chosen because its degradation is entirely due to hydroxyl radical oxidation. The degree to which 1,4-dioxane degradation occurs is an indicator of the influence of the water matrix on OH-radical production and scavenging. The degradation of 1,4-dioxane was studied in MP UV collimated beam experiments in milliQ, CSF and IX-UF treated water. The irradiance time was calculated according to Bolton et al. (Bolton et al., 2002). In milliQ water, in the absence of H_2O_2, no degradation of 1,4-dioxane was observed (figure 3.6, panel A). The 1,4-dioxane degradation in CSF and in IX-UF treated water without H_2O_2 dosage was due to photo induced OH-radical formation (figure 3.6, panel B, C).

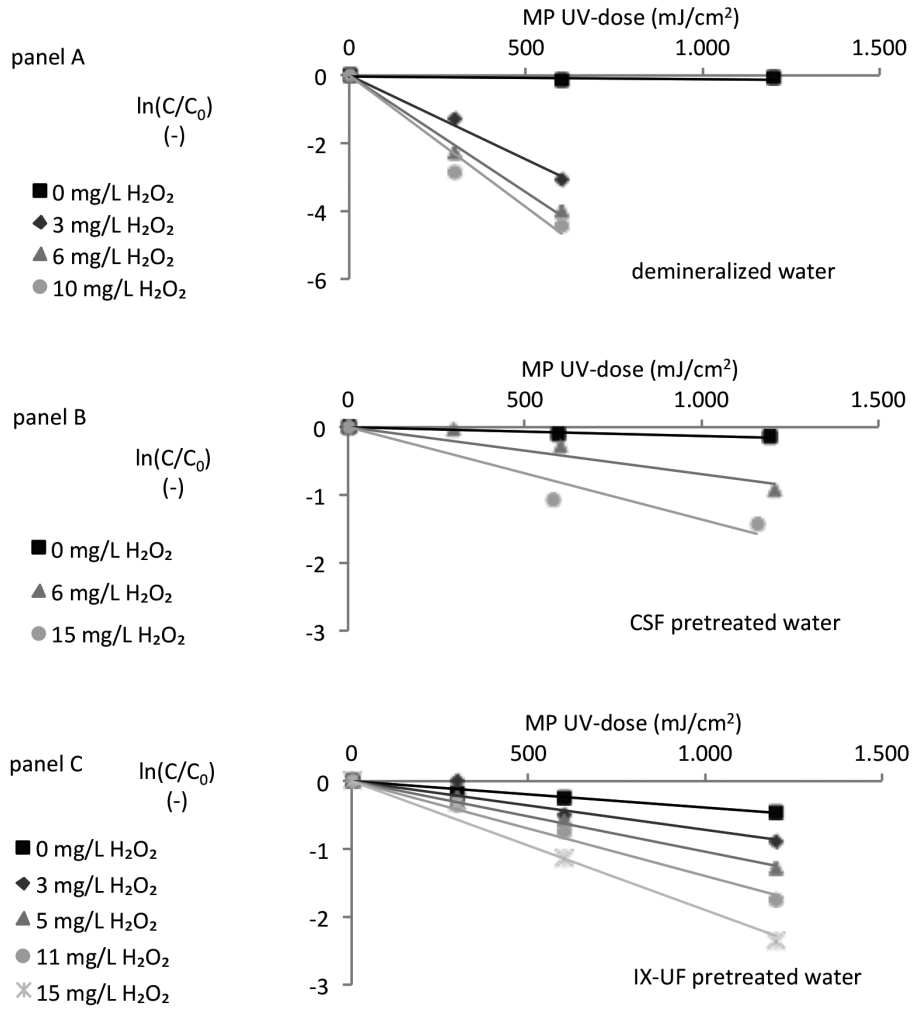


Figure 3.6: 1,4-Dioxane degradation in milliQ (panel A), in CSF (panel B) and IX-UF treated water (panel C) as a function of the MP UV-dose for a range of H_2O_2 concentrations (MP UV collimated beam experiments)

The primary reason for the observed increased 1,4-dioxane degradation in IX-UF treated water compared to CSF treated water was the restricted OH-radical scavenging (figure 3.6, panel B and C). In MP UV pilot experiments, for a range of H_2O_2 dosages, the required EEO of 1,4-dioxane degradation in IX-UF treated water was determined. For a single H_2O_2 dosage, the EEO for the degradation of 1,4-dioxane in CSF treated water was calculated as well (figure 3.7).

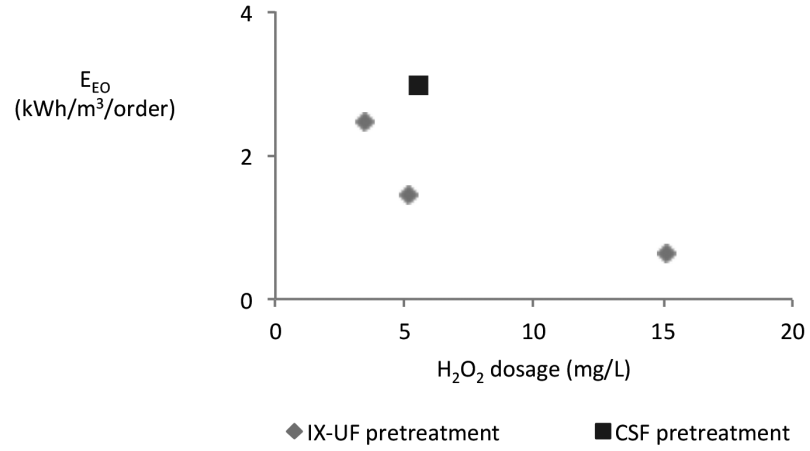


Figure 3.7: Electrical energy per order for 1,4-dioxane degradation by MP UV/H_2O_2 treatment as function of H_2O_2 concentration for CSF and IX-UF treated water (pilot experiments)

Contrary to the NDMA data, in IX-UF pretreated water the H_2O_2 dosage showed a substantial impact on the EEO for 1,4-dioxane degradation, ranging from 2.5 kWh/m^3 at 3.5 mg/L H_2O_2 to 0.6 kWh/m^3 at 15 mg/L H_2O_2 (figure 3.7). For one H_2O_2 dosage (5 mg/L), the EEO for 1,4-dioxane degradation in CSF treated water was determined as well. Comparing this EEO (3.0 kWh/m^3) to the EEO in IX-UF treated water (1.4 kWh/m^3), showed a reduction with a factor of 2.1. This improvement by pretreatment was due to both less competition for UV light for OH-radical formation and reduced OH-radical scavenging.

3.4.3 $R_{OH,UV}$ modelling for effect of pretreatment

For both 1,4-dioxane and para-chlorobenzoic acid (pCBA), the $R_{OH,UV}$ concept, developed by Rosenfeldt et al. (Rosenfeldt et al., 2007) was applied. The $R_{OH,UV}$ concept is defined as the experimentally determined OH• radical exposure per UV fluence. $R_{OH,UV}$ was determined by examining the degradation of both 1,4-dioxane and pCBA as probe compounds. The equation for $R_{OH,UV}$ was developed by Rosenfeldt:

$$R_{OH,UV} = \frac{\int_0^t [\bullet OH]dt}{H} = \frac{k_T^{'D} - k_d^{'D}}{k_{OH,probe}} \qquad \text{(Eq. 3.1)}$$

where

H is UV fluence

$k_d^{'D}$ is the fluence based rate constant of the probe destruction by MP UV photolysis;

$k_T^{'D}$ is the fluence based rate constant of the probe destruction by both OH-radical oxidation and MP UV photolysis;

$k_{(OH)}$ is the rate constant for the probe destruction by OH-radical oxidation;

in which:

$k_{OH,pCBA}$ is $5{,}0*10^9$ M^{-1} s^{-1} (Rosenfeldt et al., 2007)

$k_{OH,1,4\text{-dioxane}}$ is $2{,}8*10^9$ M^{-1} s^{-1} (Stefan et al., 1998)

Figure 3.8 shows the $R_{OH,UV}$ in milliQ as function of the H_2O_2 concentration, using both 1,4-dioxane and pCBA as probe (MP UV collimated beam experiments). Probe selection did not influence the $R_{OH,UV}$ significantly. Also plotted in figure 3.8 are the results for $R_{OH,UV}$ in milliQ from Rosenfeldt, using pCBA as a probe (MP UV collimated beam experiments).

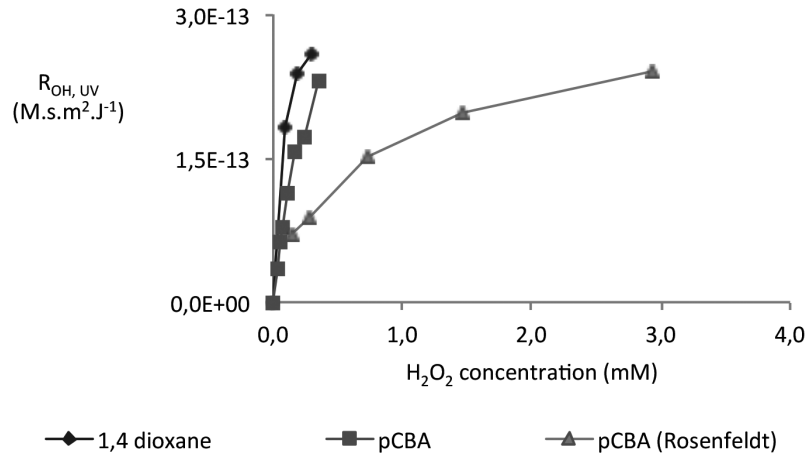


Figure 3.8: $R_{OH,UV}$ for 1,4-dioxane and pCBA in milliQ, compared to pCBA literature values (Rosenfeldt et al., 2007)

$R_{OH,UV}$ values measured by Rosenfeldt et al. (Rosenfeldt et al., 2007) are in the same order of magnitude. The $R_{OH,UV}$ for milliQ, CSF treated water and IX-UF treated water for a range of H_2O_2 concentrations (up to 0,5 mM H_2O_2) (MP UV) is presented (figure 3.9). The fluence based rate constants (table 3.6) are derived from collimated beam experiments (figure 3.6).

Table 3.6: Pseudo first order rate constants for MP UV photolysis and the combination of MP UV photolysis and hydroxyl radical oxidation for 1,4-dioxane and pCBA in milliQ (6 mg/L H_2O_2), CSF (6 mg/L H_2O_2) and IX-UF treated water (5 mg/L H_2O_2)

	k'^D_d (m^2 J^{-1})	k'^D_d (m^2 J^{-1})	k'^D_T (m^2 J^{-1})	k'^D_T (m^2 J^{-1})
	1,4-dioxane	pCBA	1,4-dioxane	pCBA
milliQ	$7.94*10^{-6}$	$3.21*10^{-5}$	$6.73*10^{-4}$	$8.22*10^{-4}$
IX-UF	$1.23*10^{-5}$	n.a.	$1.12*10^{-4}$	n.a.
CSF	$1.22*10^{-5}$	n.a.	$8.16*10^{-5}$	n.a.

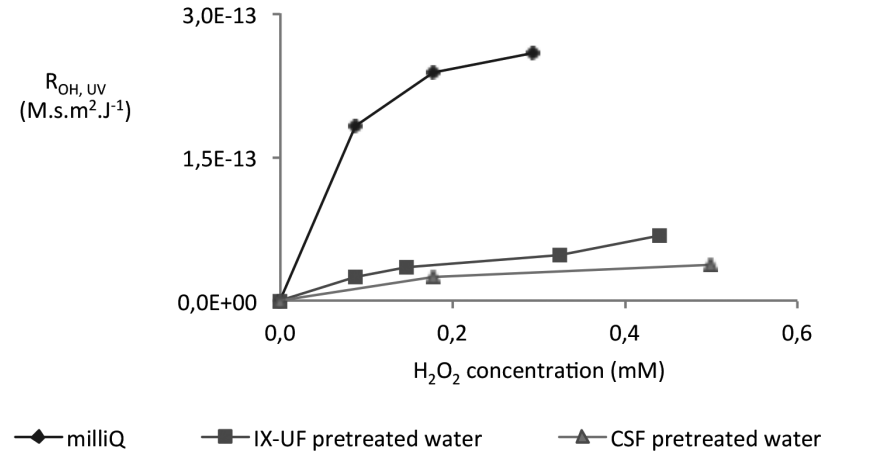


Figure 3.9: $R_{OH,UV}$ for 1,4 dioxane in milliQ, CSF and IX-UF treated water as a function of the H_2O_2 concentration

Relative to the $R_{OH,UV}$ in milliQ, $R_{OH,UV}$ values for CSF and IX-UF treated water illustrated a substantial impact of the water matrix. Comparing the $R_{OH,UV}$ at a H_2O_2 concentration of 0.15 mM (5 mg/L) for CSF and IX-UF treated water to the EEO results from the pilot experiments (figure 3.7), showed similar behaviour.

The impact of the dominant water matrix constituents, DOC and nitrate, on the $R_{OH,UV}$ were studied in MP UV collimated beam experiments, using pCBA as a probe. MilliQ was spiked with a range of DOC and nitrate concentrations at 6 mg/L H_2O_2. The $R_{OH,UV}$ for 6 mg/L H_2O_2 in miliQ water is shown as a reference (figure 3.10).

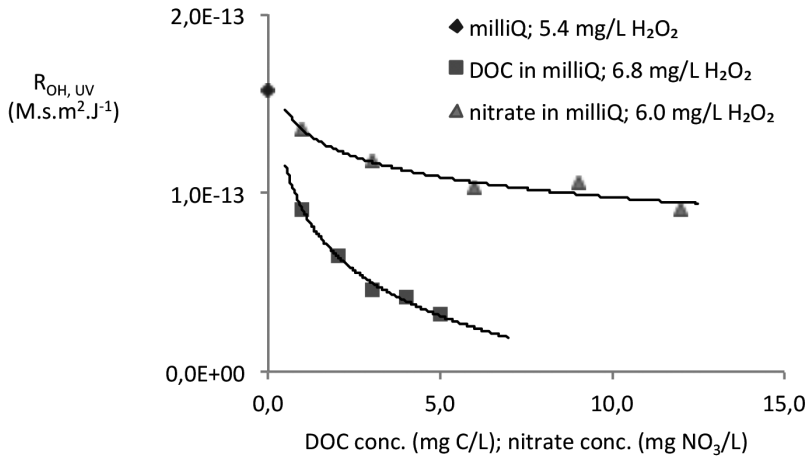


Figure 3.10: $R_{OH,UV}$ for pCBA in milliQ as a function of the DOC concentration and NO_3 concentration, (MP UV collimated beam experiments, 6 mg/L H_2O_2)

The impact of DOC over the observed annual range in CSF treated water (3-5 mg C/L) and IX-UF treated water (2-4 mg C/L) was predominantly determining the capacity of the OH-radical oxidation. The impact of nitrate was less pronounced. From this perspective a further decrease of DOC by the IX-UF process should be pursued (i.e. by selection of process conditions for IX-UF).

3.5 Conclusions

In view of the current IX-UF pretreatment at WTP Andijk, the effect of this pretreatment on the in 2004 installed MP UV/H_2O_2 process was studied in comparison to the former pretreatment based on CSF. Compared to CSF, IX-UF improved the water quality in terms of UV transmittance by extended DOC removal (to 1.0 mg/L) and significant nitrate removal (to 0.2 – 4.0 mg nitrate/L). This improved the energy requirements for post MP UV/H_2O_2 treatment. The scavenging of OH-radicals was reduced so for the application of MP UV-lamps, the generation of OH-radicals by MP UV photolysis of H_2O_2 became more favourable.

NOM (DOC) and nitrate were the predominant matrix constituents, influencing the MP UV/H_2O_2 process. CSF pretreatment removed 30% DOC and did not affect the nitrate concentration. For the tested IX-UF process settings, DOC was removed for 50% and nitrate for 70%, while a more extended removal could be pursued.

Based on UV absorbance (200-300 nm) by the water matrix and H_2O_2, treatment with IX-UF resulted in an increased availability of UV light for H_2O_2 by a factor 2, relative to CSF pretreatment (table 3.4). This behaviour was confirmed in more detail by calculating the absorbed photon flow taking the MP UV lamp emission spectrum into account (table 3.3). The relative absorbance of UV light by H_2O_2 at a wave length of 240 nm is higher than at 254 nm.

For the MP UV collimated beam and pilot experiments, NDMA was used to monitor photolytic degradation and 1,4-dioxane and pCBA were used to monitor OH-radical oxidation. Based on pilot work, the electrical energy per order (EEO) was calculated. For NDMA the EEO was independent of the H_2O_2 dosage. In IX-UF treated water the EEO was 0.41 kWh/m^3, in CSF treated water 1.24 kWh/m^3. For 1,4-dioxane the EEO was strongly dependent on the H_2O_2 dosage. In CSF treated water the EEO was 3.0 kWh/m^3 at a H_2O_2 dosage of 5 mg/L. At the same H_2O_2 dosage (5 mg/L) in IX-UF treated water an EEO of 1.4 kWh/m^3 was found.

The quantum yield for NDMA, determined in MP UV collimated beam experiments, was 0.4. Our experimental values were 15% higher, mainly due to a difference in the used molar absorption coefficient. In pilot experiments, the NDMA degradation by MP UV photolysis was confirmed. IX-UF pretreatment compared to CSF pretreatment reduced the EEO by 30%. The $R_{OH,UV}$ concept was used to compare the OH-radical oxidation in the water matrices. The OH-radical oxidation, measured by the increase of $R_{OH,UV}$, improved with 40%, applying IX-UF instead of CSF. In pilot plant experiments, a reduction of the EEO by a factor 2 was observed when CSF was replaced by IX-UF.

Additional MP UV collimated beam results, using pCBA as probe, in DOC and nitrate spiked milliQ, were used to determine the dominant water matrix constituent for the OH-radical oxidation. DOC had the strongest impact on the efficacy of the OH-radical oxidation. In view of the potentially very high removal of DOC by IX-UF, this treatment looks very promising as pretreatment for MP UV/H_2O_2 treatment.

Abstract

Since awareness of the production of trihalomethanes by drinking water chlorination, reaction product formation by chemical disinfection/oxidation has been thoroughly investigated. Originally the focus was on the formation of individual organic reaction products. After chlorination trihalomethanes (THMs), haloacetic acids (HAAs) and many other halogenated compounds were found. After ozonation, ultraviolet (UV) disinfection and advanced oxidation, biodegradable organic reaction products such as carboxylic acids were identified. All reaction products were formed by reaction with the organic water matrix (natural organic matter). Formation of reaction products from the inorganic water matrix proved to be an important issue as well. By ozonation and ozone based advanced oxidation processes bromate was formed in bromide rich water. By MP UV photolysis and MP UV/H_2O_2 treatment, nitrite was formed in nitrate rich water, especially when MP UV lamps were applied. In addition to chemical characterization of individual reaction products the side effects of disinfection/ oxidation were investigated by genotoxicity testing. After chlorination a high response was found in the standard Ames test. After ozone and ozone based advanced oxidation a decrease of the response in the standard Ames test was observed, while after MP UV disinfection and MP UV based advanced oxidation no significant effect was found. Modified genotoxicity testing such as the Ames-II testing was developed and applied on MP UV/H_2O_2 treated water. Initially, no significant genotoxic response was observed after MP UV/H_2O_2 treatment in a system equipped with natural quartz sleeves. However a substantially higher response was found after MP UV/H_2O_2 treatment in a system equipped with synthetic sleeves with a higher transmittance at lower wavelengths. The genotoxic response and the nitrite formation increased in the same order of magnitude, suggesting a relationship with the MP UV photolysis of nitrate. This was confirmed by MP UV photolysis of natural organic matter (NOM) and nitrate containing reconstituted water. No response in the Ames test was observed after post treatment with granular activated carbon (GAC) filtration and/or dune infiltration.

This chapter is derived from: Martijn, A.J., Kruithof, J.C., 2012. UV and UV/H_2O_2 treatment: the silver bullet for by product and genotoxicity formation in water production. *Ozone Science and Engineering* 34, 92-99

Chapter 4 MP UV photolysis and MP UV/H_2O_2 treatment: the silver bullet for reaction product and genotoxicity formation in water production

4.1 Introduction

Since the beginning of the last century, chlorine has been used for drinking water disinfection. Originally, the use of chlorine, "a poisonous chemical", was accepted with great reluctance, but soon drinking water chlorination was generally applied without any concern about harmful aspects (McGuire, 2008). This situation changed completely when Rook (1974), followed by Bellar et al. (1974) showed the production of trihalomethanes (THMs), suspect human carcinogens by drinking water chlorination. Since Rook's discovery, formation of disinfection by-products (DBPs) and their potential threat for public health have been a major concern. After Rook showed THM formation by chlorination, numerous halogenated DBPs have been found such as haloacetic acids (HAAs), haloacetonitriles (HANs), etc. (Cooney, 2008). To characterize the health risk genotoxicity testing (i.e., standard Ames testing) was carried out. Standards have been set for THMs (worldwide) and HAAs (North America). To restrict chlorination by-product formation, measures have been taken. In The Netherlands, originally maintenance of chlorination was pursued, restricting the DBP formation by optimizing the chlorine dose and by removing the produced DBPs by granular activated carbon (GAC) filtration. In a later phase, much attention has been paid to the removal of DBP precursors by (enhanced) coagulation and GAC filtration (Kruithof, 1986). In The Netherlands, gradually the use of chlorine for both primary and post-disinfection has come to a complete stop.

For primary disinfection a shift from chlorine to ozone (O_3) use took place. For ozonation, formation of numerous organic reaction products such as aldehydes and carboxylic acids was established. These organic reaction products were biodegradable rather than harmful for public health. For many years ozone was accepted as the best available alternative for chlorine as the primary disinfectant. This situation changed after (Kurokawa et al., 1990) showed that the inorganic DBP bromate, formed by ozonation of bromide-containing water, was a suspect human carcinogen. Much research has been carried out to control bromate formation. A significant reduction was achieved, but bromate formation could not be avoided completely (Kruithof and Kamp, 1997). The detection of *Cryptosporidium* oöcysts in drinking water sources had a strong impact on drinking water disinfection. Under practical conditions chlorine was unable to inactivate *Cryptosporidium*. Ozone was able to inactivate *Cryptosporidium* but in many cases bromate formation was prohibitive.

It was shown that UV disinfection inactivated *Cryptosporidium* already at a very low UV dose (Bolton et al., 1998). Until now after UV treatment, no individual harmful organic DBPs have been found.

Besides disinfection, organic contaminant control has gained a lot of interest caused by the increasing presence of pesticides, endocrine disruptors, pharmaceuticals, algae toxins, solvents, etc. in drinking water sources (Kruithof et al., 2007). Much attention has been paid to the application of ozone, either in combination with GAC filtration (O_3-GAC) or with H_2O_2 and GAC (O_3/H_2O_2-GAC). No harmful organic DBPs have been established until now, but once again bromate formation caused a switch from ozone to UV-based processes (UV-GAC, UV/H_2O_2-GAC) (Kruithof, 2005). Much research has been carried out into the field of bromate, nitrite and genotoxicity formation. Bromate is not produced by UV-based processes. Nitrite formation may be a concern in nitrate-rich water, especially when MP UV lamps are applied. This chapter describes research carried out in The Netherlands. The results of two Dutch drinking water supply companies, EVIDES and PWN Water Supply Company North Holland, are presented, focusing on the formation of individual organic and inorganic DBPs, and especially the formation of genotoxicity (Martijn et al., 2007)

4.2 Plant information

The treatment schemes of the Berenplaat (EVIDES) and the Andijk (PWN) water treatment plants (WTPs) before implementation of MP UV, respectively, MP UV/H_2O_2 treatment are presented in figure 4.1.

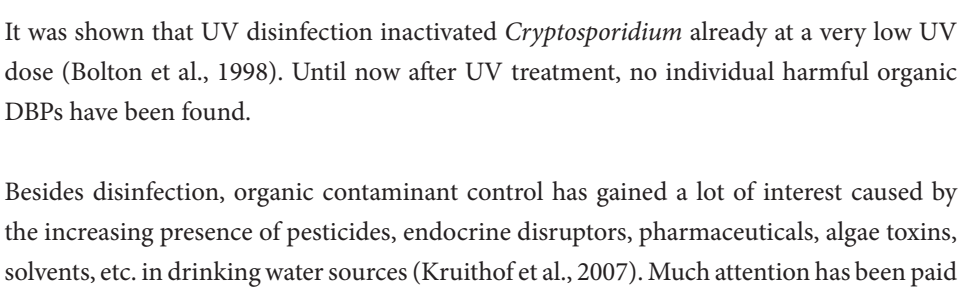


Figure 4.1: Process schemes at WTP Berenplaat and WTP Andijk before retrofit

At WTP Berenplaat river Meuse water after storage in the Biesbosch reservoirs was treated by breakpoint chlorination, coagulation, sedimentation and filtration (CSF) and postchlorination. For organic contaminant control the media in the rapid filtration step were replaced by GAC. At WTP Andijk IJssel Lake (River Rhine) water was treated after storage on site by breakpoint chlorination, CSF, GAC filtration and ClO_2 dosage. Both water supply companies decided to upgrade primary disinfection and organic contaminant control (Kamp et al., 1997). EVIDES investigated replacement of breakpoint chlorination by ozonation and installation of GAC filtration with a longer empty bed contact time (EBCT). PWN focused on the implementation of O_3/H_2O_2 treatment. Both processes were not implemented because of bromate formation. Subsequently, EVIDES pursued the application of UV disinfection once again followed by GAC filtration with a longer EBCT. PWN focused the research on the feasibility of UV/H_2O_2 treatment. Both treatment options have been installed in 2006 (Berenplaat) and 2004 (Andijk), respectively (see figure 4.2).

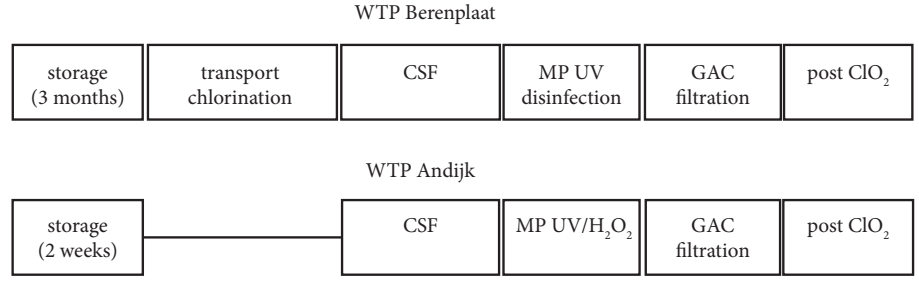


Figure 4.2: Process schemes at WTP Berenplaat and WTP Andijk after retrofit

DBP formation by reaction of both the organic and inorganic water matrix and genotoxicity testing were investigated for the original plants, the research phase and the retrofit plants. Some water matrix parameters relevant for DBP formation in both pre-treated Biesboschwater (WTP Berenplaat) and IJssel Lake water (WTP Andijk) are presented in table 4.1.

Table 4.1: Water quality parameters for WTP Berenplaat and WTP Andijk

Parameter	WTP Berenplaat	WTP Andijk
DOC (mg/L)	3.2	3.0
Nitrate (mg NO_3/L)	3.0	2-13
Bromide (µg/L)	150	300-500
UVT_{254} (cm^{-1})	88%	85%

4.3 Disinfection byproducts

4.3.1 Formation of reaction products from the organic matrix

Major chlorination reaction products such as THMs are produced by reaction of chlorine with the organic water matrix (natural organic matter). Since 2006 the Dutch standard for

individual THM's in drinking water is 10 µg/L, and the total THMs (TTHMs) standard is 25 µg/L (Drinkwaterregeling, 2011). Profiles for the average TTHM content at both WTP Berenplaat (EVIDES) and WTP Andijk (PWN) during the application of breakpoint chlorination are presented in figure 4.3.

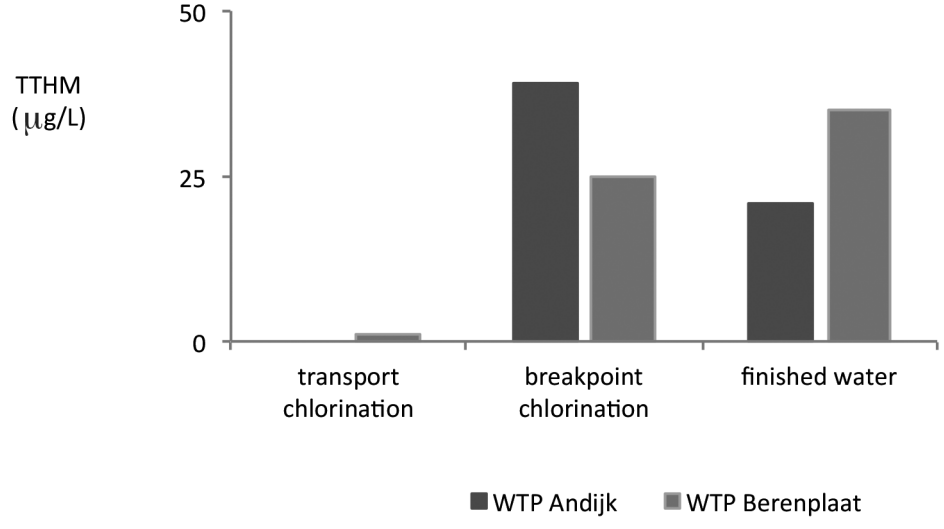


Figure 4.3: Average TTHM content before and after breakpoint chlorination and in the finished water (data WTP Berenplaat and WTP Andijk)

Figure 4.3 shows that, at both plants, the revised standard for the TTHM content in the finished water was exceeded. Lowering this TTHM content was one of the objectives of both water supply companies. By both O_3 and O_3/H_2O_2 numerous aldehydes, aldehydic acids and carboxylic acids were formed by degradation of the organic water matrix. These compounds are biodegradable rather than harmful for public health, as can be seen by the strong increase of assimilable organic carbon (AOC) content. The same types of compounds were produced by MP UV/H_2O_2 treatment in concentrations of the same order of magnitude. By MP UV radiation only, the formation of these types of compounds was much less although still significant. No individual genotoxic compounds were identified. Summarizing, it can be said that organic reaction product formation was based on reaction of the disinfectants / oxidants with the organic water matrix (natural organic matter). Chlorination caused the formation of halogenated compounds such as THMs. All other processes, O_3, O_3/H_2O_2, UV and UV/H_2O_2, reacted with the organic water matrix under formation of biodegradable rather than harmful compounds.

4.3.2 Formation of reaction products from the inorganic matrix

Formation of inorganic compounds by chlorination was insignificant. However, the bromide content of the water played an important part in the composition of the organic DBPs. In bromide rich water more brominated THMs were formed. The amount of more brominated species increased as a function of the bromide/DOC ratio (Kruithof, 1986). The bromide content also played an important part for ozone-based processes. Ozonation of bromide rich water caused the formation of bromate. EVIDES studied the application of ozone for the primary disinfection of pretreated Biesbosch water. The bromate formation as a function of the ozone dose is presented in figure 4.4.

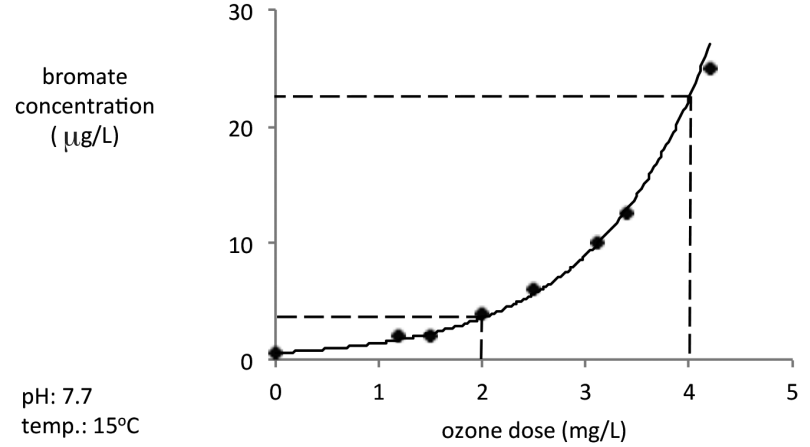


Figure 4.4: Bromate formation as a function of the ozone dose (pilot experiments WTP Berenplaat, bromide content 150 µg/L)

For a 2 log *Giardia* inactivation an ozone dose of about 2 mg/L was required causing a bromate formation of 3 µg/L lower than the Dutch drinking water standard of 5 µg/L. However, for a 2 log *Cryptosporidium* inactivation, an ozone dose of 4 mg/L was required, causing a bromate content of about 25 µg/L; much higher than the bromate standard. The feasibility of O_3/H_2O_2 treatment for pretreated IJssel Lake water was investigated. The required pesticide degradation of 80% could be achieved by ozone/hydrogen peroxide treatment at economically feasible O_3/DOC and H_2O_2/O_3 ratios. In view of the high bromide concentration of the water (300–500 µg/L), bromate formation was studied extensively. Figure 4.5 shows the dominant role of the water temperature on the bromate formation. At low H_2O_2/O_3 ratio the synergistic effect of ozone and hydrogen peroxide on the bromate formation, as previously shown by Von Gunten and Hoigné (1994), was confirmed (see figure 4.5).

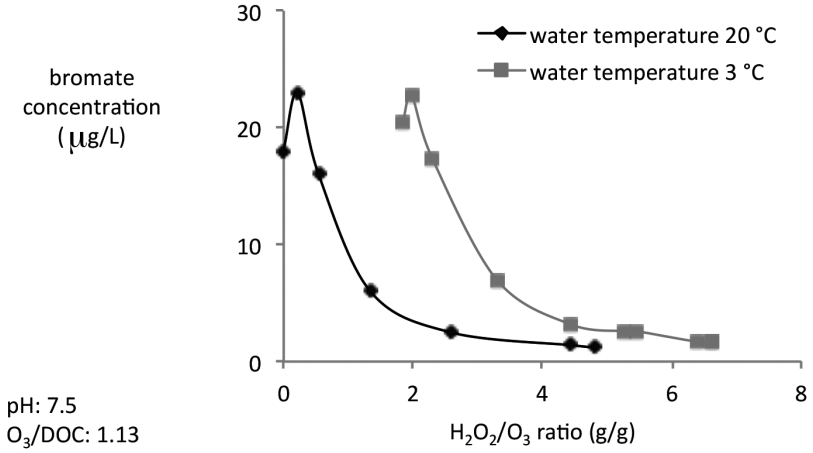


Figure 4.5: Bromate formation by O_3/H_2O_2 treatment as a function of the H_2O_2/O_3 ratio in pretreated IJssel Lake water (pilot plant data)

A bromate guideline value of 0.5 µg/L was exceeded for all H_2O_2/O_3 ratios at water temperatures of 3 and 20 degrees Celsius, while a pH increase did not lower the bromate formation significantly (Martijn et al., 2006). Therefore, the application of O_3/H_2O_2 treatment was not pursued. Because of potential bromate problems, no full scale drinking water ozone projects were realized in The Netherlands the last 20 years. The feasibility of UV disinfection and UV/H_2O_2 treatment was investigated. Nitrite formation may be an important issue for UV treatment of nitrate-rich water. Nitrite formation was very restricted when low pressure (LP) UV lamps were used, but was significant when MPUV lamps in combination with natural quartz GE214 were used (see figure 4.6).

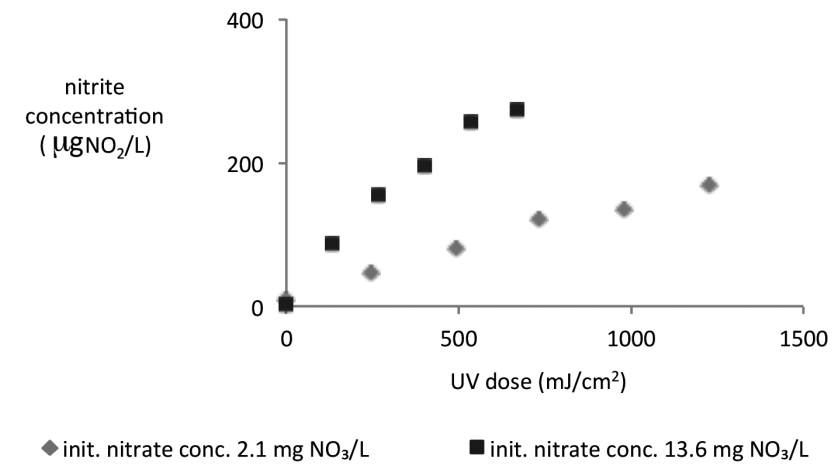


Figure 4.6: Nitrite formation by MP UV treatment of pretreated Biesbosch water for two nitrate concentrations (pilot plant data WTP Berenplaat)

Figure 4.6 presents the nitrite formation in pretreated Biesbosch water with nitrate concentrations of 2.1 and 13.6 mg/L. For MP UV disinfection with a UV dose of 90 mJ/cm^2, the nitrite concentration did not exceed the E.C. standard of 0.1 mg/L. For advanced oxidation by MP UV/H_2O_2 treatment (UV dose 600 mJ/cm^2, H_2O_2 dose 6 mg/L) the nitrite standard was exceeded significantly, so post-treatment was necessary to lower the nitrite concentration. An option to achieve this goal was chemical oxidation (i.e., by chlorine). At WTP Andijk, biological activated carbon filtration (BACF) was selected to reoxidize nitrite to nitrate. For virgin carbon, nitrite oxidation proved to be problematic at low water temperature. By BAC filtration in a biologically active mode with an empty bed contact time of 30 min, nitrite was converted to concentrations below the EC standard of 0.1 mg/L, even at low water temperatures (see figure 4.7).

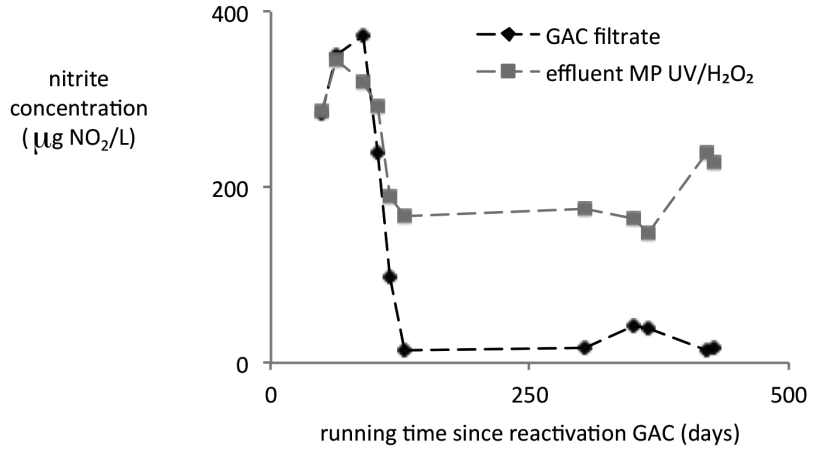


Figure 4.7: Nitrite formation by MP UV/H_2O_2 treatment and re-oxidation to nitrate by BACF in pretreated IJssel Lake water (pilot plant data)

In summary, besides the organic water matrix the inorganic water matrix played an important part in reaction product formation. This concerned especially the bromide and nitrate content. High bromide levels caused the formation of more brominated DBPs by chlorination. No inorganic DBPs were produced by chlorination. In some cases bromate was found in the finished water caused by impurity of the used sodium hypochlorite (Hutchison, 1993). When applying ozone and UV-based disinfection/oxidation, a number of individual inorganic DBPs deserved special attention: bromate and nitrite. A major concern in view of the suspect carcinogenic properties was the formation of bromate by O_3 and O_3/H_2O_2. No bromate was produced by UV disinfection and UV/H_2O_2 treatment. For these processes nitrite formation was a relevant issue for nitrate rich water, especially when MPUV lamps were applied.

4.4 Genotoxicity

4.4.1 Standard Ames testing

Worldwide genotoxicity research is carried out to characterize raw water quality and effects of treatment, specifically oxidative treatment and disinfection. The most commonly applied method for genotoxicity testing is the standard Ames test (Ames et al., 1972). The standard Ames test is an in vitro test, using bacteria to detect DNA damage (point mutation). The test is performed with and without metabolic activation to mimic the effect of the liver, detecting both direct genotoxic compounds and compounds that become genotoxic after passing the liver. Results from the Ames test cannot be translated directly into a human health risk. Chemical identification of the (group of) formed compounds is necessary for that. KWR Water Cycle Research Institute developed a methodology for standard Ames testing of concentrated water samples, based on isolation onto XAD resins at two pH values. In the isolates, standard Ames testing was applied with two bacterial strains, TA98 and TA100 with and without metabolic activation by adding S9 mix (Noordsij et al., 1999). The TA98 strain detects frame shift mutations, the TA100 strain base pair substitutions. Applying the standard Ames test with strain TA100 on chlorinated, CSF pretreated river Rhine water showed an increase of the genotoxic effect. The strongest genotoxic effect was observed in the neutral pH isolate for strain TA100 with metabolic activation (Kruithof, 1986; figure 4.8). The observed genotoxicity was much higher than the genotoxic response caused by the present THMs. By GAC filtration all genotoxic response was removed.

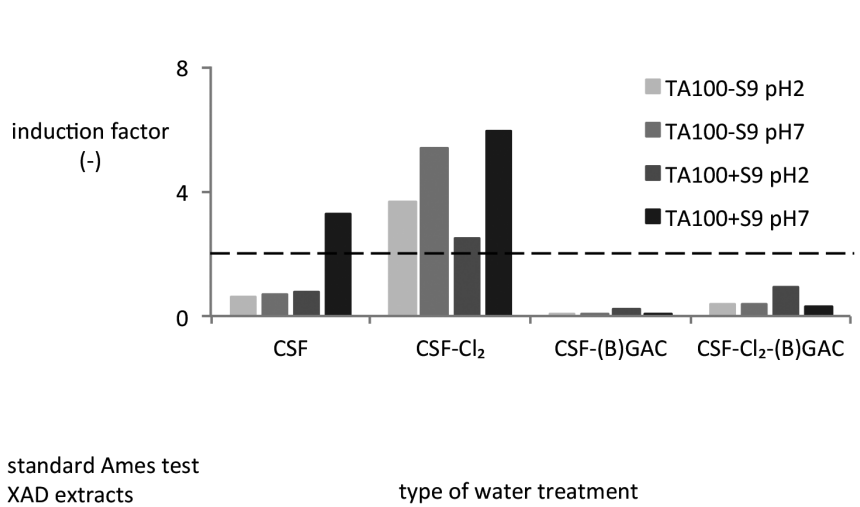


Figure 4.8: Response in the standard Ames test for strain TA100 caused by chlorinated pretreated river Rhine water, pretreated river Rhine water followed by GAC filtration and chlorinated pretreated river Rhine water followed by GAC filtration

Standard Ames tests have also been carried out in O_3 and O_3/H_2O_2 treated water. In view of the applied isolation method the effect of bromate was not detected. The found genotoxic effect was caused by the isolated organic fraction. For standard process conditions a significant decrease in genotoxic response for strain TA98 was observed (Zoeteman et al., 1982). By ozonation organic compounds were degraded into more polar, lower molecular weight compounds. For example aromatic compounds were degraded into aldehydes, carboxylic acids etc. These compounds are biodegradable rather than genotoxic. Standard Ames tests have also been carried out to determine the effect of UV-disinfection. In concentrates of water samples from the Biesbosch reservoir after CSF and GAC treatment for both LP and MP UV radiation (UV-dose 32 mJ/cm^2) the genotoxic activity was insignificant (Kruithof et al., 1992). More recently, the response in the standard Ames test was determined in concentrates of CSF pretreated Biesbosch water after UV disinfection using MP UV lamps and natural quartz GE214 sleeves (UV-dose 70 mJ/cm^2). In all samples, the response for strain TA100 was very low, while for strain TA98 the response was low but still insignificant. The strongest response was observed in strain TA98 without metabolic activation (see figure 4.9). However, the response was not regarded as a genotoxic effect since the ratio of induced over spontaneous revertants was <2.

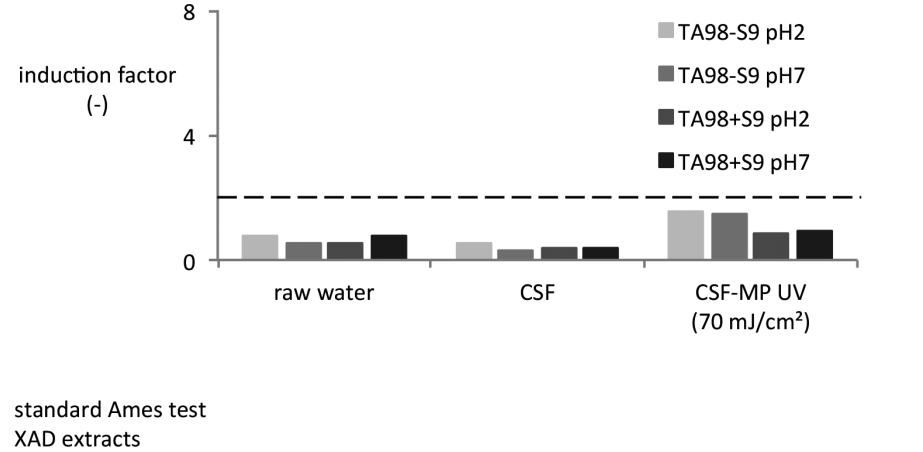


Figure 4.9: Response in the standard Ames test for strain TA98 in raw water and before and after MP UV disinfection (70 mJ/cm^2) of CSF pretreated Biesbosch water (pilot plant data)

A restricted number of standard Ames tests with strain TA98 was carried out in concentrates of CSF pretreated IJssel Lake water after MP UV/H_2O_2 treatment with MP UV lamps and natural quartz GE214 sleeves (UV dose 540 mJ/cm^2, H_2O_2 dose 5.0 mg/L). Once again, no significant increase was observed (see figure 4.10).

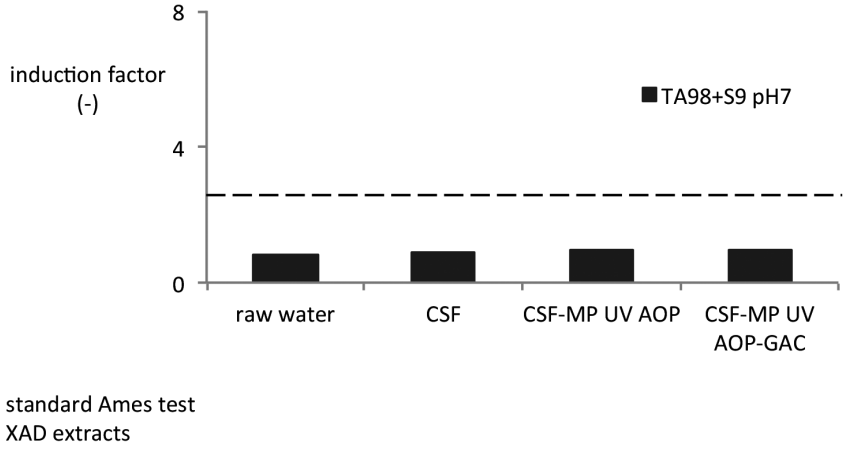


Figure 4.10: Response in the standard Ames test for strain TA98+S9 at pH7 in CSF pretreated IJssel Lake water before and after MP UV AOP and after MP UV AOP-GAC treatment (pilot plant data)

The genotoxic response in strain TA98 for a MP UV dose of 540 mJ/cm^2 combined with a H_2O_2 dose of 5.0 mg/L was either lower or in the same order of magnitude as the response caused by a MP UV dose of 70 mJ/cm^2 only. Summarizing, it can be said that chlorination caused a strong genotoxic effect in the standard Ames test, especially for strain TA100 with metabolic activation. Ozone caused a significant decrease of the response in the standard Ames test. The response observed for UV-based processes was low but not significant. For medium pressure UV lamps and natural quartz GE214 sleeves a slightly higher but insignificant response was observed than for low pressure UV lamps. There was no significant difference between disinfection conditions (70 mJ/cm^2) and advanced oxidation conditions (540 mJ/cm^2 and 5.0 mg/L H_2O_2).

4.4.2 Advanced genotoxicity testing

To characterize the potential harmful side effects of UV disinfection and UV-based AOP treatment, similar to the efforts to establish the harmful side effects of chlorination, the standard Ames test was applied (figure 4.9 and figure 4.10). No significant response was observed. The applied methodology, based on the plate incorporation method in combination with XAD sample concentration (10,000–40,000 times), may not be sensitive enough to detect MP UV- and MP UV/H_2O_2-induced genotoxicity. One of the reasons may be the inadequate adsorption by the applied XAD isolation of (hydrophilic) reaction products formed by MP UV and/or MP UV/H_2O_2 treatment. Therefore the isolation method was modified by applying solid phase extraction with standard cartridges from OASIS (Heringa et al., 2011). In addition, a modified Ames test, Ames II, was applied, a micro-array technique based on colour change of the micro wells where the standard Ames test is based on plate counting (Flückiger-Isler et al., 2004). The induced genotoxic response in the Ames II test was determined in chlorinated Dutch and UK tap water (figure 4.11).

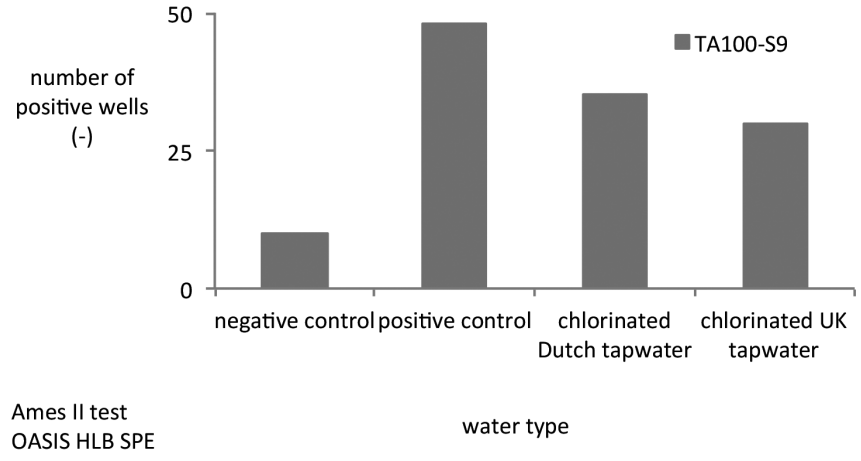


Figure 4.11: Response in the Ames II test with strain TA100-S9 for chlorinated Dutch and UK tap water (bench scale experiments)

In agreement with the standard Ames test results a significant response was observed for strain TA100 in both Dutch and UK chlorinated water samples. For IJssel Lake water after CSF treatment, after UV/H_2O_2 treatment (MP UV lamps and natural quartz GE214 sleeves) and after GAC filtration, Ames II testing with TA98 with and without metabolic activation was performed on concentrated water samples (figure 4.12).

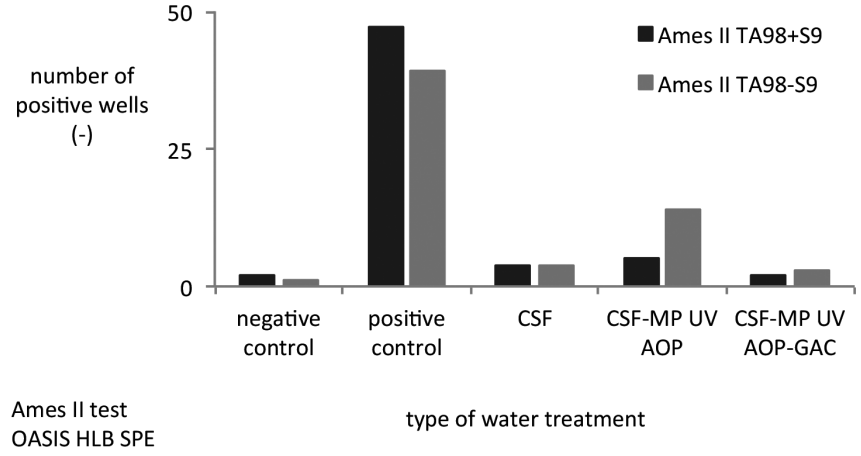


Figure 4.12: Response in the Ames II test for strain TA98 with and without metabolic activation in CSF pretreated IJssel Lake water before and after MP UV/H_2O_2 treatment (UV-dose 540 mJ/cm^2) and after GAC filtration (WTP Andijk)

Relative to the negative control a small but significant genotoxic response was observed after MP UV/H_2O_2 treatment (natural quartz sleeves) in the TA98 strain without metabolic activation, For the Ames II strain TA98 with metabolic activation, no significant increase was observed. The genotoxic response was removed by biologically active GAC filtration (EBCT 30 minutes).

Additional Ames II experiments were performed at the full-scale Heemskerk plant. The response in Ames II TA98-S9 was determined after each UV reactor using MP UV lamps and synthetic quartz sleeves with a cut-off at a lower wavelength than natural quartz GE214 sleeves (figure 4.13).

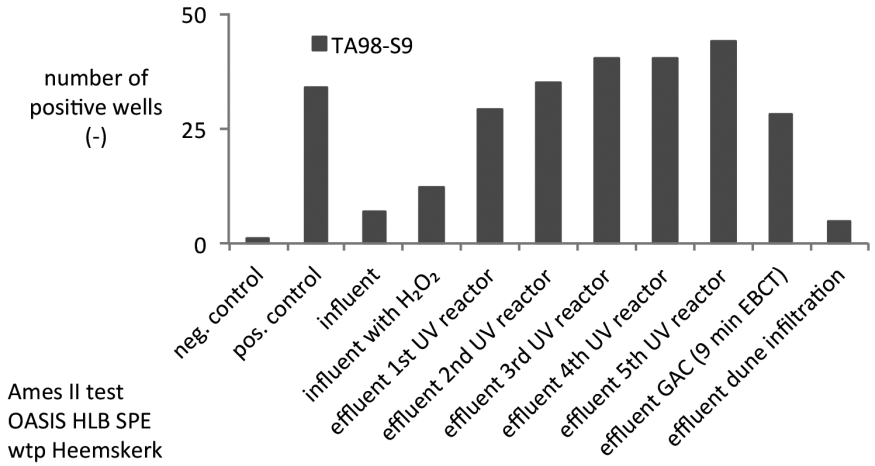


Figure 4.13: Response in Ames II test with strain TA98 without metabolic activation in concentrated water samples after MP UV/H_2O_2 treatment for several EEDs, GAC filtration and dune infiltration (WTP Heemskerk)

Although the MP UV/H_2O_2 treatment process conditions were the same as for the experiments presented in figure 4.12, figure 4.13 shows a substantially higher response in the Ames II TA98. The only difference between these experiments was the application of natural quartz GE214 sleeves at the Andijk plant (Figure 4.12) opposed to synthetic quartz sleeves at the Heemskerk plant (figure 4.13). Post-treatment at the Heemskerk plant consisted of high surface load (50 m/h) GAC contacting (EBCT 9 min) to catalytically quench the excess H_2O_2 and dune infiltration to obtain biological stability. These combined post-treatment steps removed the generated response in the Ames II test completely (figure 4.13). The electrical energy dose (EED) for the MP UV/H_2O_2 process at PWN was 0.56 kWh/m^3. At this EED the nitrite formation in the MP UV/H_2O_2 system with synthetic quartz sleeves was substantially higher than the nitrite formation when natural quartz GE214 sleeves were applied (figure 4.14). With the application of natural quartz GE214 sleeves, 160 µg NO_2/L was formed, while application of synthetic quartz sleeves resulted in the formation of 480 µg NO_2/L for an EED of 0.56 kWh/m^3.

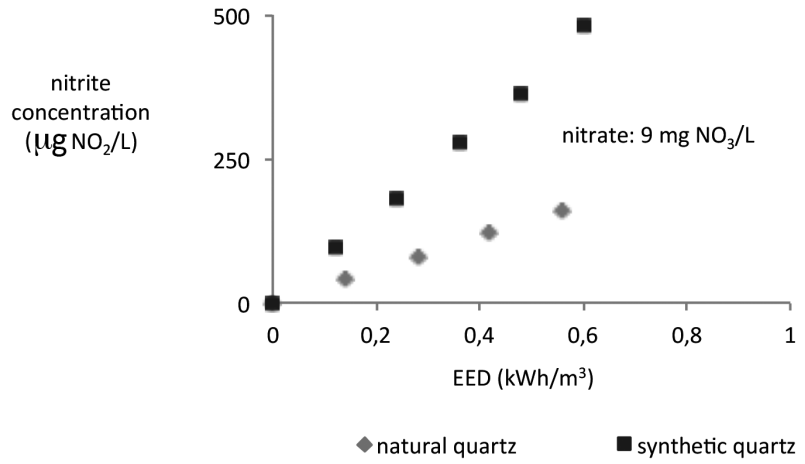


Figure 4.14: Nitrite formation by MP UV treatment as a function of the electrical energy dose in full-scale MP UV equipment for natural and synthetic quartz sleeves (WTP Heemskerk, nitrate content 9 mg NO_3/L)

The response in the Ames II test increased in the same order of magnitude as the nitrite formation (figure 4.15). The data was derived from MP UV equipment with both natural quartz GE214 sleeves and synthetic quartz sleeves. The same trend in the nitrite formation and the Ames II test response suggests that the increase in the Ames II results is somehow related to the nitrate photolysis by MP UV light causing the formation of genotoxic organic compounds in addition to the formation of nitrite.

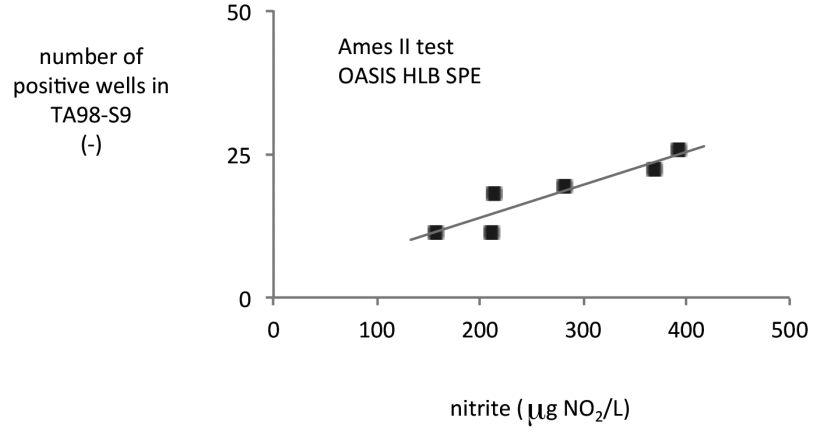


Figure 4.15: Response in the Ames II test in concentrated water samples of MP UV/H_2O_2 treated pretreated IJssel Lake water as a function of the nitrite formation for both natural GE214 and synthetic quartz sleeves (WTP Heemskerk)

Collimated beam experiments on reconstituted water were performed. NOM (Nordic Lake NOM, IHSS, 3 mg C/L) was dissolved in demineralized water with nitrate (11 mg NO_3/L) and without nitrate. MP UV/H_2O_2 process conditions were an UV dose of 500 mJ/cm^2 and a H_2O_2 dose of 6.5 mg/L. The Ames II test with and without metabolic activation was applied on concentrated water samples (figure 4.16).

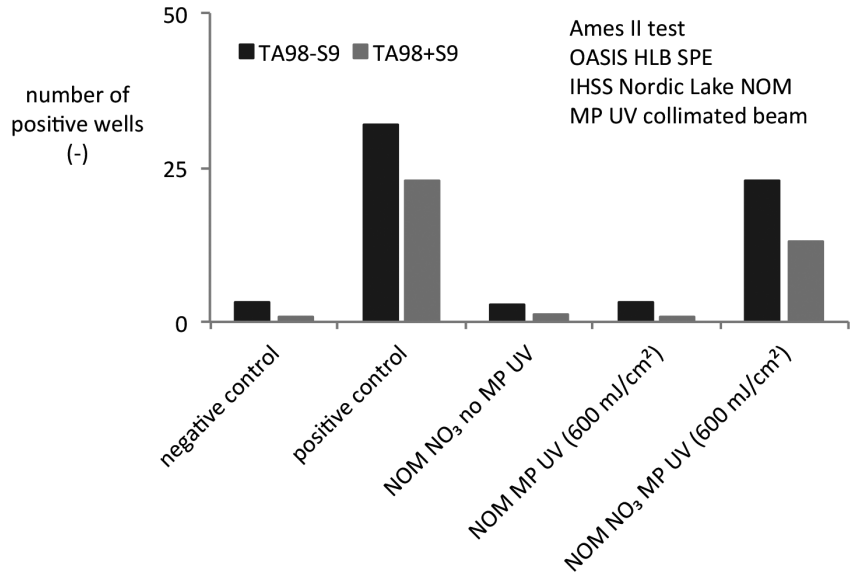


Figure 4.16: Response in the Ames II strain TA98 with and without metabolic activation in concentrated water samples before and after MP UV photolysis of reconstituted water (bench-scale experiments)

MP UV photolysis of NOM containing water in the absence of nitrate showed no elevated response in the Ames II test, relative to the blank and the negative control. In the presence of nitrate, a significant increase in the Ames II test response was observed after MP UV photolysis (figure 4.16). MP UV photolysis of nitrate in the presence of NOM caused the response in the Ames II strain TA 98 in concentrated water samples. This suggests the formation of N-DBPs by the reaction of the organic matrix and nitrogen containing intermediates formed by the MP UV photolysis of nitrate. The formation of individual N-DBPs was not studied in this research effort, but was pursued in follow-up studies (Kolkman et al., 2015).

In summary, no genotoxicity in the standard Ames test was detected after MP UV and MP UV/H_2O_2 treatment when natural quartz GE214 sleeves were used. Introduction of the Ames II test in combination with a modified concentration method showed a genotoxic response in strain TA100 after chlorination in the same order of magnitude as earlier found in the standard Ames test. When synthetic quartz sleeves were used, the Ames II test with strain TA98 without metabolic activation showed a significant response after MP UV/H_2O_2 treatment. The genotoxic response increased as a function of the MP UV dose in the same order of magnitude as the nitrite content. This suggests that the genotoxic response is related to the MP UV photolysis of nitrate. This was confirmed in collimated beam experiments with NOM and nitrate containing reconstituted water. All responses were removed by biological GAC filtration and/or dune infiltration.

4.5 Perspective chemical disinfection/oxidation

In the last 40 years, extensive research into the application of chemical disinfection and oxidation to restrict/avoid DBP formation has been carried out. Originally for both primary and post-disinfection chlorine was used. Formation of THMs, other organohalogens and a genotoxic response in the standard Ames test stopped the absolute confidence in chlorine. Drinking water standards have been set for DBPs especially THMs and HAAs. In The Netherlands the use of chlorine has been stopped completely. In Dutch surface water treatment post-disinfection is avoided or a low ClO_2 dose is applied. Also worldwide the chlorine use is restricted, i.e., by replacing post-chlorination by chloramination. After chlorination, THMs and HAAs represent the two major classes of DBPs. The use of alternative disinfectants such as chloramines and ozone minimized the formation of regulated THMs and HAAs but several other priority DBPs are formed such as iodinated THMs, nitromethanes, acetamides and brominated furanones (Krasner et al., 2006). Therefore the formation of DBPs by chlorine and alternative disinfectants is a continuing concern. In many cases for primary disinfection chlorine has been replaced by ozone.

Ozonation and O_3/H_2O_2 treatment are also interesting options for organic contaminant control. Application of ozone and O_3/H_2O_2 was hampered by the formation of bromate. Concerns about the lowering of the bromate standard prevented realization of full-scale drinking water ozone projects in The Netherlands in the last 20 years. Worldwide, lowering the bromate standard from 10 to 5 µg/L may cause problems as well. Recently relevant issues have been presented to reconsider the bromate standard. Important aspects are reduction of bromate in the stomach (low pH), in the liver and in blood (reduction by H_2S, thiols, etc.). Only a very restricted part of all consumed bromate reaches the kidneys, where tumour formation may occur. In addition, there are indications that the human body itself produces low concentrations of bromate in the same order of magnitude as the intake by the consumption of ozonated drinking water (Cotruvo et al., 2008). The results of this research effort and the subsequent strategy of USEPA and WHO will be crucial for future applications of ozone. Stabilizing or relaxing the bromate standard may have a very positive impact on future ozone projects both for primary disinfection and organic contaminant control by O_3/H_2O_2. Recently UV-based technologies were introduced for the inactivation of *Cryptosporidium* (UV) and control of organic micropollutants (UV/H_2O_2). No harmful individual organic DBPs have been detected yet. Application of MP UV caused nitrite formation in nitrate containing water. Especially for MP UV/H_2O_2 treatment, nitrite formation may exceed the drinking water standard. Produced nitrite can be removed by chemical oxidation and by biological post-treatment (BACF). After MP UV photolysis and MP UV/H_2O_2 treatment no significant response in the standard Ames test was found when natural quartz GE214 sleeves were used. However, recent results from Ames II tests in combination with a modified isolation method showed a genotoxic response after MP UV treatment utilizing synthetic quartz sleeves with a cut off at lower wave lengths than the natural quartz GE214 sleeves. The more open synthetic quartz sleeves caused a stronger nitrate photolysis for the same UV dose. This effect will be stronger using Supracil sleeves with an even lower cut off wave length. These sleeves are applied in practise but were not used in this research effort. The same trend of the nitrite formation and the Ames II test response suggests that the increase in the Ames II results is somehow related to the nitrate degradation by MP UV photolysis causing the formation of genotoxic organic compounds by reaction of MP UV photolysis intermediates with the organic water matrix in addition to the formation of nitrite. All responses in both the standard Ames test and the Ames II test were removed by GAC filtration and/or dune infiltration. The reaction product formation will be decreased by an improved pretreatment, removing both NOM and nitrate. PWN has implemented pretreatment by ion exchange and ceramic microfiltration in 2014 improving both the economics and the DBP formation of the MP UV/H_2O_2 treatment (Martijn et al., 2010). Therefore, in an integrated treatment approach MP UV photolysis and MP UV/H_2O_2 treatment are reliable barriers for primary disinfection and organic contaminant without a prohibitive reaction product formation.

Abstract

Genotoxic compounds were produced by full scale medium pressure (MP) ultra violet hydrogen peroxide (UV/H_2O_2) treatment of pretreated nitrate rich surface water. This chapter describes that MP UV nitrate photolysis caused an increase in the Ames test response, indicating the formation of genotoxic compounds. This formation was caused by reaction of nitrate photolysis intermediates with natural organic matter (NOM). An increase in the Ames test response was also found after MP UV photolysis of water containing Pony Lake NOM from the International Humic Substances Society (IHSS) and nitrate while no increase in the Ames test response was found when nitrate was absent. The same trend in Ames test response and nitrite formation was observed for both nitrate rich pretreated surface water and reconstituted water containing NOM and nitrate. The conversion of nitrate by MP UV photolysis was studied in several water types. In organic free water, nitrate was completely converted into nitrite, while no inorganic nitrogen was lost. Also, in nitrate rich surface water nitrite was found as the single inorganic reaction product, while a small decrease of the inorganic nitrogen content was observed. Orienting Liquid Chromatography-Organic Carbon Detection (LC-OCD) measurements showed some nitrogen incorporation in the organic water matrix but individual nitrogen containing compounds were not be identified. To study incorporation of inorganic nitrogen in the organic water matrix by MP UV nitrate photolysis, NOM was replaced by phenol. In this system, MP UV photolysis caused less nitrite formation and a large decrease of the inorganic nitrogen content. The formation of the nitrated phenol derivatives: 2- and 4-nitrophenol, and 4-nitrocatechol was observed with the highest concentrations under practical MP UV-treatment conditions. Under the applied MP UV/H_2O_2 process conditions (0.54 kWh/m^3; 6 mg/L H_2O_2), formation of genotoxic compounds occurred in water types containing both nitrate and NOM. Bench scale studies with reconstituted water with Pony Lake NOM and nitrate showed that a significant formation of genotoxic compounds already took place under MP UV disinfection conditions (40 mJ/cm^2).

This chapter is derived from: Martijn, A.J., Boersma, M.G., Vervoort, J.M., Rietjens, I.M.C.M., Kruithof, J.C., 2014. Formation of genotoxic compounds by medium pressure ultraviolet treatment of nitrate-rich water. *Desalination and Water Treatment,* 52 (34-36), pp. 6275-6281

Chapter 5 Formation of genotoxic compounds by MP UV/H_2O_2 treatment of nitrate rich water

5.1 Introduction

Since the water treatment industry became aware of the formation of trihalomethanes (THMs) by drinking water chlorination (Rook, 1974), reaction product formation by chemical disinfection/oxidation was investigated thoroughly. THMs and many other halogenated reaction products were formed by reaction of chlorine with the organic water matrix. Ozonation and ozone based advanced oxidation caused formation of biodegradable compounds such as carboxylic acids, once again by reaction with the organic water matrix. In addition, the inorganic water matrix played an important part. Kurokawa (Kurokawa et al., 1990) showed that bromate, formed by ozonation of bromide rich water, is a suspect human carcinogen. UV disinfection and UV based advanced oxidation caused formation of biodegradable compounds and in nitrate rich waters nitrite was formed, especially when MP UV lamps were applied.

In addition to a chemical characterization, the side effects of disinfection by-products were often determined by genotoxicity testing such as the Ames test. After chlorination a high response in the Ames test was observed, while after ozone and UV based processes no significant effect was found [Kruithof, 1986; Kruithof et al., 1995). For many years no formation of genotoxic compounds was observed for UV based processes. However when for MP UV treatment natural quartz sleeves were replaced by synthetic quartz sleeves with a stronger transmission at wavelengths <240 nm, a significant Ames test response was found in nitrate rich water (Martijn and Kruithof, 2012).

In this chapter, the research on the formation of genotoxic compounds by MP UV treatment is described. In literature the mechanism of the formation of genotoxic compounds by a practical MP UV application of nitrate rich water is not described yet. It is proposed that this formation is caused by the reaction of MP UV photolysis intermediates of nitrate with the organic water matrix producing nitrated organic compounds. The role of MP UV photolysis of nitrate on the genotoxicity formation and the production of nitrated reaction products was investigated for practical and reconstituted water matrices. To confirm the incorporation of inorganic nitrogen in the organic water matrix, experiments were carried out with an organic model compound phenol.

5.2 Nitrate and nitrite photolysis by UV light in organic free water

Nitrate has two absorption bands in the UV region, one in the near UV region from 260 nm to 350 nm with a maximum at 300 nm and a much more intensive band below 260 nm with a maximum at 200 nm (Krishnan and Guha, 1934). Nitrite formation by application of low pressure (LP) UV in water treatment is insignificant due to the low absorbance of nitrate at the major LP UV emission wavelength of 254 nm. Polychromatic MP UV light with wavelengths between 200 and 300 nm, causes a significant nitrate photolysis, resulting in a substantial nitrite formation. Especially the emitted UV wavelengths <250 nm, cause a strong nitrite formation (Mack and Bolton, 1999). Photolysis of nitrate takes place according to two pathways (Goldstein and Rabani, 2007):

$$NO_3^- \xrightarrow{h\nu} \cdot NO_2 + O\cdot^- \qquad \Phi = 0.08 \qquad \text{(Eq. 5.1)}$$

$$O\cdot^- + H_2O \rightarrow \cdot OH + OH^- \qquad \text{(Eq. 5.2)}$$

$$NO_3^- \xrightarrow{h\nu} ONOO^- \qquad \Phi = 0.48 \qquad \text{(Eq. 5.3)}$$

In view of the quantum yield Φ = 0.48 M E^{-1} the major intermediate reaction product of nitrate photolysis is peroxynitrite, giving the following consecutive reaction:

$$NO_3^- \leftarrow ONOO^- \underset{+H^+}{\overset{-H^+}{\longleftrightarrow}} ONOOH \rightarrow NO_3^- + H^+ \qquad \text{(Eq. 5.4)}$$

$$\cdot NO + O_2^- \qquad \cdot NO_2 + \cdot OH$$

$$\cdot NO + \cdot NO_2 \rightarrow N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+ \qquad \text{(Eq. 5.5)}$$

$$\cdot NO_2 + O_2^- \rightarrow O_2NOO^- \rightarrow NO_2^- + O_2 \qquad \text{(Eq. 5.6)}$$

Disproportionation of peroxynitrite produces nitroso-, nitro- and hydroxyl radicals as intermediates. The stable inorganic end product of nitrate photolysis by MP UV light is nitrite. Mack and Bolton (Mack and Bolton, 1999) give a detailed description of UV photolysis of nitrite. The major reactions are:

$$NO_2^- + H_2O \xrightarrow{h\nu} NO^\cdot + OH^\cdot + OH^- \qquad \text{(Eq. 5.7)}$$

$$NO_2^- + OH^\cdot \rightarrow NO_2^\cdot + OH^- \qquad \text{(Eq. 5.8)}$$

$$2NO_2^\cdot + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \qquad \text{(Eq. 5.9)}$$

UV photolysis of nitrite causes the formation of nitrate as stable end product. However the intermediate nitro- and nitroso radicals produced by both nitrate and nitrite photolysis may also react with the organic water matrix to form nitrogenous organic reaction products.

5.3 Photolysis of nitrate and nitrite in presence of NOM

Many authors have described the impact of nitrate/nitrite photolysis in natural waters. Their focus was on nitrate/nitrite photolysis as a potential source for hydroxyl radical formation rather than nitration or nitrosation reactions. Only Thorn and coworkers (Thorn and Mikita, 2000; Thorn and Cox, 2012) studied nitration and nitrosation reactions. Thorn and Mikita (Thorn and Mikita, 2000) showed nitrite fixation by humic substances applying ^{15}N Nuclear Magnetic Resonance. Thorn and Cox (Thorn and Cox, 2012) showed that nitrate nitrogen were incorporated in aquatic natural organic matter by irradiation of ^{15}N-labeled nitrate in aqueous solution. Because hardly any nitrosation/nitration reactions of natural organic matter are described in literature, a review was carried out on the effect of nitrate/nitrite photolysis in the presence of low molecular organic compounds. The photolysis of nitrate and nitrite in the presence of low molecular weight organics was studied for a broad variety of compounds. In particular the interaction with aromatics such as benzene (Vione et al., 2004), biphenyl (Suzuki et al., 1987), and phenol (Niessen et al., 1988; Machado and Boulé, 1995) was studied. Hydroxylation, nitration and nitrosation were observed when nitrate and/or nitrite were photolysed in the presence of these organic substances. For radiation with wavelengths < 260 nm hydroxylation and nitration intensified. The mechanism of the reactions is not completely clear yet, although a radical pathway for both hydroxylation and nitration was hypothesized. A strong mutagenicity was attributed to some nitrated compounds (Suzuki et al., 1982). In this research effort phenol was selected as a representative model compound for the natural organic water matrix to show the possibility of nitration and nitrosation of the organic water matrix.

5.4 Materials and methods

5.4.1 Full-scale experiments

The full scale MP UV/H_2O_2 installation at WTP Andijk in The Netherlands, consisted of three parallel UV process trains. Each UV train consisted of four UV reactors. Each UV reactor was equipped with sixteen 12 kW MP UV lamps. Immediately downstream of each UV reactor, a sample port was installed. MP UV/H_2O_2 treatment was applied with an electrical energy consumption of 0.54 kWh/m^3, corresponding with a UV dose of 560 mJ/cm^2, combined with a dosage of 6 mg/L H_2O_2 to meet a treatment target of 80% atrazine degradation.

5.4.2 Collimated beam experiments

Irradiations were performed in a collimated beam (CB) apparatus equipped with a 3 kW medium pressure Hg lamp. The UV dose delivered to the solution was calculated using

the UV dose calculation method developed by Bolton and Linden (Bolton and Linden, 2003). A sample volume of 55 mL was irradiated in a 60 x 35 mm crystallizing dish. The irradiation path length was 19.5 mm. For the Ames test, multiple samples were irradiated under identical conditions, utilizing an automated sample carrousel, placed under the collimated beam apparatus.

5.4.3 Ames testing

The Ames II™ Assay was used, based on the same principle as the standard Ames Assay (Ames et al., 1972). The Ames II test with strain TA98 without metabolic activation (-S9) was conducted on concentrated water samples extracted by solid phase extraction (SPE) according to the method described by Heringa et al. (Heringa et al., 2011), yielding 20,000-fold concentrated extracts. Quality control of the Ames II test was ensured by the positive control (0.5 µg/mL 4-nitroquinoline-1-oxide plus 2.0 µg/mL 2-nitrofluorene) and a solvent (dimethylsulfoxide, DMSO) control was included in the assay. Mineral water was added as an additional procedure control. The positive control for the test strain TA98 in the absence of metabolic activation must be equal to or larger than 25 positive wells. The solvent control should result in 8 or less positive wells.

5.5 Results

5.5.1 Ames test response for pretreated surface water samples

The Ames II test was conducted on concentrated water samples, taken from a full scale MP UV/H_2O_2 treatment train utilizing synthetic quartz sleeves allowing passage of UV light with wavelengths <240 nm (figure 5.1). The influent of the MP UV/H_2O_2 treatment step was surface water from the IJssel Lake, pretreated by conventional surface water pretreatment including: coagulation, sedimentation and filtration.

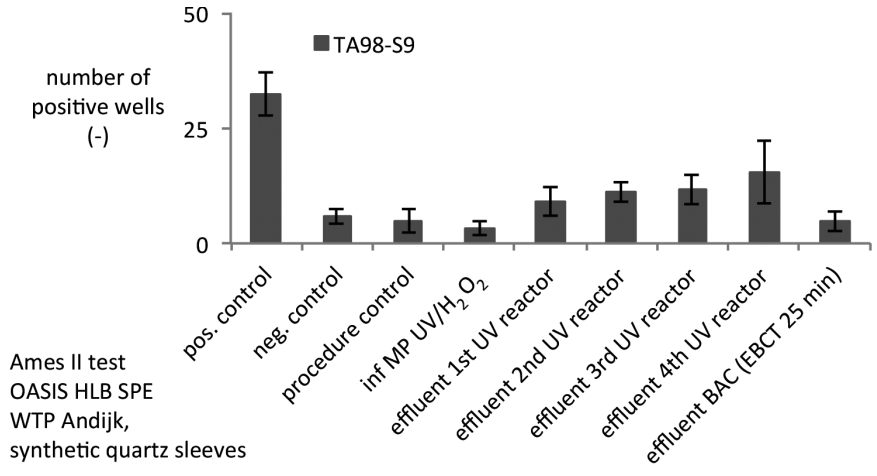


Figure 5.1: Ames test response (n=3) in strain TA98 (-S9) after consecutive UV reactors in a MP UV/H_2O_2 treatment train at WTP Andijk

After each UV reactor with increasing UV dose, a higher response in the Ames test was observed. In the general approach for genotoxicity in the Ames test, a sample is genotoxic when the response exceeds two times the procedure control. According to this approach, the response after UV reactor 4 was genotoxic. After MP UV/H_2O_2 treatment, the response in the Ames test was removed to the level of the procedure control by post treatment with biological activated carbon filtration.

To investigate the role of nitrate, MP UV collimated beam experiments in reconstituted water using 2.5 mg C/L IHSS Pony Lake NOM without nitrate and in the presence of 0.2 mmole/L nitrate were conducted. Three UV treatment conditions were applied: MP UV disinfection with a UV dose of 40 mJ/cm^2, MP UV photolysis with a UV dose of 600 mJ/cm^2 and MP UV/H_2O_2 treatment with a UV dose of 600 mJ/cm^2 in combination with 6 mg/L H_2O_2. As shown in figure 5.2, a response in the Ames test was found, confirming the effect observed when nitrate was photolysed in the presence of IHSS Nordic Lake NOM (Martijn and Kruithof, 2012).

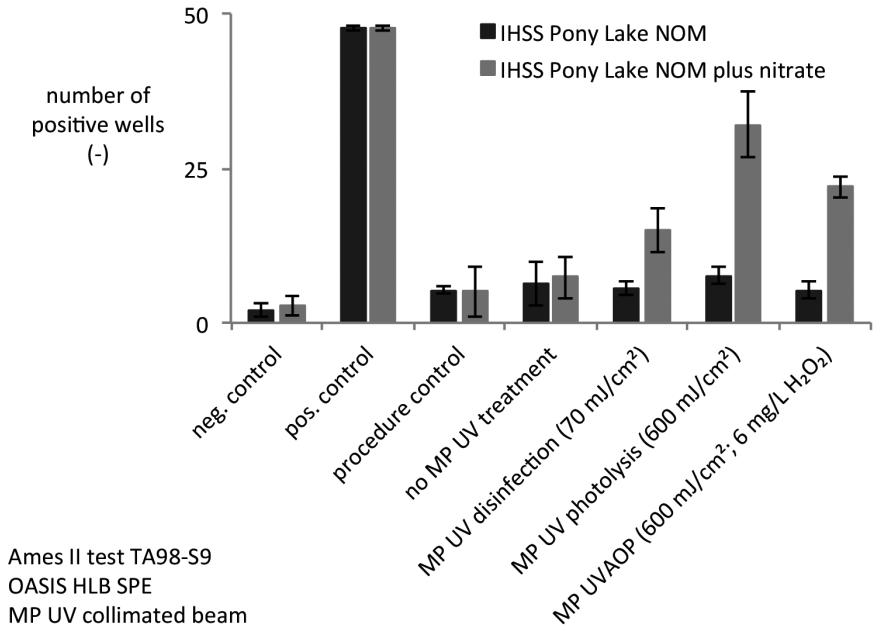


Figure 5.2: Ames test response (n=3) in strain TA98 (-S9) in MP UV CB experiments with reconstituted water IHSS Pony Lake NOM (2.5 mg C/L) without and with nitrate (0.2 mmole /L nitrate)

In the presence of nitrate, the number of positive wells increased with the UV dose. The observed response in the Ames test was in the same order of magnitude as the full scale results. A UV dose of 40 mJ/cm^2, generally applied for disinfection, generated a significant Ames test response relative to the procedure control. MP UV photolysis with 600 mJ/cm^2 caused the highest number of positive wells, while after MP UV/H_2O_2 treatment the number of positive wells was significantly lower than after MP UV photolysis only. In the absence of nitrate, no increase in the number of positive wells in the Ames test was observed, regardless of the MP UV treatment conditions, supporting the crucial role of nitrate photolysis. Figure 5.3 shows the observed response in the Ames II test for both full scale and collimated beam data as a function of the formed nitrite by MP UV irradiation.

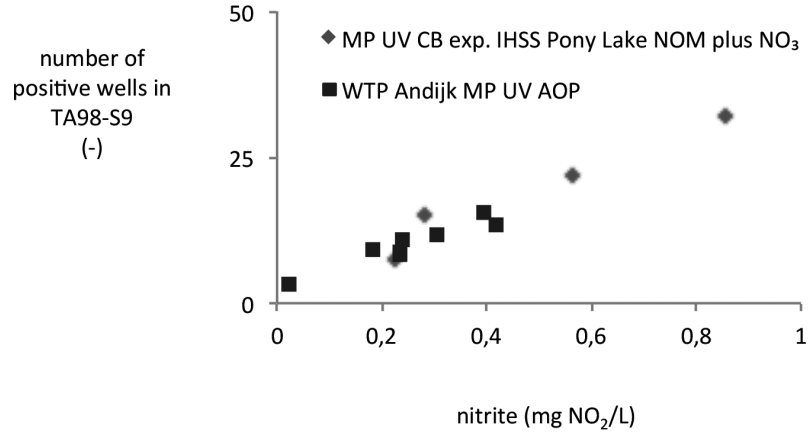


Figure 5.3: Ames test response in water samples (20,000 cf) as a function of the nitrite formation by MP UV treatment at WTP Andijk and in CB experiments with IHSS Pony Lake NOM

A clear relation was observed between the Ames II test response and the nitrite formation, confirming the impact of nitrate photolysis on both nitrite formation and Ames test response. Therefore MP UV photolysis of nitrate was studied in both organic free water and water containing a natural and synthetic organic water matrix.

5.5.2 MP UV photolysis of nitrate and nitrite in organic free water

Nitrate photolysis experiments in organic free water were carried out to investigate the formation of stable reaction products and the possible loss of inorganic nitrogen from the system. In figure 5.4 top panel, the results for nitrate photolysis and in figure 5.4 bottom panel the results for nitrite photolysis, both in organic free water, are shown. Irradiation times of about 10 minutes were applied, representative for practical UV doses for MP UV/H_2O_2 treatment.

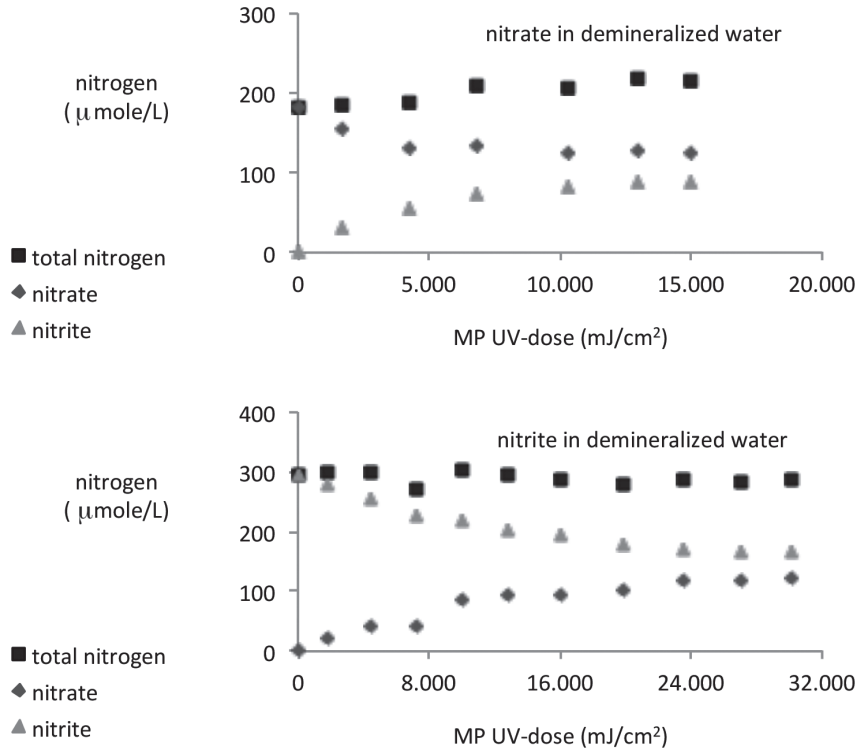


Figure 5.4: Top panel: MP UV photolysis of a nitrate solution (180 µmole/L); bottom panel: MP UV photolysis of a nitrite solution (290 µmole/L). Collimated beam experiments: irradiance in the centre of the beam 1.93 mW/cm^2, irradiation times 30, 60, 180 and 420 minutes

By the UV regimes used in practice, MP photolysis of nitrate caused a linear increase of the nitrite concentration as a function of the irradiation time (UV dose). At long irradiation times, nitrite formation from nitrate photolysis and nitrate formation from nitrite photolysis reached an equilibrium (figure 5.4 top panel). MP UV photolysis of a nitrite solution initially caused a linear increase of the nitrate concentration as a function of the MP UV dose. At long irradiation times, the nitrite and nitrate concentration reached an equilibrium (figure 5.4 bottom panel). In both experiments no nitrogen was lost from the system, confirming that the only two stable reaction products from nitrate and nitrite photolysis were nitrite and nitrate.

5.5.3 Inorganic nitrogen loss by MP UV photolysis of nitrate in the presence of an organic matrix

It was investigated if the total inorganic nitrogen content was affected by full scale MP UV/H_2O_2 treatment. Because no inorganic nitrogen was lost by irradiation of organic free water, a gap in the inorganic nitrogen mass balance suggests incorporation of inorganic nitrogen in the organic water matrix. The initial nitrate content (155 mmole/L) represented a high

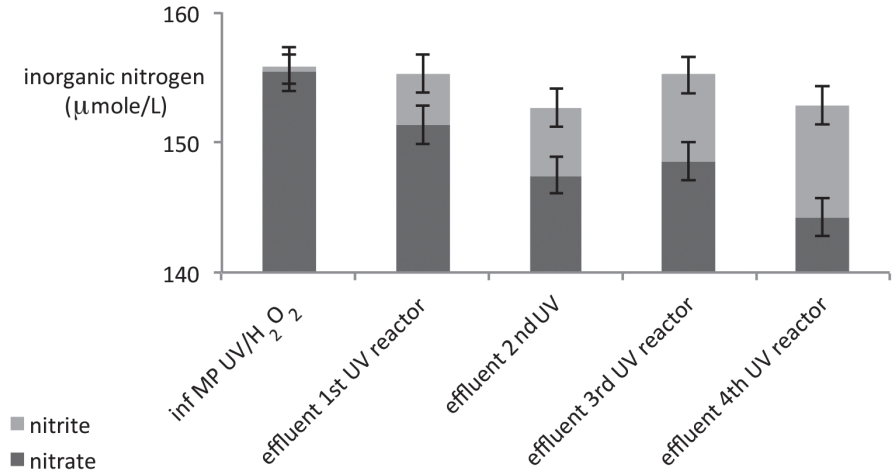


Figure 5.5: Concentration of nitrate, nitrite and total inorganic nitrogen after consecutive MP UV reactors at WTP Andijk

After each MP UV reactor, with increasing MP UV dose, the nitrite concentration increased. The total inorganic nitrogen content, combined nitrate and nitrate, showed a small, although statistically not significant, decrease of approximately 4 µmole/L nitrogen (Figure 5.5). This suggests the formation of nitrated and / or nitrosated compounds by the incorporation of inorganic nitrogen into NOM. Liquid Chromatography-Organic Carbon Detection-Organic Nitrogen Detection (LC-OCD-OND) analysis confirmed the incorporation of inorganic nitrogen in the organic water matrix (data not shown). However characterization of individual reaction products was not achieved and is an ongoing research effort.

The results showing a small but not significant decrease in inorganic nitrogen content and LC-OCD-OND measurements showing some incorporation of inorganic nitrogen in the organic water matrix were not conclusive so further research was needed. Therefore MP UV collimated beam experiments were performed with an aromatic model compound, phenol, to investigate the potential loss of inorganic nitrogen caused by nitration of the aromatic ring. Figure 5.6 shows the nitrate and nitrite concentration as a function of MP UV irradiation times in collimated beam experiments with phenol / nitrate solutions.

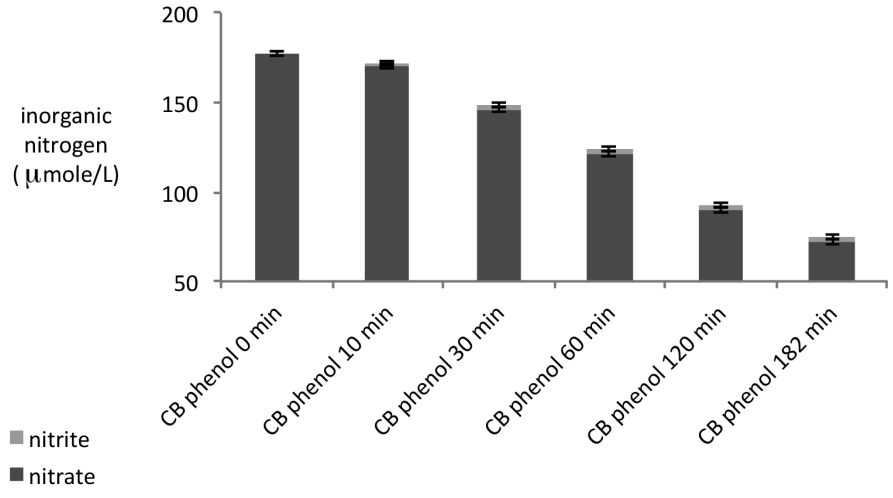


Figure 5.6: Concentration of nitrate, nitrite and total inorganic nitrogen after MP UV CB experiments with phenol (250 µmole/L) and nitrate (180 µmole/L); Collimated beam experiments: irradiance in the centre of the beam 1.4 mW/cm^2

With increasing irradiation times, the total inorganic nitrogen concentration, represented by nitrate and nitrite, decreased strongly. This suggests substitution of inorganic nitrogen onto the phenol ring. Therefore, the identification and formation rate of nitrated reaction products was pursued by Ultra Performance Liquid Chromatography (UPLC).

5.5.4 Nitrated reaction product formation in the presence of phenol

The formation of nitrophenols was investigated in collimated beam experiments as a function of the MP UV irradiation time for a solution with initial phenol and nitrate concentrations of 0.25 mmole/L and 0.18 mmole/L respectively (figure 5.7).

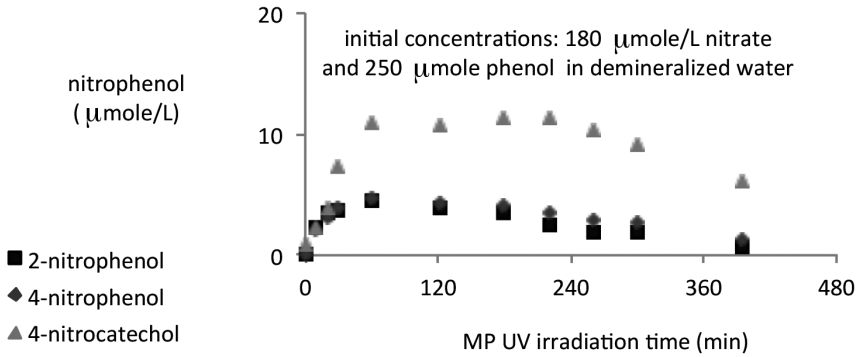


Figure 5.7: Formation of nitrophenols by MP UV treatment in a solution containing concentrations of phenol (250 µmole/L) and nitrate (180 µmole/L) in demineralised water (Collimated beam experiments)

With a MP UV irradiance of 1.4 mW/cm^2, formation of nitrophenols was observed after short irradiation times. A UV-dose applied for practical MP UV/H_2O_2 treatment resulted in the strongest formation. Under these conditions 2.2 µmole/L 2-nitrophenol, 2.1 µmole/L 4-nitrophenol and 2.4 µmole/L 4-nitrocatechol were found next to 1.7 µmole/L nitrite. Approximately 3%, respectively 5% of the original reactants nitrate and phenol was converted into nitroaromatics.

5.6 Discussion

Until recently it was generally accepted that no formation of genotoxic compounds was caused by MP UV applications. Toxicity was observed by a combined application of UV irradiation and chlorine dosage (Rosenkranz and Mermelstein, 1985). The focus of this chapter was on the formation of genotoxic compounds in nitrate rich water by the application of MP UV treatment. An increase in the Ames test response, indicating the formation of genotoxic compounds, was observed after full scale MP UV/H_2O_2 treatment of nitrate rich surface water utilizing synthetic quartz sleeves, allowing a significant UV irradiation with wavelengths < 240 nm. The Ames test response was related with the nitrite formation as shown before by Martijn and Kruithof (Martijn and Kruithof, 2012).

Collimated beam experiments with reconstituted water containing NOM in the presence and absence of nitrate were conducted. MP UV irradiation of NOM only did not show an increase in the Ames test response, while MP UV irradiated water samples with NOM in the presence of nitrate did show a response already at a MP UV dose of 40 mJ/cm^2. So already at a MP UV dose commonly applied for disinfection, a positive Ames test response was observed, contrary to the general opinion about reaction product formation by MP UV disinfection. With increasing MP UV dose, the Ames test response increased. MP UV photolysis of nitrate and NOM with a MP UV dose of 600 mJ/cm^2 in the presence of 6 mg/L H_2O_2 caused a lower Ames test response than irradiation with the same MP UV dose without H_2O_2 dosage.

The reaction mechanism of nitrate degradation by MP UV photolysis involves the formation peroxynitrite as the major intermediate reaction product. Peroxynitrite decomposes into nitro- and nitroso radicals producing nitrate and nitrite as stable end products (Mack and Bolton, 1997; Goldstein and Rabani, 2007). This research showed that, in organic free water, after MP UV irradiation, all inorganic nitrogen in the system was found back as nitrate and nitrite, so no inorganic nitrogen was lost from the system. Upon MP UV irradiation of a practical water matrix containing nitrate and NOM, a small but not significant decrease in inorganic nitrogen content (nitrate plus nitrite) was observed, due to incorporation

of inorganic nitrogen in the organic matter. By MP UV irradiation of phenol in nitrate rich water, a strong decrease of the inorganic nitrogen content was achieved, indicating a significant incorporation of inorganic nitrogen in the organic water matrix.

Thorn and Cox (Thorn and Cox, 2012) showed incorporation of inorganic nitrogen in the organic water matrix, but did not identify any low molecular nitrogen containing organics. It was tried to identify low molecular nitrogen containing organic compounds after MP UV/H_2O_2 treatment of NOM containing water, initially without success. Therefore, within the framework of this research effort the focus was on the formation of nitrated organics by MP UV/H_2O_2 treatment of an aromatic model compound, phenol, in nitrate rich water. Niessen et al (Niessen et al., 1988) and Machado and Boulé (Machado and Boulé, 1995) showed the formation of nitrated and nitrosated reaction products by MP UV photolysis of high phenol concentrations under extreme conditions. In this research effort the focus was on process conditions more suitable for UV disinfection with a MP UV dose of 40 mJ/cm^2 and MP UV photolysis with a MP UV dose of 600 mJ/cm^2, applied for advanced oxidation conditions. Under both conditions hydroxylation and nitration of phenol was confirmed. The strongest formation of hydroxylated, and nitrated phenol was found for a UV dose of 600 mJ/cm^2.

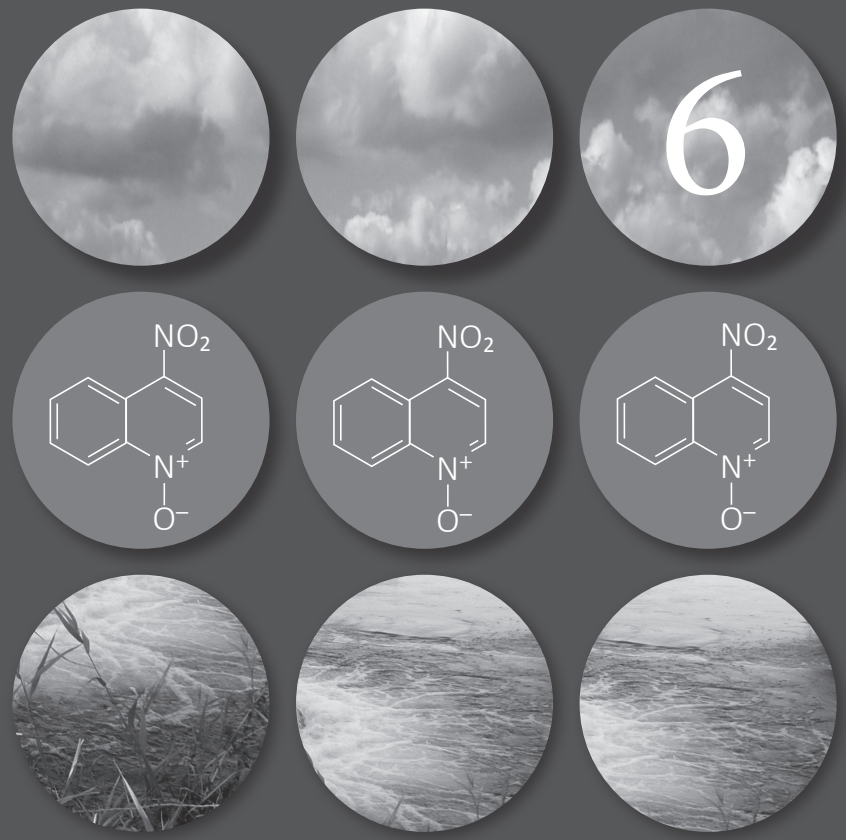
Formation of nitrated aromatic compounds by MP UV photolysis is most probably the cause of the observed Ames test response, because nitroaromatics are well known genotoxic and potentially carcinogenic compounds (Habermeyer et al., 2015). Wollin and Dieter (Wollin and Dieter, 2005) derived for a number of nitroaromatics health based drinking water guidelines. For these genotoxic substances estimations of excess lifetime cancer risk were derived. For instance for 2,6 dinitrotoluene a toxicologically based drinking water guideline value of 50 ng/L (0.27 nM) was derived for an additional 5.86 10^{-6} cancer risk over a life span of 70 years.

Summarizing, it can be concluded that utilizing synthetic quartz sleeves under the applied MP UV/H_2O_2 process conditions (0.54 kWh/m^3; 6 mg/L H_2O_2), formation of genotoxic compounds occurred in nitrate rich water types. Bench scale studies on reconstituted water with Pony Lake NOM and nitrate showed that the formation of genotoxic compounds already took place under MP UV disinfection conditions (40 mJ/cm^2). The Ames test response suggested a potential health risk. After identification and quantification of formed nitrated organics from the organic water matrix by MP UV treatment under practical conditions, risk assessment for these compounds must be carried out to quantify the possible health impact.

Abstract

An approach to enable a preliminary risk assessment of unknown genotoxic compounds formed by MP UV/H_2O_2 treatment of nitrate rich water, is described. Since the identity and concentration of specific genotoxic compounds was not established yet, a compound specific risk assessment cannot be performed. This limitation was circumvented by introducing a toxic equivalency factor, converting the concentration of unknown genotoxic compounds expressed by an Ames II test response into equivalent concentrations of 4-nitroquinoline -1-oxide (4-NQO), to enable a preliminary risk assessment. Based on the obtained 4-NQO equivalent concentrations for the tested water samples and 4-NQO carcinogenicity data, an indication of the associated risk of the by MP UV/H_2O_2 treatment produced nitrated genotoxic compounds was obtained via the margin of exposure (MOE) approach. Based on a carcinogen data, a body weight of 70 kg and a drinking water consumption of 2 litres per day, the 4-NQO equivalent concentration, associated with a negligible risk, should not exceed 80 ng/L. Applying this approach on samples from MP UV/H_2O_2 treated water of a full scale drinking water production facility, a 4-NQO equivalent concentration of 107 ng/L was established. These results indicate a safety concern in case this water would be distributed as drinking water without post treatment.

This chapter is derived from: Martijn, A.J., Van Rompay A.R., Penders, E.J.M., Alharbi, Y., Baggelaar, P.K., Kruithof, J.C., Rietjens, I.M.C.M., 2015. Development of a 4-NQO toxic equivalency factor (TEF) approach to enable a preliminary risk assessment of unknown genotoxic compounds detected by the Ames II test in UV/H_2O_2 water treatment samples. *Chemosphere,* 2015 Sep 14;144:338-345. doi: 10.1016/j.chemosphere.2015.08.070. [Epub ahead of print]



Chapter 6 Development of a 4-NQO toxic equivalency factor (TEF) approach to enable a preliminary risk assessment of unknown genotoxic compounds detected by the Ames II test in UV/H_2O_2 treated water samples

6.1 Introduction

The presence of organic micropollutants such as pesticides, pharmaceuticals, algae toxins and others in sources for drinking water requires barriers to decrease the levels of these contaminants, in order to meet regulations and to maintain customer confidence. The implementation of barriers for organic micropollutants can be achieved with retention by reverse osmosis, adsorption by activated carbon filtration or degradation by oxidation such as advanced oxidation (Snyder et al., 2007). Issues associated with the application of advanced oxidation processes (AOP) for organic micropollutant control are the formation of metabolites from the target compounds (Escher and Fenner, 2011) and the formation of undesired reaction products from the water matrix (Richardson et al., 2007; Reckhow et al., 2010). A specific type of AOP is treatment by ultraviolet (UV) light in combination with hydrogen peroxide (H_2O_2) to generate hydroxyl (OH) radicals as a non-selective barrier against (most) organic micropollutants (Kruithof et al., 2007).

One commonly applied UV technology is based on the utilization of the full UV spectrum, described as medium pressure (MP) UV (Bolton, 2010). A reaction product formed by MP UV light is nitrite, formed when nitrate is present in the water (Mack and Bolton, 1999; Sharpless and Linden, 2001). Nitrate photolysis causes the formation of nitrite via intermediates such as nitro-radicals (Goldstein and Rabani, 2007), potentially also forming nitrated organic compounds by reaction with natural organic matter (NOM) present in the water (Suzuki et al., 1985). Incorporation of nitrogen in the organic water matrix via MP UV photolysis of nitrate (Thorn and Cox, 2012) was confirmed under practical water treatment conditions (Kolkman et al., 2015), although, only some nitroaromatics, but no genotoxic compounds were identified yet. However, formation of genotoxic compounds by full scale MP UV/H_2O_2 application was shown applying Ames testing (Ames et al., 1972) with strain TA98 and TA100 on concentrated water samples (Heringa et al., 2011) and Comet assay analysis on gill tissue of mudminnow in in vivo experiments (Penders et al., 2012). The Ames II test results were confirmed after MP UV/H_2O_2 treatment of a synthetic water sample containing Nordic Lake NOM and nitrate while no positive Ames test response

was observed after MP UV/H_2O_2 treatment of a synthetic water sample containing Nordic Lake NOM only (Martijn and Kruithof, 2012). This observation suggests the formation genotoxic nitrated compounds. These results urge a risk assessment on the observed effect (Habermeyer et al., 2015).

This chapter describes the development of a method to convert concentrations of unknown genotoxic compounds present in MP UV/H_2O_2 treated water samples into 4-nitroquinoline -1-oxide (4-NQO) equivalent concentrations, using a toxic equivalency factor (TEF) approach. The use of the TEF concept is widely accepted for risk assessment of mixtures of compounds with a similar mode of action such as for example dioxins (Safe, 1992; Hong et al., 2009) and is thus also used for a preliminary risk assessment on mixtures of unknown compounds with a genotoxic mode of action causing the positive Ames test response in this study. 4-NQO is a generally accepted model compound for UV associated genotoxicity and nitrated organic compounds (Ikenaga et al., 1975; Bailleul et al., 1989; Purohit and Basu, 2000; Miranda et al., 2011; Downes et al., 2014). Based on the obtained 4-NQO equivalent concentrations for the tested water samples and 4-NQO carcinogenicity data, an indication of the associated risk of the by MP UV/H_2O_2 induced nitrated genotoxic compounds was obtained via the margin of exposure (MOE) approach (EFSA, 2005). These values were benchmarked against the maximum estimated daily intake (EDI) allowing negligible risk, based on the benchmark dose giving 10% extra cancer incidence ($BMDL_{10}$) obtained from tumour data for 4-NQO using the MOE approach.

Conversion into 4-NQO equivalent concentrations enables a direct quantitative comparison of Ames test responses and a preliminary risk assessment of the measured effect caused by MP UV/H_2O_2 treatment of nitrate rich water. The 4-NQO equivalency indicates the potency of the formed unknown genotoxic compounds. It should be noted that converting the Ames test response into a 4-NQO equivalent concentration is only a start for a preliminary subsequent risk assessment for human health using the MOE approach and 4-NQO carcinogenicity data.

6.2 Materials and methods

6.2.1 Experimental set up Ames II testing

The first objective of this study was to evaluate the genotoxic potential of water extracts due to MP UV/H_2O_2 treatment. To measure these effects, in this research effort, the Ames II test using reverse mutations in *Salmonella* Typhimurium strain TA98 in the absence of a rat liver metabolic activation system (S9), was applied. The second objective of the study was to determine (i) the concentration-Ames II test response relationship of 4-NQO, (ii) the concentration factor- Ames II test response relationship of water samples before and after MP UV/H_2O_2 treatment and (iii) the conversion of the concentration-factor dependent concentration of the water samples showing a positive Ames II test response into a 4-NQO equivalent concentration.

Water samples were taken before and after full scale MP UV/H_2O_2 treatment at water treatment plant (WTP) Heemskerk. The influent of the MP UV/H_2O_2 installation was eutrophic surface water from the IJssel Lake conventionally pretreated by coagulation, sedimentation and rapid sand filtration (CSF) (figure 6.1). The MP UV/H_2O_2 installation was equipped with synthetic quartz sleeves, allowing utilization of UV light from the fully emitted UV spectrum. The applied MP UV/H_2O_2 conditions were an electric energy input of 0.54 kWh/m^3 in combination with a hydrogen peroxide dosage of 5 mg/L. In the influent of the MP UV/H_2O_2 treatment, the dissolved organic carbon concentration and the nitrate content were 2.6 mg C/L and 1.8 mg NO_3/L respectively. MP UV/H_2O_2 treatment caused the formation of 140 µg NO_2/L (Martijn et al., 2015). After MP UV/H_2O_2 treatment, granular activated carbon (GAC) filtration was applied with an empty bed contact time (EBCT) of 9 minutes to achieve a 2.5 log H_2O_2 degradation.

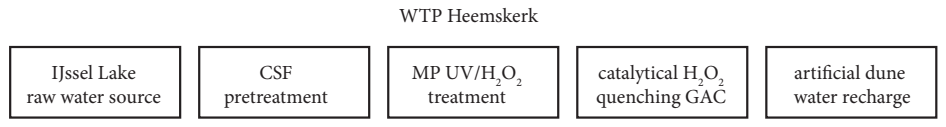


Figure 6.1: Process scheme at WTP Heemskerk with conventionally pretreated IJssel Lake water, MP UV/H_2O_2 treatment, granular activated carbon filtration and artificial dune water recharge

Water samples (before and after MP UV/H_2O_2 treatment) were extracted and concentrated by solid phase extraction (SPE) to prepare 30,000 and 20,000-fold concentrated extracts, respectively. The two extracts were diluted to determine the Ames II test response for a series of concentration factors (figure 6.2).

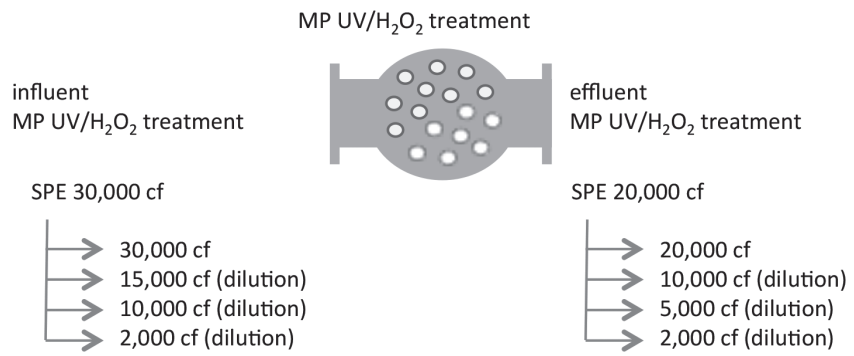


Figure 6.2: Location of water samples, the applied SPE concentration factor (cf) and dilutions before applying Ames II testing

All Ames II test experiments were performed on the same day with the same stock of overnight grown bacteria to reduce experimental bias. Next to the two dilution series of water samples, three dilution series with 4-NQO were prepared. Ames II testing of both the water sample series and the 4-NQO series were performed in triplicate to allow statistical evaluation. Figure 6.3 gives an overview of the performed Ames II testing and the use of multiwell plates.

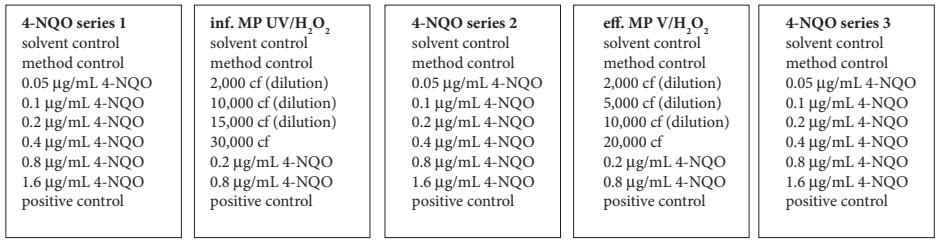


Figure 6.3: Experimental lay out for the Ames II test of three 4-NQO dilution series and two water extract dilution series including controls and internal standards (see paragraph 2.4 Ames II test procedure for definition of the controls and the internal standards used)

6.2.2 Sample preparation and 4-NQO concentration series for Ames II testing

The sample preparation procedure was based on the method described by Heringa (Heringa et al., 2011). To avoid any contamination during the extraction procedure, only glass, Teflon and stainless steel equipment was used. All materials were washed with water and then rinsed with acetone (SupraSolv, Merck) and n-hexane (LicroSolv, Merck) before use, except for the Teflon tubes, which were rinsed with ethyl acetate (Emprove, Merck). Before extraction, the water samples were stored cold (4-8 °C). The two samples (4x approximately 1,000 mL) and two blanks (2x approximately 1,000 mL) were extracted by SPE with 4x and 2x OASIS® HLB 5cc LP glass cartridges (Waters Corporation) respectively. Before extraction, the samples and blanks were acidified to pH 2.3 with a 15% ultrapure HCl-solution (SupraPur®, 30%, Merck) in Spa bottled water. Glass filtration columns (empty 6 mL glass column with frit, Macherey-Nagel) were prepared with sea sand (Sigma, Art. 18649). The filtration and SPE columns were rinsed twice with full column volumes of 20 % methanol (Licrosolve, Merck) in acetonitrile (ULCMS, Biosolve), dried and rinsed with two full column volumes of Spa bottled water acidified to pH 2.3. The columns were subsequently filled with fresh Spa bottled water of pH 2.3 and the filtration columns were mounted on the SPE columns. The air cleaning columns, OASIS ® HLB 5cc LP glass cartridges, were conditioned with one volume of ethyl acetate (Emprove, Merck), dried and mounted onto the sample bottles.

Elution was performed with 3 serial additions of 2.5 mL of 20 % methanol in acetonitrile with 1 min incubation. For each sample the 4x 7.5 mL eluates were collected in glass test tubes, combined into 1 eluate (30mL) and stored at -18 °C until further processing. For each blank the 2x 7.5 mL eluates were collected in glass test tubes, combined into 1 eluate (15mL) and stored at -18 °C until further processing. All extracts were evaporated under a gentle stream of nitrogen at 56 °C to a volume of 0.5 mL and transferred to a pre-weighted glass conical vial. The test tubes were rinsed with 0.5 mL of acetonitrile, which was added to the extract. The acetonitrile was further evaporated to approximately 100 µL under a nitrogen stream at 56 °C. DMSO was added as final solvent, and the remaining methanol/ acetonitrile was evaporated under a nitrogen stream of 56 °C in 10 minutes.

Co-evaporated DMSO was replenished by weight, yielding 30,000-fold concentrated extracts for the influent MP UV/H_2O_2 treatment sample (approx. 120 µL) and the procedure control for the influent MP UV/H_2O_2 treatment sample (approx. 60 µL) and yielding 20,000-fold concentrated extracts for the effluent MP UV/H_2O_2 treatment sample (approx. 180 µL) and for the procedure control for the effluent MP UV/H_2O_2 treatment sample (approx. 90 µL). In the Ames II assay according to the Xenometrix protocol, the above concentrations factors were lowered by a factor 25 realizing the concentration in the well (table 6.1).

Table 6.1: Applied concentration factors on water samples and procedure controls obtained by SPE and conversion to a concentration in the well for the Ames II test

Sample description	concentration factor by SPE (-)	concentration factor (cf) in well (-)
Influent MP UV/H_2O_2 treatment	30,000	1,200
	15,000	600
	10,000	400
	2,000	80
Effluent MP UV/H_2O_2 treatment	20,000	800
	10,000	400
	5,000	200
	2,000	80

6.2.3 Ames II test procedure

The Ames II assay is a screening version of the Ames assay. A validation paper published by Gee et al. (Gee et al., 1998) reported an 88% overall agreement on positive or negative classification with the regulatory Ames assay for 30 tested compounds. A study performed

by Miller et al. (Miller et al., 2005) showed a correlation of 100 % between the Ames II assay and the battery of standard Ames reversion strains using a variety of 45 compounds. Furthermore, a multicenter assessment study (Flückiger-Isler et al., 2004) reported an inter-laboratory consistency of 89.5% for 10 tested compounds.

Approximately 10^7 His- bacteria were exposed to the extracts for 90 minutes in medium containing sufficient histidine to support approximately two cell divisions. After 90 minutes, the exposed cultures were diluted in a pH indicator medium lacking histidine, and aliquoted into 48 wells of a 384-well plate. Within two days, cells that had undergone the reversion to histidine prototrophy had grown into colonies. Metabolism by the bacterial colony reduced the pH of the medium, changing the colour of that well. The number of wells containing revertant colonies were counted and compared to these values for a zero dose (solvent) control. The Ames II™ Assay 1 Sample Kit (Art. No. E01-213) was obtained from Xenometrix.

A solvent control, a method control and a positive control were part of the experimental procedure. Positive controls comprised procedures with a combination of 2-nitrofluorene (2-NF, final concentration 2 µg/mL) and 4-nitroquinoline-1-oxide (4-NQO, final concentration 0.5 µg/mL) for an assay without S9.

The 'fold induction over the baseline', the ratio of the mean number of positive wells for the test item divided by the zero-dose baseline, was calculated. The zero-dose baseline was obtained by adding one standard deviation to the mean number of positive wells of the procedure control. In case the mean revertants of procedure control was less than 1, this value was set to 1. Fold inductions in revertant numbers over the zero-dose baseline less than 2.0 are generally not considered as genotoxic. A compound that shows a fold increase greater than 2.0, is classified as genotoxic.

6.2.4 Controls and internal standards

Solvent control (100% DMSO), and method control (sample preparation using bottled water from the Spa source) and positive controls with a combination of 2-nitrofluorene (2-NF) and 4-NQO were included in the Ames II assay for the three 4-NQO dilution series and for the two dilution series from the water extracts. For the two dilution series of the water extracts, two internal standards were added to the well plates: 0.2 µg 4-NQO/mL and 0.8 µg 4-NQO/mL. Purpose of these two internal 4-NQO standards was to verify the response between the different well plates used for the dilution series.

6.2.5 Statistical evaluation Ames II test results

All samples were tested in triplicate in the Ames II test to diminish the effects of noise. To simplify the modelling of the Ames II test response of the 4-NQO dilution series, the statistical analysis was performed on the total number of positive wells of the three tests. Equivalent concentrations of 4-NQO were estimated by inversely using a regression model for the concentration-response relation of 4-NQO, derived from Ames II test-responses of three 4-NQO dilution series. The model was derived as a non-linear regression model after a logit-transformation of the relative response (the summed response divided by 144 , the total number of wells of the triplicate experiment). The logit-transformation was applied to better approach the assumption of model residuals coming from a normal distribution. The logit transformation was represented by:

$$r_i^* = \ln \frac{r_i}{1 - r_i} \qquad \text{(Eq. 6.1)}$$

Where r_i^* is the logit transformation of the relative response, r_i is the relative response, the total number of positive responses of the three tests divided by 144.
The non-linear model of the logit of the relative response for the three 4-NQO dilution series is represented by:

$$r_i^* = b_1 + \frac{b_2 \cdot x_i}{b_3 + x_i} + e_i \qquad \text{(Eq. 6.2)}$$

Where b_1, b_2 and b_3 represent the model parameters, x_i the 4-NQO concentration and e_i the model residual. Assuming independent samples and experiments in the dilution series of the water samples, a weighted combination of the estimated 4-NQO equivalent concentrations was applied for each of the influent and effluent samples. This approach resulted in an estimated 4-NQO equivalent concentration and its 95% confidence interval for each of the extracted water samples. All results of water samples and the 4-NQO concentration series were obtained with the same strain and in the same week.

6.2.6 Bench Mark Dose Modelling

The Benchmark Dose (BMD) approach was proposed as a better model for analysis of data from dose-response curve than the NOAEL (No Observed Adverse Effect Level) approach (U.S. EPA, 2012; EFSA 2005). Using the BMD method, the lower confidence limit of the benchmark dose (BMD) giving 10% extra cancer incidence ($BMDL_{10}$) can be determined. BMD modelling was applied on the in vivo dose-response curves for 4-NQO induced tumour formation obtained from literature (Tang et al., 2004) to obtain a $BMDL_{10}$ value. The $BMDL_{10}$ was used as point of departure for risk assessment by the MOE approach. BMD modelling was performed using all models for dichotomous data of the

U.S. Environmental Protection Agency (U.S. EPA)'s Benchmark Dose Software (BMDS) version 2.6 applying default settings. All models that adequately met the requirements for acceptance of the model fit were considered for the determination of $BMDL_{10}$ choosing the lowest adequate $BMDL_{10}$ value for further assessment.

6.2.7 Margin of Exposure

The MOE was suggested by the European Food Safety Authority (EFSA) as an approach to be applied for risk assessment of substances or impurities, that are both genotoxic and carcinogenic, allowing comparison between compounds to support prioritizing risk management action (EFSA, 2005; Barlow et al., 2006). The MOE was calculated by dividing the lower confidence limit of the benchmark dose that gives 10% extra cancer incidence ($BMDL_{10}$), by the EDI by humans. To evaluate the possible health risk and priority for risk management actions an MOE value of 10,000 was applied. This value of 10.000 includes a factor 100, consisting of a factor of 10 for possible inter-species differences, and a factor of 10 for differences between human individuals. Furthermore, the factor of 10,000 contains an additional factor of 10 to account for inter-individual human variability in cell cycle control and DNA repair and a factor 10 because the MOE is based on the $BMDL_{10}$ which is not a no effect level (EFSA, 2005). The value of the MOE of 10,000, means that the amount consumed is ten thousand times lower than the lower confidence bound of the dose that causes 10% extra tumour incidence above background levels in an animal bioassay. An MOE value of 10,000 or higher, based on animal cancer bioassay data, is considered to be a low concern from a public health standpoint and a low priority for risk management actions (EFSA, 2012). A value lower than 10,000 might raise a potential concern for human health (EFSA, 2005).

6.3 Results

6.3.1 Ames II test results

All Ames II test series of the extracts of the water samples and of the 4-NQO concentration series contained a procedure control, a positive control and two internal standards of 0.2 and 0.8 µg 4-NQO/mL. For all series the procedure controls and the positive controls met the quality control criteria. The internal standards, 0.2 and 0.8 µg 4-NQO/mL, resulted in similar responses for the three 4-NQO series and the two series with extracted water samples. This illustrates that the Ames II test set-up behaved similar in all five independent series, so Ames II test responses from the extracted water samples can be correlated with the Ames II test responses from the 4-NQO series (figure 6.4).

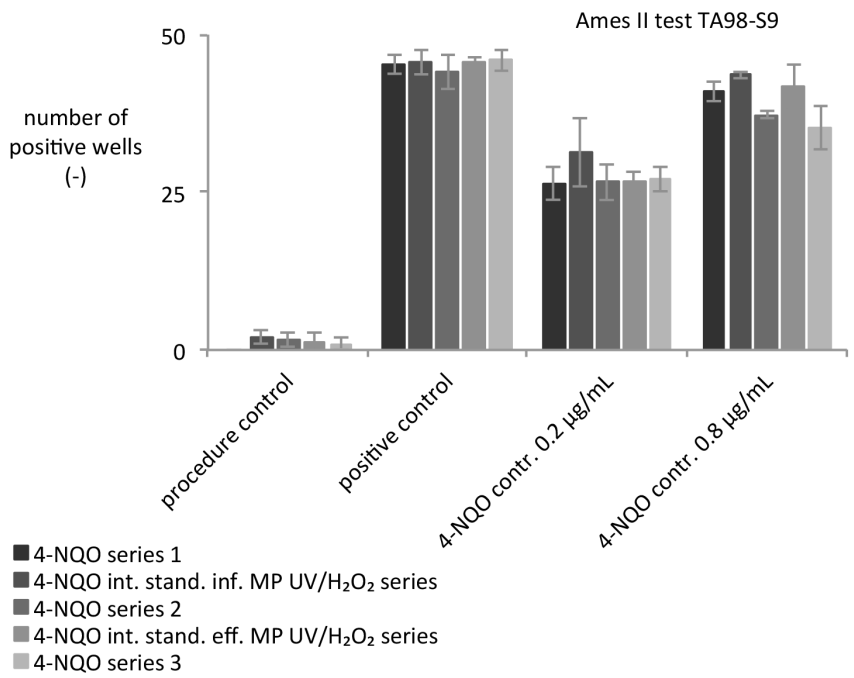


Figure 6.4: Ames II test response with standard deviation for the controls and internal standards (TA 98-S9)

Figure 6.5 shows the Ames II test responses (number of positive wells) as a function of the concentration factor in the well for the extracts of the water samples before (panel A) and after (panel B) MP UV/H_2O_2 treatment. In addition, the two-fold induction over the baseline is depicted in panel A and panel B, indicating the threshold for genotoxicity in this Ames II assay. In the applied range of concentration factors in the well (cf 80 – cf 1,200), the extracts of the water samples before MP UV/H_2O_2 treatment, did not show an increasing Ames II test response with increasing concentration factor (panel A). Furthermore, all extracts of water samples before MP UV/H_2O_2 treatment showed a fold induction factor lower than 2, not exceeding the threshold for genotoxicity in this Ames II assay (panel A). The water samples collected after MP UV/H_2O_2 treatment with the lowest two concentration factors in the well (cf 80 and cf 200) showed an increased but not significant Ames test response while for a concentration factor in the well of 400 and 800, the water extracts after MP UV/H_2O_2 treatment resulted in a response above the 2-fold threshold value and were thus considered genotoxic (panel B).

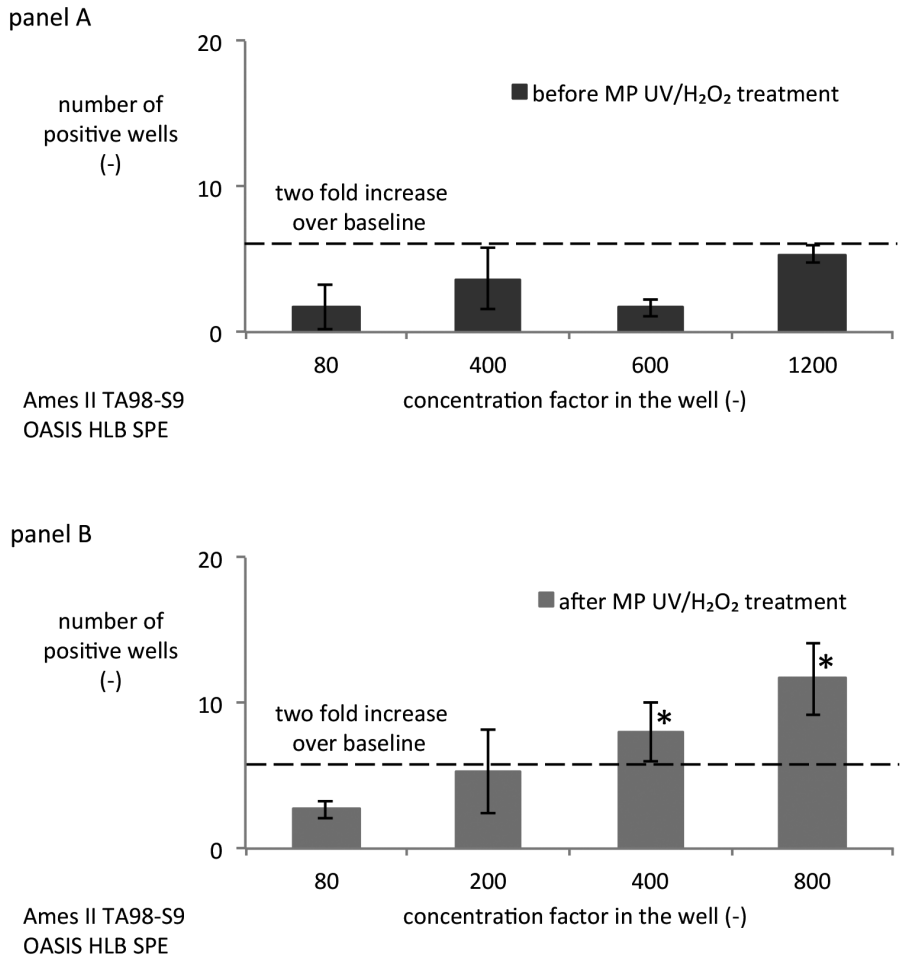


Figure 6.5: Ames II test responses in the concentration series of the extracted water samples before (panel A) and after (panel B) MP UV/H_2O_2 treatment. An asterisk indicates genotoxicity, the line at a fold increase of 2 indicates the level above which the method defines a fold increase genotoxic

The mean value of triplicate Ames II testing and the standard deviation for the 4-NQO concentration series, the basis for the reference curve to relate the Ames II test response obtained for the concentrated water samples to a 4-NQO equivalent concentration, are presented in figure 6.6. All applied 4-NQO concentrations caused genotoxicity in the Ames II assay. The variance between the three independent series was small, only the 0.4 µg/mL concentration of 4-NQO from series 3 showed a slightly larger variance. With increasing 4-NQO concentration in the well, the Ames II test response increased up to a concentration of 0.8 µg 4-NQO/mL. The non-linear dose-effect behaviour in the concentration range 0.05-0.8 µg 4-NQO/mL was attributed to the increasing probability of multiple colonies causing formation of one positive well. This is contrary to the standard Ames test where the response, measured as colonies, increases linearly with the concentration. The decrease of the response at 1.6 mg 4-NQO/mL is caused very likely by cytotoxicity and therefore not further considered.

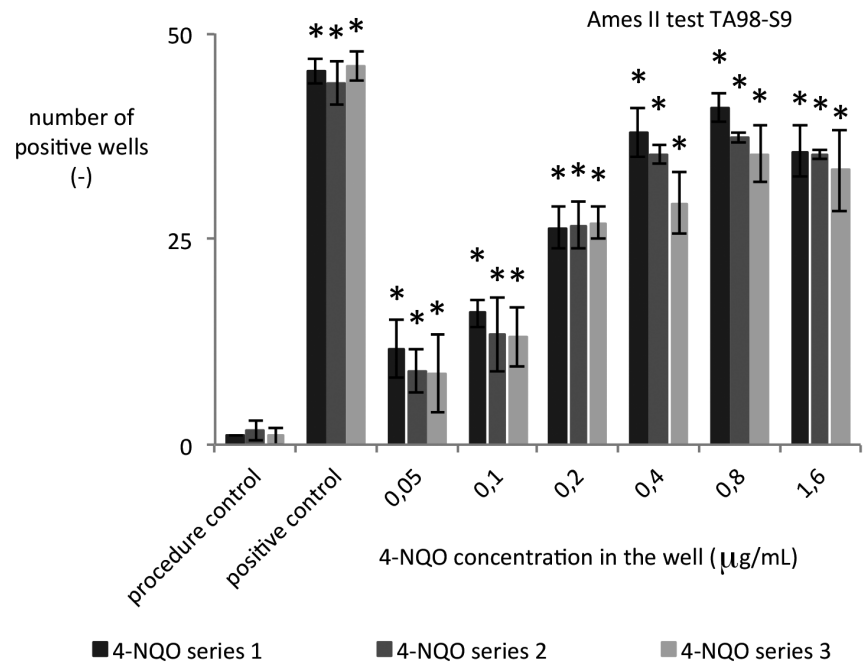


Figure 6.6: Ames II test responses (mean values and standard deviations) in the three 4-NQO concentration series. An asterisk indicates genotoxicity

6.3.2 Conversion Ames II test responses into 4-NQO equivalent concentrations

The three 4-NQO concentration series were modelled with a non-linear model. The triplicate responses for each 4-NQO series were summed, logit transformed and then modelled over three concentration ranges. The resulting model parameters are presented in table 6.2.

Table 6.2: Model parameters and variance of non-linear model of the logit of the relative response for three 4-NQO dilution series, modelled for three concentration ranges

4-NQO range	b_1	b_2	b_3	R^2
0-0.8 µg 4-NQO/mL	-4.0052	5.7816	0.0712	95.7%
0-0.4 µg 4-NQO/mL	-4.0128	5.6945	0.0679	95.0%
0-0.2 µg 4-NQO/mL	-4.0300	5.2932	0.0557	94.1%

The model, for the 0-0.8 µg 4-NQO/mL range gave the highest R^2 and was therefore selected for further model development. The assumption that the model residuals come from a normal distribution was not rejected with the Shapiro-Wilk test (p-value = 0.64). Figure 6.7, panel A shows the summed Ames II test responses of the triplicate results for the three 4-NQO dilution series, the model of the concentration-effect relation and the upper and lower limits of the 95% prediction interval.

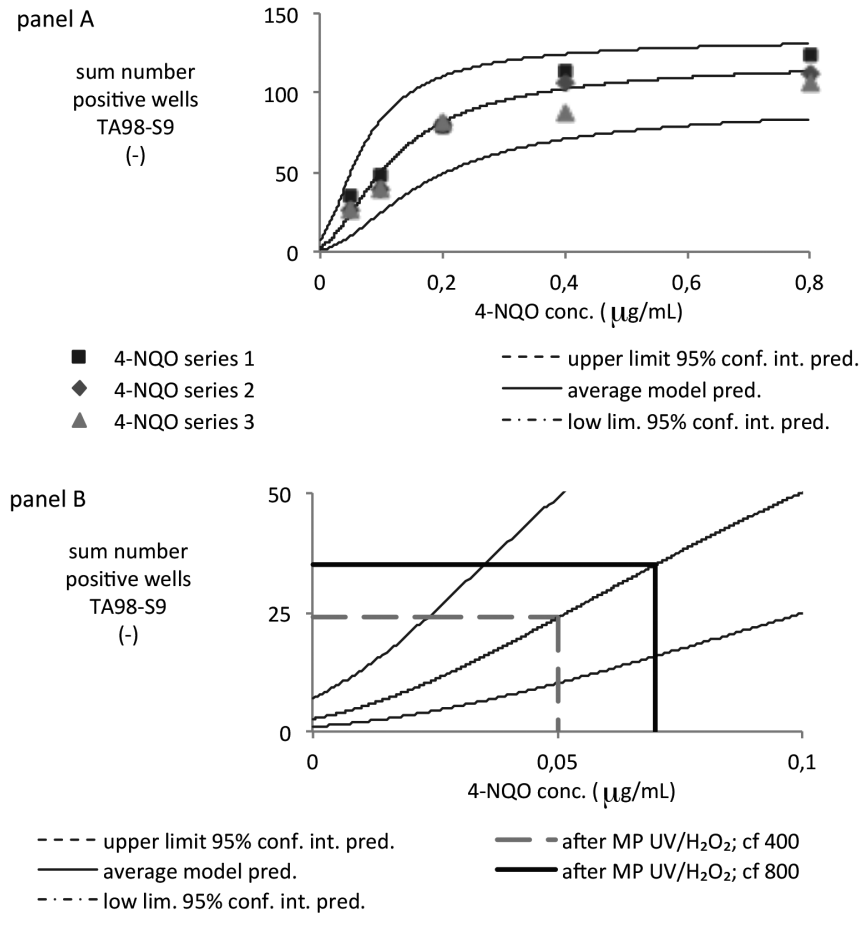


Figure 6.7: **Panel A:** Summed triplicate Ames II test responses for three series of 4-NQO conc. (data points) and non-linear model of summed Ames II test responses for three 4-NQO conc. series. **Panel B:** Detail of non-linear model with projection of summed Ames II test response of water sample extracts (400 and 800 cf in well) after MP UV/H_2O_2 treatment and corresponding 4-NQO conc.in the well

A conversion of the Ames II test response of the extracted water samples into 4-NQO equivalent concentrations was achieved by projecting each summed triplicate Ames II test response onto the horizontal 4-NQO axis, using the non-linear dose-response model. Figure 6.7, panel B illustrates this procedure for the extracted water samples collected after MP UV/H_2O_2 treatment that showed a fold induction over the baseline of >2 (cf 400 and 800). The obtained 4-NQO concentrations refer to concentrations in the well and must be divided by the concentration factor to obtain the concentration in the water samples.

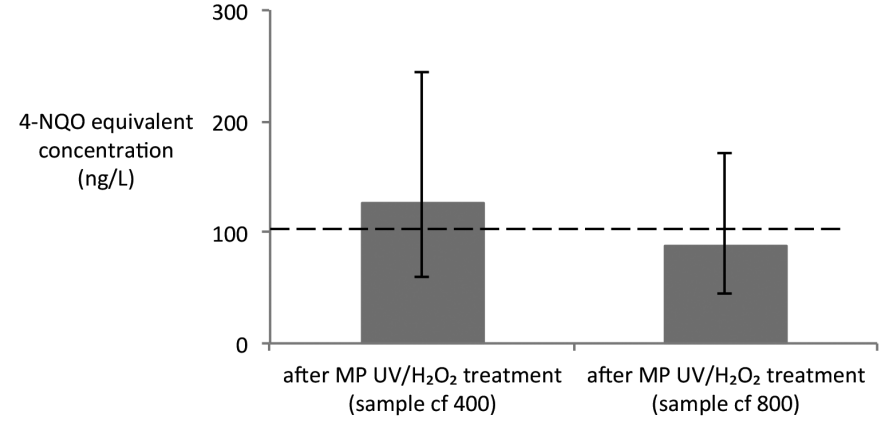


Figure 6.8: 4-NQO equivalent concentrations in the water sample after MP UV/H_2O_2 treatment, corrected for the concentration factor applied in the extraction procedure for the two genotoxic extracts (dilution with a cf 400 and cf 800) of samples obtained after MP UV/H_2O_2 treatment. The dotted line refers to the average 4-NQO equivalent concentration of the two genotoxic samples (cf 400 and cf 800) of the extract after MP UV/H_2O_2 treatment (107 ng/L)

The two genotoxic extracts of water samples, after MP UV/H_2O_2 treatment with concentration factors of 400 and 800, resulted in 4-NQO equivalent concentrations of 0.05 and 0.07 mg/mL respectively (figure 6.7, panel B). After correction for the concentration factor, the 4-NQO equivalent concentrations in the MP UV/H_2O_2 treated water samples were 126 ng/L and 88 ng/L, respectively (figure 6.8). The error bars in figure 8 show the 95% confidence interval, as determined by inversely using the non-linear dose-response model and its 95% prediction interval. The average value for the 4-NQO equivalent concentration in the water sample after MP UV/H_2O_2 treatment of 107 ng/L, is used in the subsequent risk evaluation.

6.3.3 Bench mark dose modelling and Margin of Exposure

The $BMDL_{10}$ for tumour formation upon 4-NQO exposure was derived using data from a study by Tang (Tang et al., 2004), applying different doses (20, 50, and 100 µg/mL) of

4-NQO in drinking water and two exposure scenarios for 4-NQO (8 weeks exposure with 16 weeks subsequent observation and 16 weeks exposure followed by 8 weeks subsequent observation). Exposure to 4-NQO concentrations of both 50 and 100 μg/mL resulted in significant lesions on the tongues; however, only the 100 μg/mL treatment for 8 or 16 weeks induced significant numbers of esophageal lesions. For BMD modelling, the 4-NQO concentrations in drinking water were converted into a dose consumed by the animals expressed in mg/kg bw/day. To this end a factor of 0.15 L/kg bw/day was applied (EFSA, 2009), to convert the 4-NQO concentration in drinking water into a dose. In addition, to correct for the duration of the study being shorter than the standard life span for the animals (2 years), the observed tumour incidence is corrected using the following multiplication factor (w1treatment/104) x (w2observed/104) (ECHA, 2008), resulting in the dose levels used for the BMD modelling. Table 6.3 presents the data as reported by Tang (Tang et al., 2004) and the dose consumed.

Table 6.3: Data used for the BMD modelling consisting of the incidence of oral tumours in female CBA mice from the use (ad libitum) of 4-NQO in drinking water (Tang et al., 2004) and the corresponding dose levels resulting from conversion of the 4-NQO concentrations in drinking water. The mice were treated with 4-NQO in drinking water for 8 or 16 weeks and then observed for another 16 or 8 weeks (total 24 weeks). The tongues and esophagi of mice were examined

No. of mice at week 0	Weeks of treatment	4-NQO conc. (μg/mL)	4-NQO dose (mg/kg bw/day)[c)]	Incidence esophageal lesions[a)]
5	16	0	0	0%
5	16	100	0.533	100%
5	8	100	0.266	75%
5	8	50	0.133	33%
5	8	20	0.053	0%[b)]

a) Lesions include papilloma, papilloma-carcinoma, and invasive squamous cell carcinoma.

b) Epithelial hyperplasias and dysplasias were seen, but no papillomas or invasive squamous cell carcinomas.

c) calculated by multiplying the 4-NQO conc in drinking water by 0.15 L/kg bw/day (EFSA, 2009) and by (w1treatment/104) x (w2observed/104) (ECHA, 2008) to correct for the short life span.

The dataset adapted from Tang (Tang et al., 2004), providing three different doses tested against a control, meeting the criteria for U.S. EPA BMD modelling. The U.S. EPA BMD Analysis Framework (Davis et al., 2011) was followed, resulting in a number of good fitting models, providing a slightly diverging set of $BMDL_{10}$ values (table 6.4), varying from 0.014 to 0.032 mg/kg bw/day, with an average of 0.023 mg/kg bw/day.

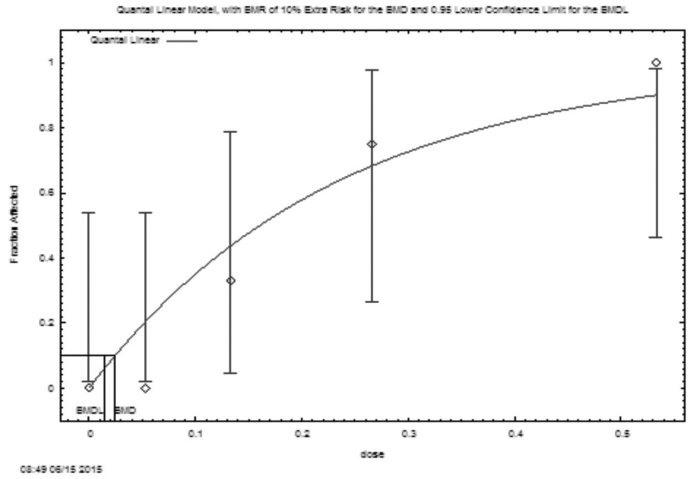
Table 6.4: BMD analysis for the incidence of oral cavity carcinogenesis in female CBA mice upon exposure to different 4-NQO concentrations via drinking water for 8 or 16 weeks. The data are analysed using BMDS software version 2.6, a BMD of 10% extra risk and default settings. The data from Tang (Tang et al., 2004) (Table 6.3) are used as input for the BMD analysis

Model	No. of parameters	p-value	Accepted	BMD (mg/kg bw / day)	$BMDL_{10}$ (mg/kg bw / day)
Null	1				
Full	5				
Gamma	2	0.95	Yes	0.091	0.029
Logistic	2	0.76	Yes	0.098	0.051
Log Logistic	2	0.91	Yes	0.094	0.038
LogProbit	2	0.96	Yes	0.094	0.041
Multistage	1	0.96	Yes	0.073	0.023
Multistage-Cancer	1	0.96	Yes	0.073	0.023
Probit	2	0.83	Yes	0.095	0.048
Weibull	2	0.92	Yes	0.085	0.026
Quantal-linear	1	0.45*	Yes	0.024	0.014

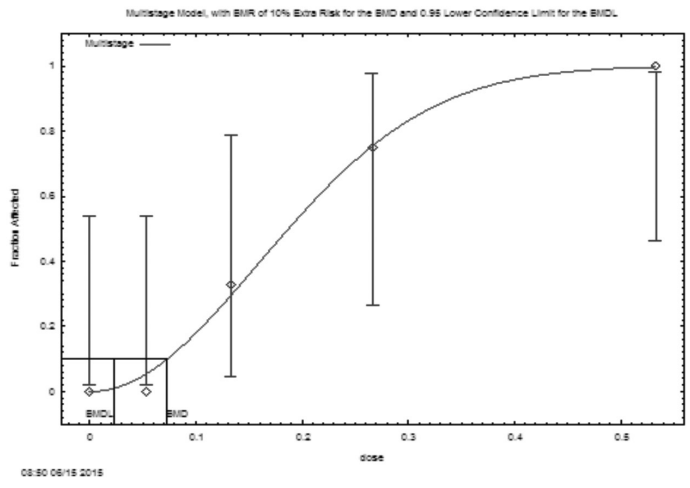
* The fit of the Quantal-linear model was less adequate giving a supralinear instead of a sub-linear curve (Figure 5A) and a far lower P value and is therefore not taken for the final $BMDL_{10}$.

The USEPA BMD Analysis Framework prescribes to select the lowest $BMDL_{10}$ for further analysis. Figure 6.9A presents the graph of the Quantal Linear model with 95% confidence interval. The supralinear curve fits the data less well than that of the Multistage and Multistage Cancer model (figure 6.9B and 6.9C which give rise to a sublinear curve and a higher p value). Therefore the $BMDL_{10}$ from the Multistage and Multistage Cancer model of 0.023 mg/kg bw/day 4-NQO was used to determine the MOE.

panel A



panel B



panel C

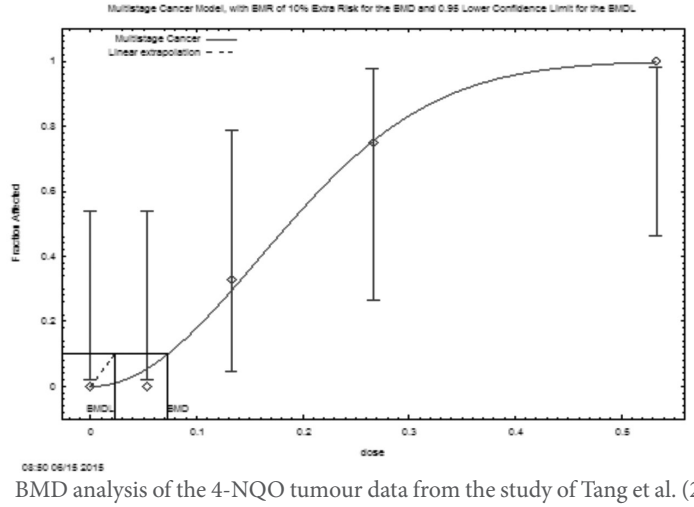


Figure 6.9: BMD analysis of the 4-NQO tumour data from the study of Tang et al. (2004) (see Table 6.3) using: A) the Quantal Linear, B) Multistage and C) Multistage-Cancer model with default settings and BMD software version 2.6

For a negligible risk, the MOE should be > 10,000. Based on the $BMDL_{10}$ value of 0.023 mg/kg bw/day and a body weight of 70 kg for a person, the EDI associated with a negligible risk should be lower than 160 ng/person/day. Assuming a drinking water consumption of 2 litres per person per day (U.S. EPA, 2004), the 4-NQO concentration should not exceed 80 ng/L in order to maintain an MOE > 10,000. The observed 4-NQO equivalent concentration in MP UV/H_2O_2 treated water for drinking water application, 107 ng 4-NQO/L eq./L, exceeds the EDI for a negligible risk of 80 ng/L per day. Upon consumption of 2L of drinking water containing 107 ng 4-NQO/L eq. /L by a 70 kg person the MOE would amount to 7,524 which is lower than 10,000 raising a safety concern in case this water would be made available as drinking water without post treatment.

6.4 Discussion

MP UV/H_2O_2 treatment, applied in water treatment of eutrophic surface water to produce drinking water, was shown to form genotoxic compounds, measured via the Ames II test (Heringa et al., 2011; Martijn and Kruithof, 2012). The origin of these genotoxic compounds is formation of nitrated organic compounds, formed via nitrate photolysis induced radical reactions (Mack and Bolton, 1999; Goldstein and Rabani, 2007; Reckhow et al., 2010; Thorn and Cox, 2012; Kolkman et al., 2015). However, identification and concentration of the formed genotoxic compounds was not reported.

At the current state of the art, a positive result in the Ames test is considered a concern in human safety assessment although the Ames test is applied with a prokaryotic organism and the fact that genotoxic compounds may not be carcinogenic. Rationale for this is that other genotoxicity tests, even those with mammalian / eukaryotic cells, often predict genotoxicity and carcinogenicity with less accuracy (Kirkland et al., 2005; Kirkland et al., 2014). Furthermore, given that for genotoxic carcinogens, epidemiological data are often lacking and human data cannot be generated for ethical reasons, in vitro and experimental animal data obtained from genotoxicity and rodent carcinogenicity assays are generally used to perform human risk assessment on genotoxic carcinogens.

Since the formation of specific genotoxic compounds was not established yet, a compound specific risk assessment could not be performed. Similar to the approach proposed by Fassbender (Fassbender et al., 2012), this limitation was circumvented by introducing a toxic equivalency factor (TEF), converting concentrations of unknown compounds resulting in positive Ames II test responses, into equivalent concentrations of a reference genotoxic compound. In the present study 4-NQO was selected as the genotoxic reference compound. 4-NQO is a water soluble nitroaromatic compound with genotoxic and

carcinogenic properties (Bailleul et al., 1989; Purohit and Basu, 2000; Downes et al., 2014). 4-NQO has been widely tested as a chemical carcinogenic model compound in rodent bioassays to provide information about the process of carcinogenesis in order to establish methods for the early diagnosis of cancer and its prevention (Miranda et al., 2011). Furthermore, 4-NQO is used to mimic UV induced DNA damage (Ikenaga et al., 1975). Therefore 4-NQO was selected as the reference compound to convert the concentrations of unknown genotoxic compounds in MP UV/H_2O_2 treated water samples measured by the Ames II test responses, using a 4-NQO TEF approach.

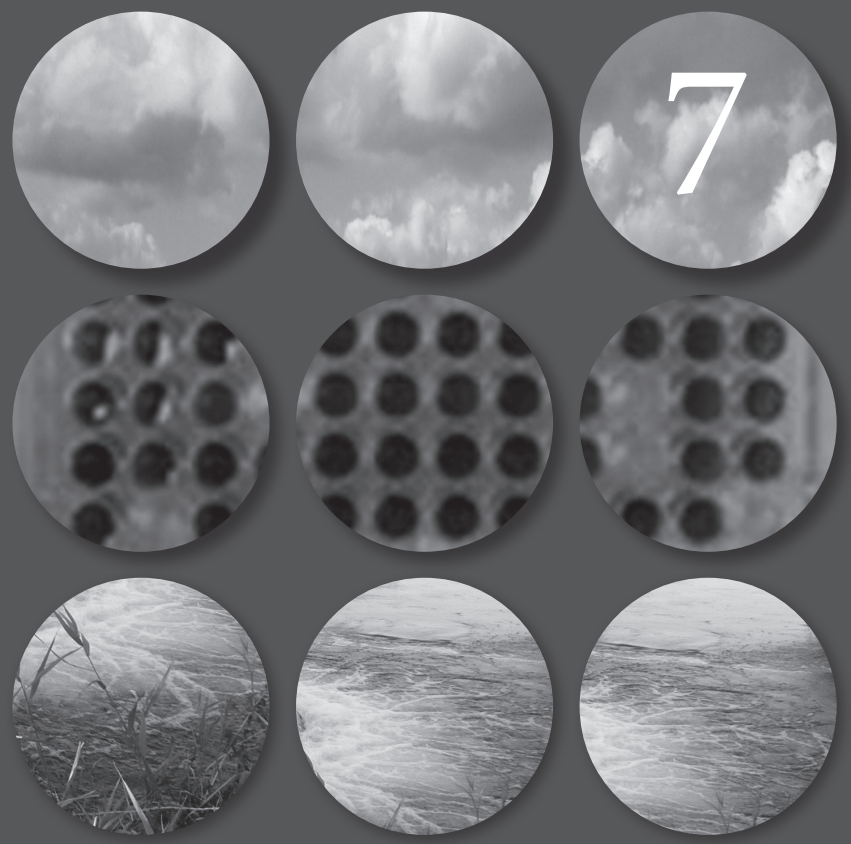
To determine the TEF, Ames II testing was performed for a series of concentrated water samples before and after full scale MP UV/H_2O_2 treatment. Simultaneously the Ames II test response for a series of 4-NQO concentrations was determined. Using the logit transformation (Baum et al., 2008), the dose-effect relationship of the 4-NQO concentration and the Ames II test response was modelled with a 95% confidence interval. This model was used to express the concentrations of unknown genotoxic compounds in the water samples before and after MP UV/H_2O_2 treatment in 4-NQO equivalent concentrations. Assuming a daily consumption of two litres of drinking water by an adult of 70kg, the EDI expressed as a 4-NQO equivalent concentration was estimated for the different water samples. The applied MOE approach for risk assessment was based on this EDI and 4-NQO cancer data modelled according to the bench mark dose (BMD) method to define the lower confidence bound of the benchmark dose causing 10% extra cancer risk above background ($BMDL_{10}$). The MOE, defined as the ratio between the $BMDL_{10}$ and the EDI (EFSA, 2005), was used to estimate the priority for risk management.

It was found that the tested MP UV/H_2O_2 treated water samples exhibit a 4-NQO equivalent concentration, exceeding the EDI associated with a level of no concern. Therefore, the observed 4-NQO equivalent concentration indicates a level of concern and urge for a compounds specific risk assessment. It is recommended that the observed effect should be investigated in further detail. Under the current multibarrier approach for water treatment, combining MP UV/H_2O_2 treatment with biological and adsorptive processes (Kruithof and Martijn, 2013), the nitrated organic genotoxic compounds are removed with post treatment by biological GAC filtration or dune passage, thus eliminating the risk. The mechanisms involved in the apparent biological mitigation of the genotoxic effect require further testing as well.

Abstract

Just like after chlorination, at UV dosages applied for disinfection purposes, MP UV treatment caused formation of genotoxic compounds, measured by the Ames test. By lowering the nitrate and DOC content, substantial reduction of the Ames test response was achieved. The impact of MP UV photolysis of nitrate on the formation of genotoxic compounds was confirmed. A representative organic micropollutant selection had no significant impact on the Ames test response. Formation of genotoxic compounds was found after MP UV disinfection, photolysis and advanced oxidation of pretreated groundwater. In addition to the nitrate and DOC content, aromaticity of organic matter had a strong impact. Ames test responses were converted into 4-nitroquinoline-1-oxide equivalent concentrations to enable quantitative comparison and to apply a simple risk assessment. Based on the threshold for toxicological concern, already at doses applied for MP UV disinfection the equivalent concentration of formed genotoxic compounds in nitrate rich water, exceeded the limit of no risk.

This chapter is derived from: Martijn, A.J., Kruithof, J.C., Hughes, R.A.M., Mastan, R.A., Van Rompay, A.R., Malley jr., J.P., 2015. Induced genotoxicity in medium pressure UV treated nitrate rich water, *Journal - American Water Works Association,* 107 (6), 301-312



Chapter 7 Induced genotoxicity in nitrate rich water treated with MP ultraviolet processes

7.1 Introduction

To cost effectively inactivate *Cryptosporidium* and *Giardia* as well as to avoid disinfection by-product (DBP) formation by chlorination and ozonation, the application of ultraviolet (UV) technology has increased significantly (Malley 2002, Kruithof et al. 1992). Since the discovery of the formation by chlorination of harmful disinfection by-products such as trihalomethanes (THMs), haloacetic acids (HAAs) and other halogenated reaction products (Bellar et al. 1974, Rook 1974), research into the formation of harmful by-products by oxidative drinking water treatment has intensified. Oxidative treatment via processes such as chlorination, ozonation, or advanced oxidation results in the formation of numerous reaction products from the organic and inorganic water matrix (Krasner et al. 2006) and metabolites from micropollutants (Esplugas et al. 2007). Chemical identification of individual reaction products and determination of their potential health impact is laborious and does not give information on the synergistic effect of the formed reaction products. Application of effect-derived methods, for instance the Ames test (Ames et al. 1972), to determine DNA damage induced by genotoxic reaction products overcomes this limitation.

The Ames test is a screening method to evaluate the genotoxic properties of test items such as water samples. This bioassay uses amino acid (histidine)–dependent, genetically modified strains of *Salmonella* Typhimurium and is based on reverse mutation. Colony growth can only occur after mutation of bacteria, revertants which overcome their histidine deficiency. Spontaneous revertants are measured as procedure control. Genotoxic compounds are evaluated relative to this background level. Different types of Salmonella strains, for instance TA98 and TA100, are used to detect different types of genotoxic compounds. The benefit of the Ames test is that it is a generally accepted standard screening method for detection of genotoxic compounds, allowing high throughput of samples. The disadvantage of this method is that the DNA damage is measured in bacteria, which does not allow a direct translation to human health effects, nor does it give any information about the character of the genotoxic compounds since only an effect is measured. In water practice, the Ames test has been used for a long time to screen water samples for the presence or absence of genotoxic compounds. Sample preparation, such as a concentration step, influences detection of genotoxic compounds in a sample and the level of the response of the Ames test. In this chapter, the Ames test was used as a screening method. The measured effect was converted into an equivalent concentration of a known genotoxic compound, 4-nitroquinoline-1-oxide (4-NQO), to enable quantitative comparison and to apply simple risk assessment.

7.1.1 Reaction products and metabolites

After chlorination, an increase in Ames test response was found (AwwaRF, 1986) indicating the formation of genotoxic compounds. Ozonation caused the formation of biodegradable organic reaction products such as aldehydes, ketones and carboxylic acids (AwwaRF, 1991). No increase in Ames test response was found after ozonation while in most water types a decrease in Ames test response was observed (Zoeteman et al., 1982). However, in bromide containing water, ozonation caused formation of bromate (Von Gunten and Hoigné, 1994), a suspect human carcinogen (Kurokawa et al., 1990; Cotruvo et al., 2008). For medium pressure ultraviolet (MP UV) technology, formation of the inorganic reaction product nitrite in nitrate rich waters has been widely reported (Mack and Bolton, 1999; Sharpless and Linden, 2001). The formation of harmful organic reaction products by UV based photolysis and advanced oxidation process (AOP) of organic micropollutants in synthetic water was investigated by many researchers (Shemer and Linden, 2007; Esplugas et al., 2007). However, in natural waters, formation of harmful organic reaction products has not been documented yet. No Ames test response was found before and after low pressure UV treatment (Heringa et al., 2011). In preliminary research efforts, no positive Ames test response was found after MP UV/H_2O_2 treatment utilizing natural quartz sleeves (type GE214) with a cut-off of UV-light below 240 nm of IJssel Lake water, pretreated by coagulation, sedimentation and rapid sand filtration (Penders et al., 2012). However, MP UV/H_2O_2 treatment utilizing synthetic quartz sleeves that did not cut-off UV light at wavelengths below 240 nm caused a significant response. (Heringa et al., 2011; Martijn and Kruithof, 2012). The difference was caused by the photolysis of nitrate by the low wavelength UV light. To study this effect all experiments described in this chapter were carried out utilizing synthetic quartz. The Ames test responses observed after MP UV/H_2O_2 treatment were lower, but still significant compared to the observed Ames test response after chlorination (Kruithof, 1986). However, a direct assessment of health impact could not be made since only a response was measured, not providing information about the character of the compounds causing the response.

7.1.2 Nitrate photolysis in the presence of natural organic matter (NOM)

MP UV/H_2O_2 treatment of synthetic water containing Nordic Lake NOM only, did not cause an increase in Ames test response. However when nitrate was added to the NOM solution a significant increase in the Ames test response was observed after MP UV treatment under disinfection, photolysis and AOP conditions (Martijn and Kruithof, 2012), establishing the impact of nitrate photolysis on the formation of genotoxic compounds. In the USA the nitrate content of natural groundwater is 4-9 mg NO_3-N/L while in surface water the nitrate content usually is <4 mg NO_3-N/L. In 3% of the water sources the nitrate content is >20 mg NO_3-N/L while in 40 surface water supplies and 568 groundwater supplies the nitrate content is higher than 10 mg NO_3-N /L (US EPA, 1987). In Europe, the nitrate content is in the same order of magnitude, while agricultural activities may cause nitrate contents up to several hundreds mg/L (WHO, 1985). Therefore model waters were prepared and a number of Dutch groundwater and surface water samples with representative nitrate concentrations were selected.

Liquid chromatography-organic carbon detection (LC-OCD) with additional organic nitrogen detection showed incorporation of nitrogen in the organic matrix. This gave rise to the assumption that intermediates from nitrate photolysis, such as nitroradicals, react with organic constituents from the water matrix. It was hypothesized that these reaction products, i.e. nitroaromatics may be causing the observed Ames test responses. Besides the high molecular weight organic substances from the water matrix, organic micropollutants may play a role in the formation of genotoxic compounds by MP UV based AOP as well. Many researchers have shown the formation of harmful metabolites from micropollutants and the health impact of these compounds (Bolong et al., 2007; Shemer and Linden, 2007). However, the contribution of micropollutants and their metabolites to the Ames test response after MP UV AOP treatment, relative to the Ames test response caused by reaction products of NOM after MP UV AOP treatment in nitrate rich water, was not studied yet and is explored in this chapter.

7.1.3 Ames test responses in natural waters

Until now the research into Ames test responses was focussed on MP UV AOP treatment of conventionally pretreated surface water (Heringa et al., 2011; Martijn and Kruithof, 2012) and on synthetic water matrices containing NOM and nitrate (Martijn and Kruithof, 2012). Natural water matrices, varying in DOC content and composition and nitrate concentration were applied in this research. The effect of MP UV treatment under disinfection, photolysis and AOP conditions on the formation of genotoxic compounds was studied in eutrophic surface water after two types of pretreatment and in two types of groundwater originating from an aerobic and anaerobic aquifer. This chapter describes the effect of conventional pretreatment with coagulation, sedimentation and rapid sand filtration and advanced pretreatment with ion exchange followed by ceramic microfiltration on the Ames test response after MP UV treatment under all previously described processes. In addition, the effect of MP UV treatment on two types of pretreated groundwater is described. Previous research on heavily contaminated groundwater (Haider et al., 2002) showed formation of genotoxic compounds by LP UV disinfection. In our research, pretreated anaerobic and aerobic groundwater, were selected to illustrate the importance of nitrate and DOC content on the Ames test response after MP UV treatment under disinfection, photolysis and AOP conditions. The impact of the character of the organic water matrix is illustrated

by the relation between the Ames test response and nitrite formation. In addition, the impact of the characteristics of the organic water matrix, determined by parameters such as total organic nitrogen (TON), LC-OCD, UV absorption and specific UV absorbance (SUVA) on the Ames test response after MP UV treatment has been pursued.

7.1.4 4-Nitroquinoline-1-oxide (4-NQO) equivalent concentrations

Commonly, Ames test responses are reported in number of revertants, positive wells or induction factors without reporting the applied concentration factors to prepare the samples. To address the question 'what is the health concern of an Ames test response in concentrated water samples?', in this approach Ames test responses are converted into 4-NQO equivalent concentrations. 4-NQO is a genotoxic compound, with a positive response in the Ames test and is used to mimic effects of UV radiation and photoproducts on DNA in cells of organisms (Ikenaga et al., 1975). Conversion into 4-NQO equivalent concentrations enables a direct quantitative comparison of Ames test responses and a preliminary risk assessment of the measured effect caused by MP UV treatment of nitrate rich water. The 4-NQO equivalent concentration methodology indicates the potency of the formed genotoxic compounds. However, it should be noted that converting the Ames test response into a 4-NQO equivalent concentration does not allow direct extrapolation of a 4-NQO dose to human health effects.

7.1.5 Threshold for toxicological concern

A preliminary risk assessment based on the threshold for toxicological concern (TTC) (Kroes et al., 2000) is carried out. The TTC is a concept to establish a human exposure threshold value below which no appreciable health risk occurs. The TTC concept may be applied when the presence of a new contaminant in food is observed, for which no toxicological information is available. It could also be useful in setting priorities for testing large groups of functionally similar chemicals to which exposure is generally very low, such as flavourings (Barlow, 2005) or deriving target values for drinking water contaminants (Mons et al., 2013). These conditions also apply for genotoxic compounds formed by MP UV treatment of nitrate rich pretreated surface and groundwater. The formation of a large number of similar, unknown genotoxic compounds at very low concentrations, from which toxicity data are lacking, requires a preliminary risk assessment. Application of the TTC concept can demonstrate whether or not the formation of genotoxic compounds by MP UV treatment under disinfection, photolysis and AOP conditions poses an acceptable risk.

7.1.6 Margin of Exposure approach

The Margin of Exposure (MOE) approach was suggested by the European Food Safety Authority (EFSA) as an approach which can be applied for risk assessment of both genotoxic and carcinogenic substances or impurities, for which toxicological data is available. The MOE approach is an alternative for the TTC concept that may be applied when the presence of a new contaminant in food is observed, for which no toxicological information is available. However, it is important to realize that certain classes of genotoxic carcinogens have been excluded from application of the Threshold of Toxicological Concern (TTC) concept, including for example high-potency carcinogens such as aflatoxins, azoxy compounds, N-nitroso compounds, benzidines, hydrazines and compounds with an unknown chemical structure. The MOE approach allows comparison between compounds to support prioritisation for risk management action (Barlow, 2005) (EFSA, 2005). The MOE can be used by risk managers to determine the priority of concern for public health, to prioritise the possible actions required. An MOE value gives an indication of the level of concern, but is not a precise quantification of risk. Also, MOE values depend on the carcinogenicity data selected to be used in a Bench Mark Dose (BMD) approach to determine the BMD giving 10% extra cancer incidence ($BMDL_{10}$) and the estimation of human dietary exposure. For a negligible risk, the MOE should be > 10,000. Assuming a drinking water consumption of 2 litres per person per day (U.S. EPA, 2004), the 4-NQO concentration should not exceed 80 ng/L in order to maintain an MOE > 10,000 (chapter 6).

7.2 Materials and methods

7.2.1 Natural water sources

Experiments with natural waters were performed with two types of pretreated surface water and two types of pretreated groundwater. These water sources are representative for eutrophic surface water in moderate climates and for a range of aerobic and anaerobic groundwaters. The raw surface water originating from the IJssel Lake, contained 5 mg C/L and 2.6 mg NO_3-N/L. Two types of pretreatment were applied: coagulation with ferric chloride (20 mg/L Fe), sedimentation and rapid sand filtration (CSF) and anion exchange (Lewatitt VPOC, 15 g/L resin) followed by ceramic microfiltration (IX-MF). CSF pretreated IJssel Lake water contained 2.5 mg C/L and 2.6 mg NO_3-N/L. IX-MF pretreated IJssel Lake water contained 1.7 mg C/L and 0.68 mg NO_3-N/L. The two groundwaters were anaerobic and aerobic groundwater after aeration and filtration, with a high and a low nitrate content respectively. The TOC and nitrate content of the treated aerobic groundwater were 1.3 mg C/L and 0.50 mg NO_3-N/L while the treated anaerobic groundwater contained 1.6 mg C/L in combination with 5.17 mg NO_3-N/L.

7.2.2 Chemicals and chemical analysis

Nordic Lake and Pony Lake NOM representative for aquatic (1R108N) and fulvic acid

NOM (1R109F), respectively were obtained from the International Humic Substances Society (IHSS) to prepare the synthetic water samples. The nitrogen content of Nordic Lake NOM was 1.1% (w/w) and the SUVA was 1.3 L mg^{-1} m^{-1}. The nitrogen content of Pony Lake NOM was 6.5% (w/w), the highest nitrogen content of the available IHSS NOM isolates. The SUVA was 3.6 L mg^{-1} m^{-1}. Sodium nitrate (Sigma Aldrich) was used to achieve the required nitrate concentration in synthetic water. Representative micropollutants for the IJssel Lake (isoproturon, carbendazim, sulfamethoxazole, hydrochlorothiazide, carbamazepine, AMPA, glyfosate, amidotrizoic acid, iomeprol, DEPH, DBPH, caffeine, metformine and EDTA) were obtained from Ehrerstorfer and Sigma-Aldrich. The presence and concentrations of micropollutants in surface water from the IJssel Lake are representative for the river Rhine, placing the findings in a worldwide context. Stock solutions and synthetic water were prepared by HWL Laboratory (Haarlem, The Netherlands) using demineralized water.

Characterization of NOM was carried out by HWL Laboratory. The methods used were LC-OCD, also measuring TON, UV absorbance at 254 nm and aromaticity as SUVA (Huber et al., 2011). Nitrate and nitrite were measured using ion chromatography.

7.2.3 Ames testing and sample preparations

The Ames II Assay (Xenometrix), based on a liquid microplate format, was used. This assay is a screening version of the Ames assay (Ames et al., 1972). For Ames II testing, water samples were extracted by Solid Phase Extraction (SPE) with OASIS HLB 5cc LP glass cartridges (Waters Corporation) at a pH of 2.3 by adding 15% ultrapure HCl-solution (Suprapur, 30%, Merck, diluted with Spa bottled water). Elution was performed with 20% methanol in acetonitrile (Lichrosolve, Merck and ULCMS, Biosolve). With a gentle stream of nitrogen at 56°C, the volume of the eluate was reduced to approx. 100 µL. DMSO (Anhydroscan, LabScan) was added as a final solvent achieving a concentration factor of circa 10,000 and 20,000, resulting in a concentration factor in the liquid microplate well of circa 400 and 800 respectively. Applying these concentration factors, a semi-quantitative comparison of the Ames test results can be obtained. The genotoxic potential of these extracts to induce reverse mutations in *Salmonella* Typhimurium strain TA98 in the absence of a rat liver metabolic activation system (S9) was explored. SPE of the water samples and Ames tests were performed by Vito Laboratory in Belgium. Procedure / solvent and positive controls were performed in triplicate for each natural water source and for the synthetic waters (Table 7.1).

In addition to the assessment of the genotoxic potential of the concentrated water samples, the genotoxic potential of 4-NQO was determined, using the Ames II Assay protocol (Flückiger-Isler et al., 2004). Ames test responses were generated for a series of 4-NQO concentrations, enabling a more quantitative comparison by converting Ames test responses into 4-NQO equivalent concentrations. In order to apply the TTC methodology on the MP UV treated water samples, responses of the extracts were converted into equivalents via a dose-response curve for 4-NQO concentrations ranging from 0 to 0.8 µg/mL. The calibration curve was generated with the same batch of TA98 *Salmonella* bacteria used for the concentrated water samples. To correct for the sample preparation procedure using SPE, the exact concentration factors in the liquid microplate well for the synthetic and natural waters are required . The applied concentration factors are summarized in table 7.1.

Table 7.1: Concentration factor in the well, procedure and positive controls of the Ames test after SPE of synthetic and natural waters for the three MP UV treatments

Practical water matrix	Conc. factor MP UV/H_2O_2	Conc. factor MP UV photolysis	Conc. factor MP UV disinfection	Procedure / negative control	Positive control
CSF pretreated surface water	788	793	801	3.00 +/- 2.00	48.00+/- 0.00
IX-MF pretreated surface water	390	414	400	2.67 +/- 1.53	48.00 +/- 0.00
Aerobic groundwater (low nitrate)	800	794	792	2.00 +/- 2.65	48.00 +/- 0.00
Anaerobic groundwater (high nitrate)	794	797	792	2.33 +/-2.52	48.00 +/- 0.00
IHSS Pony Lake NOM plus nitrate	396	383	371	2.67 +/- 1.53	47.67 +/- 0.58

7.2.4 Medium pressure UV collimated beam experiments

The irradiations were performed in a collimated beam apparatus equipped with a 3 kW medium pressure Hg lamp (Trojan Rl-01) with a well-defined spectral output. An example of the emission spectrum of a MP UV lamp is given in figure 7.1 together with the molar absorption coefficient of nitrate and hydrogen peroxide. This figure shows the impact in particular of low wavelength light on the photolysis of both hydrogen peroxide and nitrate.

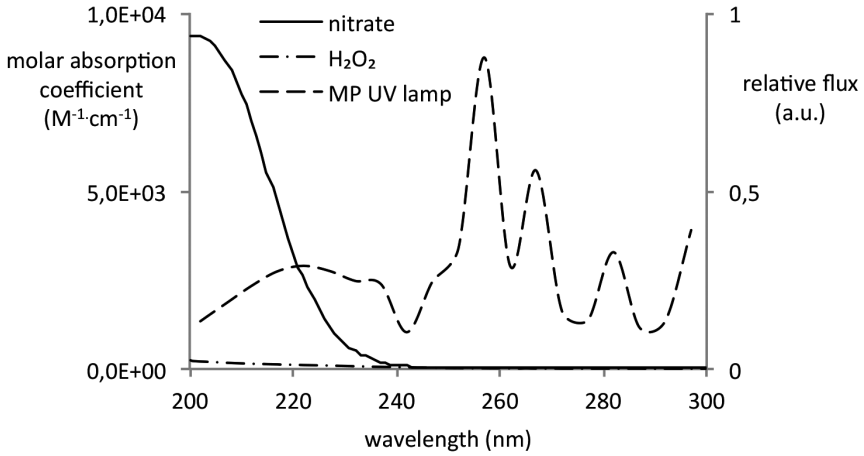


Figure 7.1: Emission spectrum of a MP UV lamp and molar absorption of nitrate and hydrogen peroxide

The light passed through a synthetic quartz window before reaching the solution, allowing utilization of low wavelength radiation. The irradiance readings were taken with an IL research Radiometer, with a SED240/W detector calibrated every 2 nm within the 200-320 nm range. Absorption spectra were recorded in a 1 cm path length quartz cell, with a Hach-Lange DR5000 spectrophotometer. The irradiation time was accurately monitored for each sample and the UV dose delivered to the solution was calculated using the UV dose calculation method developed by Bolton and Linden (2003). The collimated beam experiments were carried out under conditions to guarantee representative results for the full scale MP UV/ H_2O_2 installation. The representativeness of the collimated beam experiments for full scale effects is shown by comparison of both nitrite formation and Ames test results for both systems (data not shown). For the irradiations, 60 x 35 mm crystallizing dishes were employed with a sample volume of 55 mL. The irradiation path length was approx. 1.95 cm. The crystallizing dish was placed on a stirring plate, and the solution was continuously stirred with a small magnetic stir bar.

The applied process conditions were:

- For MP UV disinfection: 40 mJ/cm^2
- For MP UV photolysis: 600 mJ/cm^2
- For MP UV/H_2O_2 treatment: 600 mJ/cm^2 combined with 6 mg/L H_2O_2

7.3 Results

7.3.1 The Ames test response in synthetic water for practical MP UV treatment conditions

To confirm the formation of an Ames test response in nitrate rich water, collimated beam experiments with synthetic water containing IHSS Pony Lake NOM, in the presence and absence of nitrate were carried out under several MP UV process conditions. In addition to MP UV AOP treatment with a UV dose of 600 mJ/cm^2 in combination with 6 mg/L H_2O_2, MP UV photolysis (UV dose 600 mJ/cm^2) and MP UV disinfection (UV dose 40 mJ/cm^2) were applied. The IHSS Pony Lake NOM content was 2.5 mg C/L and the nitrate concentration was 2.71 mg NO_3/L. Figure 7.2 shows the results of the Ames tests (TA98-S9) of SPE concentrated water samples. The response in the samples 'before UV treatment' did not differ significantly from the procedure control measurement carried out in triplicate (see Table 7.1 in Materials and Methods section).

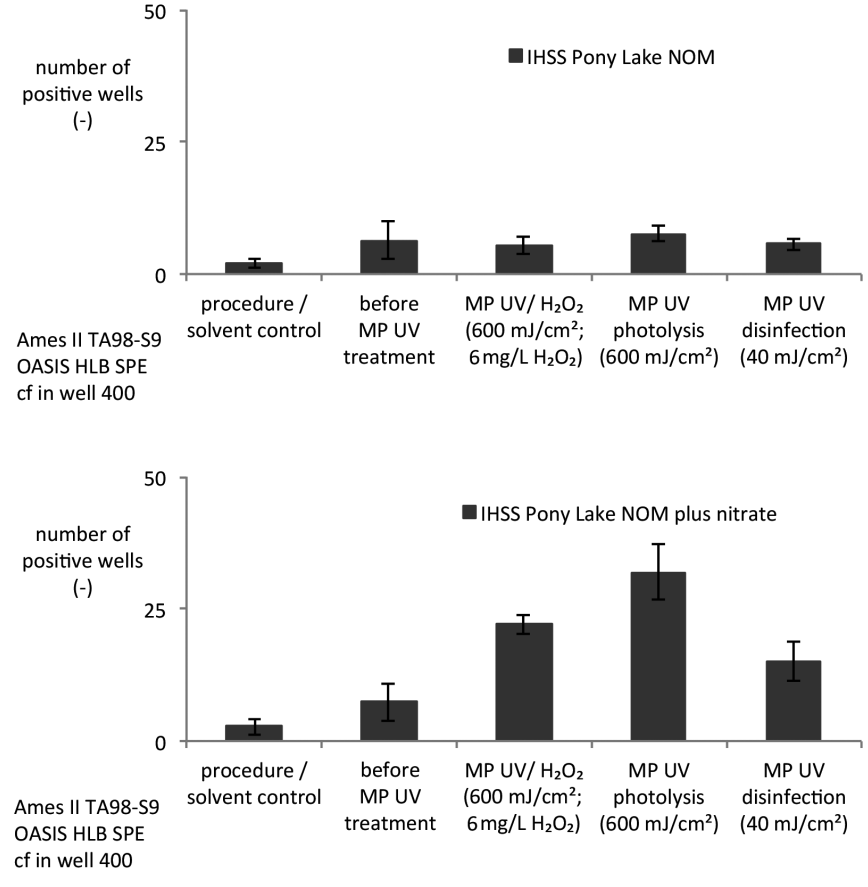


Figure 7.2: Ames test response in strain TA98-S9 before and after MP UV AOP treatment, MP UV photolysis and MP UV-disinfection of IHSS Pony Lake NOM (2.5 mg C/L) in the presence (2.71 mg NO_3/L) and absence of nitrate; concentration factor in the well: 400

In synthetic water with IHSS Pony Lake NOM only, the Ames test response was not affected by the MP UV based water treatment steps. However, in synthetic water containing both NOM and nitrate an increase in the Ames test response was observed already at a MP UV dose of 40 mJ/cm^2 applied for disinfection. Compared to MP UV disinfection, the Ames test response after MP UV photolysis was two times higher. MP UV AOP treatment generated a significantly lower response in the Ames test than MP UV photolysis, despite the fact that the same UV dose was applied.

7.3.2 Contribution of micropollutants and their metabolites to the Ames test response

The contribution of micropollutants and their metabolites to the Ames test response is not well documented in literature. Therefore, the impact of micropollutants representative for the catchment area of the IJssel Lake (Table 7.2) was investigated. The dosed levels of the priority compounds were representative of actual concentrations in the IJssel Lake. The total micropollutant concentration was 9.5 µg/L.

Table 7.2: Selection of relevant priority compounds, their actual concentration in the IJssel Lake and the concentration applied in MP UV/H_2O_2 bench scale experiments for Ames testing

Type of micropollutant	Compound	Actual concentration-*µg/L*	Experimental concentration-*µg/L*
Complexing agent	EDTA	5.22	5.0
Herbicide	Isoproturon AMPA Glyphosate	0.0115 0.252 Sporadic	0.1 0.5 0.5
Biocide	Carbendazim	0.0135	0.1
X-ray contrast medium	Amidotrizoic acid Iomeprol	0.227 0.282	0.5 0.5
Antibiotic	Sulphametoxazole hydrochlorotriazide	0.016 0.012	0.1 0.1
Endocrine disruptor	DEPH DBPH	0.309 0.608	0.5 0.5
Other	Caffeïne Carbamazepine Metformine	0.107 0.082 0.338	0.5 0.1 0.5

The Ames tests were performed on SPE samples of untreated and MP UV/H_2O_2 treated water of five synthetic water types: micropollutants only in demineralized water; IHSS Nordic Lake NOM (2.5 mg C/L) with and without micropollutants; and IHSS Nordic Lake NOM (2.5 mg C/L) plus nitrate (2.71 mg NO_3/L) with and without micropollutants. The results are presented in Figure 7.3.

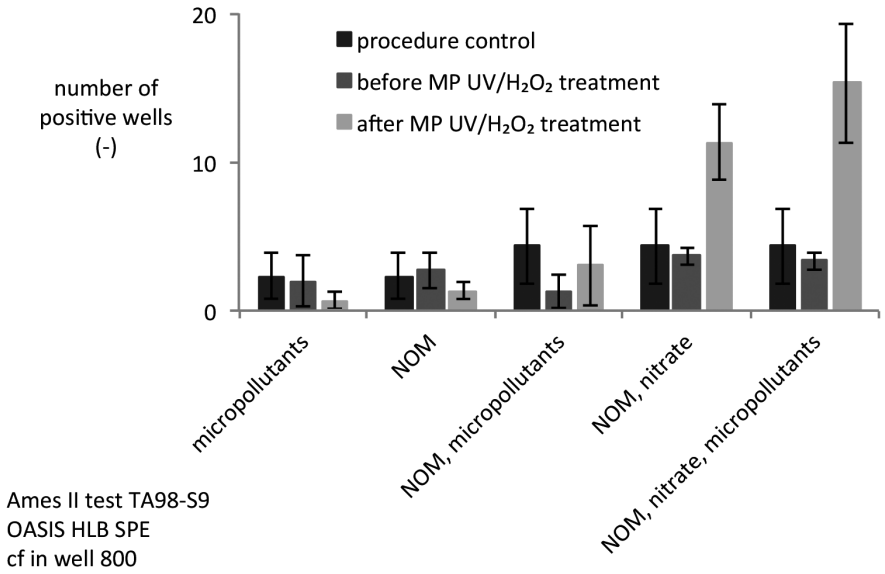
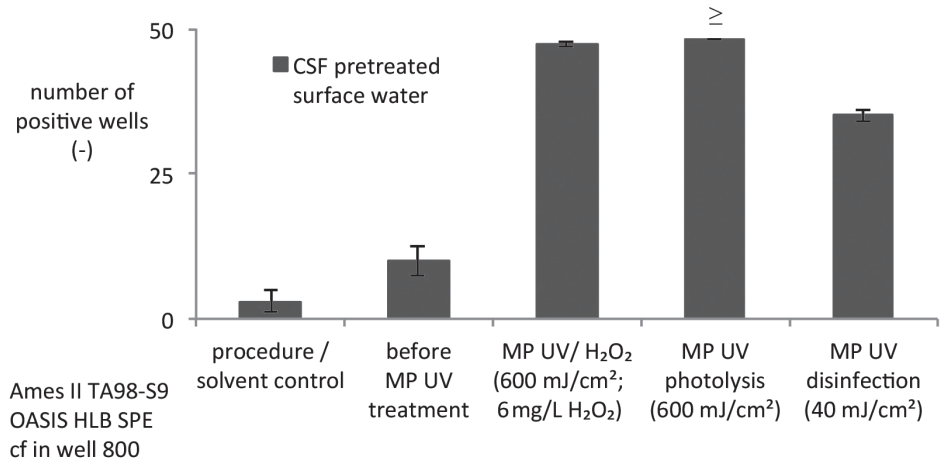


Figure 7.3: Ames test response in strain TA98-S9, of the procedure control and concentrated water samples before and after MP UV/H_2O_2 treatment (600 mJ/cm^2; 6 mg/L H_2O_2) of several combinations of a representative selection of micropollutants, IHSS Nordic Lake NOM and nitrate. Concentration factor in the well: 800

The Ames test response in the samples before MP UV AOP treatment did not differ from the procedure control (triplicate) (table 7.1), indicating that prior to MP UV AOP treatment neither NOM, nor nitrate nor the selection of organic micropollutants nor the combination of these pollutants generated a significant genotoxic response in the Ames test. In addition, MP UV AOP treatment of the water samples containing micropollutants, NOM and the combination of NOM with the selection of micropollutants did not cause a significant increase in Ames test response. However, when nitrate was present, MP UV AOP treatment of NOM generated genotoxic compounds, shown by a significant increase in the Ames test response. In nitrate rich water, the Ames test response of MP UV AOP treated water containing micropollutants and NOM did not differ significantly from the MP UV AOP treated water containing NOM only. Therefore, the Ames test response was not significantly impacted by the presence of this representative selection of organic micropollutants and /or their metabolites.

7.3.3 Natural water sources

Ames test responses were determined before and after MP UV treatment for a number of natural water sources: two types of pretreated surface water and two types of pretreated groundwater. Experiments were conducted using a MP UV collimated beam set up under the following conditions: UV disinfection (40 mJ/cm^2), UV photolysis (600 mJ/cm^2) and UV/H_2O_2 (600 mJ/cm^2 plus 6 mg/L H_2O_2).



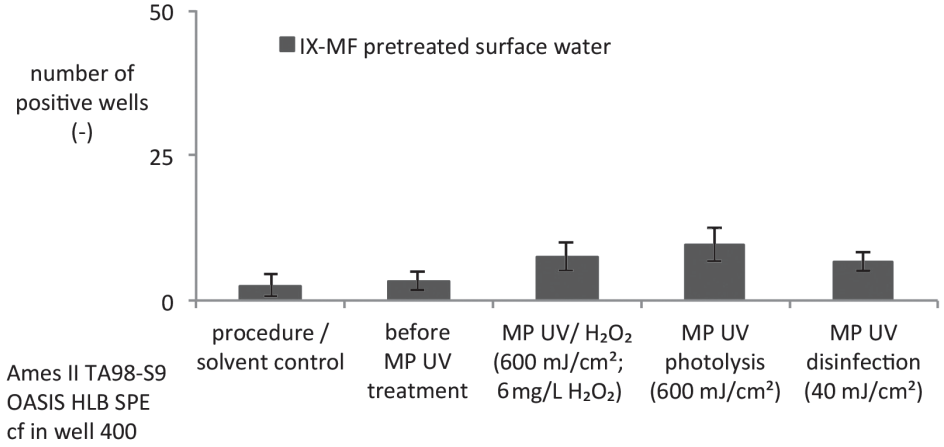


Figure 7.4: TA98-S9 Ames II test response before and after MP UV H_2O_2 treatment, MP UV photolysis and MP UV-disinfection of CSF pretreated IJssel Lake water (2.5 mg C/L; 2.62 mg NO_3/L); cf in the well: 800 and of IX-MF pretreated IJssel Lake water (1.7 mg C/L; 0.68 mg NO_3/L); cf in the well: 400

The Ames test response after the three types of MP UV treatment of pretreated surface water is presented in Figure 7.4. After all three UV treatments of CSF pretreated surface water, a significant increase in the Ames test response was observed. The response after MP UV photolysis was equal to or higher than the maximum test response (indicated by ≥ in Figure 7.4), making a quantitative comparison between the three MP UV treatments impossible. However, the increase after MP UV photolysis and MP UV AOP treatment was substantially higher than the increase caused by MP UV disinfection. In the IX-MF pretreated surface water with an improved DOC and additional nitrate removal, the Ames test response after MP UV AOP treatment, MP UV photolysis and MP UV disinfection did show a small increase relative to the Ames test response before UV treatment but this increase was not high enough to consider the samples as genotoxic.

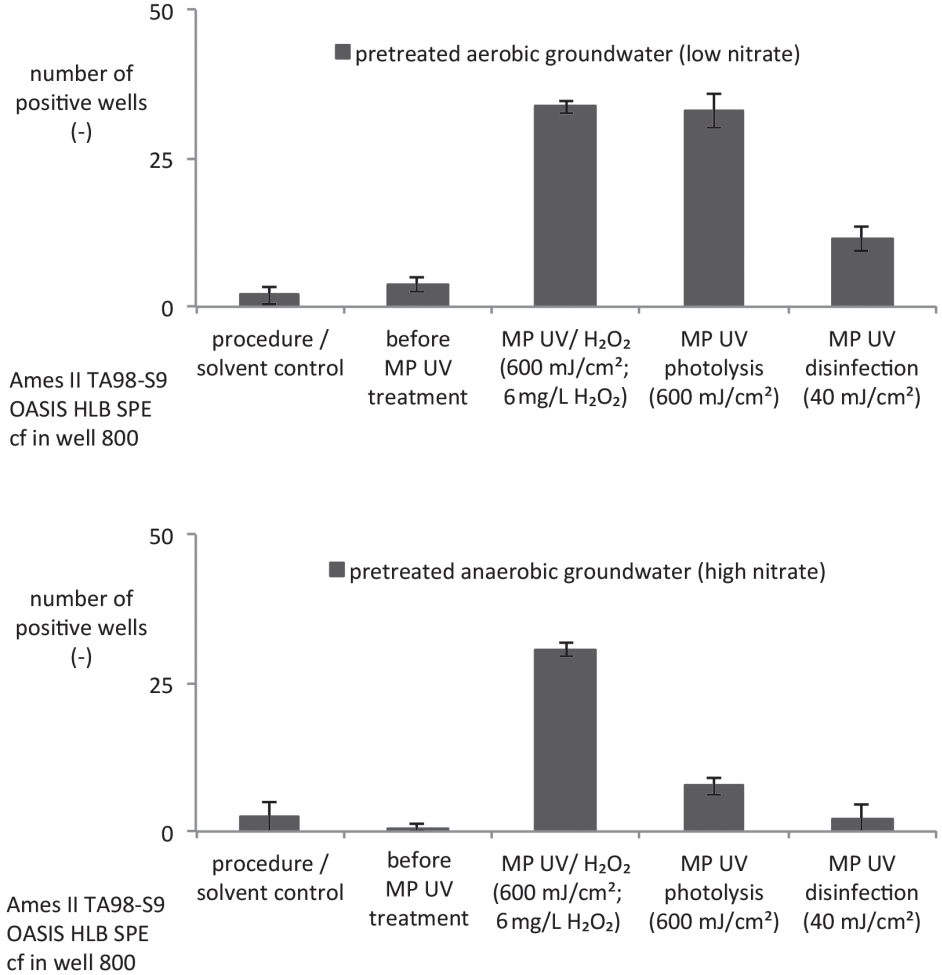


Figure 7.5: Ames test response in strain TA98-S9 before and after MP UV AOP treatment, MP UV photolysis and MP UV-disinfection of pretreated groundwater with a low nitrate content (1.3 mg C/L; 0.50 mg NO_3/L) and a high nitrate content (1.6 mg C/L; 5.17 mg NO_3/L); cf in the well: 800

MP UV AOP treatment and MP UV photolysis caused a strong increase in Ames test response for the aerobic groundwater after aeration and filtration with a low nitrate concentration (0.50 mg NO_3/L), while after MP UV disinfection a lower but still significant response was observed (Figure 7.5). No significant Ames test response was observed after MP UV disinfection of aerated and filtered anaerobic groundwater with a high nitrate concentration (5.17 mg NO_3/L). In this water type, the highest Ames test response was found after MP UV AOP treatment while unexpectedly MP UV photolysis showed a response that barely differed from the procedure control.

7.3.4 Impact of content and character of the water matrix on Ames test response

Figure 7.6 shows the relation between the nitrite formation and the Ames test response for two natural water sources after MP UV disinfection, MP UV AOP treatment and MP UV photolysis. Aerobic pretreated groundwater (1.3 mg C/L; 0.50 mg NO_3/L) and IX-MF pretreated surface water (1.7 mg C/L; 0.68 mg NO_3/L) with similar DOC content and nitrate concentration showed a similar nitrite formation. However, the Ames test response after MP UV irradiation of aerobic groundwater was substantially higher than the Ames test response in MP UV irradiated IX-MF pretreated surface water.

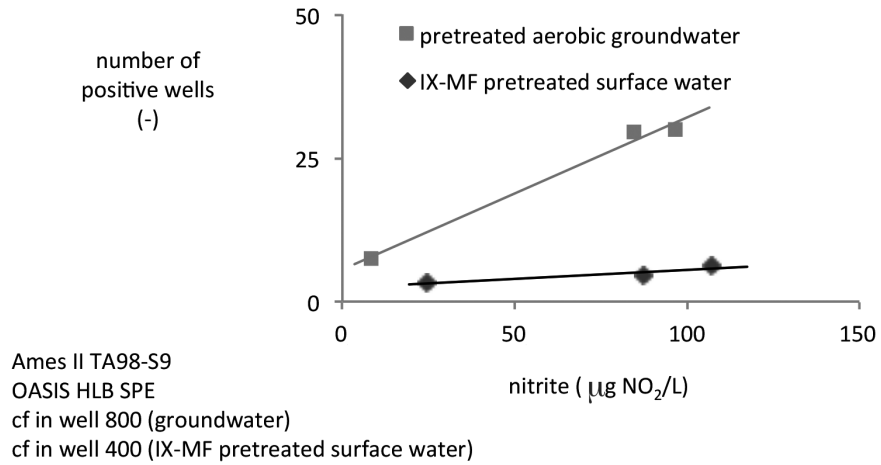


Figure 7.6: Nitrite formation and Ames II test response (TA98-S9) for two natural water sources: aerated / filtered aerobic groundwater (cf 800) and IX-MF pretreated surface water (cf 400) after MP UV AOP treatment, MP UV photolysis and MP UV-disinfection

The Ames test response seemed to be dependent on the characteristics of the NOM. TON, UV absorption, aromaticity (SUVA254) and LC-OCD analysis were measured for both water types. No significant differentiation was found for TON, UV absorption and LC-OCD while significant differences were found for SUVA254. The aerobic groundwater and the IX-MF pretreated surface water sample had a SUVA254 of 3.7 L mg^{-1} m^{-1} and 2.0 L mg^{-1} m^{-1}, respectively.

A mass balance for inorganic nitrogen, based on the sum of the nitrate and nitrite content, was determined for CSF pretreated IJssel Lake water before and after MP UV treatment (Figure 7.7A). Despite a significant Ames test response in the MP UV treated water, no significant nitrogen deficit was observed. Available analytical techniques proved insufficiently sensitive to detect any loss of inorganic nitrogen, indicating that only very low concentrations (likely in the nanogram per litre range) of nitrated genotoxic compounds may be formed. In a first broad screening research effort no nitrated organic reaction products were identified.

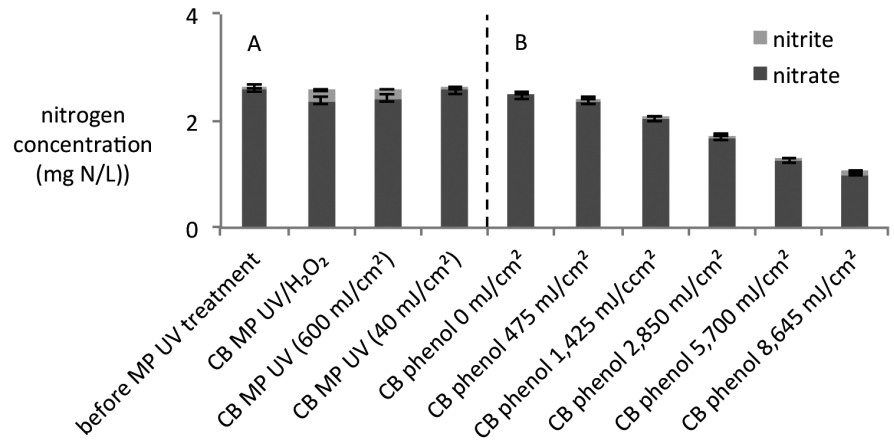


Figure 7.7: Nitrate and nitrite content before and after MP UV AOP treatment, MP UV photolysis and MP UV-disinfection of conventionally pretreated IJssel Lake water (A) and in synthetic water with phenol (0.25 mM) and nitrate (0.18 mM) (B) in collimated beam experiments (radiometer reading: 1.1 mW/cm^2)

However, experiments with the organic model compound phenol and nitrate showed the development of a large gap in the inorganic nitrogen balance (Figure 7.7B), indicating a strong incorporation of inorganic nitrogen in the organic matrix (phenol). In a second broad screening research effort with synthetic water containing IHSS Pony Lake NOM and ^{15}N nitrate, the formation of about one hundred mainly nitroaromatics in low concentrations was observed. This observation suggests that the Ames test response may be caused by the produced nitroaromatics.

7.3.5 Converting the Ames test response to a 4-NQO equivalent concentration

The measured Ames test response clearly indicated the presence of genotoxic compounds in concentrated (SPE) water samples after MP UV treatment under practical conditions. In order to quantify the measured genotoxic effect, the Ames test response was converted into a 4-NQO equivalent concentration.

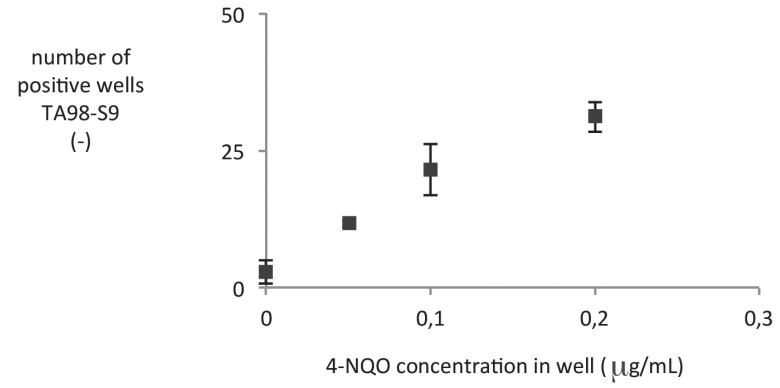


Figure 7.8: 4-NQO calibration curve for the Ames II test response (strain TA98-S9) as a function of the 4-NQO concentration

Figure 7.8 shows the calibration curve for the number of induced positive wells as a function of the 4-NQO concentration. Using the calibration curve, the measured Ames test responses of natural and synthetic water were converted into 4-NQO equivalent concentrations in the well. As an example, the conversion of the Ames test results for synthetic water with IHSS Pony Lake NOM (2.5 mg C/L) and nitrate (2.71 mg NO_3/L), using the applicable concentration factors (table 7.1) and 4-NQO calibration curve (figure 7.8) is illustrated in figure 7.9.

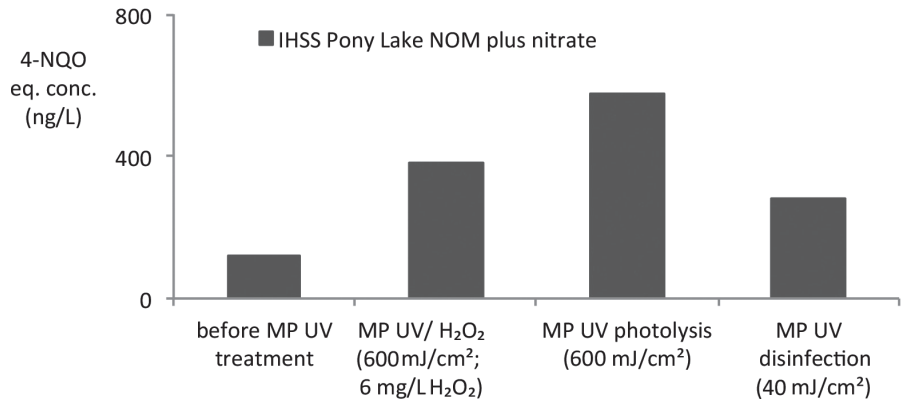


Figure 7.9: 4-NQO equivalent concentrations (ng/L) for MP UV treated synthetic water with IHSS Pony Lake NOM (2.5 mg C/L) and nitrate (2.71 mg NO_3/L) using the 4-NQO calibration curve (Figure 7.8) and the applied concentration factor (Table 7.1)

The Ames test responses for the natural water sources were converted into 4-NQO equivalent concentrations and presented in Table 7.3 in the discussion.

7.4 Discussion

7.4.1 Genotoxicity after MP UV treatment of nitrate rich water in the presence of NOM

The previously found Ames test responses after MP UV treatment of CSF pretreated surface water (Heringa et al., 2011; Martijn and Kruithof, 2012) and synthetic water with Nordic Lake NOM and nitrate (Martijn and Kruithof, 2012) were confirmed for synthetic water with IHSS Pony Lake NOM and nitrate. The Pony Lake NOM had a high nitrogen content, originating from algae, showing similarities with the organic matter in CSF pretreated IJssel Lake water and was therefore selected for the experiments with synthetic water. No genotoxic compounds were found after MP UV treatment when nitrate was absent, so the nitrogen already present in the IHSS Pony Lake NOM did not cause an Ames test response (figure 7.2). In the presence of nitrate, MP UV treatment of synthetic water with IHSS Pony Lake NOM showed a significant Ames test response already at a typical MP UV disinfection dose of 40 mJ/cm^2. The formation of an Ames test response proved to be UV dose dependent. After MP UV photolysis with a UV dose of 600 mJ/cm^2 the number of positive wells was roughly twice as high as after MP UV disinfection. However, after MP UV AOP with the same UV dose of 600 mJ/cm^2 combined with a H_2O_2 dose of 6 mg/L, a significantly lower Ames test response was observed than after MP UV photolysis (figure 7.2). For this water type, this suggests a modification of the NOM by MP UV AOP treatment, causing a decrease of the number of positive wells compared to MP UV photolysis. This is a first indication that, besides nitrate and NOM content, the NOM character has also an impact on the Ames test response.

7.4.2 Effect of low concentrations of organic micropollutants on the Ames test response

MP UV AOP treatment for the degradation of organic micropollutants, causes the formation of metabolites. Although advanced oxidation processes for organic contaminant control should be applied at process conditions where only harmless, biodegradable reaction products are formed, much research has been focused on the formation of potentially harmful reaction products (Esplugas et al., 2007; Shemer and Linden, 2007; Escher and Fenner, 2011). The research described in this chapter shows that in the absence of nitrate, water samples containing micropollutants and/or NOM did not show an Ames test response after MP UV AOP treatment (figure 7.3). In the presence of

nitrate, micropollutants, metabolites and nitrated reaction products originating from the micropollutants, did not give a significant increase compared to the Ames test response after MP UV AOP treatment of NOM only. This observation shows that under the applied MP UV AOP conditions (UV dose: 600 mJ/cm^2, H_2O_2 dose: 6 mg/L) the produced metabolites and nitrated reaction products do not significantly contribute to the Ames test response (figure 7.3). Nitrated reaction products from the micropollutants may have genotoxic properties but the produced quantities are very low so that no contribution to the Ames test response was observed. Therefore genotoxic compounds were primarily produced from NOM in nitrate rich water.

7.4.3 Role of water constituents on the Ames test response after MP UV treatment

To further study the impact of the water constituents, MP UV treatment of four different natural water sources was tested: two pretreated surface waters and two pretreated groundwaters. Surface water originating from the same source and pretreatment consisting of CSF, resulted in a relatively high nitrate and DOC content, while after IX-MF pretreatment reduced levels of DOC and nitrate were observed. The groundwaters originating from an aerobic and an anaerobic source, resulted in respectively a low and a high nitrate content while the DOC concentration was similar. After MP UV treatment of CSF pretreated surface water the Ames test responses exceeded the observed responses in nitrate containing synthetic water, despite similar levels of DOC and nitrate (figure 7.2, figure 7.4). The difference in applied sample concentration factor, 800 for the CSF pretreated surface water samples and 400 for synthetic water containing Pony Lake NOM and nitrate, was the main cause for this difference. When the impact of the concentration factor was ruled out, the caused effect in MP UV treated CSF pretreated surface water and in synthetic water was similar. Applying the same concentration factor of 400, the Ames test response in MP UV treated IX-MF pretreated surface water was substantially lower than the response observed in synthetic water , caused by the lower nitrate and DOC content (figure 7.2, figure 7.4). These results confirm the impact of the DOC content and especially the nitrate concentration on the Ames test response. MP UV treated pretreated aerobic groundwater roughly showed the same trend in the generated Ames test response as MP UV treated surface water. Once again, already for MP UV disinfection conditions a significant Ames test response was observed (figure 7.5). Highest responses were found after MP UV photolysis and MP UV AOP treatment. Compared to MP UV treatment of IX-MF pretreated surface water, with roughly the same DOC and nitrate content in pretreated aerobic groundwater, the Ames test response was significantly higher, accentuating the part played by the character of the NOM. Completely different Ames test responses were observed after MP UV treatment of pretreated anaerobic groundwater. Although the nitrate content of the water was 5.17 mg NO_3/L the Ames test response after MP UV disinfection was negligible, while the response after MP UV photolysis was hardly significant. Only after MP UV AOP treatment a significant Ames test response was observed. It seems that the organic water matrix from anaerobic groundwater must be modified by MP UV AOP treatment before a significant response in the Ames test was achieved, once again accentuating the part played by the character of the NOM. IX-MF pretreated surface water and pretreated aerobic groundwater roughly had the same nitrate and DOC content (figure 7.6). Nevertheless after MP UV treatment, the relationship between nitrite formation and Ames test response differed strongly. Much higher Ames test responses were observed for the aerobic pretreated groundwater. This once again suggests that in addition to the nitrate and DOC content the character of the NOM was of importance for the level of the Ames test response after MP UV treatment. Therefore the character of the NOM was determined by parameters such as LC-OCD, TON, UV absorbance and SUVA254. No differentiation between the water matrices was found for the parameters LC-OCD, TON and UV absorbance. Significant differences in SUVA254 were observed between the different water matrices. Pretreated aerobic groundwater had a SUVA254 of 3.7 L mg^{-1} m^{-1}, while IX-MF pretreated surface water had a SUVA254 of 2.0 L mg^{-1} m^{-1}. The SUVA254 is a measure of the aromaticity. The results show that the NOM fraction of the pretreated aerobic groundwater had the highest aromaticity. This high aromaticity may be the cause for the formation of a high Ames test response, because especially aromatic structures are susceptible for nitration.

7.4.4 Formation of nitrated organics

It was hypothesized that the Ames test response induced by MP UV treatment was initiated by the photolysis of nitrate in the presence of an organic water matrix. The formed intermediates such as nitroradicals should react with the organic water matrix producing nitrated organics. Machado and Boule (Machado and Boule, 1995) and Vione et al. (Vione et al., 2004) showed that especially aromatic organics are susceptible for nitration. Reckhow et al. proposed a mechanism that photo nitration causes formation of new nitro organics during UV treatment (Reckhow et al., 2010). Thorn and Cox (Thorn and Cox, 2012) showed the same for the aromatic fraction of NOM. These observations support the impact of the aromaticity of the organics on the formation of nitrated compounds. Our results showed the highest Ames test responses in water with the highest aromaticity such as synthetic water with IHSS Pony Lake NOM and nitrate and CSF pretreated surface water with an aromaticity of 3.6 and 3.2 L mg^{-1} m^{-1}, respectively. By MP UV AOP treatment the aromaticity is lowered to about 2.7 L mg^{-1} m^{-1}. This may explain the lower Ames test response in MP UV AOP treated water in comparison with water after MP UV photolysis. Nitration of the organic water matrix should result in a wide range of nitroaromatics in low

concentrations. This formation should cause a deficit in the inorganic nitrogen balance. In our experiments this gap was not observed, while in a first broad screening research effort no nitroaromatics were found after MP UV treatment of CSF pretreated IJssel Lake water. However, when the organic water matrix was replaced by phenol a large gap in the nitrogen balance was observed and formation of a number of nitrophenols was found (Martijn et al., 2014). In a second broad screening research effort with synthetic water containing IHSS Pony Lake NOM and ^{15}N nitrate the formation of over one hundred nitrated organic compounds in low concentrations was observed (Vughs, 2014; Kolkman et al., 2015). Our research effort shows that MP UV treatment of nitrate rich water causes the formation of nitrated compounds and especially nitroaromatics. The character of the water matrix, especially the aromaticity, seems to have an impact on the formation of these nitroaromatics.

7.4.5 Risk identification

Formation of nitrated aromatic compounds initiated by MP UV photolysis of nitrate may well be the cause for the observed Ames test response since nitroaromatics are well known genotoxic compounds (Rosenkranz and Mermelstein, 1985). Wollin and Dieter (2005) derived for a number of nitroaromatics health based drinking water guidelines. For these genotoxic substances estimates of excess lifetime cancer risk were applied. For instance, for 2,6 dinitrotoluene a toxicologically based drinking water guideline value of 50 ng/L (0.27 nM) was derived for an additional 5.86 $x10^{-6}$ cancer risk over a life span of 70 years. To convert a response in the Ames test measured in concentrated water samples into an equivalent concentration of a known genotoxic compound, 4-NQO, was selected as reference compound (figure 7.8). 4-NQO has an aromatic structure, contains a nitrogroup and is widely used as a positive control in the Ames test. Furthermore, 4-NQO has similar mutagenic behaviour towards bacteria as UV radiation (Kondo et al., 1970; Ikenaga et al., 1975), making this a relevant reference compound for MP UV induced genotoxicity. The 4-NQO equivalent concentrations after MP UV AOP, photolysis and disinfection of the four natural water sources are presented in table 7.3. In general, the highest 4-NQO equivalent concentrations were found after MP UV photolysis of all water types. Relative to the applied UV dose, CSF pretreated surface water and IX-MF pretreated surface water showed a high 4-NQO equivalent concentration for MP UV disinfection conditions. The 4-NQO equivalent concentration for the anaerobic groundwater showed a different response.

Table 7.3: 4-NQO equivalent concentrations after MP UV AOP, MP UV photolysis and MP UV disinfection of four natural water sources

Practical water matrix	**MP UV/H_2O_2 treatment** *4-NQO eq. concentration (ng/L)*	**MP UV photolysis** *4-NQO eq. concentration (ng/L)*	**MP UV disinfection** *4-NQO eq. concentration (ng/L)*
CSF pretreated surface water	304	>307	221
IX-MF pretreated surface water	135	161	115
Aerobic groundwater (low nitrate)	213	211	73
Anaerobic groundwater (high nitrate)	196	49	13

To assess the relevance of the 4-NQO equivalent concentration levels as presented in Table 7.3, the Threshold of Toxicological Concern (TTC) methodology, proposed by the International Life Sciences Institute (Barlow, 2005), and the Margin of Exposure approach (EFSA, 2012) were applied. The TTC derived decision tree (Kroes et al., 2000) applies a threshold of 0.15 µg/L per person per day intake of genotoxic compounds before a compound specific risk assessment should be performed. Assuming a daily consumption of 2 litres drinking water leads to 75 ng/L as a threshold concentration for unknown genotoxic compounds. This threshold is derived from the carcinogenic potency database collected by Cheeseman et al. (Cheeseman et al., 1999), based on structural alerts. The work by Cheeseman resulted in five groups of compounds from which a number of chemicals still may be of concern at an intake of 0.15 µg/person/day. One of these groups are the nitroso-compounds that may be formed by MP UV treatment of nitrate rich water containing organic matter, making the TTC methodology less suitable for a preliminary risk assessment in this situation. In chapter 6, based on the MOE approach, an EDI of a 4-NQO equivalent concentration of 160 ng/person/day was derived from tumour data (Tang et al., 2004) and BMD software version 2.6.

The level of no concern based on the TTC methodology and the MOE approach result in a similar concentration, 75 ng 4-NQO eq./L and 80 ng 4-NQO eq./L respectively. Relating these values to the obtained 4-NQO equivalent concentrations after MP UV water treatment of nitrate rich water, Table 7.3 clearly illustrates that a compound specific risk analysis is necessary before MP UV treatment can be applied to these types of waters without post treatment, for example GAC filtration, to remove these compounds (Martijn and Kruithof, 2012). Based on a preliminary risk assessment, it is clear that already at doses applied for MP UV disinfection the equivalent concentration of formed genotoxic compounds in nitrate rich water, exceeded the level of no concern.

Chapter 8 Discussion

8.1 Introduction

In this chapter an overview and discussion of the main findings of the research described in this thesis is presented, followed by perspectives and suggestions for future research.

In advanced drinking water treatment, organic micropollutant control is a major issue (Glaze et al., 1987). The presence of organic micropollutants such as pesticides, endocrine disruptors and pharmaceuticals in surface water sources for drinking water production (Pieters et al., 2004; Houtman, 2010), requires implementation of multiple barriers for organic contaminant control, for instance a combination of advanced oxidation treatment followed by granular activated carbon (GAC) filtration. The effect of advanced oxidation processes is based on the formation and reaction of hydroxyl radicals. Hydroxyl radicals are rather non selective agents, reacting with most organic compounds, including natural organic matter (NOM), by adding to aromatic or unsaturated structures or by abstracting hydrogen atoms. In principle a complete mineralization can be achieved by advanced oxidation (Stefan and Bolton, 1996). However, under economically feasible conditions this does not take place so reaction products are formed. Two commonly applied advanced oxidation technologies are ozone / hydrogen peroxide (O_3/H_2O_2) treatment and ultra violet/hydrogen peroxide (UV/H_2O_2) treatment. In the nineties of the last century O_3/H_2O_2 treatment was pursued. After extensive research, in The Netherlands application of this type of advanced oxidation was rejected because of the formation of bromate, a suspect human carcinogen (Kurokawa, 1990), which could not be avoided (completely) (Von Gunten and Hoigné, 1994). At the present state-of-the-art two types UV based advanced oxidation are commonly applied in practice: low pressure ultra violet (LP UV)/H_2O_2 treatment and medium pressure ultra violet (MP UV)/H_2O_2 treatment (Bolton, 2010). In the research effort described in this thesis the focus was completely on the application of MP UV/H_2O_2 treatment. The aim of this research was to determine the impact of both the organic and inorganic water matrix on the efficacy of MP UV/H_2O_2 treatment for the removal of organic micropollutants and on the formation of reaction products with a possible health concern (Schriks and Heringa, 2010). In water treatment practice, the Ames test has been used for a long time, screening water samples for the presence of genotoxic compounds (Ames, 1972). In the research described in this thesis, the concentrations of unknown genotoxic compounds causing a measurable response were converted into an equivalent concentration of a genotoxic reference compound using a toxic equivalency factor approach to enable a preliminary risk assessment.

8.2 MP UV/H_2O_2 treatment for organic contaminant control

Chapter 2 of the thesis describes the research into the feasibility of MP UV/H_2O_2 treatment for organic contaminant control. In a standard pilot MP UV reactor, developed for disinfection purposes, experiments were carried out to investigate the degradation of a representative selection of pesticides. It was observed that MP UV photolysis, without H_2O_2 addition, already resulted in a substantial degradation of herbicides such as atrazine and diuron, while compounds such as bentazone and bromacil primarily were oxidized by hydroxyl radicals, produced by combined application of MP UV and H_2O_2. Addition of H_2O_2 increased the decay rate of all selected herbicides. Based on computational fluid dynamics, irradiance profiles and kinetic modelling, an optimized MP UV pilot reactor for advanced oxidation was developed. The optimized MP UV reactor achieved an improvement of the electric energy consumption by circa 50% compared to the standard reactor for disinfection purposes. At process conditions 0.56 kWh/m³ and 6 mg/L H_2O_2, more than 80% degradation was achieved for most pesticides, commonly found in surface water. A similar degradation was observed for most pharmaceuticals, endocrine disrupting compounds, algae toxins and industrial compounds. The combination of MP UV photodegradation and oxidation by OH radicals produced by MP UV photolysis of H_2O_2 proved to be an effective and non selective barrier for these types of organic micropollutants. Based on the results of this research effort, two full scale MP UV/H_2O_2 installations were designed and implemented. For atrazine, a good agreement was observed between the achieved degradation, the degradation calculated by the developed model and the degradation predicted by the installed monitoring system. The system has proven to provide a robust and reliable barrier against organic micropollutants such as pesticides and pharmaceuticals. However the economic feasibility would increase significantly with a further reduction of the energy consumption, making MP UV/H_2O_2 treatment even more attractive to solve a wide range of water treatment problems.

8.3 Impact of the water matrix on the efficacy of MP UV/H_2O_2 treatment

Chapter 3 describes the impact of the water matrix on the efficiency of MP UV/H_2O_2 treatment. Advanced pretreatment plays an important role to achieve the goal of reduced energy consumption. A significant reduction of the energy consumption of MP UV/H_2O_2 treatment is achieved by removing UV absorbing constituents in the pretreatment, thereby reducing the competition for UV light. In addition, the removal of these compounds reduces the scavenging of OH-radicals, produced by the photolysis of H_2O_2. Major constituents of the water matrix influencing the efficacy of the MP UV/H_2O_2 process are NOM and nitrate. Nitrate absorbs MP UV light at the same wavelength as H_2O_2 and has a higher molar absorption, causing competition for photons. NOM is also a strong UV absorber and is the most important OH-radical scavenger. Replacing conventional pretreatment with Coagulation, Sedimentation and rapid sand Filtration (CSF) by advanced pretreatment, based on Ion eXchange followed by MicroFiltration (IX-MF) caused an improved removal of NOM and nitrate. Nevertheless even after IX-MF pretreatment more than 80% of the photon flow is absorbed by the water matrix with a dominant effect of NOM. MP UV collimated beam experiments with nitrosodimethylamine (NDMA) and 1,4-dioxane were carried out to determine the impact of advanced pretreatment on the degradation by MP UV photolysis and hydroxyl radical oxidation. Replacing CSF by IX-MF caused a decrease of the electric energy per order (EE/O) for the photolytic degradation of NDMA from 1.2 kWh/m³ to 0.4 kWh/m³, while for the OH radical oxidation of 1,4-dioxane the EE/O dropped from 3.0 kW/m³ to 1.4 kWh/m³ when 5 mg/L H_2O_2 was dosed. So a significant reduction of the energy consumption was achieved by replacing CSF by advanced pretreatment with IX-MF, although still only a maximum of 20% of the photon flow was utilized by the target compounds NDMA and H_2O_2. Therefore further optimization of the pretreatment should be pursued.

8.4 Side effects MP UV/H_2O_2 treatment

Chapter 4 describes the reaction product formation of oxidation / disinfection technologies in water treatment. Reaction products are primarily formed by reaction with both the organic and inorganic water matrix. Disinfection byproducts from chlorination such as trihalomethanes, haloacetic acids, nitrogeneous disinfection byproducts and their health impacts were extensively studied since the 1970's. Formation of the suspect human carcinogen bromate by ozonation of bromide containing water types for drinking water production is currently limiting the applicability of ozone based processes, both for disinfection and advanced oxidation. At the start of this research, for MP UV treatment, only the formation of nitrite in nitrate rich water was reported. Applying MP UV/H_2O_2 treatment with natural quartz sleeves with a wavelength cut off at 240 nm, no Ames test response was observed in concentrated water samples. When the natural quartz sleeves were replaced by synthetic quartz sleeves, enabling utilization of low wavelength (200-240 nm) UV light, formation of genotoxic compounds by MP UV treatment of nitrate rich water was observed. It was hypothesized that the increase in the Ames test response was caused by nitrated organic compounds, formed via MP UV nitrate photolysis in the presence of NOM. This was confirmed in MP UV/H_2O_2 experiments with reconstituted water containing NOM in the presence and absence of nitrate.

8.5 Mechanism of reaction product formation by MP UV/H_2O_2 treatment

The mechanism of the reaction product formation upon MP UV based treatment is elucidated in chapter 5. Formation of nitrite by MP UV based processes in nitrate rich water was reported in literature. The MP UV emission spectrum has a substantial emission in the wavelengths (<240 nm) where nitrate has a high molar absorption coefficient. Especially at these emitted lower wavelengths, MP UV photolysis causes a strong nitrite formation (Mack and Bolton, 1999). MP UV photolysis of nitrate produces nitroso-, nitro- and hydroxyl radicals as intermediates. These radicals cause formation of nitrite and nitrogeneous reaction products by interaction with organic compounds. In synthetic, nitrate rich water an Ames test response was observed after MP UV disinfection, MP UV photolysis and MP UV/H_2O_2 treatment. Applying MP UV based treatment, formation of nitrated aromatic compounds was most probably the cause of the observed Ames test response since nitroaromatics are well-known genotoxic compounds. MP UV treatment of CSF pretreated water resulted in a small but not significant decrease of the inorganic nitrogen content. To confirm the possible incorporation of inorganic nitrogen in the organic water matrix, MP UV photolysis collimated beam experiments were conducted with organic model compound phenol and nitrate in demineralized water. The initial inorganic nitrogen content decreased strongly with the increase of the MP UV dose, indicating formation of nitrated organic compounds. From a range of hydroxylated and nitrated reaction products detected in the model compound experiments, 2-nitrophenol, 4-nitrophenol and 4-nitrocatechol were identified. In both pretreated surface water and in synthetic water genotoxic nitrated compounds were not identified in this research effort.

8.6 Preliminary risk assessment side effects MP UV/H_2O_2 treatment

The development of an approach to enable a preliminary risk assessment of unknown genotoxic compounds is described in chapter 6. Since the identity and concentration of specific genotoxic compounds were not established yet, a compound specific risk assessment could not be performed. Similar to the approach proposed by Fassbender et al. (Fassbender et al., 2012) this limitation was circumvented by introducing a toxic equivalency factor (TEF) approach. Herewith the concentration of unknown compounds in concentrated water samples, causing a positive Ames test response, were converted into an equivalent concentration of a selected genotoxic compound, 4-nitroquinoline-1-oxide (4-NQO). This facilitates a preliminary risk assessment. Based on the obtained 4-NQO equivalent concentrations for the tested water samples and 4-NQO carcinogenicity data, an indication of the associated risk of the by MP UV/H_2O_2 produced nitrated genotoxic compounds was obtained via the margin of exposure (MOE) approach. For a negligible risk, the MOE should be > 10,000. Based on a carcinogenicity study of 4-NQO reported by Tang et al., (2004) and a body weight of 70 kg for a person, the estimated daily intake (EDI) associated with a negligible risk should not exceed 160 ng/person/day. Assuming a drinking water consumption of 2 litres per person per day (U.S. EPA, 2004), the 4-NQO equivalent concentration should not exceed 80 ng/L in order to maintain an MOE > 10,000. Application of this approach on samples from MP UV/H_2O_2 treated water from a full scale drinking water production facility, resulted in a 4-NQO equivalent concentration of 107 ng/L. These results from a full scale drinking water treatment plant suggest that a safety concern could exist in case this water would be distributed as drinking water without post treatment.

8.7 Genotoxicity after MP UV based treatment of natural water matrices

The induced genotoxicity by MP UV based treatment, MP UV disinfection, photolysis and advanced oxidation, in a number of surface waters and ground waters is described in chapter 7. Herewith the methodology for a preliminary risk assessment described in chapter 6 is applied. To investigate the potential hazardous effect of micropollutants present in natural water types, the Ames test response after MP UV/H_2O_2 treatment of a representative selection and concentration of micropollutants present in the tested surface water types, was determined. In the presence of nitrate, metabolites and nitrated reaction products produced by MP UV/H_2O_2 treatment of micropollutants did not give a significant contribution to the Ames test response. Therefore genotoxic compounds were primarily produced from NOM in nitrate rich water, confirming the findings of chapter 4.

For a selection of natural waters containing NOM and nitrate, the Ames test responses due to MP UV based treatment were converted into 4-NQO equivalent concentrations. The selected water types were two pretreated surface waters and two pretreated ground waters. Surface water originating from the same source, resulted in a relatively high nitrate and DOC content after CSF pretreatment, while reduced levels of DOC and nitrate were observed after IX-MF pretreatment. The ground waters originating from an aerobic and an anaerobic source, resulted in respectively a low and a high nitrate content while the DOC concentration was similar. In addition to MP UV/H_2O_2 treatment, MP UV disinfection using a low UV dose and MP UV photolysis using the same UV dose as applied for MP UV/H_2O_2 treatment but without H_2O_2 dosage, were tested in a bench scale set-up. Different relationships between Ames test response and nitrite concentration were found for the four water types. In general the Ames test response became higher with increasing nitrate concentration and aromaticity of the organic matter. Table 7.3 presents the obtained

4-NQO equivalent concentrations for the four selected natural water types after MP UV treatments, indicating that already for MP UV disinfection of pretreated surface water the level of no concern was exceeded. Using the risk assessment based on the MOE approach, the 4-NQO equivalent concentration should not exceed 80 ng/L, assuming a water consumption of 2L per day by a person with a body weight of 70 kg. Three of the four MP UV treated water types resulted in 4-NQO equivalent concentrations, substantially exceeding this value representing the level of no concern. Therefore, the observed 4-NQO equivalent concentrations indicate the need for post MP UV treated water and urge for a compounds specific risk assessment.

8.8 Perspectives

An important finding of the research described in this thesis is that MP UV/H_2O_2 treatment has proven to be an effective, non selective barrier for organic micropollutants such as herbicides, pharmaceuticals, endocrine disrupting compounds, algae toxins etc. Although the required energy consumption by MP UV/H_2O_2 treatment is substantial, the process has proven to be feasible for NDMA degradation (Plumlee et al., 2008) and taste and odour removal (Scheideler et al., 2015). However, not all classes of organic micropollutants are degraded by MP UV/H_2O_2 treatment. The class of perfluorated compounds are degraded by neither MP UV photolysis nor hydroxyl radical oxidation. Therefore for the removal / degradation of this type of micropollutants multiple barriers are required i.e., a combination of MP UV/H_2O_2 treatment and GAC filtration.

Reduction of the energy consumption should make MP UV/H_2O_2 treatment more attractive to solve a wide range of organic contaminant control problems. For that reason, natural quartz sleeves were replaced by synthetic quartz sleeves, enabling utilization of low wavelength UV light. Hereby a 20% reduction of the energy consumption was achieved. However low wavelength UV light caused a significant increase in nitrite formation and Ames test response. This makes this option less attractive especially when MP UV is applied as a final treatment step. Replacing CSF by IX-MF pretreatment increased the fraction of the UV light absorbed by 6 mg/L H_2O_2 from circa 5% to 20%. Still about 80% of the photon flow was absorbed by the water matrix. Further improvement of the pretreatment should be pursued i.e. by increasing the removal of NOM and nitrate by adjusting the IX-MF conditions.

In this thesis the Ames II test (Flückiger et al., 2004) was applied as a screening method to detect genotoxicity. A TEF approach was developed to convert the concentration of unknown genotoxic compounds in samples, resulting in a positive Ames test response, into an equivalent concentration of a genotoxic compound. In parallel research not described in this thesis, in vivo genotoxicity tests in Eastern mudminnow fish (*Umbra pygmaea*) using a Sister Chromatid Exchange (SCE) and a Comet assay on isolated gill cells were performed on full scale MP UV/H_2O_2 treated surface water (Penders et al., 2012). No significant increases in SCEs were observed, but gill cells isolated from fish exposed to water sampled immediately after MP UV/H_2O_2 treatment showed significantly increased DNA damage in the Comet assay. So the Ames II test results described in this thesis were confirmed by DNA damage observed in the Comet assay. Further research with alternative effect-based screening tools and chemical analytical methods should be pursued for a better characterisation of the observed genotoxic effect.

In the research described in this thesis no identification of the nitrogeneous reaction products formed upon MP UV/H_2O_2 treatment of surface water after CSF pretreatment was realized. In follow up research, identification of nitrated reaction products was further pursued in reconstituted water containing NOM and nitrate, using ^{15}N labelled nitrate (Kolkman et al. 2015). Application of this method showed that nitrogen originating from nitrate is indeed incorporated in nitrogeneous reaction products resulting in detection of 84 nitrogen containing organic reaction products. The summed equivalent concentration of these compounds was about 1.2 µg bentazon eq./L and 69 ng atrazine eq./L for negative and positive ionization in the LC-Orbitrap method, respectively. However, by comparison with commercially available standards, only 4-nitrophenol, 4-nitrocatechol and 2-methoxy-4,6-dinitrophenol could be identified. No genotoxic compounds were identified yet. The research should be continued to identify nitrated reaction products focussing on the formation of genotoxic compounds to enable a compound specific risk assessment. In addition, analysis of precursors for genotoxic compounds in the organic water matrix should be pursued to enable identification of 'risk matrices' (Lee et al., 2013, Chon et al., 2013).

For a better perspective it is relevant to benchmark the level of concern derived from MOE results for MP UV based treatment based on the TEF approach as presented in chapter 6. For example the results could be compared to an MOE based evaluation of the level of bromate formation upon ozonation of bromide containing water.

Table 8.1: Data used for the BMD modelling consisting of the incidence of dysplastic foci, kidney carcinoma, thyroid carcinoma and mesothelioma in male F244 rats from the use (ad libitum) of $KBrO_3$ in drinking water (Kurokawa et al., 1986)

Number of animals (-)	Duration of treatment (months)	$KBrO_3$ dose (mg/kg bw/ day)	Incidence dysplastic foci (-)	Incidence kidney carcinoma (-)	Incidence thyroid carcinoma (-)	Incidence mesothelioma (-)
19	24	0	0	0	0	0
19	24	0.7	1	0	0	0
20	24	1.3	5	0	0	3
24	24	2.5	6	1	1	4
24	24	5.6	12	5	0	2
20	24	12.3	19	5	3	3
20	24	33	19	9	n/a	n/a

In the study by Kurokawa (Kurokawa et al., 1986), an overview of bromate carcinogenicity data is given resulting from animal tests treating 22-24 male F344 rats with different concentrations of $KBrO_3$ administered in drinking water for two years. Although the interpretation of the health impact of bromate formation in drinking water treatment has evolved, incorporating metabolism in the body (Cotruvo et al., 2012), the tumour study by Kurokawa (Kurokawa et al., 1986) was used to determine a $BMDL_{10}$ for tumour formation by bromate and perform a subsequent MOE based risk assessment of the levels of bromate formation reported upon ozonation of bromide containing water.

The dataset adapted from Kurokawa (Kurokawa et al., 1986), providing six different doses tested against a control, meeting the criteria for U.S. EPA BMD modelling. The U.S. EPA BMD Analysis Framework (Davis et al., 2011) was followed, resulting in a number of good fitting models for incidence of dysplastic foci, kidney carcinoma, thyroid carcinoma and mesothelioma, providing a set of $BMDL_{10}$ values for each of the types of incidences. The qualifying models and the associated $BMDL_{10}$ values per type of incidence are summarized in table 8.2. The lowest $BMDL_{10}$ value was obtained for dysplastic foci and therefore presented in detail in table 8.3 and figure 8.1.

Table 8.2: BMD analysis with lowest $BMDL_{10}$ values for the incidence of dysplastic foci, kidney carcinoma, mesothelioma and thyroid carcinoma in F344 male rats upon exposure to different $KBrO_3$ concentrations via drinking water for 24 months. The data are analysed using BMDS software version 2.6, a BMD of 10% extra risk and default settings. The data from Kurokawa (Kurokawa et al., 1986) (Table 8.1) are used as input for the BMD analysis.

Incidence type	Model	No. of param.	p-value	Accepted	BMD (mg/kg bw /day)	$BMDL_{10}$ (mg/kg bw /day)
Dysplastic foci	**Log Logistic**	**2**	**0.374**	**Yes**	**0.988**	**0.568**
Kidney carcinoma	LogProbit	2	0.834	Yes	4.441	2.425
Mesothelioma	Quantal-linear	2	0.041	Yes	3.452	2.37
Thyroid carcinoma	Quantal-linear	1	0.875	Yes	9.55	6.03

Table 8.3: BMD analysis for the incidence of dysplastic foci in F344 male rats upon exposure to different $KBrO_3$ concentrations via drinking water for 24 months. The data are analysed using BMDS software version 2.6, a BMD of 10% extra risk and default settings. The data from Kurokawa (Kurokawa et al., 1986) (Table 8.1) are used as input for the BMD analysis.

Model	No. of parameters	p-value	Accepted	BMD (mg/kg bw / day)	$BMDL_{10}$ (mg/kg bw /day)
Null	1				
Full	7				
Gamma	1	0.293	Yes	0.755	0.590
Logistic	2	0.000	No	-	-
Log Logistic	**2**	**0.374**	**Yes**	**0.988**	**0.568**
LogProbit	2	0.375	Yes	0.967	0.582
Multistage	1	0.293	Yes	0.755	0.590
Multistage-Cancer	1	0.293	Yes	0.755	0.590
Probit	2	0.000	No	-	-
Weibull	1	0.293	Yes	0.755	0.590
Quantal-linear	1	0.293	Yes	0.755	0.590

The USEPA BMD Analysis Framework prescribes to select the lowest $BMDL_{10}$ for further analysis. Therefore the $BMDL_{10}$ from the Log Logistic model of 0.568 mg/kg bw/day $KBrO_3$ was used to determine the MOE. Figure 8.1 presents the graph of the Log Logistic model with 95% confidence interval.

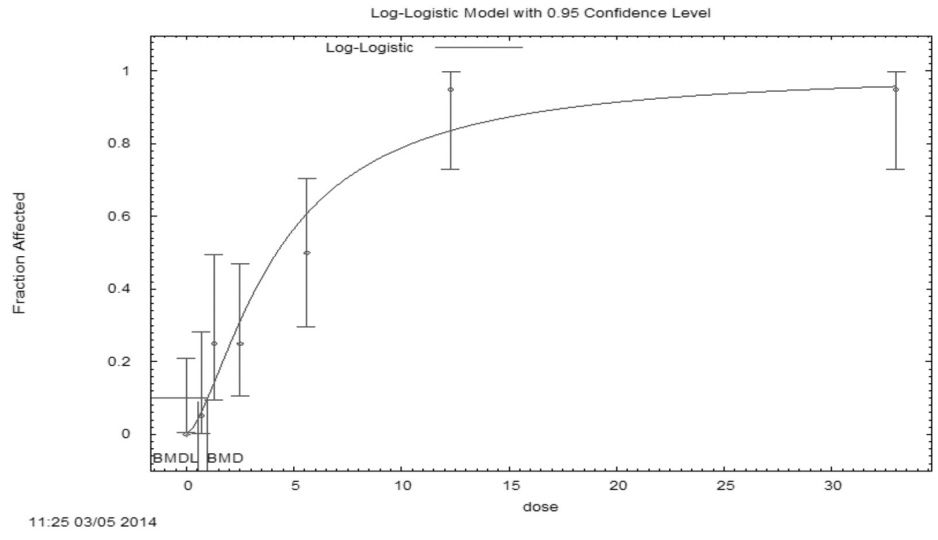


Figure 8.1: BMD analysis of the $KBrO_3$ dysplastic foci data from the study of Kurokawa et al. (1986) (table 8.1) using the Log Logistic model with default settings and BMD software version 2.6 (table 8.3)

For a negligible risk, the MOE should be > 10,000. Based on the $BMDL_{10}$ value of 0.568 mg/kg bw/day and a body weight of 70 kg for a person, the EDI associated with a negligible risk should be lower than 3.98 µg/person/day. Assuming a drinking water consumption of 2 litres per person per day (U.S. EPA, 2004) and drinking water being the only source for bromate uptake, the $KBrO_3$ concentration should not exceed 2 µg/L in order to maintain an MOE > 10,000.

Current Dutch regulations for bromate in drinking water allow 5 µg/L bromate when ozone is applied for disinfection (Drinkwaterregeling, 2011). Furthermore, a guideline value for bromate of 1 µg/L is set when ozone or ozone based AOPs are applied for organic contaminant control. Application of ozone based processes for drinking water production was restricted in the last fifteen years due to risks and regulation regarding bromate formation. At the same time UV based technologies were perceived as causing no harmful side effects. These results reveal that although the identity of the nitrogeneous compounds with carcinogenic and/or genotoxic properties formed by MP UV based treatment of nitrate rich water under practical conditions was not established yet, based on a TEF and the MOE approach, the associated risks of these byproducts may raise a level of concern similar to the risk associated with bromate.

In water treatment, advanced oxidation processes such as MP UV/H_2O_2 treatment and O_3/H_2O_2 treatment are often followed by post treatment with processes such as biological activated carbon filtration to decompose H_2O_2 and O_3 and to biologically degrade reaction products (Martijn et al., 2007). The carcinogenic byproduct from O_3 based processes, bromate, is not removed by commonly applied post treatment technologies in drinking water production (Kruithof et al. 1995). A preliminary investigation into the effects of post treatment on the by MP UV/H_2O_2 treatment formed genotoxic reaction products, showed that biological post treatment was capable to remove the compounds responsible for the Ames test response (Kolkman et al., 2015). This offers an opportunity to mitigate the MP UV/H_2O_2 treatment induced reaction products and emphasizes the essential value of an integrated treatment approach using multiple barriers for the most important treatment objectives. Alternatively, LP UV technologies can be applied to avoid the formation of genotoxic nitrogeneous reaction products by MP UV technology.

This thesis illustrates the technological aspects to the application of MP UV/H_2O_2 treatment as a non-selective barrier for organic micropollutants in advanced drinking water production from eutrophic and polluted surface water. Furthermore, the mechanism and the preliminary risk of MP UV nitrate photolysis induced reaction product formation was studied. The TEF concept, a widely accepted approach for risk assessment of mixtures of compounds with a similar mode of action, was used in this study to perform an MOE based preliminary risk assessment of the mixture of unknown compounds with a genotoxic mode of action, causing the positive Ames test response in MP UV treated water. The preliminary risk assessment based on the MOE approach, using the developed 4-NQO based TEF, exceeded the level of no concern and justifies further study to establish the identity of the MP UV formed genotoxic compounds in nitrate rich water containing natural organic matter. To relate the results of the preliminary risk assessment of the reaction products formed by MP UV treatment of nitrate containing water to the risk of a known and well-studied ozone based water treatment byproduct, bromate, the MOE approach was applied on the Kurokawa study (Kurokawa et al., 1990). It was observed that the the level of no concern, based on the MOE approach for bromate formation in ozone based processes, was exceeded, although the bromate standard was met. This thesis shows that MP UV/H_2O_2 treatment as part of an integrated multibarrier water treatment scheme provides a robust barrier for organic micropollutants, forming nitrated organic byproducts to levels exceeding the level of no risk in nitrate containing water types, a risk that can be mitigated by post treatment.

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Samenvatting

Het wijdverspreide gebruik van, en een grote variëteit aan, chemische stoffen in een moderne maatschappij, vormt een voortdurende bedreiging voor drinkwaterbronnen. Klimaatverandering beïnvloedt algenbloei en kan er de oorzaak van zijn dat de afvoer van rivieren sterkere seizoensfluctuaties laat zien, waarmee bij lage rivierafvoeren, hoge concentraties van stoffen aangetroffen worden. Deze ontwikkelingen maken een waterzuiveringsproces, gebaseerd op meerdere barrières voor stoffen met verschillende eigenschappen, noodzakelijk. Dit proefschrift laat de haalbaarheid van toepassing van middendruk ultraviolet licht met waterstof peroxide (MP UV/H_2O_2) behandeling als barrière voor organische microverontreinigingen in geavanceerde drink-waterproductie uit een eutrofe en verontreinigde bron zien. Incidenten en lozingen van microverontreinigingen in bronnen voor drinkwater maar ook proefinstallatie onderzoek naar de robuustheid van zuiveringstechnologieën, laten zien dat, ondanks het steeds veranderende karakter van vóórkomende verontreinigingen, de combinatie van MP UV fotolyse en MP UV/H_2O_2 behandeling gevolgd door actieve koolfiltratie (adsorptie en biologische omzetting van reactieproducten) een robuuste zuiveringsstrategie is ten aanzien van microverontreinigingen, gebaseerd op meerdere barrières.

Bij de toepassing van MP UV/H_2O_2 technologie is de concentratie en samenstelling van de water matrix van groot belang voor de efficiëntie van de UV fotolyse en de hydroxyl radicaal oxidatie. Substantiële energiebesparing voor MP UV/H_2O_2 behandeling kan bereikt worden door een verbetering van de UV transmissie van het water met geavanceerde voorzuivering. De belangrijkste UV absorberende stoffen in het IJsselmeer zijn DOC en nitraat, waarvan de concentratie verlaagd kan worden door voorzuivering. Momenteel wordt de conventionele voorzuivering (CSF) van pompstation Andijk vervangen door een geavanceerde voorzuivering gebaseerd op ionenwisseling gevolgd door microfiltratie (IX-MF). In vergelijking met de CSF voorzuivering, is de voor het MP UV/H_2O_2 proces benodigde energie (EEO) tot 50% verlaagd door toepassing van een IX-MF voorzuivering.

Vorming van genitreerde organische reactieproducten ten gevolge van toepassing van MP UV technologie op nitraathoudend water is recent aangetoond. In dit proefschrift zijn de resultaten van onderzoek naar het mechanisme van de vorming van deze stoffen en het mogelijke risico, beschreven. De genotoxiciteit is steeds beoordeeld aan de hand van de Ames test bioassay omdat de identiteit van de gevormde genotoxische reactieproducten niet bekend is. Vervolgens is de respons in de Ames test omgezet in

4-nitroquinoline 1-oxide (4-NQO) equivalenten op basis van de Toxic Equivalency Factor (TEF) methode. In deze studie werden de resultaten van de TEF methode gebruikt in een Margin of Exposure (MOE) benadering, een geaccepteerde methode in de risicobeoordeling van stoffen met een vergelijkbaar gedrag. De resultaten van de voorlopige risicobeoordeling op basis van de MOE benadering, geven aan dat de 4-NQO TEF het niveau van 'verwaarloosbaar risico' overschrijdt. Deze bevinding rechtvaardigt verder onderzoek naar de identiteit van de ten gevolge van MP UV behandeling gevormde genotoxische stoffen in nitraatrijk natuurlijk water.

Ter vergelijking is een MOE risicobeoordeling toegepast op 10 µg/L bromaat, een carcinogeen reactieproduct ontstaan bij ozonbehandeling van bromidehoudend water. Dit leidde tot een risicoprofiel dat vergelijkbaar is met het risicoprofiel veroorzaakt door de door MP UV behandeling gevormde, als 4-NQO TEF uitgedrukte, genotoxische stikstofhoudende organische reactieproducten.

Dit proefschrift illustreert de rol van MP UV/H_2O_2 behandeling in een robuuste, op meerdere barrière voor organische microverontreinigingen gebaseerde drinkwaterzuivering. Vorming van genitreerde organische reactieproducten ten gevolge van MP UV behandeling van nitraathoudend natuurlijk water leidt tot een niet verwaarloosbaar risico in een voorlopige risicobeoordeling, een risico dat kan worden verlaagd door bijvoorbeeld nazuivering met actieve koolfiltratie.

Summary

The widespread use of a large variety of chemicals in the community is a continuous threat to contaminate drinking water sources. Climate changes influencing algae blooms or affecting the discharge of rivers may also cause contaminants to emerge. These developments require a water treatment strategy based on multiple barriers, able to deal with a wide range of chemical contaminants with different degradation and removal properties. This thesis shows the feasibility of the application of medium pressure ultraviolet/hydrogen peroxide (MP UV/H_2O_2) treatment as a non-selective barrier against organic micropollutants in advanced drinking water production from eutrophic and polluted surface water. Incidents and spillage of micropollutants in the raw water source and challenge tests on pilot scale show that, although the composition of emerging contaminants is changing continuously, the combination of MP UV photolysis and hydroxyl radical oxidation by MP UV/H_2O_2 treatment followed by granular activated carbon (GAC) filtration providing adsorption and biodegradation of the reaction products, guarantees a robust treatment approach for organic contaminant control, based on multiple barriers.

In the application of MP UV/H_2O_2 treatment, both competition for UV radiation (NDMA) and competition for OH-radical scavenging (1,4-dioxane) are heavily impacted by the water matrix. A significant reduction of the energy consumption by MP UV/H_2O_2 treatment can be achieved by a strong improvement of the UV-transmittance applying advanced pretreatment. Dominant UV absorbing constituents in raw IJssel Lake water are DOC and nitrate and their content can be lowered by pretreatment. Currently, the conventional pretreatment (CSF) is replaced by ionexchange followed by microfiltration (IX-MF) pretreatment. Compared to CSF, the EEO for IX-MF treated water was reduced with about 50%.

Recently, it was shown that genotoxic nitro-organic reaction products are formed in water treatment when MP UV technology is applied on nitrate containing water types. In this thesis, the mechanism and the preliminary risk of MP UV nitrate photolysis induced reaction product formation was studied. The genotoxicity was characterized by the Ames test bioassay and because the identity of the genotoxic reaction products was not established yet, converted into 4-nitroquinoline -1-oxide (4-NQO) equivalents, using the Toxic Equivalency Factor (TEF) methodology. In this study, the TEF concept, a widely accepted approach for risk assessment of mixtures of compounds with a similar mode of action, was used to perform a Margin of Exposure (MOE) approach. The preliminary risk assessment based on the MOE approach, showed that the 4-NQO based TEF exceeded

the level of no concern. These findings justify further study to establish the identity of the genotoxic compounds produced by MP UV treatment in nitrate rich natural waters.

To relate the preliminary risk assessment results of the reaction products formed by MP UV treatment of nitrate containing water to the risk of a known and well-studied ozone based reaction product, bromate, the MOE approach was applied on available bromate carcinogenicity data from animal tests. It was observed that regulatory acceptable levels of bromate formation and levels of nitrogenous MP UV induced genotoxic reaction products, expressed as 4-NQO equivalent concentration, have a similar risk profile.

This thesis shows that MP UV/H_2O_2 treatment as part of an integrated multibarrier water treatment scheme provides a robust barrier for organic contaminant control. The formation of nitrated organic reaction products exceeds the level of no risk in nitrate containing water types, a risk that is mitigated by post treatment.

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Publications

This thesis

Kruithof, J.C., Martijn, A.J., 2013. UV/H_2O_2 treatment: an essential process in a multi barrier approach against trace chemical contaminants, *Water Science & Technology: Water Supply,* 13(1): 130-138

Martijn, A.J., Fuller, A.L., Malley, J.P., Kruithof, J.C., 2010. Impact of IX-UF pretreatment on the feasibility of UV/H_2O_2 treatment for degradation of NDMA and 1,4 dioxane. *Ozone Science and Engineering* 30 (6), 383-390

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Martijn, A.J., Boersma, M.G., Vervoort, J.M., Rietjens, I.M.C.M., Kruithof, J.C., 2014. Formation of genotoxic compounds by medium pressure ultraviolet treatment of nitrate-rich water. *Desalination and Water Treatment,* 52 (34-36), pp. 6275-6281

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Martijn, A.J., Kruithof, J.C., Hughes, R.A.M., Mastan, R.A., Van Rompay, A.R., Malley jr., J.P., 2015. Induced genotoxicity in medium pressure UV treated nitrate rich water, *Journal - American Water Works Association*, 107 (6), 301-312

Other publications

Martijn A.J., Veer, van der A.J., Kruithof J.C., 2007. Byproduct formation in ozone and UV based processes a critical factor in process selection, *Water Practice* 1(3), 1-14

Penders, E.J.M., Martijn, A.J., Spenkelink, A., Alink, G.M., Rietjens, I.M.C.M., Hoogenboezem, W., 2012. Genotoxicity testing of samples generated during UV/H_2O_2 treatment of surface water for the production of drinking water using the Ames test in vitro and the Comet assay and the SCE test in vivo. *Journal of Water Supply: Research and Technology – AQUA* 61(7), 435.

Kolkman A., Martijn B.J., Vughs D., Baken K.A., van Wezel, A.P., 2015. Tracing nitrogenous disinfection by-products after medium pressure UV water treatment by stable isotope labeling and high resolution mass spectrometry. *Environmental Science and Technology* 49(7), 4458-4465

Overview of training activities

Courses

Toxicogenomics, Maastricht University, February 27th – March 2th, 2012
Mutagenesis Carcinogenesis, Leiden University, February 4th – February 8th, 2013
Ecotoxicology, Utrecht University and Wageningen University August 19th – 30th, 2013
Advanced Food Analysis, Wageningen University, January 26th – 30th, 2015

General courses

Risk communication, Wageningen University, May 21st-25th, 2012
Philosophy and Ethics in Food Science and Technology, Wageningen University, January 12th, 19th, 26th, February 2nd, 9th, 16th, 2012
Risk assessment, Leiden University, October 15th – 21st, 2013
Applied statistics, Wageningen University, August 26th and 27th, 2014
PhD workshop carousel, Wageningen University, June 2nd, 2014
Master class: How to develop effective interventions in public health practice?, Wageningen University, October 26th and 27th, 2015

Optionals

Preparation PhD research proposal
Photochemistry and UV basics literature review, University of New Hampshire, August 2012
Chemical analyses and UV collimated beam experiments, University of Arizona, November 2012
Isotope labelling study, KWR Watercycle Research Institute, 2013

Meetings

4th World Congress International Ultra Violet Association, IUVA, September 21st and 22nd, 2009, Amsterdam, The Netherlands (oral presentation)

3rd International Symposium on Genotox in Aquatics, Freiburg Universuty Medical Center, September 22nd- 24th, 2010, Freiburg, Germay

Combined International Ultra Violet Association and International Ozone Association World Congress, IUVA / IOA, May 23rd-27th, 2011, Paris, France (oral presentation)

IWA Micropol specialist conference on micropollutants, International Water Association, July 11th – 13th, 2011, Sydney, Ausltralia (oral presentation)

IWA NOM specialist conference on Natural Organic Matter in water, International Water Association, July 27th – 29th, 2011, Los Angeles, USA (oral presentation)

American Chemical Society National Meeting & Exhibition, ACS, March 25th-29th, 2012, San Diego, USA (oral presentation)

Water Quality and Technology Conference, American Water Works Association, November 4th- 8th, 2012, Toronto, Canada (oral presentation)

Combined International Ultra Violet Association and International Ozone Association World Congress, IUVA / IOA, September 22nd – 26th, 2013, Las Vegas, USA (oral presentation)

IWW Disinfection Byproducts, IWW Zentrum Wasser Mülheim, October 27th – 29th, 2014, Mülheim a/d Ruhr, Germany (oral presentation)