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Assimilable Organic Carbon Formation and Disinfection during Oxidative Drinking Water Treatment

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Auf keine Weise also können diese irgend etwas anderes für das Wahre halten, als die Schatten jener Kunstwerke?

Wenn einer entfesselt wäre und gezwungen würde, sogleich aufzustehen, den Hals herumzudrehen, zu gehen und gegen das Licht zu sehn, und, indem er das täte, immer Schmerzen hätte und wegen des flimmernden Glanzes nicht recht vermöchte, jene Dinge zu erkennen, wovon er vorher die Schatten sah: was, meinst du wohl, würde er sagen, wenn ihm einer versicherte, damals habe er lauter Nichtiges gesehen, jetzt aber, dem Seienden näher und zu dem mehr Seienden gewendet, sähe er richtiger, und, ihm jedes Vorübergehende zeigend, ihn fragte und zu antworten zwänge, was es sei? Meinst du nicht, er werde ganz verwirrt sein und glauben, was er damals gesehen, sei doch wirklicher als was ihm jetzt gezeigt werde?

Platon, Politeia 7. Buch, übersetzt von F. Schleiermacher

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TABLE OF CONTENTS

Zusammenfassung

In dieser Arbeit wurden die Inaktivierung von natürlichen Bakterienpopulationen und die Bildung von assimilierbarem organischem Kohlenstoff (engl. assimilable organic carbon (AOC)) während der oxidativen Behandlung von Trinkwasser mit Ozon, Chlor, Chlordioxid, Permanganat und Ferrat untersucht.

Die Desinfektion ist einer der wichtigsten Schritte in der Trinkwasseraufbereitung. Dazu können Oxidationsmittel wie Ozon, Chlor und Chlordioxid eingesetzt werden. Die Methoden und Richtlinien, mit denen die hygienische Qualität des Trinkwassers heute bewertet wird, prüfen nur das Vorhandensein von Indikatororganismen (*E. coli* und *Enterokokken*) und von aeroben mesophilen Keimen, was für eine umfassende Beurteilung der Desinfektionsleistung der Aufbereitung ungeeignet ist. Die vorliegende Arbeit hatte deshalb zum Ziel, die Desinfektionskinetik von natürlich im Trinkwasser vorkommenden Bakterien durch verschiedene Oxidationsmittel mittels Durchflusszytometrie (engl. Flow-Cytometry (FCM)), einer neuen und praktisch anwendbaren, mikrobiologischen Methode, zu untersuchen.

Um die Kinetik dieser Desinfektionsprozesse zu untersuchen, wurden die Bakterien verschiedenen Expositionen an Ozon, Chlor, Chloridoxid, Chloramin, Permanganat und Ferrat ausgesetzt und anschliessend mit zwei fluoreszierenden DNA-Farbstoffen angefärbt. Mittels FCM konnte so die Konzentration der Bakterien in der Wasserprobe bestimmt werden und gleichzeitig zwischen Bakterien mit intakter und mit beschädigter Zellmembran unterschieden werden. Bakterien mit beschädigter Zellmembran wurden als inaktiviert bewertet. Die Methode hat den Vorteil, dass die Kinetik der Desinfektion durch verschiedene Oxidationsmittel als Schädigung der Zellmembrane gemessen wird und somit nicht auf Indikatororganismen wie *E. coli* angewiesen ist. Mit Hilfe des Chick-Watson Modells konnten die Geschwindigkeitskonstanten der Zellmembranschädigung durch die Oxidationsmittel bestimmt werden. Die ermittelten Geschwindigkeitskonstanten variierten über sieben Grössenordnungen und nahmen in folgender Reihenfolge ab: Ozon >> Chlor > Chlordioxid ≈ Ferrat > Permanganat > Chloramin. Zwei durch FCM unterscheidbare Bakteriencluster, Bakterien mit hohem und niedrigem Gehalt an Nukleinsäuren (engl. high nucleic acid (HNA) und low nucleic acid (LNA) content cells), zeigten bezüglich der

Kinetik ihrer Membranschädigung durch die untersuchten Desinfektionsmittel folgendes Verhalten: grosse Unterschiede der beiden Cluster waren bei Chlordioxid und Permanganat, geringe Unterschiede bei Chlor und Chloramin und keine Unterschiede bei Ferrat feststellbar. Da beide Cluster sehr schnell mit Ozon reagierten, konnte keine Aussage über mögliche Unterschiede bei der Reaktion mit Ozon gemacht werden. Basierend auf der unterschiedlichen Reaktivität der eingesetzten Oxidationsmittel ergibt sich die Hypothese, dass Unterschiede in der chemischen Zusammensetzung der HNA- und LNA-Zellmembrane bestehen. Dabei sind bei HNA-Zellen möglicherweise mehr verschiedene und/oder für Oxidationsmittel besser zugängliche funktionelle Gruppen in den Membranen enthalten.

Oxidationsmittel reagieren jedoch auch mit Komponenten der Wassermatrix und können dabei sogenannte Desinfektionsnebenprodukte (DBPs, engl. disinfection by-products) bilden, die zum Teil gesundheitsgefährdend sind. Daher wird die Exposition der Oxidationsmittel im Allgemeinen möglichst gering gehalten. Häufig wird deshalb auch ganz auf einen Netzschutz verzichtet. Dazu muss das Trinkwasser eine Qualität aufweisen, die das Aufwachsen von Mikroorganismen einschränkt. Der kritische Faktor ist dabei oft der assimilierbare organische Kohlenstoff (AOC), der in den meisten Fällen der wachstumsbegrenzende Nährstoff für Mikroorganismen ist. Gemessen wird die AOC Konzentration im Wasser anhand der Anzahl Bakterien, die in einer Wasserprobe unter definierten Bedingungen aufwachsen können. Zusätzlich zum natürlicherweise vorhandenen AOC wird AOC teilweise auch während der oxidativen Trinkwasseraufbereitung gebildet. Da die Kinetik und die Mechanismen der damit verbundenen Prozesse nur ungenügend bekannt sind, war ein Ziel der vorliegenden Arbeit, die AOC Bildungsprozesse durch ausgewählte Oxidationsmittel zu untersuchen.

Die Kinetik der AOC Bildung durch Ozon, Chlor, Chlordioxid, Permanganat und Ferrat wurde in filtrierten Seewasserproben ohne und mit Zusatz von Cyanobakterienzellen (*Aphanizomenon gracile*, Geosmin-Produzenten) untersucht. Die Ozonung der Wassermatrix führte zu einer schnellen und signifikanten AOC Zunahme, die mit Zusatz der Cyanobakterienzellen noch vergrössert wurde. Aufgrund der hohen Reaktivität von Ozon wurde dieses schnell gezehrt, was zu einer deutlichen Veränderung der organischen Wassermatrix hin zu kleineren Molekülgrössen führte. In Anwesenheit von

Cyanobakterien konnte zudem Geosmin nachgewiesen werden. Dies bestätigt, dass Ozon die Membranen der Cyanobakterien so beschädigen kann, dass Geosmin und intrazelluläres Material (teilweise AOC) aus der Zelle austreten können.

Bei der Behandlung des Wassers mit Chlor und Chlordioxid konnte weder ohne noch mit Zusatz von Cyanobakterien eine AOC Zunahme gemessen werden. Die Freisetzung von Geosmin zeigte jedoch, dass auch Chlor und Chlordioxid die Zellen der Cyanobakterien schädigen können. Dies führt aber entweder nicht zur Freisetzung von intrazellulärem AOC oder die AOC Messung wird durch den Einsatz von Chlor und Chlordioxid gehemmt. Ferrat führt sowohl ohne als auch mit Cynabakterienzellen zu einer signifikanten Zunahme des AOC. Die AOC Bildung durch Permanganat ist typischerweise gering, da Permanganat in den untersuchten Wässern sehr stabil ist. In Anwesenheit von Cyanobakterien konnte aber eine deutlich erhöhte AOC Konzentration gemessen werden.

Als generelles Produkt der Reaktion der Oxidationsmittel mit der organischen Matrix wurde Oxalat bestimmt. Es wurde ein linearer Zusammenhang zwischen der konsumierten Menge der Oxidationsmittel und der Oxalatkonzentration gefunden. Da Oxalat in verschiedenen Oberflächengewässerproben nur in geringen Konzentrationen gemessen wurde (< 10 µg/L), könnte die Oxalatkonzentration nach der chemischen Desinfektion eine Abschätzung über die konsumierte Oxidationsmittelmenge ermöglichen.

Um die Prozesse bei der Reaktion von Ozon mit dem natürlichen organischen Material (NOM) zu simulieren, wurde Phenol als Modellsubstanz eingesetzt, da es eine häufig vorkommende Struktureinheit im NOM darstellt. Phenol und dessen primäre und sekundäre Reaktionsprodukte wurden in Batchsystemen mit Ozon behandelt, um die Reaktionsmechanismen aufzuklären. Zudem wurde die Kinetik der Abnahme von Phenol und der Bildung der Produkte während der Ozonung mit Hilfe eines kontinuierlichen Durchfluss-Systems (engl. continuous quench flow (CQF)) im Bereich von einigen Zehntelsekunden bis zu einigen Sekunden untersucht. Es zeigte sich, dass Benzochinon sehr schnell gebildet wird und bei pH 7 das Hauptprodukt der Ozonung von Phenol ist. Ein weiteres wichtiges Produkt ist Catechol, das unter typischen Bedingungen einer Ozonung sehr rasch weiterreagiert. So konnte gezeigt werden, dass innerhalb weniger Sekunden die Anionen von kleinen organischen Säuren, wie Formiat, Acetat und Oxalat gebildet wurden, obwohl diese sekundäre oder tertiäre Produkte der Phenoloxidation darstellen. Diese

organischen Säuren können einen bedeutenden Teil des durch Ozonung entstandenen AOCs ausmachen, was bei der Ozonung von Zürichseewasser bestätigt werden konnte.

Zusammengefasst liefert die vorliegende Arbeit einen wichtigen Beitrag zur Beurteilung der Wasserqualität bei der Desinfektion. Einerseits wurde die Inaktivierung von natürlichen Bakterienpopulationen quantifiziert, andererseits die Bildung von AOC beurteilt. Um die Wasseraufbereitung zu optimieren, müssen beide Prozesse verstanden werden, da sie einen gegenläufigen Effekt auf die Trinkwasserqualität haben. Das Verständnis der Reaktivität der eingesetzten Oxidantien erlaubte es zudem, Hypothesen zu den elementaren Prozessen bei der Desinfektion und der AOC Bildung aufzustellen.

Summary

This thesis investigates inactivation of native drinking water bacteria and assimilable organic carbon (AOC) formation during oxidative water treatment with ozone, chlorine, chlorine dioxide, permanganate, and ferrate.

Disinfection is one of the most important steps in drinking water treatment. Oxidants such as ozone, chlorine, and chlorine dioxide are often applied for this purpose. Currently, methods to assess the drinking water quality rely on indicator organisms and aerobic mesophilic bacteria that are measured by cultivation techniques. However, these methods are not suitable for a broad assessment of the disinfection efficiency during water treatment. Therefore, one objective of this thesis was to measure the inactivation kinetics of natural bacteria by the selected six oxidants with flow-cytometry (FCM), an innovative and applicable microbiological method.

The inactivation kinetics of indigenous drinking water bacteria by the selected oxidants were determined as a function of the oxidant exposure. After fluorescent staining of the cells, their concentration could be measured with FCM. Additionally, cells with intact and with damaged cell membranes could be distinguished and enumerated. Cells with damaged membranes were assumed to be inactivated. This method had the advantage that inactivation efficiency is measured without relying on cultivation techniques and pure cultures of indicator organisms such as *E. coli*. By applying the Chick-Watson Model, the rate constants for disinfection by the six oxidants could be determined. The rate constants varied over seven orders of magnitude and decreased in the following order: ozone $\gg$ chlorine > chlorine dioxide $\approx$ ferrate > permanganate > chloramines.

Two bacterial clusters could be distinguished in FCM: cells with a high (HNA) and low (LNA) nucleic acid content. The inactivation kinetics was different for these two clusters during oxidation with chlorine dioxide and permanganate, while only a small difference was found during chlorination and chloramine application and no difference was observed during oxidation with ferrate. Ozone reactions with both clusters were too fast to resolve differences. Based on the observed range of reactivities for the selected oxidants it was hypothesized that differences in the composition of the cell membranes of HNA and LNA

cells exist. HNA cell membranes probably contain more diverse chemical functional groups or they are more accessible to the oxidants than LNA cell membranes.

In addition to the inactivation of microorganisms, oxidants can react with water matrix components to form disinfection by-products (DBPs). Because some DBPs have adverse health effects, oxidant exposure during water treatment should be optimized to guarantee disinfection while minimizing DBP formation. In many cases a residual disinfectant in the distribution system can be abandoned if the distributed water is of a quality that limits microbial regrowth. Since carbon is usually the growth-limiting nutrient for microorganisms in treated water the concentration of AOC is a crucial parameter. AOC is measured by the number of bacterial cells that can grow in a sterilized water sample under defined conditions. AOC was shown to be produced during oxidative treatment in previous studies, however, the kinetics and mechanisms of AOC formation are mostly unknown.

The kinetics of AOC formation by ozone, chlorine, chlorine dioxide, permanganate, and ferrate were investigated in lake water samples with and without cyanobacterial cells (*Aphanizomenon gracile*, geosmin producing cyanobacteria). Ozonation of the water matrix led to a fast and significant increase of AOC that was even more pronounced in water containing cyanobacterial cells. Due to the high reactivity of ozone, the oxidant concentration decreased rapidly and the water matrix composition changed towards higher concentrations of low molecular weight compounds. In samples containing cyanobacterial cells, geosmin was released. This confirmed that ozone damaged the cells to such an extent that geosmin and intracellular material (partly AOC) could leach into the water.

Oxidation of lake water with or without cyanobacterial cells by chlorine and chlorine dioxide did not increase the AOC concentration. However, geosmin release showed that the two oxidants were able to damage the cells with no AOC leaching into the water. Alternatively, the two oxidants had a negative impact on AOC measurements by hindering the growth of the bacteria in the assay.

Ferrate application increased the AOC concentration in water with and without cyanobacterial cells significantly. Formation of AOC by permanganate was low. This could be explained by the low reactivity of this oxidant. Nevertheless, an AOC increase could be observed in water containing cyanobacterial cells after oxidation with permanganate.

The reaction of the selected oxidants with the water matrix components resulted in the formation of oxalate which was only found in small concentrations (< 10 µg/L) in three surface water samples prior to oxidation. A linear increase of the oxalate concentration as a function of the oxidant consumption was observed for all oxidants. Therefore, oxalate might be used as an estimate for the consumed amount of oxidant during disinfection.

To investigate the reaction mechanisms of ozone with natural organic matter (NOM), phenol was chosen as a surrogate compound for NOM. The reaction pathways could be determined by oxidizing phenol and its primary and secondary products with ozone in batch systems. Additionally, the kinetics of phenol degradation and product evolution was measured with a continuous quench-flow system at reaction times between 0.06 and 23 seconds. Benzoquinone was the major primary product at pH 7 and was formed faster than the available experimental time resolution. Catechol was another important primary product which continued reacting quickly with ozone. It could be shown that within a few seconds small organic acids such as formate, acetate, and oxalate were formed although they were secondary or tertiary products from phenol ozonation. These acids were also formed during ozonation of Lake Zurich water, and contribute significantly to AOC.

Overall, this study provides an important contribution to the assessment of the water quality during disinfection. The inactivation of indigenous bacteria was quantified as well as the formation of AOC. To further optimize chemical water treatment, both processes have to be understood since they have an opposing effect on the water quality. Based on the knowledge of the reactivity of the selected oxidants hypothesis about basic processes during disinfection and AOC formation could be formulated.

1

INTRODUCTION

1.1 Drinking Water Treatment

The goal of each drinking water provider is to deliver a safe (chemically and hygienically) and enjoyable (color, taste and odor) product to the consumers. However, our water resources are affected by diverse natural processes and anthropogenic activities and therefore vulnerable to pollution. In recent years, microbial or chemical contaminants are present in many drinking water resources (e.g Kilchmann et al. (2009)). Scheme 1.1 illustrates some examples of chemical contaminants and pollution sources. To protect the consumers from negative impacts of chemically and biologically contaminated water, treatment prior to use is often required.
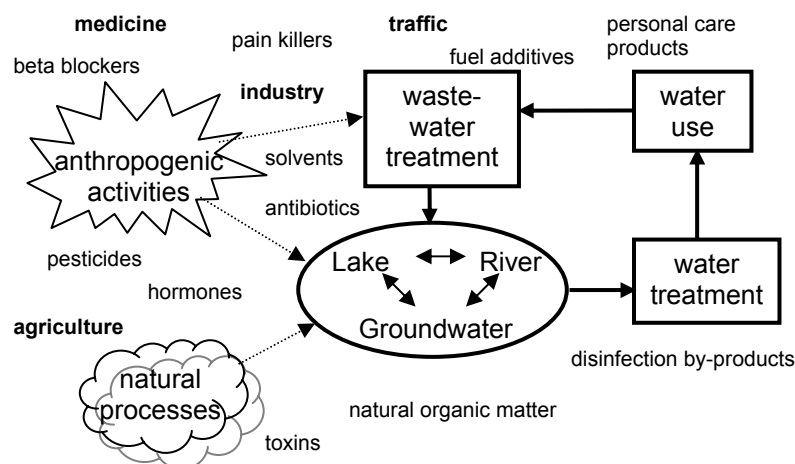
Scheme 1.1 *Extract of the anthropogenic water cycle with examples of classes of organic micropollutants and their origin that pose a threat to water quality.*

The awareness of this need developed in the second half of the nineteenth century when the connection between microorganisms and illnesses and their spread via the fecal-oral route and via water was recognized. Since it became accepted that human waste is a major source for pathogens, the presence of *Escherichia coli* in water resources and drinking water was implemented as an indicator for microbial contamination. Up to date drinking water regulations still rely on the concept of the presence or absence of indicator organisms to control hygienic safety, as is shown in Table 1.1 for Switzerland. An observation that gave reason for questioning the suitability of coliforms as an indicator for hygienic safety of water was the presence of protozoa in water after 1980. These microorganisms are

zoonotic (i.e. they also infect animals). Therefore, they do not only originate from human wastewater and consequently *E. coli* is not always a good tracer for these pathogens (Crittenden et al., 2005). Hence, the presence of coliforms in water indicates contamination, but the absence of coliforms does not ensure water free of contamination. Furthermore, between 1940 and 1960 it was found that viruses can transmit infectious diseases through drinking water as well, and that they can survive much longer than members of the coliform group. Beside viruses more pathogens were found that are less susceptible to chemical inactivation than *E. coli* (e.g. protozoan cysts or oocysts). Therefore, coliforms are not a safe criterion to judge the efficacy of disinfection during water treatment. Accordingly, outbreaks of water born diseases transmitted by drinking water have been reported even though hygienic standards were met, e.g. an outbreak of Cryptosporidiosis infection in Milwaukee (USA) in 1993 (Mac Kenzie et al., 1994) or a Norwalk-like virus infection in La Neuveville (Switzerland) in 1998 (Widmer et al., 2002). Therefore, hospitals are concerned that their patients, who often have a compromised immune system, can be infected by pathogens in drinking water that are not usually monitored (Widmer et al., 2002). However, assessment of biological parameters related to pathogens is in general time consuming and for many purposes no simple and applicable method exists. Therefore, new methods need to be developed to control disinfection efficacy and gain a better understanding of the disinfection process.

Table 1.1. Drinking water standards for microbiological parameters regulated for drinking water in Switzerland (EDI, 2009).

Compound	Value (mg/L)	Remark
Escherichia coli	nn/100 mL	
enterococci	nn/100 mL	
mesophilic aerobic bacteria	20/mL	after water treatment
	100/mL	at water catchment for untreated water
	300/mL	in distribution system
Pseudomonas aeruginosa	nn/100 mL	only for water filled into containers and for mineral water sources

As outlined, the major goal of water treatment is disinfection. Several methods for water treatment are in use and fulfill a range of purposes besides disinfection. Removal or transformation of chemical micropollutants, as exemplified in Scheme 1.1, and assuring

aesthetic quality of the water should be mentioned. Table 1.2 gives a non-exhaustive overview of some parameters and methods relevant in drinking water treatment. The basic options for purifying water are transformation of the contaminant (e.g. oxidation) and removal of the contaminant by adsorption (e.g. activated carbon filtration) or exclusion (e.g. membrane filtration).

Table 1.2. Methods applicable to remove a selection of water constituents for drinking water production (adapted from Crittenden et al. (2005)).

Constituents to remove	Method
particles / turbidity	flocculation, sedimentation, sand filtration, membrane filtration (microfiltration (MF), ultrafiltration (UF))
iron ions, manganese ions	aeration, oxidation with permanganate (or ozone, chlorine dioxide or chlorine, though chlorine reacts slowly with manganese) and subsequent sedimentation or filtration, reverse osmosis filtration (RO)
dissolved organic carbon (DOC)	coagulation, biological filtration (slow sand filtration, activated carbon filtration), membrane filtration (nanofiltration (NF), RO)
chemical micropollutants	coagulation, adsorption, oxidative treatment (chlorine, chlorine dioxide, ozone, advanced oxidation with hydroxyl radicals, UV light)
pathogens (bacteria, viruses, protozoan cysts)	chemical disinfection (chlorine, chlorine dioxide, ozone), UV disinfection, membrane filtration (UF, NF, RO), also coagulation, sedimentation, filtration

In the USA a conventional water treatment train consists of coagulation, flocculation, sedimentation, granular media filtration, and disinfection, often with chlorine (Crittenden et al., 2005). However, depending on the raw water quality this train might not satisfy all requirements. For example chemical micropollutants might not be removed well enough without oxidative treatment and/or adsorption.

Treatment with oxidative reagents is a key technology for disinfection but also for removal or transformation of chemical contaminants (Itoh et al., 2008). Since the beginning of the twentieth century chlorine and ozone have been applied primarily as disinfectants (Crittenden et al., 2005). However, their oxidative property became more important with the increasing number of chemical contaminants due to growing chemical industries. Yet, since 1974 it is known that oxidative treatment also produces disinfection by-products (DBP) with adverse health effects (Rook, 1974). Since then it is obvious that the idea "the

more the better" does not apply. Nevertheless, disinfection has to warrant hygienically safe drinking water to the consumer's tap. An overview of several disinfectants and oxidants will be given in Section 1.2. Section 1.3 will focus on the subject of microbial growth in the distribution system.

1.2 Oxidative Water Treatment

1.2.1 Reaction kinetics

In the following Chapters the term "oxidant exposure" will often appear and will be explained in this section. To do this, a brief introduction into reaction kinetics is indispensable.

The rate of a chemical reaction is defined as the change of the concentration of a reactant or product per time (Oxtoby et al., 1999). The reaction rate depends on the concentration of the reactants and on the temperature. For an elementary reaction (i.e. a reaction directly caused by the collisions of atoms, ions, or molecules), the rate at a certain temperature can be derived from the known reaction stoichiometry. In the following example a bimolecular elementary reaction is given:

$$A + B \rightarrow C + D \qquad (1.1)$$

$$-\frac{\delta A}{\delta t} = -\frac{\delta B}{\delta t} = \frac{\delta C}{\delta t} = \frac{\delta D}{\delta t} = k[A][B] \qquad (1.2)$$

where k is the rate constant which is concentration independent but not temperature independent. However, if only the stoichiometry (eq. 1.3) but not the mechanism of a reaction is known, one has to be aware that a combination of multiple elementary reactions (e.g. including unstable intermediate compounds) might lead to the observable reaction rate

$$eE + fF \rightarrow gG + hH \qquad (1.3)$$

with e, f, g, and h being the stoichiometric coefficients. The corresponding rate expression can be written as follows:

$$-\frac{1}{e}\frac{\delta E}{\delta t} = -\frac{1}{f}\frac{\delta F}{\delta t} = \frac{1}{g}\frac{\delta G}{\delta t} = \frac{1}{h}\frac{\delta H}{\delta t} = k[E]^m[F]^n \qquad (1.4)$$

The two new parameters, the powers m and n, give the order of the reaction: the reaction is m-th order in E, n-th order in F and the order of the overall reaction is m+n. In an elementary reaction (1.1) the order of the reaction can be derived from the stoichiometric coefficients. In eq. 1.1 the coefficients were 1 for A and B and accordingly the reaction was first-order in A, first-order in B, and overall it was a second-order reaction. In a non-

elementary reaction the order cannot be derived from the balanced reaction equation. Therefore, m and n in eq. 1.4 remain unknown and can only be determined experimentally.

In environmental systems the elementary reactions are often not known. Nevertheless, one needs to be able to compare the rates of the reactions of, for example, two compounds (K). Therefore, the reaction rate constants k_K of these compounds have to be determined.

$$K + O \xrightarrow{\ k_J\ } products \tag{1.5}$$

Luckily, many reactions are experimentally found to be second-order reactions, first-order in both K and O. Therefore, the rate for reaction 1.6 can be written according to eq. 1.4 and after multiplication with δt and division by [K] as given in eq. 1.6:

$$\frac{1}{[K]} \delta K = -k_K [O] \delta t \tag{1.6}$$

Since the concentration of K is dependant of the concentration of O and the concentration of O is dependant of the concentration of K, this equation system is not easy to resolve for the concentrations. However, to facilitate the determination of the second-order rate constant k_K certain experimental methods can be applied, whereof one is presented here.

1.2.2 Pseudo first-order reaction conditions

During the experiment performed to determine k_K the concentration of the reactant O can be applied in large excess to the concentration of reactant K. Under these circumstances its concentration change can be considered negligible and it can be assumed that the concentration [O] remains constant over time. Therefore [O] can be "included" in k giving an apparent rate constant k_{app}. The resulting rate law now shows a pseudo first-order reaction: first-order in K, but zero-order in O:

$$\frac{1}{[K]} \delta K = -k_{app} \delta t \tag{1.7}$$

Integration of eq. 1.7 gives eq. 1.8.

$$\ln(\frac{[K]}{[K]_0}) = -k_{app} t \tag{1.8}$$

If [K] can be determined experimentally at several reaction times the apparent rate constant k_{app} can be easily determined from plotting $\ln(\frac{[K]}{[K]_0})$ versus time. The second order rate constant k_K can now be obtained by dividing k_{app} by the concentration [O].

1.2.3 The exposure concept

In real water matrices the concentration of the oxidant [O] often decreases due to reactions with matrix components. The concentration [O] cannot be considered constant any more, but if both concentrations [K] and [O] can be determined experimentally at a sufficient number of time points during the reaction of K with O, the introduction of k_{app} is not necessary. Instead, eq. 1.6 can be integrated and gives eq. 1.9.

$$\ln(\frac{[K]}{[K]_0}) = -k_K \int [O]\delta t \qquad (1.9)$$

The integral $\int [O]\delta t$ can be obtained from the measurement of the concentration [O] as a function of time. With O being an oxidant, this integral defines the oxidant exposure (von Gunten and Hoigne, 1994). If the oxidant exposure can be determined experimentally the reaction rate k_K can be derived from the slope of the linear plot of $\ln(\frac{[K]}{[K]_0})$ versus the oxidant exposure.

During water treatment the oxidant or disinfectant exposure is crucial. Changes in water conditions (e.g. temperature, pH, water matrix) can change the decomposition rate of oxidants significantly. Therefore, the oxidant dose is not a suitable parameter to ensure a certain effect on pathogens or micropollutants. The oxidant exposure includes the oxidant decomposition over time and thus presents a measure of the transient oxidant concentration acting during a certain reaction time (von Gunten and Hoigne, 1994). For inactivation of pathogens disinfection requirements are hence given in disinfectant exposures rather than disinfectant concentrations or doses. Table 1.3 shows the required oxidant exposures to achieve a 4-log inactivation of viruses and a 3-log inactivation of *Giardia* cysts by four disinfectants. Further examples can be found in Chapter 2. It has to be noted that within the engineering community the parameter ct is often used instead of the oxidant exposure. Ct

stands for "concentration x time" and has a slightly different meaning than exposure as illustrated by Figure 1.1. Since it is labor intensive for water utilities to determine the exposure $\int [O] \delta t$ - the oxidant concentration has to be determined at several time or reactor points - in water treatment a minimum estimated exposure for a reactor is calculated and referred to as ct. The ct is determined by multiplying the time it takes for the water to flow through the reactor, with the remaining oxidant concentration measured at the reactor outflow.
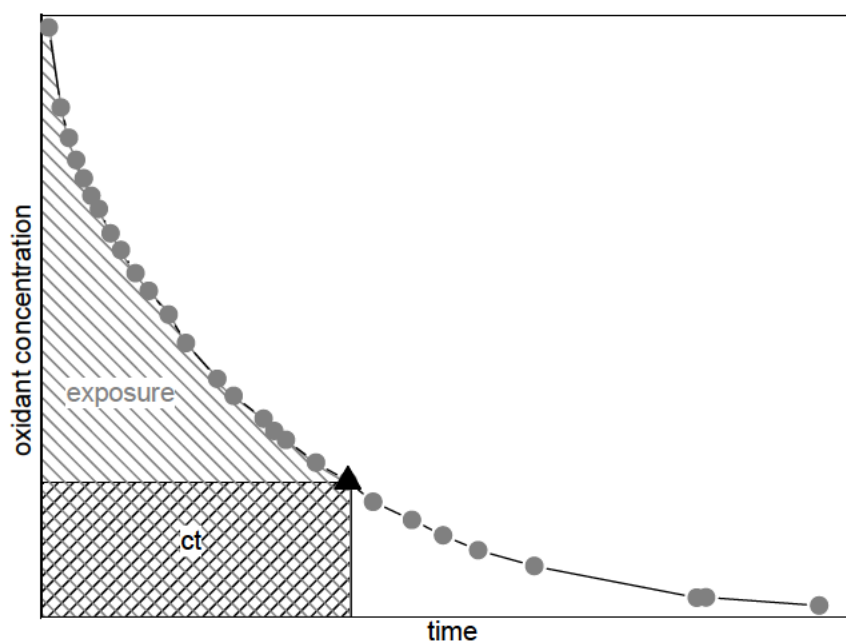
Figure 1.1 *Illustration of the ct (marked with black lines) calculated from a one-point measurement of the oxidant concentration after a certain time and the exposure $\int [O] \delta t$ (marked with grey lines) after the same reaction time.*

From the data in Table 1.3 it is obvious that temperature plays a major role in disinfection kinetics and the four oxidants exhibit different disinfecting strength. The different oxidants will be discussed in the following sections.

Table 1.3 Disinfectant exposures (min*mg/L) for 4-log inactivation of viruses and 3-log inactivation of *Giardia* cysts at pH 6-9, adapted from Crittenden et al. (2005).

Inactivation by	virus		Gardia cysts	
	10 °C	20 °C	10 °C	20 °C
free chlorine	6	3	140	70
chloramines	1500	750	1850	1100
chlorine dioxide	25	13	23	15
ozone	1.0	0.5	1.4	0.7

1.2.4 Chlorine and chloramine

Chlorine is the most common disinfectant and has been used for more than a century for drinking water treatment. It is relatively cheap and easy to handle. It can either be applied as hypochlorite salt ($NaOCl$) or as chlorine gas (Cl_2) (Crittenden et al., 2005). Chlorine dissolved in water rapidly hydrolyses to hydrochloric acid and hypochlorous acid, eq. 1.10. Table 1.4 gives the names and formulas of different chlorine species mentioned in this Chapter.

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \qquad (1.10)$$

Hypochlorous acid undergoes acid base speciation (eq. 1.11) with a $pK_a = 7.54$. The hypochlorous acid is more reactive than the hypochlorite ion and acts as the actual disinfecting species. Therefore, the disinfection power of chlorine is significantly pH-dependent.

$$HOCl + H_2O \rightarrow OCl^- + H_3O^+ \qquad (1.11)$$
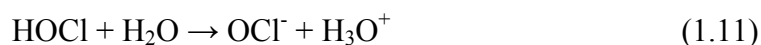
In the following discussion and throughout the whole thesis the term 'chlorine' will be used to summarize hypochlorite and hypochlorous acid, although this is chemically incorrect.

In general, hypochlorous acid reacts fast with deprotonated amines and ammonia. In addition, its reaction with deprotonated dihydroxybenzenes and, to a lesser extent, phenolates is fast. However, the reaction with protonated phenols is much slower (Deborde and von Gunten, 2008; Gallard and von Gunten, 2002). Since chlorine is mainly present as hypochlorite ion at pH values where (substituted) phenols are deprotonated (pK values roughly between 7 and 11), the overall reaction of phenolic compounds with chlorine is

moderate. Table 1.5 gives an overview of the reaction constants of the oxidants discussed in this work at a pH relevant for water treatment for several compounds with specific functional groups. Due to the relatively low reactivity of chlorine with organic compounds other than amines, chlorine is relatively stable.
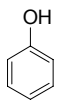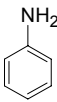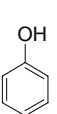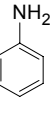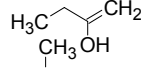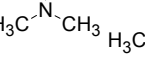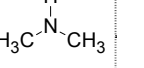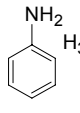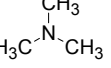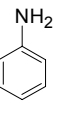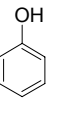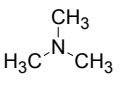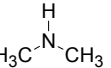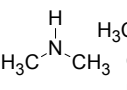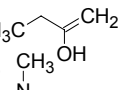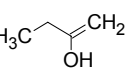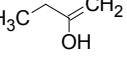
Table 1.4 Names, formulas, and chlorine oxidation states of different chlorine species relevant in drinking water.

Name	Formula	oxidation state of Cl
chloride / hydrochloric acid	Cl^- / HCl	-1
free chlorine	Cl_2	0
hypochlorite / hypochlorous acid	OCl^- / HOCl	+1
chlorite / chlorous acid	ClO_2^- / $HClO_2$	+3
chlorate / chloric acid	ClO_3^- / $HClO_3$	+5
chlorine dioxide	ClO_2	+4
monochloramine	NH_2Cl	+1

The fact that chlorine is relatively stable in water is an advantage that leads to the use of chlorine as residual disinfectant in the water distribution system to avoid bacterial and biofilm growth in pipes (see section 1.3). However, the major drawbacks of chlorine are: (1) it is not very efficient in inactivating protozoa (e.g. *Cryptosporidium parvum* oocysts) and (2) it forms chlorinated, brominated, and iodinated disinfection by-products (DBP) of which some cause taste and odor problems and some are classified as carcinogens (Richardson et al., 2007).

A selection of DBPs are regulated in Switzerland, maximum allowed concentrations are given in Table 1.6 for trihalomethanes (THMs). Table 1.7 gives some known by-products formed from the reaction of several oxidants with dissolved organic matter (DOM). Today more than 600 DBPs have been identified for commonly applied oxidants in drinking water (Richardson et al., 2007). To reduce the DBP formation, DOM has to be removed as much as possible prior to chlorination. A second strategy is to reduce the chlorine exposure to the minimum necessary for disinfection. To still maintain a disinfectant residual, in the USA ammonia is often added to the chlorine containing water before it is released into the distribution system. The reaction of chlorine with ammonia is fast, as mentioned before, and leads to the formation of chloramines which prohibit microbial growth, too.

Table 1.5 Second order rate constants for the reaction of selected oxidants with different compounds with distinctive functional groups at pH 8, adapted from Lee and von Gunten, (2010).

rate constant $[M^{-1}s^{-1}]$	ozone	chlorine dioxide	chlorine	ferrate
10^9				
10^8				
10^7	phenol (OH), aniline (NH_2)			
10^6		phenol (OH), aniline (NH_2)		
10^5	2-buten-2-ol (H_3C–C(OH)=CH_2)		glycine (–COOH, NH_2)	
10^4	trimethylamine (H_3C–N(CH_3)CH_3), dimethylamine (H_3C–NH–CH_3)		ammonia (H–NH–H), aniline (NH_2), dimethylamine (H_3C–NH–CH_3)	
10^3	glycine (–COOH, NH_2)	trimethylamine (H_3C–N(CH_3)CH_3)		aniline (NH_2)
10^2			phenol (OH), trimethylamine (H_3C–N(CH_3)CH_3)	phenol (OH), glycine (–COOH, NH_2)
10^1				
10^0	ammonia (H–NH–H)	dimethylamine (H_3C–NH–CH_3)		dimethylamine (H_3C–NH–CH_3), 2-buten-2-ol (H_3C–C(OH)=CH_2), trimethylamine (H_3C–N(CH_3)CH_3), ammonia (H–NH–H)
10^{-1}				
10^{-2}			2-buten-2-ol (H_3C–C(OH)=CH_2)	
10^{-3}		2-buten-2-ol (H_3C–C(OH)=CH_2)		
10^{-6}		glycine (–COOH, NH_2), ammonia (H–NH–H)		
10^{-7}				

Three terms are used in water treatment to differentiate between the chlorine species: free chlorine ($HOCl/OCl^-/Cl_2$), combined chlorine (chloramines), and total chlorine (free chlorine + combined chlorine). By controlling the chlorine:ammonia ratio in water treatment mainly monochloramine is formed according to eq. 1.12.

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \qquad (1.12)$$
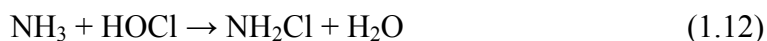
In the USA, monochloramine is often kept as a residual disinfectant in the distribution system. However, it has been found that monochloramine can form iodinated organic compounds (Bichsel and von Gunten, 2000), which might be even more toxic than brominated and chlorinated analogues (Plewa et al., 2004; Richardson et al., 2008), and other DBPs such as *N*-Nitrosodimethylamine (NDMA). NDMA is toxic and is formed from nitrogen-containing DOM, especially from dimethylamine (DMA). Hypochlorite also forms NDMA, however, the formation is approximately an order of magnitude slower than with monochloramine (Mitch and Sedlak, 2002). Fortunately, (pre-)oxidation with ozone and chlorine dioxide is able to transform significant parts of the NDMA precursors and hence reduce NDMA formation (Lee et al., 2007).

1.2.5 Chlorine dioxide

Chlorine dioxide is a stable free radical and can be kept in purified water for several weeks. However, it is a toxic, non-compressible gas that is explosive in the gas phase at concentrations >300 gm^{-3}. Therefore, it has to be produced on site and its application requires educated personnel.

Compared to chlorine it offers several advantages. Chlorine dioxide reacts generally as an electron acceptor and not by Cl-substitution. Therefore, fewer chlorinated organic DBPs and no THMs are formed (Rav-Acha, 1984). Furthermore, it does not cause taste and odor problems, it is more effective in inactivating protozoa, its disinfection efficiency is not pH dependent, and since it does not react with ammonia it is stable in water with low DOM content (Hoigné and Bader, 1994). As a consequence it is often applied as residual disinfectant in European water distribution systems. However, chlorine dioxide is mainly transformed into chlorate and chlorite, where chlorite can be formed up to 75 % from

chlorine dioxide (Crittenden et al., 2005; Hoigné and Bader, 1994). The formation of chlorite, a regulated, blood poisonous by-product limits the chlorine dioxide dose during water treatment (cf. Table 1.6). Consequently, chlorine dioxide cannot be used for disinfection purposes in water with high DOM concentrations.

As an electron acceptor chlorine dioxide reacts mainly through a one electron transfer reaction. Chlorine dioxide is a highly selective oxidant. It reacts fast with phenolates and neutral tertiary amines (Hoigné and Bader, 1994). Accordingly, a study showed that from a wide range of pharmaceuticals only those with specific reactive moieties could be degraded in drinking water treated with chlorine dioxide (Huber et al., 2005).

Table 1.6. Notification values[a] and threshold value[b] of a selection of disinfectants and disinfection by-products regulated in Swiss drinking water.

Compound	Value (mg/L)	Remark
inorganic compounds		
bromate	0.01 [a]	disinfection by-product
free chlorine	0.1 [a]	disinfectant
chlorate	0.2 [a]	disinfection by-product
chlorine dioxide	0.05 [a]	disinfectant
chlorite	0.2 [a]	disinfection by-product
ozone	0.05 [a]	disinfectant
organic compounds		
trihalomethanes		
bromodichloromethane	0.015 [b]	disinfection by-product
dibromochloromethane	0.1 [b]	disinfection by-product
tribromomethane	0.1 [b]	disinfection by-product
trichloromethane	0.04 [b]	disinfection by-product

a) Notification value: the product - in this case drinking water - is considered contaminated or reduced in quality if this maximum concentration is exceeded (EDI, 2010).
b) Threshold value: the product is considered unsuitable for human consumption if this maximum concentration is exceeded (EDI, 2010).

1.2.6 Ozone

Ozone is a strong oxidant used in most lake water treatment plants in Switzerland and the second most used disinfectant after chlorine in the USA. Ozone gas, explosive at

concentrations > 23 %, is produced on site from oxygen or dried air and often brought into the water through bubble diffusers. Ozone is a strong disinfectant also efficiently inactivating viruses and protozoa with lower exposures than any of the other disinfectants discussed in this thesis (Table 1.3).

Ozone reacts selectively and fast with various compounds, e.g. phenolic and activated aromatic compounds, deprotonated amines, olefines, and sulfides (von Gunten, 2003) (refer to Table 1.5). Ozone is efficient in removing color and many micropollutants. Ozonation products are usually smaller, more polar and therefore less volatile than the parent compounds (e.g. aldehydes, ketones, and organic acids). They can often be easier degraded by microorganisms in a subsequent biological filtration step than non-oxidized DOM, leading to a reduction of DOC. However, these easily bioavailable compounds can also enhance undesired biological growth in the distribution system (section 1.3). Unfortunately, oxidized biodegradable organic compounds are not the only products formed during ozonation. Bromate which is potentially carcinogenic is formed in bromide containing waters. Strategies to minimize bromate formation, e.g. pH depression and ammonia addition, are known (Buffle et al., 2004). Additionally, NDMA was recently found to be produced during ozonation (Schmidt and Brauch, 2008).

Table 1.7 Selected DBPs for commonly applied oxidants in drinking water (adapted from Crittenden et al. (2005); Krasner et al. (2006); Richardson et al. (2007); Richardson et al. (1994)).

Oxidant	DBPs
free chlorine	Trihalomethanes (THM), haloacetic acids, haloacetonitirles, haloketones, (halo-)aldehydes, chloral hydrates, trihalonitromethanes
chloramine	Nitrosamines, cyanogen halides, iodinated THMs, haloacetaldehyde
chlorine dioxide	Chlorite, chlorate, organic acids
ozone	Bromate, bromoform, aldehydes, aldoketoacids, carboxylic acids, *N*-nitrosodimethylamine

The high reactivity of ozone towards chemical functional groups present in DOM (i.e. phenolates, activated olefines, deprotonated amines) makes it unstable in water. Therefore, it cannot be applied as residual disinfectant in the distribution system. However, the stability of ozone in water is influenced not only by its reaction with DOM, but also its

decomposition under formation of hydroxyl radicals ($^{\bullet}$OH) in a chain reaction (Scheme 1.2) (von Gunten, 2003). Depending on the composition of the water matrix (e.g. carbonate concentration, propagating or scavenging properties of DOM) more or less ozone is consumed due to the $^{\bullet}$OH-catalyzed decomposition reaction.

Hydroxyl radicals are the strongest oxidant in water and react unselectively with high rate constants between 10^9 to 10^{10} $M^{-1}s^{-1}$, e.g. by hydrogen abstraction or addition to olefines. Therefore, during ozonation, hydroxyl radicals are responsible for the oxidation of compounds that are resistant towards ozone. This way, the radicals add to the oxidative strength of the ozonation process despite their low steady state concentration. Oxidation with hydroxyl radicals is generally referred to as an advanced oxidation process (AOP) and can be enhanced by increasing the pH or adding H_2O_2 during ozonation (promotion of reactions 1 and 2 in Scheme 1.2, respectively). Several process combinations such as H_2O_2 and UV, titanium dioxide and UV, ozone and UV with or without H_2O_2 addition, etc. are currently applied as AOPs.
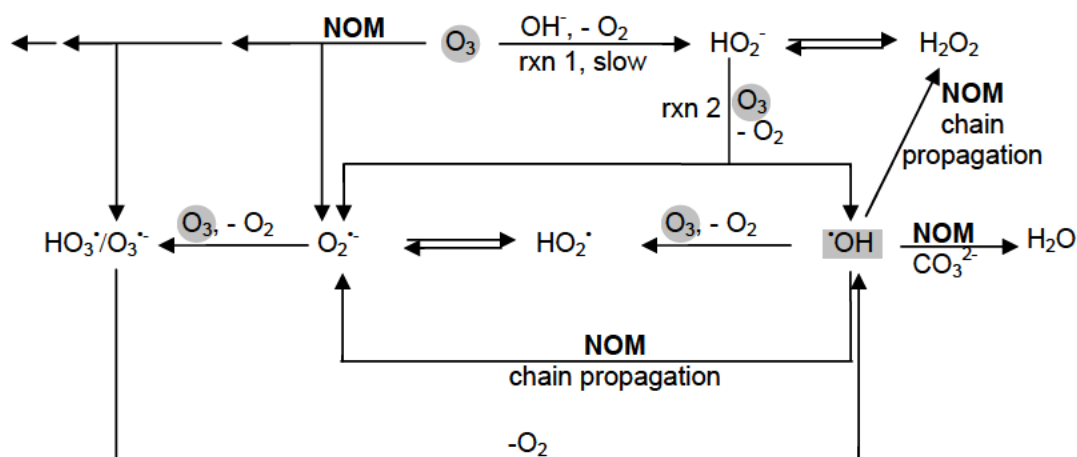
Scheme 1.2 *Formation of hydroxyl radicals (grey square background) from ozone (grey round background) in water and influence of NOM (bold) on hydroxyl radical and ozone concentrations (adapted from Buffle and von Gunten (2006) and von Gunten (2003)).*

1.2.7 Permanganate

Permanganate is more expensive than chlorine and ozone, but it requires less equipment and is simple to apply (Crittenden et al., 2005). It is sometimes used to oxidize taste and odor compounds (e.g. fishy odors produced by methyl sulfides) and THM precursors and to control growth of microorganisms. However, the reaction of permanganate with organic compounds is rather slow. It reacts with substituted phenols and olefins with rate constants between 10^{-1} - 10^2 $M^{-1}s^{-1}$ (Waldemer and Tratnyek, 2006).

The main objective of permanganate application is the removal of iron and manganese according to equations 1.13 and 1.14 (Crittenden et al., 2005).

$$3\ Fe^{2+} + MnO_4^- + 7\ H_2O \rightarrow 3\ Fe(OH)_3(s) + MnO_2(s) + 5\ H^+ \qquad (1.13)$$

$$3\ Mn^{2+} + 2\ MnO_4^- + 2\ H_2O \rightarrow 5\ MnO_2(s) + 4\ H^+ \qquad (1.14)$$
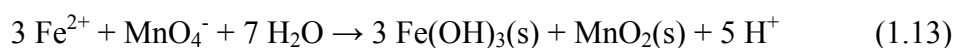
Upon oxidation of iron(II) and manganese(II), iron(III) (hydr)oxides and manganese(IV) dioxide is formed. A recent study showed that manganese dioxide might act as a catalyst for the iodination of DOM in iodide containing water under certain conditions (Gallard et al., 2009). Therefore, DBP formation should not be disregarded for permanganate application.

1.2.8 Ferrate

Ferrate (Fe(VI)) is not yet applied in water treatment, however, it has been proposed as novel process for wastewater treatment due to its ability to both oxidize micropollutants and precipitate phosphate by formation of ferric phosphates (Lee et al., 2009). At low pH values ferrate undergoes a self-decomposition reaction with the formation of oxygen and hydrogen peroxide. Therefore, aqueous solutions need to be prepared directly prior to utilization.

Ferrate has been shown to react relatively fast with phenolic, anilinic, organosulfur compounds and to a certain extent with amines, olefines, and alcohols (cf. Table 1.5). For example, phenol reacts with ferrate by a one-electron transfer and produces phenoxyl radicals through hydrogen abstraction (Lee and von Gunten (2010); Lee et al. (2005b) and references therein). At pH values relevant for water treatment $HFeO_4^-$ is the main reactive

species ($pK_{(HFeO_4^-)}$ = 7.2). The ferrate species H_2FeO_4 and FeO_4^{2-} have been shown not to contribute significantly to the reaction with phenolic compounds (Lee et al., 2005b). Hence, the reactivity of ferrate is pH dependent (Figure 1.2). Ferrate has also been tested with regard to its disinfection efficiency and has been shown to achieve a 3-log removal of *E. coli* at exposures smaller than 10 mg*min/L at pH 5.6 - 8.2 (Cho et al., 2006). In terms of *E. coli* inactivation, H_2FeO_4 was clearly more effective than $HFeO_4^-$ and FeO_4^{2-}.
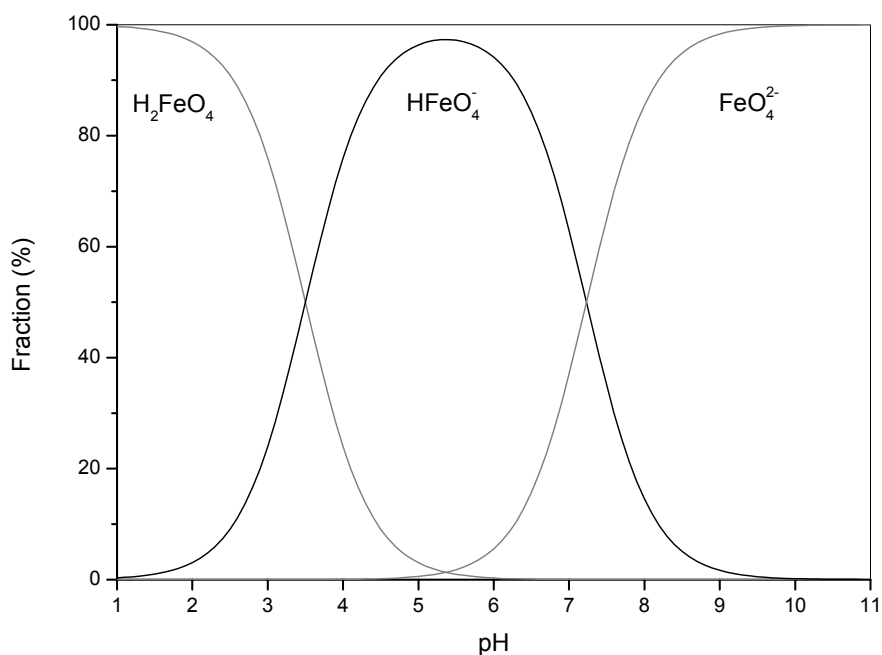
Figure 1.2 *Distribution of Fe(VI) species as a function of the pH.*

1.3 Assimilable Organic Carbon

Once the water has been treated, it needs to be delivered to households. Large distribution systems exist in towns/cities of industrialized countries that are built up by kilometers of pipelines (e.g. 1550 km pipe in the city of Zürich with 383'000 inhabitants (www.stadt-zuerich.ch)). These distribution systems can be considered as bioreactors (Huck and Gagnon, 2004). Water utilities are concerned about bacterial (re-)growth in the distribution system, especially because studies have shown that pathogens can proliferate in freshwater at moderate temperatures (Szewzyk et al., 2000; Vital et al., 2007; Vital et al., 2008) or survive in biofilms in the pipes (Lehtola et al., 2007; Norton et al., 2004). Beside hygienic problems biological processes in the distribution system may also cause aesthetic (taste and odor) or technical problems like clogging of point-of-use filters, bio-fouling of distribution pipes, and bio-corrosion (Hammes et al., 2010; Hijnen et al., 2009; Hijnen and van der Kooij, 1992; van der Kooij, 2000).

1.3.1 Control of biological growth in drinking water distribution systems

Bacterial (re-)growth can be influenced by many factors, e.g. pipe material, corrosion products, presence of biofilms, open biological niches, residual disinfectant, and availability of nutrients (Norton et al., 2004). Accordingly, two main approaches to control biological processes in distribution systems are (i) maintaining a disinfectant residual or (ii) distributing biologically stable water (Srinivasan and Harrington, 2007; van der Kooij, 2000), which is a reduction of microbial growth by nutrient limitation.

The first approach is very widespread. Chlorine and chloramine are disinfectants that exhibit certain stability in treated waters and hence can be applied to safeguard drinking water in the distribution system (Section 1.2). However, chlorination of drinking water can directly or indirectly lead to consumer complaints due to taste and odor problems (Peter and von Gunten, 2009) and disinfection by-products with e.g. carcinogenic properties. The formation of these DBPs limits the application of chlorine or chloramine. Furthermore, some studies have shown bacterial regrowth despite the presence of residual disinfectants in the distribution system (Escobar et al., 2001; Zhang and DiGiano, 2002).

It has been shown that in most cases bioavailable organic carbon or bioavailable phosphorus is growth limiting in drinking water (e.g. Escobar et al. (2001); LeChevallier et al. (1991); Lehtola et al. (2001); Polanska et al. (2005)). Therefore, reducing bacterial growth by nutrient limitation requires far reaching removal of bioavailable organic carbon during water treatment and a high quality of the pipes in the distribution system (e.g. pipes that do not leach carbon from the pipe material into the water or allow external microorganisms to enter the system through leaks) (van der Kooij, 2000).

The question of what strategy is the best, has been strongly discussed (LeChevallier, 1999; van der Kooij et al., 1999) and still under debate. Nevertheless, some European countries, such as the Netherlands, Germany, Austria, and Switzerland (Hammes et al., 2010) have used the approach to limit bacterial regrowth by reducing the amount of bioavailable organic carbon in the water. Many years of experience in producing biologically stable drinking water without residual disinfectant show that this strategy can work successfully in practice (Hammes et al., 2010; te Welscher et al., 1998).

1.3.2 Biological stability of drinking water

Biological stability of drinking water is the inability of the water to support microbial proliferation (Hammes et al., 2010). Since microbial growth in drinking water is often limited by organic carbon, the concentration of bioavailable organic carbon in the water is an indication for biostablity.

Two parameters can be assessed to measure bioavailable organic carbon, namely biodegradable dissolved organic carbon (BDOC) and assimilable organic carbon (AOC). The two parameters are determined in different ways: for BDOC measurement the sample water is exposed to bacterial cells, usually fixed on sand, and incubated for several days. The removal of the bioavailable carbon is measured by measuring the difference in DOC before and after the biological treatment (Joret et al., 1991). If this difference is very small the measurement can be delicate. AOC measurement is based on a completely different principle: the sterilized water sample is inoculated with a small amount of bacterial cells and incubated for bacterial proliferation. The final number of cells is counted and from this the amount of bioavailable carbon is calculated on the basis of a calibration that shows

how many cells of the inoculum grow on a certain amount of organic carbon (Hammes and Egli, 2005; van der Kooij et al., 1982).

Due to the different measurement methods, BDOC and AOC do not give the same results (Lebanowski and Feuillade, 2009). The AOC concentration is smaller than the BDOC concentration (Frias et al., 1995) because AOC is only one part of BDOC. AOC behaves differently from BDOC in various treatment processes. For example while nanofiltration can remove the majority of the BDOC, the majority of the AOC passes through the filter (Escobar et al., 2001). Ozonation of DOM increases the AOC concentration considerably, but it increases the BDOC concentration to a lesser extent or even insignificantly (Escobar et al., 2001). Volk and LeChevallier (Volk and LeChevallier, 2000) came to the conclusion that AOC and BDOC provide different pieces of information. They showed that the probability of coliform occurrences was associated with the AOC concentration in the water. Other studies showed a correlation between AOC and heterotrophic bacteria in distribution systems which was not found for BDOC (Escobar et al., 2001; LeChevallier et al., 1991). Therefore, maximum admissible AOC concentrations for drinking water with (LeChevallier et al., 1991) and without (van der Kooij, 1992) residual disinfectant were proposed.

1.3.3 Assimilable organic carbon

AOC is the organic carbon that is taken up by microorganisms during growth and that is converted into new cellular material (assimilation) and not used to satisfy the energy requirements of the cell by conversion into carbon dioxide (dissimilation) (van der Kooij et al., 1982). AOC consists of compounds such as amino acids, peptides, fatty acids, carbohydrates, and hydroxycarboxylic acids (van der Kooij et al., 1982). For example, acetic and oxalic acid were measured to be converted into biomass in a lab scale water pipe system (Chu and Lu, 2004; Lu and Chu, 2005), and Schechter and Singer found an increase of aldehydes during ozonation of water that correlated with the increase of AOC concentration (Schechter and Singer, 1995). Studies where DOM was fractionated confirmed that AOC is composed of rather small molecules. Many studies reported after membrane fractionation that 50 - 90 % of the AOC was related to the DOM fraction with a

molecular weight of less than 1000 Da (Hem and Efraimsen, 2001; Meylan et al., 2007; Shu et al., 2008). Lee and colleagues confirmed that relatively small and hydrophilic compounds that passed an ultra- or nanofiltration step were more biodegradable than the DOM in the feed water (Lee et al., 2005a). Furthermore, Lebanowski and Feuillade showed that the AOC concentration in the hydrophilic fraction of surface water was considerably higher than in the humic acid, transphilic, or hydrophobic fraction (Lebanowski and Feuillade, 2009). However, since the hydrophobic fraction contributed more than 50 % to the DOC compared to 22 % of hydrophilic DOC, this fraction was still the major contributor to the total AOC concentration.

Regardless of what is found to contribute to AOC it should not be forgotten that AOC is an operationally defined parameter and its meaning depends on the measurement technique. The initial method proposed by van der Kooij (van der Kooij et al., 1982) defined AOC by the growth of a pure strain of *Pseudomonas fluorescens* (*P17*) in a heat pasteurized sample water. This species was selected because of its versatility in using organic carbon compounds, its natural presence in drinking water, and its culturability. However, soon it was recognized that P17 could not assimilate oxalate and subsequently a second species (*spirillum NOX*) was incorporated in the AOC test (Huck et al., 1990). This example shows nicely how dependant the outcome of an AOC test is on the applied measurement technique.

Van der Kooij (van der Kooij, 2000) gives a historical overview on this interesting parameter and reports that measurements similar to the one used today have been proposed already in the 1890's. However, in the last 30 years a lot of research has been performed and modifications of the initial measurement technique (van der Kooij et al., 1982) have been described and discussed (e.g. Hammes and Egli (2005); Huck et al. (1990); Kang et al. (1997); LeChevallier et al. (1993); van den Broeke et al. (2008); Weinrich et al. (2009)). Four major points should be highlighted: (i) should the water be heat pasteurized or sterile filtered to remove the original cells? (ii) should one or several pure strain(s) or a natural bacterial community be used for inoculation? (iii) what are the best incubation conditions e.g. temperature and time? and (iv) how can the final bacterial cell number be determined e.g. counting cells by plating, fluorescent staining, luminescence reading of mutagenized cells, or indirectly via adenosine triphosphate or turbidity measurement? Huck and

colleagues (1990) give an overview of different techniques developed before the 1990ies. In the past twenty years the AOC method was further developed. However, only one example (Hammes and Egli, 2005) will be presented here, because this method was used to determine the data for this work. The filtered water sample is inoculated with a natural bacterial consortium, preferentially gained from the same water as the tested sample water. Such a diverse bacterial culture possesses a broader substrate range than a single pure culture as proposed earlier and therefore a more realistic AOC value is expected. However, a natural consortium cannot be measured by plating techniques. Fluorescent staining of the cells and subsequent enumeration by flow cytometry provides a simple, fast and statistically reliable solution for this problem (Hammes and Egli, 2005).

Besides information on the method development and on DOM fractions relevant for AOC, research of the past three decades provides insight into the AOC concentrations over different drinking water treatment steps of full-scale and pilot-scale treatment plants and in some distribution systems. The main results of these studies were:

- oxidation, especially ozonation, but also chlorination and treatment with hydrogen peroxide increase the AOC concentration, however, UV treatment alone does not (Escobar et al., 2001; Huck et al., 1991; Kruithof and Masschelein, 1999; Miettinen et al., 1998; Polanska et al., 2005; Price et al., 1993; van der Kooij et al., 1982)

- biological filtration, especially by activated carbon, is able to reduce the AOC concentration considerably (Huck et al., 1991; Polanska et al., 2005; Price et al., 1993; van der Kooij et al., 1982)

- in general, high AOC concentrations in the distribution system increase the bacterial concentration (Escobar et al., 2001; LeChevallier et al., 1996), but a correlation between AOC and bacterial counts could not always be found (Gibbs et al., 1993; Miettinen et al., 1997).

1.4 Thesis Outline

Oxidative treatment has both a positive and negative impact on the drinking water quality by disinfecting the water on the one hand and forming DBPs such as AOC on the other hand. To optimize the disinfection strategy a more basic understanding of both processes is needed. Therefore, the oxidants discussed in section 1.2 are compared in terms of both their disinfection and AOC formation kinetics.

Chapter 2 focuses on inactivation of bacteria by six oxidants. In section 1.1 it was mentioned that new techniques to assess disinfection are required. By choosing a disinfection criterion that can be measured by fluorescent staining and flow-cytometry, Chapter 2 proposes a method for determining bacterial inactivation without relying on single indicator organisms. The method was applied to measure the disinfection kinetics of the selected oxidants discussed in the previous section. Different disinfection kinetics were found for two bacterial clusters for some oxidants. The differences between the clusters and the oxidants could be explained on the basis of the specific reactivity of the oxidants.

Chapter 3 compares the oxidants in terms of AOC formation potential and kinetics at exposures relevant for disinfection. Ozone and ferrate were the only oxidants that formed high AOC concentrations. Size exclusion chromatography showed which parts of DOM were transformed by ozone as AOC was produced. No suitable surrogate compound for AOC formation was found: oxalate was shown not to behave exactly as AOC does during oxidative treatment of surface water.

Chapter 4 investigates the mechanisms of AOC formation by ozone with phenol as a surrogate compound for NOM. Phenol was treated with ozone and product formation was studied as a function of time. Products likely to contribute to AOC were formed at ozone exposures as low as 0.2 mg*min/L.

Chapter 5 summarizes selected results from the previous chapters and discusses them on a more general level with regard to their consequences for drinking water treatment.

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2

KINETICS OF MEMBRANE DAMAGE TO HIGH (HNA) AND LOW (LNA) NUCLEIC ACID BACTERIA IN DRINKING WATER BY OZONE, CHLORINE, CHLORINE DIOXIDE, MONOCHLORAMINE, FERRATE(VI), AND PERMANGANATE

Abstract

Drinking water was treated with ozone, chlorine, chlorine dioxide, monochloramine, ferrate(VI), and permanganate to investigate the kinetics of membrane damage of indigenous bacterial cells. Membrane damage was measured by flow cytometery using a combination of SYBR Green I (SGI) and propidium iodide (PI) as DNA stain for cells with permeabilized membranes and SGI to measure total cell concentration. SGI/PI staining revealed that the cells were permeabilized upon relatively low oxidant exposures of all tested oxidants without lag phase. However, the total cell concentration only decreased during ozonation. Rate constants for the membrane damage reaction varied over seven orders of magnitude in the following order: ozone > chlorine > chlorine dioxide ≈ ferrate > permanganate > chloramine. The rate constants were compared to literature data and were in general smaller than previously measured rate constants. This confirmed that membrane integrity is a conservative and therefore safe parameter for disinfection control.

High nucleic acid (HNA) bacterial cell membranes were damaged much faster than low nucleic acid (LNA) bacterial cell membranes during treatment with chlorine dioxide and permanganate. However, only small differences were observed during treatment with chlorine and chloramine, and no difference was observed for ferrate treatment. Based on the different reactivity of these oxidants it was suggested that HNA and LNA cell membranes have a different chemical constitution.

2.1 Introduction

Disinfection is an essential drinking water treatment step that safeguards consumers against pathogenic microorganisms. During both conventional and enhanced drinking water treatment, a chemical disinfection step using an oxidizing agent (e.g., chlorine, monochloramine, chlorine dioxide or ozone) is typically applied, while disinfection with alternative oxidants such as permanganate and ferrate is at the moment mostly limited to laboratory studies (Cho et al., 2006). All these oxidants exhibit different reactivity towards chemical functional groups (Lee and von Gunten, 2010). For example chloramine and permanganate react only slowly with organic compounds (Waldemer and Tratnyek, 2006). Chlorine reacts very quickly with primary amines but only slowly with phenolate (Deborde and von Gunten, 2008). In contrast, chlorine dioxide is unreactive towards primary amines, but reacts quickly with phenolate (Hoigné, 1985) as do ferrate and ozone (Hoigné and Bader, 1983; 1994b; Lee et al., 2009). Among these six oxidants, ozone can be considered the most reactive. The different selectivity and reactivity of the oxidants towards specific compounds can directly affect their reactivity towards microorganisms.

In addition to the reactivity of the oxidant, the composition and physiological state of the bacterial community can influence the efficiency of bacterial disinfection. Indigenous bacterial communities in natural and drinking waters comprise a range of very different bacteria, with highly variable physical (e.g., cell size and shape), chemical (e.g., membrane composition) and biological (e.g., physiological state) properties. This heterogeneity is likely to impact the efficacy of disinfection processes. For example, diverse bacteria exhibit a different susceptibility towards disinfectants. Séka and co-workers (2001) found chlorine susceptible and chlorine resistant types of 021N filamentous bacteria in activated sludge, while it is also known that different bacteria like *E. coli* and *Mycobaterium avium* require different disinfectant exposures in order to be inactivated (Luh and Mariñas, 2007; Wojcicka et al., 2007). Furthermore, cells cultivated under realistic low nutrient conditions exhibit considerably diverse physiology and different susceptibility towards disinfectants compared to cells cultivated in rich medium under laboratory conditions (Lisle et al., 1998; Berney et al., 2006; Wojcicka et al., 2007; Berry et al., 2009).

Even though disinfection of drinking water targets the entire indigenous bacterial community in the water, such communities are not commonly used in disinfection studies. Instead, previous studies on disinfection efficacy have used predominantly pure cultures of pathogens or indicator organisms (Mir et al., 1997; Lisle et al., 1998; Cho et al., 2010). Methodological limitations were one of the main reasons why native microbial communities were not normally used for disinfection studies. Conventional cultivation-based methods (plating) do not suffice for indigenous community analysis because these methods do not detect the vast majority of drinking water bacteria (Hammes et al., 2008). Fluorescent staining in combination with flow-cytometry (FCM) is a rapid, cultivation-independent approach for analysis of indigenous bacteria in drinking water on single-cell level (Hoefel et al., 2003; Hoefel et al., 2005; Berney et al., 2008; Hammes et al., 2008). FCM does not provide information on the type of species that are measured (e.g., *E. coli*, *Legionella*, *Vibrio*, non-pathogenic unknown bacteria) unless immunolabelling is applied (Hammes and Egli, 2010). However, it can provide information on the physiological state of the cells. A wide range of so-called "viability" dyes have been described previously (McFeters et al., 1995; Lisle et al., 1998; Joux and Lebaron, 2000; Berney et al., 2006). It is imperative that these dyes are understood as indicators for specific cellular functions that can be related to viability, but that they do not measure cell viability per se (Kell et al., 1998). In this respect, it is meaningful to select a dye that is generally applicable and which targets specifically the location of cell damage that is under investigation (Novo et al., 2000).

Propidium iodide (PI) is one of the most commonly used viability stains, with numerous previous applications on pure cultures and environmental samples (Bunthof et al., 1999; Schumann et al., 2003; Berney et al., 2007). PI is a large, double-positively charged molecule that binds to DNA and RNA but which is normally excluded from cells with intact cytoplasmic membranes (Joux and Lebaron, 2000; Novo et al., 2000; Shapiro, 2003). Staining of cells with PI is therefore indicative of severe membrane damage and as a result, PI is normally viewed as a conservative indicator of cell death (Lisle et al., 1999; Berney et al., 2006), although some exceptions have been noted (Novo et al., 2000; Shi et al., 2007). PI is the appropriate stain for investigating oxidative damage to cells. Firstly, it measures a

cellular function (membrane integrity) that is a specific target of the oxidants. Secondly, as a conservative estimation of cell death it is a safe indicator for disinfection processes.

In FCM analysis of indigenous aquatic bacterial communities two different clusters of cells can often be observed that differ in fluorescence intensity and cell size (Wang et al., 2009; Hammes and Egli, 2010) and are commonly referred to as high nucleic acid (HNA) and low nucleic acid (LNA) content bacteria (Gasol et al., 1999; Lebaron et al., 2001; Wang et al., 2009). HNA and LNA cell clusters have been reported for seawater, lake water, and drinking water (Bouvier et al., 2007; Wang et al., 2009) (see also Figure 2.1 and Supporting Information). Little is known about the exact nature of LNA cells. In particular, it is unclear whether LNA and HNA bacteria are different species, whether LNA bacteria can contain pathogens, and to which extent LNA bacteria are viable and active organisms (Servais et al., 2003; Longnecker et al., 2005; Phe et al., 2005; Bouvier et al., 2007; Falcioni et al., 2008; Wang et al., 2009). Preliminary measurements from full-scale drinking water plants have suggested that LNA cells behave dissimilar to HNA cells following chlorination, often appearing intact when stained with PI (Supporting Information of this study). This has driven a specific interest in following the reaction of indigenous drinking water bacteria with oxidants.

In the present study we have investigated the disinfection kinetics of native bacteria in real drinking water samples with six different oxidants, namely ozone, chlorine, chlorine dioxide, monochloramine, ferrate(VI), and permanganate. Disinfection efficacy was measured with a combination of SYBR® Green I and PI-staining to detect cells with damaged membranes - an effect likely caused by the oxidants and a widely acknowledged criterion for cell death. We have focused specifically on variations in the impact of the different oxidants on the two main bacterial groups (LNA and HNA bacteria) in drinking water that are commonly observed with flow-cytometry.

2.2 Materials and methods

2.2.1 Experimental procedure

Water samples. The water samples were collected at a drinking water tap of a distribution system fed by varying amounts of untreated groundwater (roughly 50 %), multi-step treated lake water (roughly 50 %, treated by ozonation, sand- and activated carbon filtration in Lengg, Zurich) and small amounts of spring water (roughly 1 %, treated with 0.02 mg/L chlorine). No residual disinfectant is added to the distribution system. The total organic carbon (TOC) concentration was 0.8 mg/L and the pH 8. For the experiments, the water samples were buffered at pH 8.0 with 10 mM borate. The experiments were performed over a time period of 6 months. Despite the fact that the drinking water was sampled from the same distribution system, initial cell concentrations varied by a factor of three (Table 2.1). Therefore, the cell numbers will be shown relative to the initial cell concentration. However, the fraction of intact LNA and HNA cells was always between 40 - 60 % (Table 2.1) and the fraction of PI positive cells in untreated water samples was between 7 - 20 %, similar to a previous study (Berney et al., 2008).

Table 2.1 Oxidant doses and initial cell numbers given in x 10^7 cells/L in the water samples used for experiments with different oxidants.

oxidant	oxidant dose (mg/L)	cells stained with SGI: total cells	cells stained with SGI/PI: intact + damaged cells	intact cells in % of cells stained with SGI/PI	% LNA of intact cells
ozone	0.25	14	8	88	50
chlorine dioxide	0.75 2.5[a)]	38	39	90	58
chlorine	0.77	14	14	93	63
chloramine	1.0	18	19	79	42
ferrate(VI)	1.0 5.0[b)]	25	26	81	53
permanganate	2.0	32	38	87	59

a) Chlorine dioxide exposures above 200 mg*min/L were achieved by dosing 2.5 mg/L.
b) With a ferrate dose of 1 mg/L only exposures up to 100 mg*min/L could be achieved, therefore 5 mg/L ferrate were dosed to obtain higher exposures.

Oxidant preparation. The oxidant stock solutions were prepared as described earlier in various studies. Briefly, chlorine, permanganate, and ferrate(VI) stock solutions were prepared by diluting or dissolving commercially available products, chlorine dioxide was prepared from the reaction of chlorite with peroxodisulfate (Hoigné and Bader, 1994b; Huber et al., 2005), chloramine solution was freshly prepared by mixing an HOCl and an ammonium chloride solution at pH 9.5 (Hand and Margerum, 1983), and an ozone solution was obtained from purging an ozone-oxygen mixture from an oxygen fed ozone generator through milliQ water (Elovitz and von Gunten, 1999). The concentrations of the stock solutions were: 1.4 mM ozone, 1.1 mM chlorine, 4.1 mM chlorine dioxide, 10 mM monochloramine, 5 mM permanganate, and 2 mM ferrate.

Oxidant exposure. The oxidant consumption in the water matrix was determined by adding oxidant stock solution to the stirred water sample in a bottle equipped with a dispenser system (Hoigné and Bader, 1994a). The initial oxidant doses are summarized in Table 2.1. The residual oxidant concentration was measured at 22 °C at various time intervals by stopping the reaction with a quenching agent dye that undergoes a color change upon oxidation. ABTS (2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt) was used for chlorine dioxide (Pinkernell et al., 2000), ferrate (Lee et al., 2005), and chloramine measurement (Pinkernell et al., 2000), indigotrisulfonate was used for ozone (Bader and Hoigne, 1981), and DPD (*N,N'*-diethyl-*P*-phenylenediamine) was used for chlorine measurement (Clesceri et al., 1998). The permanganate concentration was directly measured spectrophotometrically at 525 nm. Integration of the transient oxidant concentration over the reaction time gave the oxidant exposure (von Gunten and Hoigne, 1994).

Inactivation of bacteria. In addition to the aliquots taken for oxidant concentration determination, aliquots from the oxidized water samples were also taken for staining and cell counting by FCM. In these latter aliquots, all oxidants were quenched with thiosulfate (3-fold excess to monochloramine, 7-fold excess to chlorine and chlorine dioxide, and 10-fold excess to ozone, permanganate, and ferrate). However, in the experiments with ozone and chlorine, and for the exposures up to 7 mg*min/L in the case of chlorine dioxide and ferrate, separate batches were prepared for each data point and the oxidant was quenched

directly in the batch after the desired reaction time. This was done because the time intervals between two sampling points were too short to allow taking samples from a single batch only. The data points in these experiments therefore represent each an independent experiment. In general an experiment was completed within one day and the bacterial cells in the water samples were measured on the same day. However, in the case of chloramine, samples were taken over four days (See Supporting Information for detailed information concerning the repetition of experiments and controls).

2.2.2 Fluorescent staining and flow cytometry

Stock solutions of SYBR$^®$ Green I (SGI) alone and combined with propidium iodide (SGI/PI) were prepared as described previously for drinking water sample measurements (Berney et al., 2008) and stored at -20 °C until use. The bacterial cells in the sample aliquots were stained separately with 10 µL/mL SGI with addition of 10 µL/mL ethylenediaminetetraacetate (EDTA, 0.5 M) or 10 µL/mL SGI/PI and incubated at room temperature in the dark for 14 - 18 minutes prior to analysis. The stained samples were ten-fold diluted in 0.22 µm filtered bottled mineral water (EVIAN, France) and measured on a PASIII flow cytometer (FCM) (Partec GmbH, Germany) as described elsewhere (Hammes et al., 2008). The FCM was equipped with a 25 mW solid-state laser, emitting at a fixed wavelength of 488 nm, and a volumetric counting hardware, that measures the number of particles in 200 µL of each sample. Green and red fluorescence was collected on the FL1 (520 ± 20 nm) and FL3 (> 615 nm) channel, respectively. The specific instrumental gain settings were: FL1 = 540, FL3 = 800, speed = 4 (event rate < 1 000 events/s), no compensation was used and the data collection trigger was set on the green fluorescence channel (FL1). The limit of quantification is 1000 cells/mL and the instrumental error is below 5 % (Hammes et al., 2008). All data were collected as logarithmic (3 decades) signals and processed with the Flowmax software (Partec). Data presentation as FL1 vs. FL3 dot plots allowed for optimal distinction between differently fluorescing clusters of stained microbial cells and instrument noise or sample background. Electronic gating was used to quantify selected signals (Hammes et al., 2008; Hammes and Egli, 2010).

2.3 Results and discussion

2.3.1 Flow-cytometric analysis

Figure 2.1 shows the principle of the FCM analysis with representative dot plots of a drinking water sample treated with increasing chlorine dioxide exposures. Figure 2.1a shows cells that are only stained with SGI. Note that the red fluorescence (630 nm) signals in these plots stem from the tailing of the fluorescence emission spectrum of SGI (Hammes et al., 2008). The cells are clearly distinguishable from the background as indicated by the region called 'total cells'. The entire microbial community remains clearly visible throughout the experiment and in this example the total cell concentration did not change (Figures 2.1a-d). For high exposures (1400 mg*min/L, Figure 2.1d) the fluorescence intensity diminished slightly, presumably due to oxidative damage of DNA (Phe et al., 2004), which is the binding site for SGI molecules (Zipper et al., 2004). Figure 2.1b shows the same samples stained with a combination of SGI and PI, allowing cells with damaged membranes to take up red fluorescent PI. Due to the stronger red fluorescence of these PI-positive cells with damaged membranes, they can clearly be distinguished from intact cells as indicated by the regions 'damaged' and 'intact' (for details, see Berney et al. (2007) and Supporting Information). Furthermore, among the intact cells the two main bacterial clusters, HNA and LNA cells, are clearly visible irrespective of the staining combination. Already from this example it can be observed that HNA and LNA cells respond dissimilar to chlorine dioxide exposure (Figure 2.1g). These results support the notion that analysis with SGI and SGI/PI provides valuable and clear information on damage caused to bacterial cells due to oxidative disinfection.

2.3.2 Comparison of different oxidants

FCM measurement also allows accurate quantification of the cells that are displayed in the different regions of the dot plots (e.g. Figure 2.1). The relative number of the intact cells derived from SGI/PI staining (e.g. Figure 2.1e-h) as well as the relative total cell
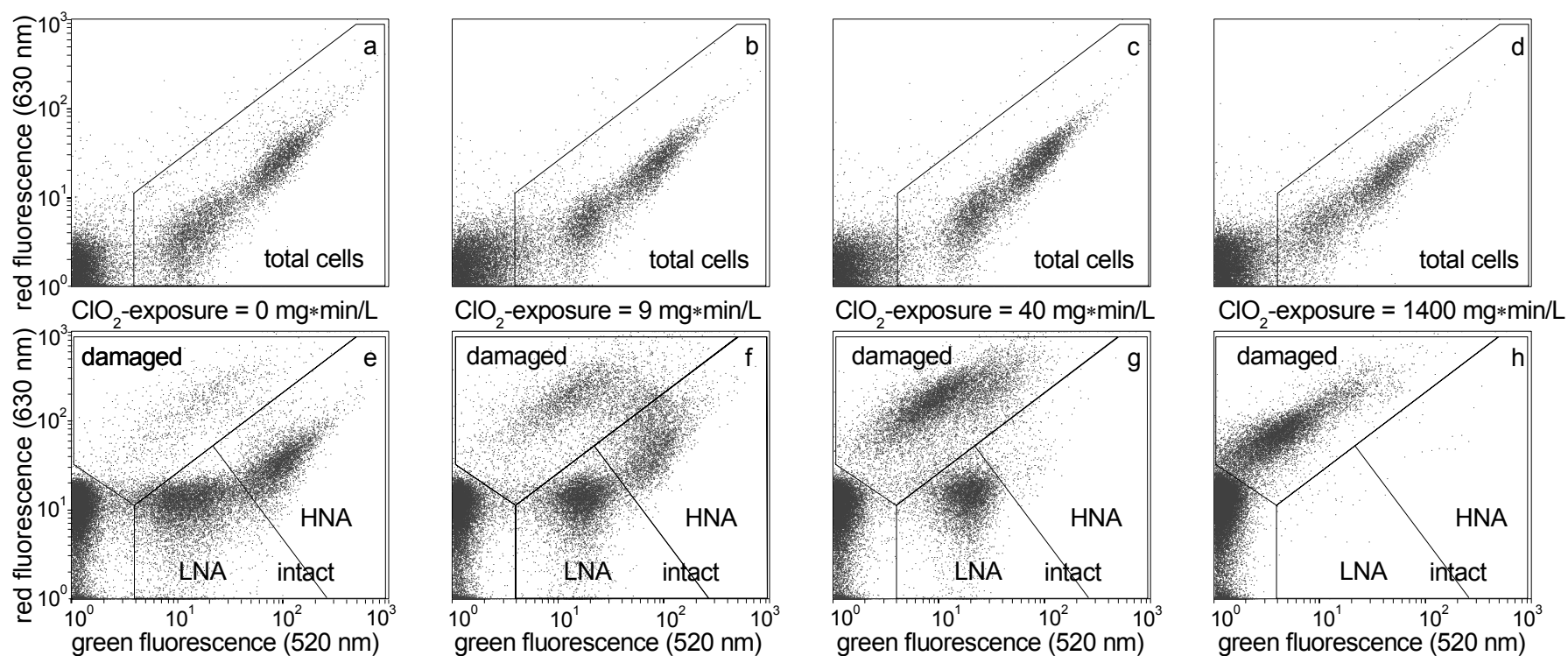
Figure 2.1 *Examples of flow-cytometric dot plots from water samples treated with chlorine dioxide that was quenched after having reached different oxidant exposures (a-d and e-h). Water samples were stained with SYBR Green I (SGI) (a-d) and a combination of SGI and propidium iodide (PI) (e-h). The regions for quantification of the cell numbers are shown: total cells = intact and damaged cells stained with SGI only, damaged = cells with a PI permeable membrane, intact = cells with a PI impermeable membrane, HNA = high nucleic acid cells, and LNA = low nucleic acid cells*

number derived from SGI staining (e.g. Figure 2.1a-d) is shown in Figures 2.2a-f for all oxidants that were investigated. Only during ozone application (Figure 2.2a) the total cell concentration decreased substantially in the range of the measured exposure. In contrast to the total cell concentration, the intact cell number decreased dramatically for all oxidants applied. The intact cell number in the case of ozone was negligible already at an ozone exposure of 0.06 mg*min/L (0.25 mg/L ozone for 15 seconds). However, despite the high reactivity of ozone the cells remained detectable as damaged cells (cf. Figure 2.2a) and were not completely destroyed. For all other oxidants, the cell membranes were damaged, i.e. the cells became PI permeable, while the total cell concentration remained constant. This shows that bacterial membrane integrity is lost during exposure to all of the oxidants before the cells loose their capability to be stained and detected (i.e. before the DNA is heavily damaged). However, these results do not exclude that the oxidants enter the cell before the membrane becomes PI permeable. Lisle and co-workers (1999) found in chlorination experiments with *E. coli* that other cellular functions ceased before the membrane integrity was lost. The results also demonstrate one of the basic differences between chemical disinfection and inactivation by UV-C light. UV-C inactivation is based on adsorption of the radiation by bacterial DNA, followed by the formation of thymine dimers (Al-Adhami et al., 2007). Nocker and co-workers reported that DNA of *E. coli* was extensively damaged by UV-C light while the membrane was still impermeable for propidium ions (Nocker et al., 2007). In contrast, sufficient exposure of *E. coli* or freshwater bacterial communities to UV-A light produced the same pattern in a FCM dot plot as obtained with the chemical disinfectants in this study, because UV-A damages also bacterial membrane proteins (Berney et al., 2007; Bosshard et al., 2010).

Monochloramine and permanganate are oxidants with relatively low reactivity towards most chemical functional groups. The data in Figure 2.2c and 2.2f show that these oxidants consequently require very high oxidant exposures to damage the cell membranes. Figures 2.2b, 2.2d, and 2.2e show that among chlorine, chlorine dioxide, and ferrate, it is chlorine that damages the cell membranes at the lowest exposure. This result is somewhat surprising since the exposures required for inactivation of many bacterial microorganisms are similar for chlorine dioxide and chlorine (USEPA, 2005; WHO, 2008), which may indicate that inactivation kinetics measured by conventional methods and membrane damage kinetics can be dissimilar. Furthermore, chlorine is in general less reactive with many functional groups compared to chlorine dioxide and ferrate (Lee and von Gunten,

2010), which is also reflected by its slower consumption by dissolved organic matter in natural waters. However, the reactivity with primary and secondary amines is higher for chlorine than for chlorine dioxide or ferrate (Lee and von Gunten, 2010).
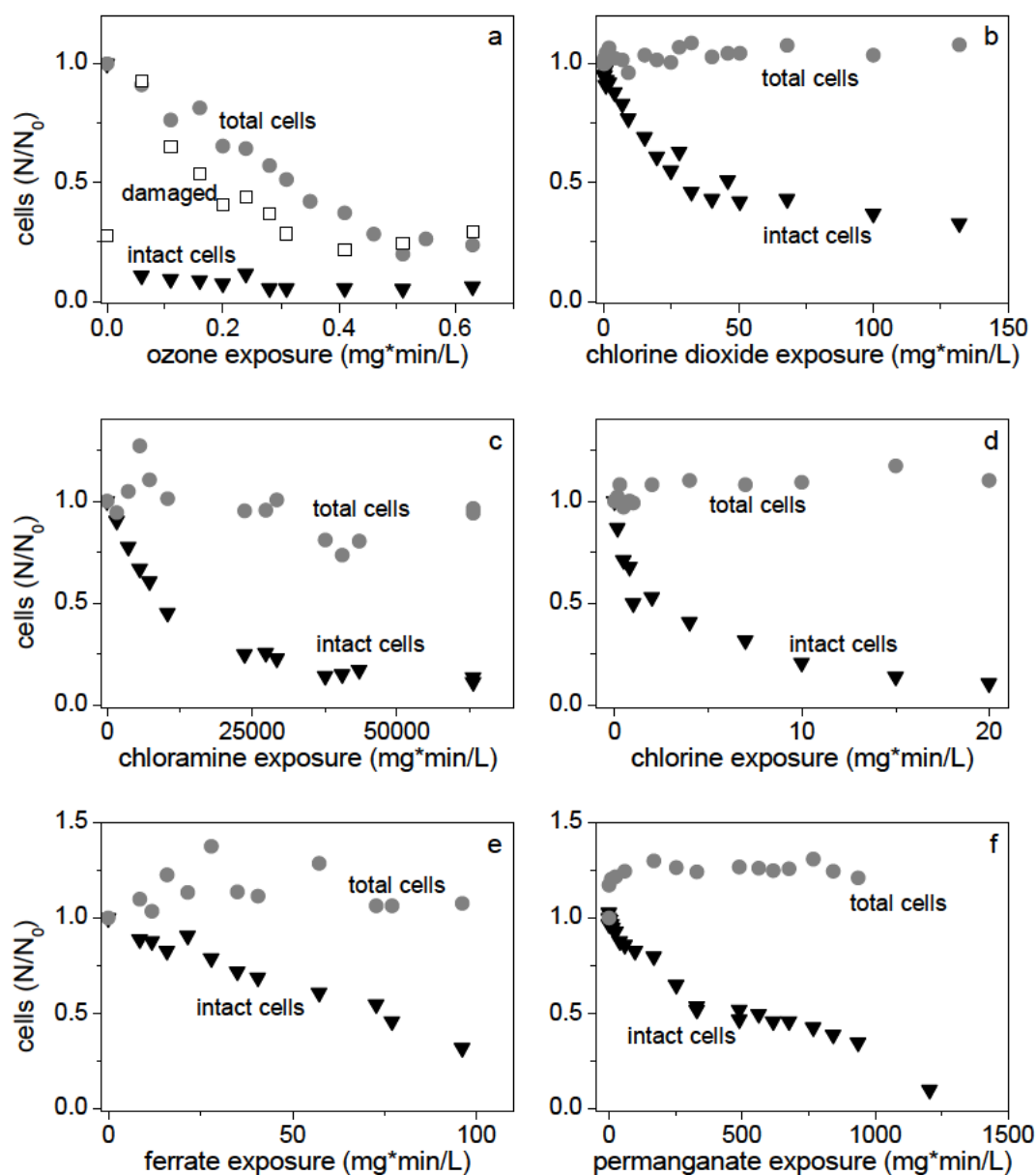
Figure 2.2 *Total cell numbers (circles) counted by staining with SGI only and intact cell number (triangles) derived from SGI / PI staining normalized to the initial cell number in drinking water treated with increasing exposures of (a) ozone, (b) chlorine dioxide, (c) chloramine, (d) chlorine, (e) ferrate, (f) permanganate. Note the different exposure scales for the various oxidants. In (a) the damaged cells (squares) are displayed normalized to the initial number of intact cells.*

This suggests that reactions with amine moieties might play an important role regarding membrane damage of a cell. Amine moieties are present in some phospholipids forming the cytoplasmic membrane. Amines also appear in the peptidoglycan (in lysine and diaminopimelic acid) forming the cell wall and periplasm of Gram-positive and Gram-negative bacteria, respectively. Furthermore some amino acids (e.g. lysine, proline) of proteins that are embedded in the cell membrane contain amines. Previous studies investigated the impact of different oxidants on amino acids and proteins by different methods and showed that the tertiary structure of proteins is important for oxidation and vice versa (Davies and Delsignore, 1987; Pattison et al., 2007; Hawkins et al., 2009). In this respect, Lapinski and co-workers (1991) mention that alteration of cell membrane proteins allows entry of PI. However, detailed information on the exact conditions when PI can permeate a cell's membrane is scarce and therefore the conclusion described above remains open to debate.

2.3.3 Difference between membrane damage of HNA and LNA bacterial cells

The relative decrease in the intact cell concentrations of HNA and LNA cells is given in Figure 2.3 for all six oxidants. A strong dependency of the reactivity of HNA and LNA cell membranes on the oxidant can be observed. While there is an obvious difference between HNA and LNA cell membrane damage kinetics during treatment with chlorine dioxide and permanganate (Figure 2.3b,f), this difference is much smaller in the case of chlorine and chloramine application (Figure 2.3c,d) and it cannot be observed at all during treatment with ferrate (Figure 2.3e). In the case of ozone application, no difference in the damage kinetics could be observed between the two clusters, because of the high reactivity of ozone and resulting low experimental resolution of the ozone exposure (Figure 2.3a).

From these results it is inferred that some characteristics of the bacterial cytoplasmic membrane are different for HNA and LNA cell membranes; these characteristics are relevant for PI permeability and show different reactivity towards the various oxidants. Unfortunately, there is no fundamental knowledge available on the compositional difference between HNA and LNA cell membranes except transmission electron microscopy images that show relatively normal Gram-negative cell membranes in a LNA bacterial pure culture (Wang et al., 2009). However, the two oxidants that caused the

strongest difference between HNA and LNA cell membrane permeabilization, namely chlorine dioxide and permanganate (Figure 2.3b,f), have a much lower reactivity with amine moieties than with other functional groups (Lee and von Gunten, 2010).
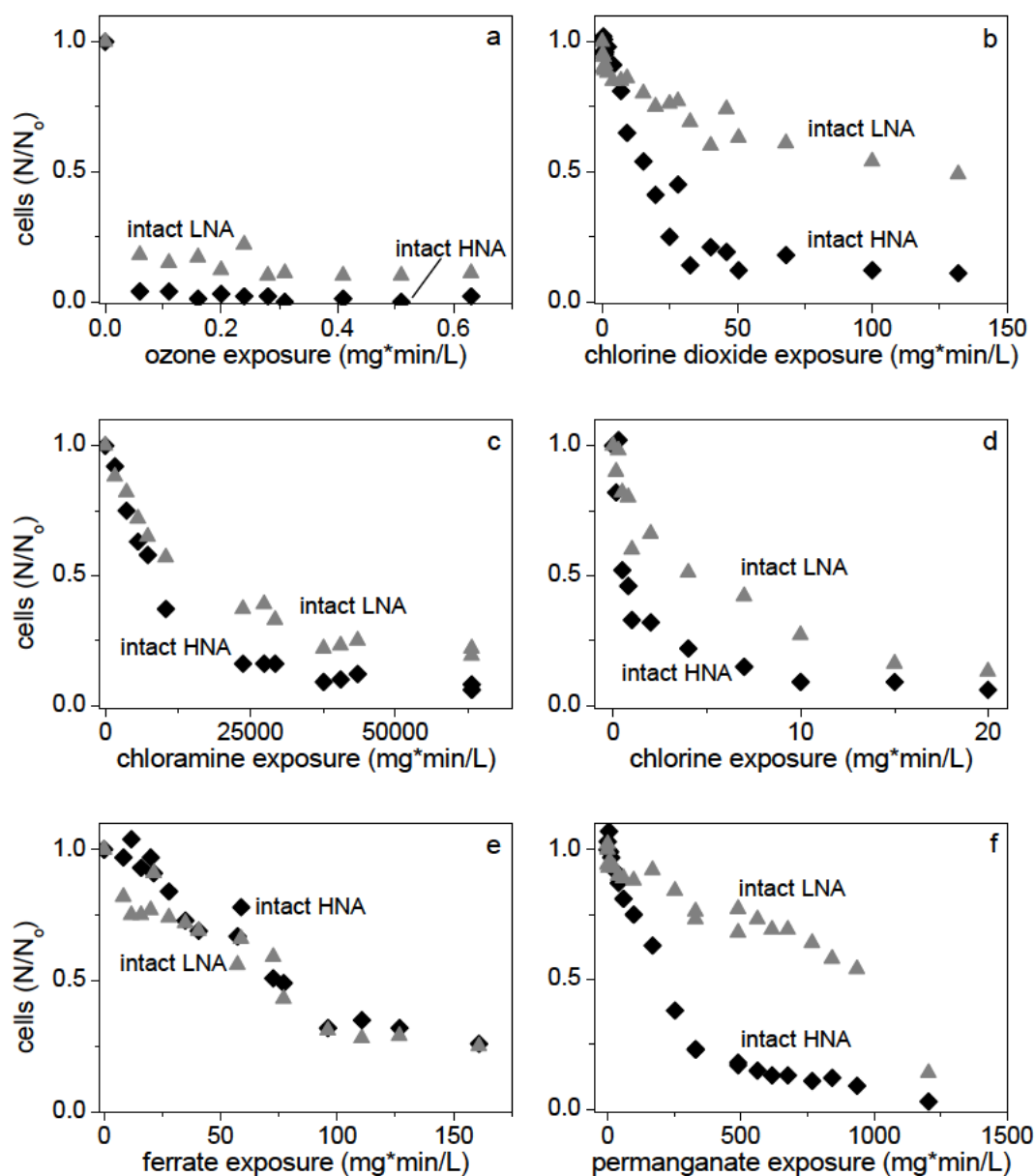
Figure 2.3 *Relative decrease of intact HNA (diamonds) and LNA (triangles) cell number determined by staining with SGI and PI after treatment with (a) ozone, (b) chlorine dioxide, (c) chloramine, (d) chlorine, (e) ferrate, and (f) permanganate. Note the different exposure scales for the various oxidants.*

In section 3.2 (above) we suggested that the reaction of amine moieties plays an important role for membrane damage. However, if an oxidant reacts much better with other functional groups than with amines, these other groups might become important as well. For example investigations of the reactivity of chlorine dioxide with amino acids showed that chlorine dioxide reacts only with 6 out of 19 amino acids but only the reaction with cysteine, tyrosine, and tryptophan was fast, i.e. with amino acids that contain a thiol-, a phenyl-, or an indole-group (Noss et al., 1986). However, the reaction with these three amino acids was sufficient to inactivate the bacterial virus f2 (Noss et al., 1986). Permanganate only changed the cysteine (amino acid containing a thiol group) in an enzyme of *E. coli*, but this change was sufficient to rapidly lose enzymatic activity (Benisek, 1971). In contrast, chlorine and ferrate react with all amino acids (Sharma and Bielski, 1991; Deborde and von Gunten, 2008) and do not differentiate greatly between HNA and LNA cell membranes (Figure 2.3d,e). Linking our results and these studies with the observation that membrane protein alteration probably allows PI to enter the cell (Lapinsky et al., 1991) a tentative conclusion can be formulated: the reaction of oxidants with amines is important for membrane damage in all cells (section 3.2). If an oxidant does not react quickly with amines, its reactivity towards other functional groups, e.g. thiols, may become important. This would suggest that the membranes of both LNA and HNA cells contain amines. However, we postulate that HNA cell membranes contain more, or more accessible, thiol- and/or other groups reactive towards chlorine dioxide and permanganate than LNA cells (section 3.3). For validation of this theory, additional research on the precise mechanisms of PI permeability across cell membranes as well as the fundamental difference between HNA and LNA cell membranes is required.

2.3.4 Kinetics of oxidative membrane damage

A way of quantifying the disinfection rate or, in our case, the rate of membrane permeabilization, has been described by Chick and Watson (Chick, 1908; Watson, 1908). The rate law is given in Equation 2.1, where [N] is the cell number per volume, [Ox] is the concentration of the oxidant, t is the time, k the rate constant, and n and m are the exponents indicating the reaction order.

$$\frac{d[N]}{dt} = -k[N]^n[Ox]^m \qquad (2.1)$$

In many cases, disinfection processes and chemical reactions are found to be first order in both reactants with n = m = 1 resulting in a second order reaction (e.g. von Gunten (2003)). Assuming such a second order reaction, Equation 2.1 can be integrated (Eq. 2.2) with $[N]_0$ being the initial cell concentration and $\int [Ox] dt$ the oxidant exposure, i.e. the integral of the oxidant concentration over time in the investigated water sample.

$$\ln \left(\frac{[N]}{[N]_0} \right) = -k \int [Ox] dt \qquad (2.2)$$

Plotting $\ln([N]/[N]_0)$ against the oxidant exposure allows determination of the second order rate constant (k) of the membrane permeabilization reaction. Figure 2.4a shows as an example the corresponding plot for chlorine dioxide (refer to the Supporting Information for the other oxidants). Clearly two phases of membrane damage can be observed, displayed with different symbols. The two linear phases show that a second-order rate law is not underlying the total cell membrane damage process. However, in the previous section the different behavior of HNA and LNA cells with regard to membrane damage upon treatment with chorine dioxide has been discussed. In Figure 2.4b the HNA and LNA cell data are plotted separately according to Eq. 2.2. In this plot, both the HNA and LNA bacteria data sets show linearity and the two phases shown in Figure 2.4a are no longer apparent. The slope of the first phase in Figure 2.4a (-0.02 L/(mg*min)) is between the slopes of the HNA cells (-0.04 L/(mg*min)) and LNA cells (-0.004 L/(mg*min)) data set in Figure 2.4b. The slope of the second phase in Figure 2.4a (-0.004 L/(mg*min)) is the same as the slope of the LNA cells data set in Figure 2.4b. This indicates that the first phase of membrane damage by chlorine dioxide represents permeabilization of HNA and LNA cell membranes, whereas the second phase is dominated by LNA cell membrane damage. Figure 2.4 reflects quantitatively the observation made from the FCM dot-plots in Figure 2.1e-h, where the cells of the LNA bacteria cluster stay visibly intact while the cells of the HNA cluster were damaged (Figure 2.1g).

The rate constants of membrane damage of HNA and LNA cells were calculated for all oxidants based on the data shown in Figure 2.3 (Supporting Information). The results are given in Table 2.2 and show that the membrane permeabilization rate is smaller for LNA cells than for HNA cells for chlorine, chlorine dioxide, chloramine, and permanganate, but not for ferrate, as discussed in the previous section. As mentioned earlier, the rate constant could not be determined for ozone because of the high reactivity, and only a minimal rate

constant was estimated. Table 2.2 also shows that the rate constants vary over more than seven orders of magnitude among the different oxidants and confirms quantitatively the qualitative statement made above that membrane damage efficacy of the oxidants increases in the order monochloramine < permanganate < ferrate ≈ chlorine dioxide < chlorine < ozone. In the case of chlorine, two phases of membrane damage kinetics have been observed for HNA cells (Supporting Information). These two phases of disinfection have been reported in earlier studies during disinfection of pure cultures (Pernitsky et al., 1995; Luh and Mariñas, 2007), which means the kinetic change does not necessarily originate from different bacterial species with different resistance towards chlorine. Comparison of the rate constants obtained for membrane permeabilization (this study) with inactivation rate constants measured with other methods in former studies (Table 2.2) shows that the rate constants obtained in this study are in most cases smaller (up to four orders of magnitude). Only the disinfection rate of *Mycobacterium avium* with chlorine was two orders of magnitude smaller than the rate found in this study.
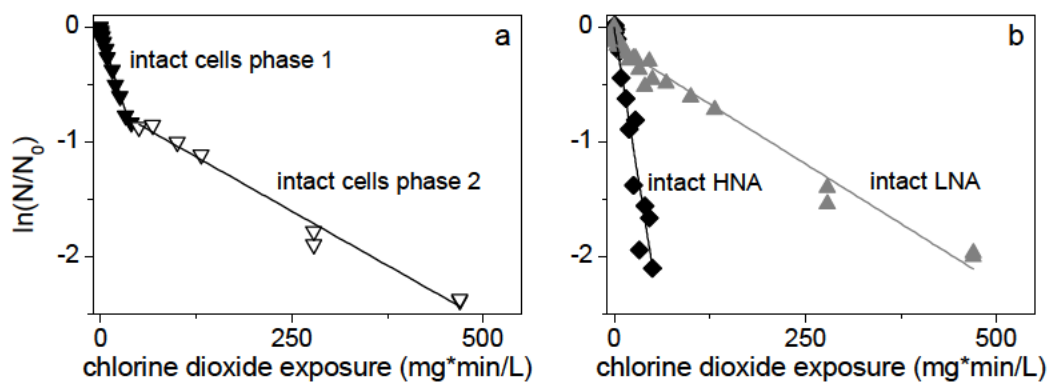
Figure 2.4 *Plot of ln(N/N$_0$) versus chlorine dioxide exposure according to equation 2.2 (a) for intact cells and (b) for the HNA (diamonds) and LNA (triangles) clusters separately. The two linear phases observed in (a) are represented by different symbols and the slope of the linear fit for the first phase is -0.02 L/(mg*min) and for the second phase -0.004 L/(mg*min). The slopes of the linear fits in (b) are -0.04 L/(mg*min) for HNA and -0.004 L/(mg*min) for LNA.*

The comparably small rate constants in this study can be explained as follows. Firstly, PI permeability is a conservative parameter for disinfection. Previous studies have used PI in combination with other dyes on pure cultures in a wide array of disinfection studies and

specifically in comparison with conventional cultivation-based methods (Lisle et al., 1999; Hoefel et al., 2003; Berney et al., 2006). For example, Lisle and co-workers (1999) reported the impact of chlorination on *E. coli* to proceed in the following order of viability indicators: (1) loss of culturability, (2) loss of substrate responsiveness, (3) loss of membrane potential, (4) loss of respiratory activity, and finally (5) loss of membrane integrity. Similar findings were reported for drinking water bacteria exposed to chloramine (Hoefel et al., 2005) and for solar disinfection of *E. coli* (Berney et al., 2006).

Table 2.2 Summary of the rate constants k in L/(mg*min) of HNA and LNA cell membrane damage obtained for six different oxidants at pH 8 and 22 °C and inactivation rate constants in L/(mg*min) from previous studies for various microorganisms.

Oxidant	*this study*		*previous studies*		
	k HNA L/(mg*min)	**k LNA** L/(mg*min)	**k** L/(mg*min)	**tested microorganism**	**reference**
$HOCl$ /OCl^-	1.1 (exposure 0-1 mg*min/L) 9.1 x 10^{-2} (exposure 1-20 mg*min/L)	1.0 x 10^{-1}	k1: 3.85 x 10^{-3} k2: 1.09 x 10^{-3} at 20°C	*Mycobacterium avium*	Luh and Mariñas (2007)
			8 - 36 at 21 °C and pH 7	*E. coli*	estimated from Wojcicka et al. (2007)
			3 - 4	HPC [a]	Pernitsky et al. (1995)
ClO_2	4.2 x 10^{-2}	4.2 x 10^{-3}	1.2 (pH 10) 3.5 (pH 6)	*Mycobacterium avium*	Vicuña-Reyes et al. (2008)
NH_2Cl	4.6 x 10^{-5}	3.6 x 10^{-5}	0.5 - 1	HPC [a]	Pernitsky et al. (1995)
			0.2 - 0.5	*E. coli*	estimated from Wojcicka et al. (2007)
			4.0 x 10^{-3}	*Nitrosomonas europaea* (PI+Syto9)	Wahman et al. (2009)
			3.0 x 10^{-3} – 1.2 x 10^{-2}	*Mycobacterium avium*	Luh et al. (2008)
O_3	> 53 [b]	> 29 [b]	8280 at 20 °C	*E. coli*	Hunt and Mariñas (1997)
FeO_4^{2-}	9.2 x 10^{-3}	9.1 x 10^{-3}	0.33	*E. coli*	Cho et al. (2006)
MnO_4^-	3.7 x 10^{-3}	5.6 x 10^{-4}			

a) Heterotrophic plate count
b) estimated from the slope of the linear fit through the origin and only the first data point at an ozone exposure of 0.06 mg*min/L according to equation 2.2.

On the one hand these results support the notion that cells that have lost their membrane integrity, are completely inactivated. On the other hand they explain why all studies performed with cultivation-based methods are likely to result in higher inactivation rates. Secondly, many studies are performed using pure cultures of bacteria grown under favorable laboratory conditions (Table 2.2). However, a natural community grown on low nutrient conditions might comprise bacteria with different tolerances towards oxidants as mentioned in the introduction. Therefore kinetic data obtained from studies with a native bacterial community should incorporate a wider range of single rate constants and are likely to differ from conventional measurements.

Table 2.3 Oxidant exposures required for 2-log (99 %) inactivation of bacteria and percentage of cells with damaged membrane at the corresponding exposures for selected oxidants.

	required exposures[a]		cells with damaged membrane[b]
Ozone	0.02	(pH 6-9, 5°C)	30%
Chlorine dioxide	0.13	(pH 7)	4-6%
	0.19	(pH 8.5)	
Chlorine	0.08	(pH 7)	32%
	3.3	(pH 8.5)	55%
Monochloramine	94	(pH 7)	0%
	278	(pH 8.5)	2%

a) required exposures ct_{99} according to the WHO guidelines (WHO, 2008) at 1 - 2 °C (if not stated otherwise). Note: with increasing temperatures the required exposures decrease.
b) Percentage was determined from this study (intact cells in Figure 2.2) at 22 °C and pH 8.

While cultivation is a proper way to assess disinfection in cultivable pure cultures, cultivation independent methods (e.g. staining, FCM) are the only mean to investigate the viability of native drinking water bacterial communities. However, a consequence of this approach is that non-conventional disinfection endpoints are measured. Phe and co-workers (2005) proposed the use of total cell numbers (based on SG II staining) to assess chlorination efficacy. The present study offers an additional alternative by using a combination of SG I and PI. PI permeability is achieved at lower oxidant exposures than a decrease of the total cell concentration or changes in the SG staining pattern (Figures 2.1 and 2). Nevertheless, it still results in data whose comparison to conventional data is challenging. A consequence of the smaller rate constants derived from a conservative parameter as endpoint (in this case membrane damage) is for example that the exposures

required to achieve this endpoint are higher. Table 2.3 gives the oxidant exposures required by the WHO guidelines (WHO, 2008) for a 99 % inactivation of bacteria and the percentage of PI permeable cells measured in this study at the corresponding exposures. No oxidant caused membrane damage of 99 % of the cells at the required oxidant exposures. The discrepancy is strongest in the cases of chlorine dioxide and monochloramine, where only 4-6 % and 0-2 % of the cell membranes were PI permeable, respectively. This, again, exemplifies that membrane damage is a conservative and therefore safe parameter to judge disinfection efficacy.

2.5 Conclusions

- The membrane damage potential of the oxidants decreased in the order ozone > chlorine > chlorine dioxide ≈ ferrate > permanganate > chloramine. The membrane damage kinetics was slower than typical disinfection kinetics in previous studies based on cultivability of pure cultures.

- For ozone, chlorine, chlorine dioxide, chloramine, ferrate, and permanganate, PI staining revealed that the membranes were already damaged at exposures where the total cell number measured by SGI staining only had not changed yet.

- In general, HNA cell membranes were damaged faster by the oxidants than LNA cell membranes. However, the difference between HNA and LNA cell membrane permeabilization was not the same for all oxidants, disappearing completely for ferrate.

- Based on the experimental evidence in this study it can be hypothesized that (i) the reactions of the oxidants with amine moieties in the cell membrane are important for enhancing PI permeability and (ii) cell membranes of HNA and LNA cells could contain chemical or structural differences, e.g. HNA cell membranes could contain more or more readily accessible oxidizable non-amine functional groups.

Acknowledgements

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SUPPORTING INFORMATION FOR CHAPTER 2

S-2.1 HNA and LNA in a drinking water treatment plant
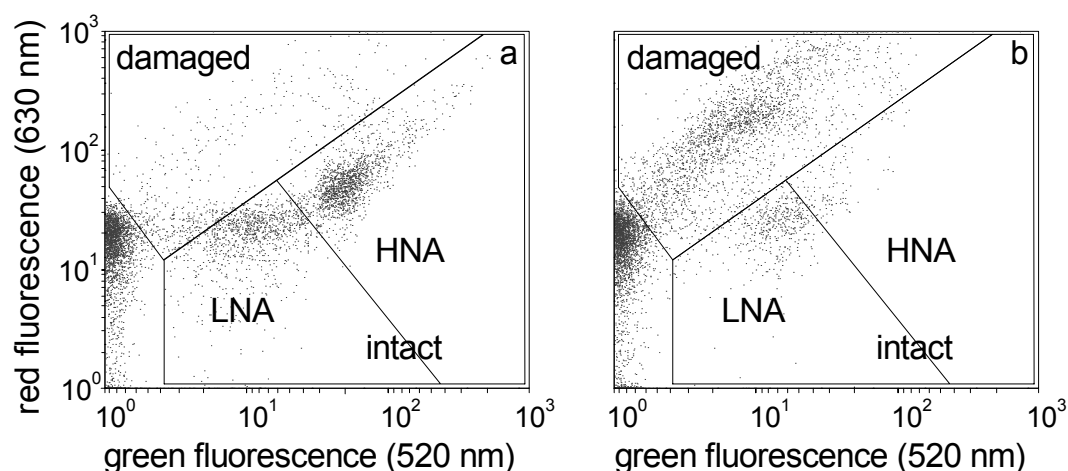
Figure S2.1 *Flow-cytometric dot plots of groundwater after activated carbon filtration (a) and subsequent ClO₂ addition (0.05 mg/L, 5h) (b). Water samples were stained with a combination of SYBR Green I (SGI) and propidium iodide (PI). The regions show cells with a PI permeable membrane (damaged), and cells with a PI impermeable membrane (intact). Intact cells are divided into high nucleic acid cells (HNA) and low nucleic acid cells (LNA).*

Groundwater is the primary drinking water source in Basel (Switzerland). After activated carbon filtration, 0.05 mg/L chlorine dioxide is added for final disinfection. Figure S2.1 shows the flow-cytometric dot plots of samples taken after activated carbon filtration and after chlorine dioxide addition that were measured 5 hours after sampling by staining with SYBR Green I (SGI) and propidium iodide (PI). Activated carbon filtered water contains intact high nucleic acid cells (HNA) as well as low nucleic acid cells (LNA) (Figure S2.1a). Chlorine dioxide addition damages the cell membranes and the cells are now visible in the region of damaged cells in the dot-plot (Figure S2.1b). However, while all HNA cells are PI permeable after five hours of chlorine dioxide exposure some LNA cells remain intact (Figure S2.1b). These results confirm qualitatively the results from the present bench scale study with the help of samples from a drinking water treatment plant with a different water source.

S-2.2 Interpretation of flow-cytometric dot plots
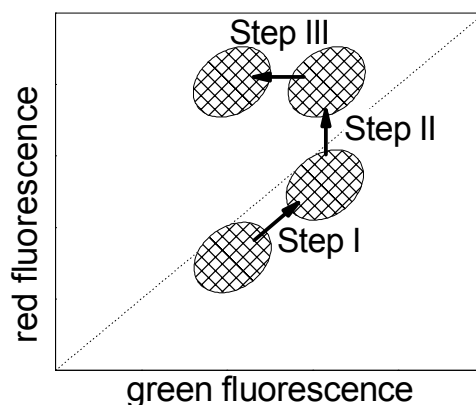
Figure S2.2 *Schematic presentation of a FCM dot plot and the shifts of the cell's signals, represented in ellipses, during membrane damage. Step I: increase of green fluorescence signal due to minor membrane damage that facilitates SYBR Green I (SGI) to enter the cell. Step II: increase of red fluorescence signal due to major membrane damage and propidium (PI) binding to DNA. Step III: decrease of green fluorescence signal due to competition of SGI and PI for binding sites.*

Figure 2.1f in the manuscript shows a typical pattern for cell membrane damage in the FCM dot plot. This pattern is described in an earlier study (Berney et al., 2007) and will be explained in this section by means of Figure S2.2.

SGI has a strong green fluorescence and a weaker red fluorescence. Therefore each cell can be represented as a dot in the red/green dot plot. Upon oxidation of the cell, the membranes first become more permeable for SGI but not yet permeable for PI. In this stage a cell contains more SGI, therefore the green and red fluorescence increase, corresponding to a diagonal upward and right shift of the dots in the dot plot (Step I in Scheme S1). Once the membrane becomes damaged to an extent that some PI is able to enter the cell, PI binds to DNA and the red fluorescence increases. In the dot plot this is observed as an upward shift of the dots (Step II in Scheme S1). When the membranes become more damaged, PI enters the cell easily and it starts to compete with SGI for the target sites. Therefore, less SGI can bind and less green fluorescence is expected. Additionally, SGI and PI interact through fluorescence resonance energy transfer (Berney

et al., 2007) what further weakens the green fluorescence signal. Consequently, the cells' signal shift to the left in the dot plot (Step III in Scheme S1). Note, that even at this moment no change can be observed when the cells are stained with SGI only (Figure 2.1a-d in the manuscript): in this case there is no competition for the target sites and SGI can stain as it could before the membranes were damaged. Only once the DNA gets damaged, fewer target sites are available and less SGI can be bound in a cell. Consequently the green and red fluorescence signal of the cells become weaker which results in a diagonal shift of the dots in the dot plot to the lower left corner. In this case one can not distinguish between HNA cells with damaged DNA and LNA cells with an intact DNA any more. Therefore the distinction between HNA and LNA cells is not made in Figure 2.1a-d.

S-2.3 Control experiments

Figure S2.3 shows the total and intact cell number as well as the HNA and LNA cell number in drinking water without oxidant addition. The data show no decrease of the intact cells (Figure S2.3a) which confirms that the decrease observed in this study after oxidant addition is due to disinfection. All experiments of this study were performed within one day, only in the case of chloramine the experiment was performed over four days. Therefore, the control was run over four days as well. Over this time period the HNA cell number increased, while the LNA cell number decreased slightly (Figure S2.3b). However, in the time frame of all other experiments (ozone, chlorine, chlorine dioxide, ferrate, and also permanganate) this growth and decrease of HNA and LNA cells, respectively, did not occur (Figure S2.3c).
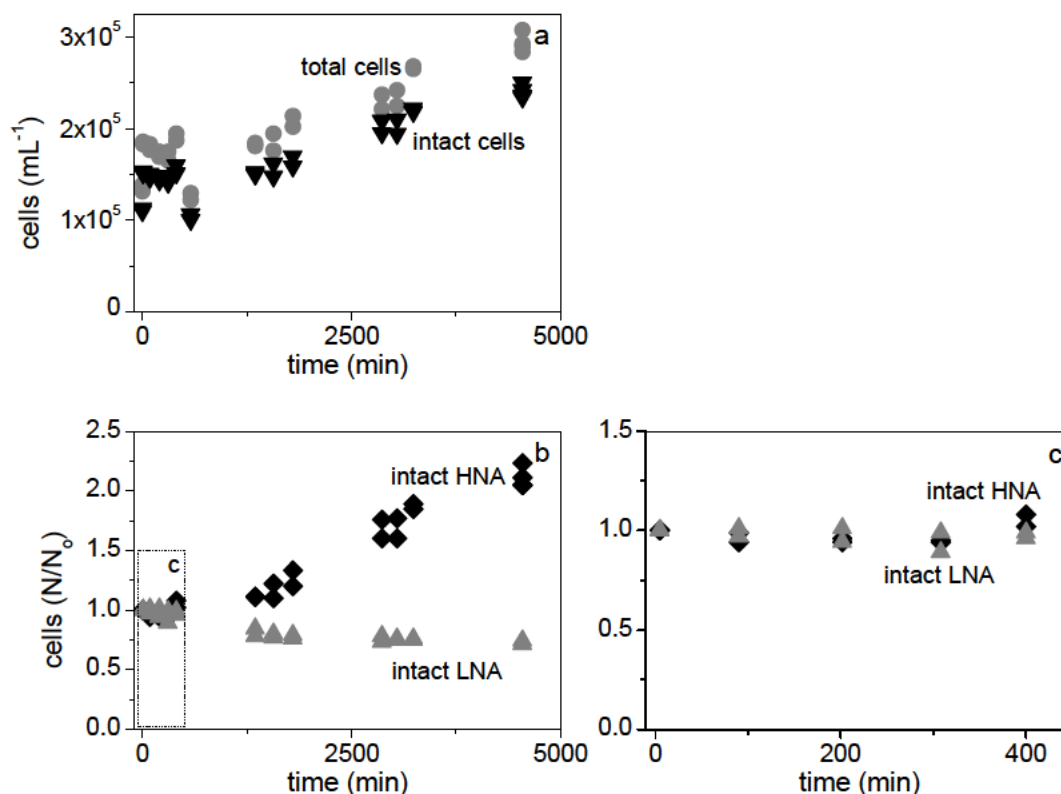
Figure S2.3 *(a) Total cell number (circles) and intact cell number (triangles) and (b) relative intact HNA (diamonds) and relative intact LNA (triangles) cell numbers in drinking water buffered with borate at pH 8 without oxidant addition. The square in (b) gives the region of the zoom presented in (c).*

S-2.4 Repetition of the experiments

In the case of the chlorination and ozonation experiments and for chlorine dioxide and ferrate exposures < 7 mg*min/L each data point shown in this study represents a single independent batch experiment. For the other oxidants and exposures the samples to measure the cell number were taken from one batch. Figure S2.4 shows the data of two repetitions of the experiments with chlorine dioxide (Figure S2.4a) and ferrate (Figure S2.4b). The data coincide well and show the reliability of the results. Figure S2.4c additionally shows the intact cell number of three batch experiments with different chloramine concentrations. There, too, the trends of the cell decrease coincide. The scatter

of the cell numbers during oxidation with ferrate (Figure S2.4b) could be explained by the iron(III) phosphate precipitation interfering with the FCM measurement because the side scatter measurement showed a strong background signal in this case.
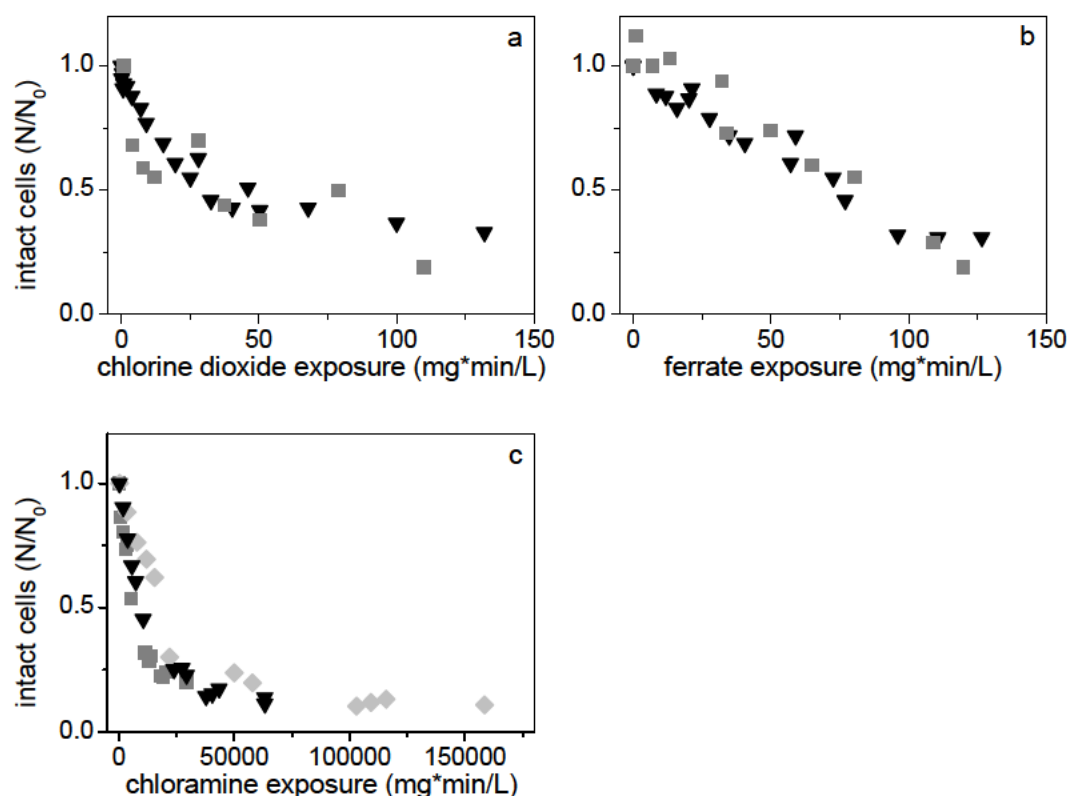
Figure S2.4 *Intact cell number of experiments performed on two different days (squares and triangles) with chlorine dioxide (a) and ferrate (b). (c) Relative intact cell number in water treated with 0.5 mg/L (squares), 1 mg/L (triangles), and 2 mg/L (diamonds) chloramine. The triangles are the data used for discussion in this study.*

S-2.5 Additional Kinetic Data

Figure S2.5 shows the experimental data for chlorine dioxide, chloramine, ferrate, and permanganate that were used to calculate the second order rate constants for membrane damage shown in Table 2 in the manuscript. The rate constants were calculated according to equation 2 in the manuscript and are given by the slopes in Figure S2.5. Further research

is needed to determine whether the assumption of second order reaction kinetics is indeed correct as suggested by the linearity obtained in Figure S2.5.
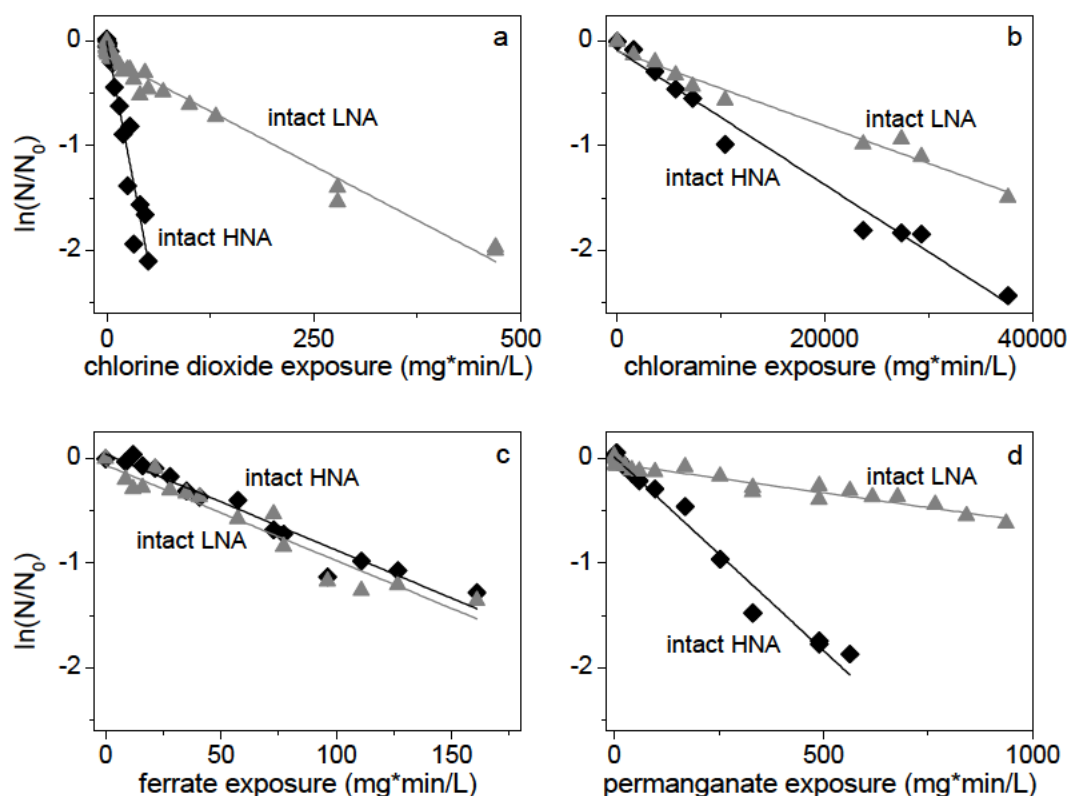
Figure S2.5 *Plot of $\ln(N/N_0)$ versus the oxidant exposure according to equation 2 in this study with N being the cell number for intact HNA (diamonds) and LNA (triangles) cells during oxidation with (a) chlorine dioxide, (b) chloramine, (c) ferrate, and (d) permanganate. The slopes and corresponding R^2-values are as follows for chlorine dioxide HNA slope = -4.2 x 10^{-2} L/(mg*min), R^2 = 0.93, LNA slope = -4.2 x 10^{-3} L/(mg*min), R^2 = 0.97, for chloramine HNA slope = -4.6 x 10^{-5} L/(mg*min), R^2 = 0.98, LNA slope = -3.6 x 10^{-5} L/(mg*min), R^2 = 0.98, for ferrate HNA slope = -9.2 x 10^{-3} L/(mg*min), R^2 = 0.95, LNA slope = -9.1 x 10^{-3} L/(mg*min), R^2 = 0.93, for permanganate HNA slope = -3.7 x 10^{-3} L/(mg*min), R^2 = 0.98, LNA slope = -5.6 x 10^{-4} L/(mg*min), R^2 = 0.94.*
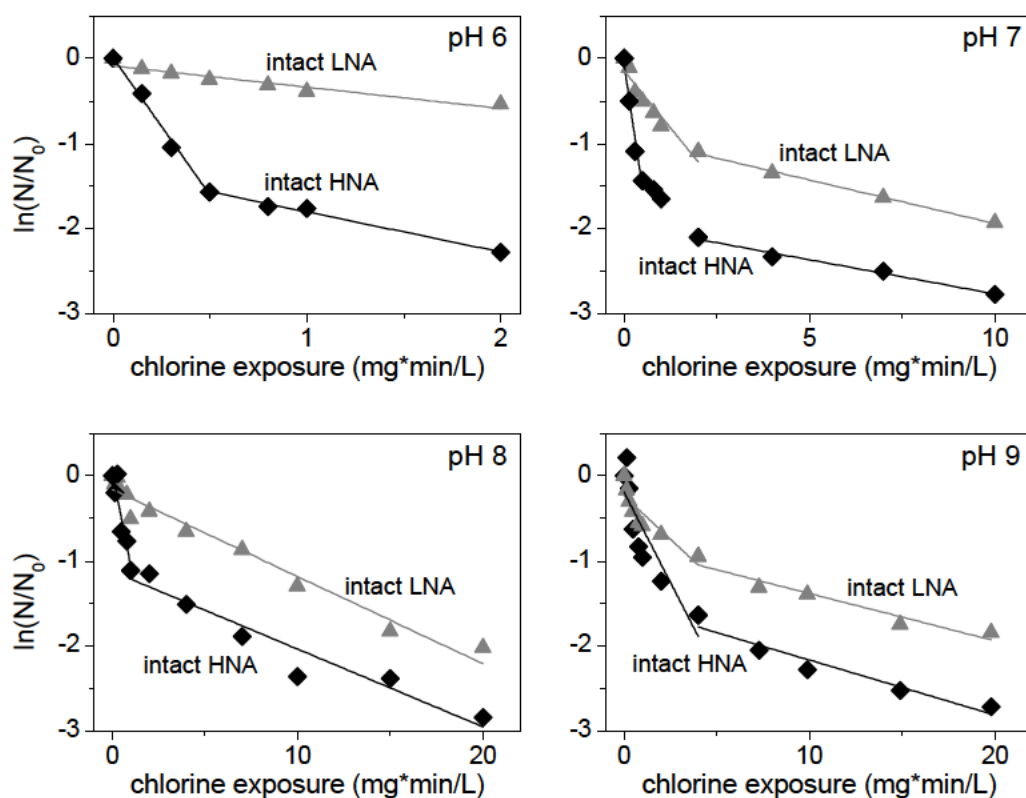
Figure S2.6 *Plot of ln(N/N₀) versus chlorine exposure according to equation 2 in this study with N being the cell number for intact HNA (diamonds) and LNA (triangles) cells at pH 6, pH 7, pH 8, and pH 9. The slopes and corresponding R^2-values are at pH 6 HNA slope of the first phase = -3.23 L/(mg*min), R^2 = 0.99, HNA slope of the second phase = -0.47 L/(mg*min), R^2 = 0.99, LNA slope = -0.25 L/(mg*min), R^2 = 0.92, at pH 7 HNA slope of the first phase = -2.94 L/(mg*min), R^2 = 0.97, HNA slope of the second phase = -0.08 L/(mg*min), R^2 = 0.99, LNA slope of the first phase = -0.54 L/(mg*min), R^2 = 0.90, LNA slope of the second phase = -0.10 L/(mg*min), R^2 = 0.99, at pH 8 HNA slope of the first phase = -1.1 L/(mg*min), R^2 = 0.88, HNA slope of the second phase = -9.1 x 10^{-2} L/(mg*min), R^2 = 0.93, LNA slope = -1.0 x 10^{-1} L/(mg*min), R^2 = 0.96, and at pH 9 HNA slope of the first phase = -0.42 L/(mg*min), R^2 = 0.78, HNA slope of the second phase = -0.06 L/(mg*min), R^2 = 0.93, LNA slope of the first phase = -0.20 L/(mg*min), R^2 = 0.80, LNA slope of the second phase = -0.06 L/(mg*min), R^2 = 0.93.*

Figure S2.6 shows the corresponding data in the case of chlorine application. Unlike the other oxidants plotting ln(N/N₀) versus chlorine exposure with N being the cell number did

not result in a linear function. The chlorination data for membrane damage can often be interpreted as showing two phases (Figure S2.6). Two phases of bacterial inactivation have been observed in former studies as mentioned in the manuscript. However, from the non-linearity of the data it could also be concluded that the permeabilization reaction of chlorine with the cellular membrane does not follow second order reaction kinetics. It remains unclear why this is only observed in the case of chlorine.

Reference

Berney, M., Hammes, F., Bosshard, F., Weilenmann, H.-U., Egli, T., 2007. Assessment and Interpretation of Bacterial Viability by Using the LIVE/DEAD BacLight Kit in Combination with Flow Cytometry. Applied and Environmental Microbiology 73 (10), 3283-3290.

3

FORMATION OF ASSIMILABLE ORGANIC CARBON DURING OXIDATION OF NATURAL WATERS WITH OZONE, CHLORINE DIOXIDE, CHLORINE, PERMANGANATE, AND FERRATE

Abstract

Five oxidants, namely ozone, chlorine dioxide, chlorine, permanganate, and ferrate were studied with regard to their stability in lake water matrices, their formation of assimilable organic carbon (AOC) in absence and presence of cyanobacteria, and their yield of oxalate formation.

The stability of the oxidants in three natural waters increased in the order ozone << ferrate < chlorine dioxide < chlorine << permanganate. Size exclusion chromatography (SEC) with organic carbon detection (OCD) showed a decrease of humic substances and other high molecular weight compounds and a formation of low molecular weight acids upon ozonation. No alteration of the matrix was observed caused by chlorine dioxide and chlorine application.

Ozone and ferrate formed significant amounts of AOC, e.g. more than 100 μg/L AOC were formed from the reaction with the water matrix at an ozone exposure of 5 mg*min/L. In the same water samples chlorine dioxide, chlorine, and permanganate produced no or only limited AOC. When cyanobacterial cells (*Aphanizomenon gracile*) were added to the water, an AOC increase was detected with ozone, permanganate, and ferrate, probably due to cell lysis. This was confirmed by the increase of extracellular geosmin, a substance usually found in the selected cyanobacterial cells. AOC formation by chlorine and chlorine dioxide was not affected by the presence of the cells.

The formation of oxalate upon oxidation was found to be a linear function of the oxidant consumption for all five oxidants. The following yields were measured in three different water matrices based on oxidant consumed: 2.4 - 4.4 % for ozone, 1.0 - 2.8 % for chlorine dioxide and chlorine, 1.1 - 1.2 % for ferrate, and 11 - 16 % for permanganate. Furthermore, oxalate was formed in similar concentrations as trihalomethanes during chlorination (yield ~ 1% based on chlorine consumed). Oxalate formation kinetics and stoichiometry did not correspond to the AOC formation. Therefore, oxalate cannot be used as a surrogate for AOC formation during oxidative water treatment.

3.1 Introduction

Drinking water needs to be delivered to the tap in a hygienically impeccable state. To minimize bacterial regrowth in distribution systems a chemical disinfectant is frequently added, which can lead to the formation of undesired disinfection by-products (Krasner et al., 2006; Richardson et al., 2007). Alternatively, the amount of assimilable organic carbon (AOC) in the water can be reduced to avoid bacterial regrowth through nutrient limitation (van der Kooij, 1992). During drinking water treatment, primary disinfection with chemical oxidants can produce AOC by transforming macromolecular dissolved organic matter (DOM) into smaller molecules, which in turn can be taken up by bacteria more easily (van der Kooij et al., 1989; Hammes et al., 2006). Therefore, oxidative water treatment, e.g. ozonation, is generally followed by a biological filtration step to remove AOC (van der Kooij et al., 1989; Lykins et al., 1994; Vahala et al., 1998).

Up to date many studies concerning AOC production have been performed in full-scale treatment systems under highly variable treatment conditions, which do not allow to compare different oxidants (Liu et al., 2002; Volk and LeChevallier, 2002; Polanska et al., 2005; Chen et al., 2007). In the present study five oxidants - ozone (O_3), chlorine dioxide (ClO_2), chlorine (Cl_2 as $HOCl$), permanganate (MnO_4^-), and ferrate (FeO_4^{2-}) - were investigated in the same water matrices with regard to their AOC formation potential and kinetics. Ozone, chlorine dioxide, and chlorine are widely applied in drinking water treatment for oxidation and disinfection purposes, permanganate is used for iron and manganese removal, and ferrate is discussed as a novel oxidant for micropollutant and phosphate removal mainly in wastewater treatment (Lee et al., 2009). These oxidants undergo various reactions with DOM and their stability differs significantly (Lee and von Gunten, 2010). A brief description of the mechanisms and kinetics of the reactions of the five oxidants with different moieties in DOM is given here.

Ozone. Ozone is the most reactive of the selected oxidants and a high AOC formation can be expected. Ozone can react in different ways with moieties present in DOM: oxygen atom transfer, electron transfer, formation of oxyl radicals, ozone insertion and ring formation (von Gunten, 2003). Ozone is a very selective oxidant and reacts mainly with phenolic and activated aromatic compounds (second order rate constants k: 10^6 - 10^9 $M^{-1}s^{-1}$), deprotonated amines (k: 10^3-10^6 $M^{-1}s^{-1}$) (Hoigné and Bader, 1983), and electron rich

olefines (k: ∼ 10^5 M^{-1}s^{-1}) (Lee and von Gunten, 2010). During ozonation, OH radicals are formed that react unselectively with nearly diffusion controlled rates. Despite the low steady-state concentration of < 10^{-12} M during ozonation of drinking waters they are responsible for the oxidation of ozone-resistant compounds (von Gunten, 2003).

Chlorine dioxide. Chlorine dioxide, a free radical, is a very selective oxidant. The rate constants for the reaction with phenolates (k: 10^6 - 10^9 M^{-1}s^{-1} (Hoigné and Bader, 1994b)), and non-protonated tertiary amines (trimethylamin 6x10^4 M^{-1}s^{-1} (Hoigné and Bader, 1994b)) are high and similar to ozone. In contrast to ozone, chlorine dioxide does not react with olefines, primary and secondary amines, and many organic and inorganic compounds (Hoigné and Bader, 1994b).

Chlorine. The reaction of chlorine with inorganic and organic compounds as well as the disinfection efficiency is pH-dependant because in most cases hypochlorous acid HOCl is more reactive than OCl$^-$ (pK$_{HOCl,25 C}$ 7.54 (Morris, 1966)). Generally hypochlorous acid can undergo oxidation reactions, addition reactions to olefines, or electrophilic substitution reactions (Deborde and von Gunten (2008) and references therein). Except for primary amines, HOCl is in general significantly less reactive than ozone. It reacts with the neutral form of amines resulting in chloramine formation (k$_{app}$ at pH 7: 10^2 - 10^4 M^{-1}s^{-1}), with aromatic compounds by chlorine substitution (reactivity is strongly substituent dependent, k$_{app}$ at pH 7: 10^{-1} - 10^4 M^{-1}s^{-1}), however, the reactivity with olefines is low (k$_{app}$ at pH 7: 1 M^{-1}s^{-1}). Due to the lower reactivity compared to ozone, chlorine is more stable in a water matrix and can therefore be applied for final disinfection in distribution systems.

Permanganate. MnO$_4^-$ is a selective oxidant mainly reacting with olefines. However, also attack of benzylic C-H bonds, single electron transfer from phenols, aromatic ring cleavage, hydrogen or hydride abstraction - especially from alcohols - have been proposed. Substituted phenols (k: 10^1 - 10^2 M^{-1}s^{-1}) and substituted olefines (k: 10^{-1} - 10^2 M^{-1}s^{-1}) react relatively fast with permanganate, although much slower than ozone. Other aromatic systems, other olefines, and any other organic compound react even slower (Waldemer and Tratnyek (2006) and references therein). Permanganate is the least reactive of the five oxidants investigated in this study.

Ferrate. FeO$_4^{2-}$ is more reactive than chlorine and permanganate, and its reaction rate constants are typically about three orders of magnitude lower than those for ozone. It reacts well with phenolic and anilinic compounds (k around 10^3 M^{-1}s^{-1} at pH 7 - 8) but its

reactivity with olefines and tertiary amines is low (k: 1 - 10 $M^{-1}s^{-1}$ at pH 7 - 8) (Lee et al., 2009). Although ferrate rate constants are smaller than ozone rate constants, similar removal of micropollutants in a wastewater matrix has been reported (Lee and von Gunten, 2010). This is due to higher stability of ferrate at neutral to high pH compared to ozone and hence higher oxidant exposure at similar oxidant doses. However, at acidic pH, the ferrate self-decomposition reaction is significant, being pH and ferrate concentration dependent (k: 3 - 250 $M^{-1}s^{-1}$ at pH 8 - 5.5) (Lee et al., 2004). Therefore, at low pH only limited ferrate exposures can be achieved.

The objective of this study was a comparison of the selected oxidants in three natural surface waters with regard to their stability and the extent of their AOC formation from oxidation of DOM in water in absence and presence of bacterial cells. Furthermore, oxalate formation during oxidative treatment was studied as a surrogate for AOC and for THM formation.

3.2 Materials and Methods

Water samples. Waters were sampled from three different surface waters. Lake Greifensee (LG) (Switzerland) is a eutrophic lake surrounded by agricultural land. Samples were taken directly at the surface of the lake, 30 m off shore in November 2008 (LGn) and January 2009 (LGj). Lake Zuerich (LZ) (Switzerland) is a mesotrophic lake and samples were taken in April 2009, 30 m below the lake surface around 600 m off shore. Chriesbach (CB) (Switzerland) is a little creek fed to a considerable part by wastewater treatment plant effluent. Water was sampled in April 2009 at the surface. Some general parameters characterizing all water samples are given in Table 3.1. The water samples were filtered through a 0.2 μm filter (regenerated cellulose, Sartorius AG, Goettingen, Germany, rinsed with 1 L of micropure water (NANOpure Diamond^{TM}, Barnstead) and stored at 4°C in the dark until use (maximum 10 days).

Table 3.1 Water quality parameters of the selected waters.

water type	DOC (mg C/L)	pH	Alkalinity (mM)	Ammonium (μM NH_4^+)
Lake Greifensee (LGn) [a]	3.6	8.2	3.4	11
Lake Greifensee (LGj) [a]	3.8 - 3.1 [b]	8.1	3.9	1.5-3.6 [b]
Lake Zürich (LZ)	1.3	8.1 [c]	2.6	0.5
Chriesbach (CB)	3.1	8.1	6.2	0.6

a) LGn: November 08 sample, LGj: January 09 sample
b) measured at the beginning (first value) and at the end (second value) of the experimental series that was performed within 10 days
c) containing 5 mM borate buffer

Oxidant stock solutions. Ozone was applied as aqueous stock solution prepared by sparging an oxygen-ozone gas mixture from an oxygen-fed ozone generator (CMG 3-3, Apaco AG, Switzerland) through ice-cooled water. The ozone concentration of the stock solution was determined spectrophotometrically with $\varepsilon(258\ nm) = 3000\ M^{-1}cm^{-1}$ (Elovitz and von Gunten, 1999) and was 1.3 - 1.5 mM. A chlorine dioxide stock solution was prepared by reaction of chlorite with peroxodisulfate and the reaction product, chlorine dioxide, was transferred by a nitrogen stream into a gas-washing bottle containing ice-cooled water (Hoigné and Bader, 1994b; Huber et al., 2005). The concentration of the stock solution was determined spectrophotometrically with $\varepsilon(358\ nm) = 1200\ M^{-1}cm^{-1}$.

Sodium hypochlorite (10% active chlorine Riedel-deHaen, Germany) was diluted in micropure water and standardized spectrophotometrically at pH 8.7 with $\varepsilon(292\ nm) = 350$ $M^{-1}cm^{-1}$ (supporting information section of Lee et al. (2008)). Potassium ferrate (K_2FeO_4) was prepared by the method of Thompson and co-workers (1951) and had a purity of 88 % as Fe(VI) (w/w). Ferrate stock solutions were prepared from solid potassium ferrate in micropure water and used immediately. The stock concentration was measured spectrophotometrically, with $\varepsilon(510\ nm) = 1150\ M^{-1}cm^{-1}$ (Lee et al., 2005). A 5 mM permanganate stock solution was prepared in water from solid potassium permanganate and standardized spectrophotometrically with $\varepsilon(525\ nm) = 2430\ M^{-1}cm^{-1}$ (cf. supporting information).

Measurement of oxidant consumption and determination of oxidant exposure. Oxidant stock solutions were added to the stirred water samples in a 250 ml Schott bottle equipped with a dispenser system (Hoigné and Bader, 1994a). At various time intervals, the residual oxidant concentration was determined by dispensing aliquots of the sample into a plastic sampling tube containing a buffer and a quenching agent dye that changes color upon oxidation. Table 3.2 summarizes the buffers, quenching agents, wavelength and absorption coefficients.

For all oxidants, the exposure (ct) was calculated as the integral of the transient oxidant concentration over the reaction time (von Gunten and Hoigné, 1994).

Measurement of oxidation products. AOC was measured as described elsewhere (Hammes and Egli, 2005). Briefly, all samples were prepared in AOC free glassware. Prior to the experiments, the water was filtered through a thoroughly rinsed 0.2 μm filter (see above) into AOC-free glassware. After the experiment the water was partitioned into two AOC free vials. Cells from a natural microbial community were added and the sample was incubated at 30°C for three days. The added natural microbial inoculum had been prepared by mixing different water samples (oxidized and untreated LZ, CB, and LG water) and had been stored at 4 °C for several days to weeks. The final cell concentration after regrowth in the actual water sample was determined by staining the cells with SYBR® Green and an addition of 5 mM ethylendiaminetetraacetate and counting the cells by flow cytometry on a PASIII flow cytometer (Partec, Germany). The AOC concentration was calculated

assuming a bacterial growth of 10^7 cells per μg assimilable organic carbon (Hammes et al., 2006). The limit of detection was 10 μg/L and the standard error of triplicate incubated samples < 10%.

Oxalate was measured using ion chromatography (Dionex ICS-3000) as described elsewhere (Hammes et al., 2006). A Dionex AS11HC column with an OH^--gradient was used for separation and the target compound was detected by conductivity measurement with a limit of quantification of 0.5 μM and the standard deviation of four repetitions of the same sample (20 μM) was <10 %.

Trihalomethanes ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$, $CHBr_3$) were measured by GC-ECD (GC 8000 and ECT 80/800, Fisons). 6 ml of the sample solution were heated at 80°C before 1 ml of the headspace was injected on a Restek RTX 624 column (Gallard and von Gunten, 2002). Detection limits were 0.1 μg/L and the standard deviation of multiple measurements of the standards used for calibration was < 3 %, except for chloroform, where it was < 15 %.

DOM fractions were measured by size exclusion chromatography (SEC) coupled to an organic carbon detector (OCD) (Meylan et al., 2007) and a UV detector (K-200, Knauer, Berlin, Germany) measuring at a fixed wavelength of 254 nm. Samples containing iron-precipitates from experiments where iron(II)chloride was used as quencher (see below) were allowed to settle and were then decanted. The SEC-OCD chromatograms were characterized according to Huber and Frimmel (1996).

Selection of quenching agent. To measure AOC after a defined oxidant exposure, the oxidants needed to be quenched. Nitrite was used to quench ozone, a freshly prepared iron(II)chloride solution was used to quench chlorine dioxide, chlorine, permanganate, and ferrate (see below). Experiments had shown that neither nitrite nor its oxidized form, nitrate, have an influence on the regrowth of the bacteria in the AOC assay (data not shown). However, nitrite reaction was only fast enough with ozone but not with the other oxidants. Thiosulfate, an often used alternative, affected the AOC measurement by amplifying the bacterial growth in some waters, e.g., in LG water by 100 - 180 %. Experiments with iron(II)chloride showed only a minor influence of the quencher on the amount of AOC measured in LG water (cf. supporting information). Also, experiments

with iron(III)chloride confirmed that the DOM precipitation with iron(hydr)oxides did not change the relative AOC and DOC concentrations (data not shown).

Table 3.2 Summary of methods used to determine the residual oxidant concentration in a water sample (refer to text for explanation).

oxidant	dye and buffer	wave-length (nm)	absorption coefficient ($M^{-1}cm^{-1}$)	reference
ozone	indigotrisulfonate in phosphate buffer	600	20 000	Bader and Hoigne (1981)
chlorine	DPD[a] in phosphate buffer spiked with EDTA[b]	551	21 000[c]	Clesceri et al. (1998)
chlorine dioxide	ABTS[d] in acetate / phosphate buffer (sample pH 6.5)	415	34 000	adapted from Pinkernell et al. (2000)
ferrate	ABTS[d] in acetate / phosphate buffer	415	34 000	Lee et al. (2005)
permanganate	direct measurement without dye	525	2 430	supporting information of this study, similar values in Reisz et al. (2008), Ahlstrom et al. (1984)

a) *N,N'*-diethyl-*P*-phenylenediamine
b) ethylenediaminetetraacetate
c) stoichiometry of 1 Cl_2 producing 2 $DPD^{\bullet+}$ included in ε
d) 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt

Experimental procedure. To determine the formation of AOC, oxalate, THM and the change in DOM fractions upon oxidation, LGj water was filtered through a 0.2 µm filter (regenerated cellulose, Sartorius AG, Goettingen, Germany, rinsed with 1 L of micropure water) into an AOC-free bottle. The decay of each oxidant in this matrix was determined in a 250 ml glass bottle equipped with a dispenser system as described above and the reaction times to achieve the desired oxidant exposures were determined. Then 20 - 50 ml of the filtered LGj water were poured into AOC-free Erlenmeyer flasks with three replicates for each designated reaction time. Oxidant stock solution was added to each flask and after the appropriate reaction time the oxidant was quenched. The oxidant doses were: 4.6 mg/L or 0.096 mM for O_3, 3 mg/L or 0.042 mM for Cl_2, 3 mg/L or 0.045 mM for ClO_2, 3.2 mg Mn/L or 0.058 mM for MnO_4^-, and 4.6 mg Fe/L or 0.083 mM for FeO_4^{2-} and the quencher was added in the following concentrations: 0.5 mM nitrite in the case of ozone, and 0.3 mM of freshly prepared $FeCl_2$ solution (pH around 1) in the case of chlorine dioxide and

ferrate, and 0.2 mM in the case of chlorine and permanganate. After quenching each water sample was partitioned into two AOC-free vials for AOC determination, one DOC-free vial for SEC measurement, after filtration through a 0.45 µm nylon syringe filter (BGB Analytik AG, Switzerland) one vial for oxalate determination, and in the case of chlorination one GC vial for THM determination. All experiments were performed at 22°C (+/-1°C) and the pH was 8.0 - 8.3.

For AOC formation calculation the difference between the mean AOC value of three replicates measured at a certain oxidant exposure and the mean AOC value of three replicates at an exposure of 0 mg*min/L was calculated. Hence the error bars were calculated according to the law of error propagation and represent $\sqrt{[std.dev(AOC_x)]^2 - [std.dev(AOC_0)]^2}$.

To measure the production of oxalate upon oxidation of different water matrices, additional experiments with LZ and CB water were preformed: the water was filtered through a 0.45 µm cellulose nitrate membrane filter (Sartorius AG, Goettingen, Germany) and 5 mM borate buffer was added to LZ water and adjusted to a pH of 8.1 +/- 0.1 with sulfuric acid. The consumption of each oxidant was measured as described above. At several time points an additional aliquot for oxalate determination was taken and quenched with an excess of nitrite in the case of ozone or thiosulfate in the case of the other oxidants. Applied oxidant doses were: 0.093 mM O_3, 0.058 mM Cl_2, 0.044 mM ClO_2, 0.071 mM K_2FeO_4, and 0.060 mM $KMnO_4$. The temperature was 20-22°C and the experiments in each water matrix were performed within three days after sampling.

Oxidation experiments in presence of Aphanizomenon gracile. The *Aphanizomenon gracile* strain SAG 31.79, which is a geosmin producing cyanobacterium, was grown on BG-11 medium and the culture stock solution had an optical density of 0.47 measured at 685 nm. The bacterial cells were washed by centrifuging and decanting the supernatant twice, resuspended in LZ water and then spiked into 0.2 µm-filtered LZ water (see above) in AOC-free glassware to reach cell concentrations similar to natural conditions. Three oxidant doses (0.01, 0.02, and 0.05 mM) of each oxidant were added to LZ water with and without cells with reaction times of 24 hours at 12°C. The lower temperature was chosen to avoid additional stress on the cells by an increased temperature. After 24 h, ozone and

ferrate were consumed completely and the remaining chlorine, chlorine dioxide, and permanganate was quenched by addition of $FeCl_2$. All samples were filtered through a 0.22 µm polyethersulfon membrane syringe filter (Millipore, Cork, Ireland) into two AOC free vials and inoculated to measure AOC as described above.

Additionally, the geosmin concentration in the solution after the oxidation process was measured by solid phase micro extraction (SPME) coupled with GC-MS as described elsewhere (Peter et al., 2009).

3.3 Results and Discussion

3.3.1 Reactivity and consumption of different oxidants in the same matrix

Based on the high variability of the reactivity of the selected oxidants with DOM moieties, large differences for their consumption rate can be expected. As a first simplified approach for the decrease of oxidants in a natural water matrix with DOM moieties in large excess, a pseudo first-order rate law can be assumed:

$$\frac{d[Ox]}{dt} = -k[Ox] \qquad (3.1)$$

where [Ox] is the concentration of the oxidant, t is the reaction time, and k is the pseudo first-order rate constant of the oxidant decrease for the specific matrix. After integration, the pseudo first-order rate constant can be obtained from a plot of $\ln([Ox]/[Ox]_o)$ versus time according to equation (3.2):

$$\ln\left(\frac{[Ox]}{[Ox]_0}\right) = -kt \qquad (3.2)$$

Figure 3.1 shows the corresponding plot for CB water and shows that the reactivity of the oxidants in the waters studied followed the order ozone >> ferrate > chlorine dioxide > chlorine >> permanganate. However, depending on the water matrix and the pH the sequence of the five oxidants may change slightly, especially among ferrate, chlorine dioxide, and chlorine.

According to Figure 3.1 a change in the pseudo first-order rate constant (slopes in Figure 3.1) could be observed during the oxidation process. Therefore, the kinetics were empirically divided into different phases. To determine the pseudo first-order rate constants of these phases, linear regression curves were fitted to the data set starting at the time point zero such that each regression had an $r^2 \geq 0.96$. Permanganate did not follow a pseudo first-order rate law at any time. The rate constants for the other four oxidants for various waters are summarized in Table 3.3. Additionally, the oxidant dose and the amount of oxidant that is consumed within the first pseudo first-order reaction phase are given. In general, the rate constants in the initial phase were similar for chlorine, chlorine dioxide, and ferrate. For longer contact times the rate of chlorine consumption decreased drastically while ferrate continued to be consumed. Ferrate is known to undergo a self-decomposition

reaction (Lee et al., 2004) and modeling this reaction showed that in all waters the major part of its concentration decrease was due to this self-decomposition reaction (cf. supporting information).
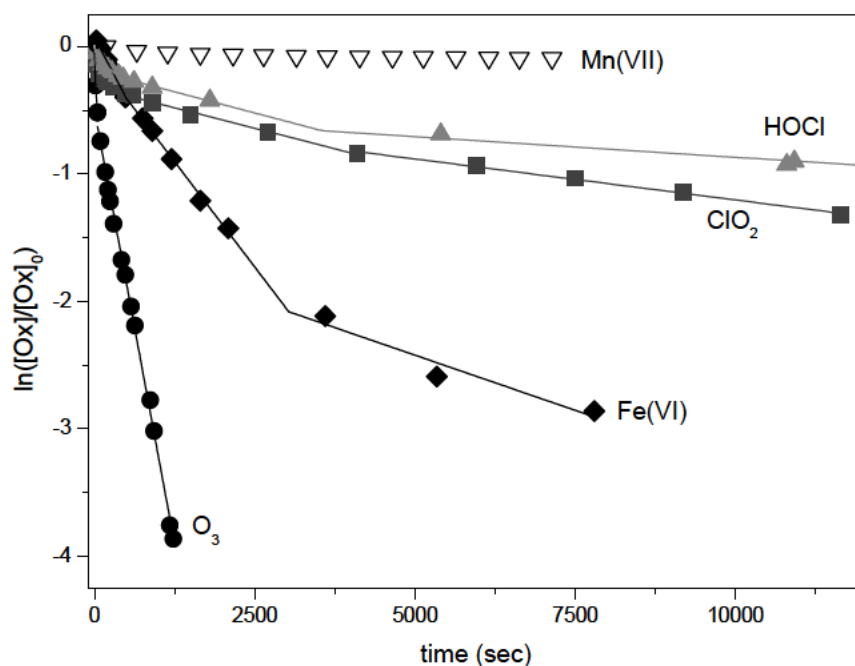
Figure 3.1 *Decrease of five oxidants in CB water. Doses were 4.6 mg/L ozone, 3 mg/L chlorine dioxide, 4 mg/L chlorine, 3.3 mg/L permanganate-manganese, and 4 mg/L ferrate-iron. The slope of the* $\ln\left(\frac{[Ox]}{[Ox]_0}\right)$ *vs. time plot corresponds to the pseudo first-order rate constant (equation 3.2).*

The change in the pseudo first-order rate constants shows that the pseudo first-order conditions were not entirely fulfilled. It points towards a depletion of certain reactive moieties in the DOM which is more pronounced for more selective oxidants. In a first phase the reactions of the oxidant with the most reactive moieties take place and the consumption of the oxidant can be very fast, as has been reported for oxidants in wastewater (Lee and von Gunten, 2010). Table 3.3 shows that the initial chlorine consumption in LG water was four-times higher in November than in January. Chlorine reacts predominantly with amines and ammonia and Table 3.1 confirms that the ammonium concentration in LG water was significantly higher in November than in

January. Additionally, Tables 3.1 and 3.3 show that the initial chlorine consumption was in the range of the ammonia concentration or slightly higher, presumably due to other amines present in the water matrix. In contrast, chlorine dioxide reacts predominantly with aromatic compounds (Hoigné and Bader, 1994b) and the similar initial chlorine dioxide consumption in November and January suggests their concentrations to be similar in both seasons. However, the initial consumptions were small. This implies that the matrix only contained small concentrations of fast reacting functional groups.

Although Figure 3.1 shows a change of the pseudo first-order rate constant for ozone, its consumption remains fast in the second phase. Beside the reaction with DOM a second mechanism enhances ozone consumption: ozone forms hydroxyl radicals that consume ozone in a chain reaction with some DOM compounds acting as chain reaction promoters. This chain reaction accelerates ozone decrease (von Gunten, 2003).

3.3.2 DOM transformation by ozone, chlorine, and chlorine dioxide

As discussed above, ozone, chlorine, and chlorine dioxide have different reactivities towards DOM, which is reflected in the change of DOM fractions during oxidative water treatment:

Figure 3.2a shows SEC-OCD difference chromatograms and Figure 3.2b SEC-UV chromatograms during oxidation of LGj water. For the OCD data the representation as difference chromatogram was chosen to better illustrate the differences of the signal for the various oxidant exposures (ct-values).

Table 3.3 Pseudo first-order rate constants $k^{a)}$ for different phases of oxidant decrease (cf. Figure 3.1 for CB water) and initial fast oxidant consumption are given for four oxidants in different waters (for details refer to text). k is given in s^{-1}.

	LGn water		LGj water		LZ water		CB water	
Ozone	initial dose (µM):	112	initial dose (µM):	96	initial dose (µM):	93	initial dose (µM):	97
	k(0-18 sec.):	$1.9 \cdot 10^{-2}$	k(0-13 sec.):	$2.6 \cdot 10^{-2}$	k(0-45 sec.):	$3.8 \cdot 10^{-3}$	k(0-15 sec.):	$2.0 \cdot 10^{-2}$
	k(18-60 sec.):	$4.8 \cdot 10^{-3}$	k(13-60 sec.):	$4.2 \cdot 10^{-3}$	k(75-255 sec.):	$1.4 \cdot 10^{-3}$	k(15-45 sec.):	$7.1 \cdot 10^{-3}$
	k(60-2790 sec.):	$1.3 \cdot 10^{-3}$	k(60-1890 sec.):	$1.4 \cdot 10^{-3}$	k(480-5220 sec.):	$6.6 \cdot 10^{-4}$	k(45-1230 sec.):	$2.7 \cdot 10^{-3}$
	oxidant consumption within		oxidant consumption within		oxidant consumption within		oxidant consumption within	
	0 - 18 sec. (µM):	32.2	0 - 13 sec. (µM):	27.6	0 - 45 sec. (µM):	14.6	0 - 15 sec. (µM):	25.5
Chlorine	initial dose (µM):	42	initial dose (µM):	42	initial dose (µM):	58	initial dose (µM):	56
	k(0-13 sec.):	$3.9 \cdot 10^{-2}$	k(0-11 sec.):	$7.9 \cdot 10^{-3}$	k(15-75 sec.):	$3.3 \cdot 10^{-4}$	k(0-26 sec.):	$2.0 \cdot 10^{-3}$
	k(40-120 sec.):	$2.2 \cdot 10^{-3}$	k(45-180 sec.):	$5.7 \cdot 10^{-4}$	k(75-600 sec.):	$8.1 \cdot 10^{-5}$	k(75-375 sec.):	$4.2 \cdot 10^{-4}$
	k(120-1500 sec.):	$6.7 \cdot 10^{-4}$	k(300-3600 sec.):	$8.5 \cdot 10^{-5}$	k(1380-10575 sec.):	$8.3 \cdot 10^{-6}$	k(375-1800 sec.):	$1.3 \cdot 10^{-4}$
	oxidant consumption within		oxidant consumption within		oxidant consumption within		k(5400-106560 sec.):	$3.2 \cdot 10^{-5}$
	0 - 13 sec. (µM):	16.7	0 - 11 sec. (µM):	3.49	15 - 75 sec. (µM):	1.15	oxidant consumption within	
							0 - 26 sec. (µM):	2.87
Chlorine dioxide	initial dose (µM):	15	initial dose (µM):	44	initial dose (µM):	44	initial dose (µM):	44
	k(0-20 sec.):	$8.7 \cdot 10^{-3}$	k(0-14 sec.):	$6.5 \cdot 10^{-3}$	k(0-165 sec.):	$3.7 \cdot 10^{-4}$	k(0-16 sec.):	$9.0 \cdot 10^{-3}$
	k(20-180 sec.):	$1.6 \cdot 10^{-3}$	k(43-300 sec.):	$3.2 \cdot 10^{-4}$	k(165-23340 sec.):	$7.6 \cdot 10^{-5}$	k(70-300 sec.):	$4.1 \cdot 10^{-4}$
	k(300-6000 sec.):	$4.4 \cdot 10^{-4}$	k(300-3600 sec.):	$1.1 \cdot 10^{-4}$	oxidant consumption within		k(495-4095 sec.):	$1.3 \cdot 10^{-4}$
	oxidant consumption within		oxidant consumption within		0 - 165 sec. (µM):	2.57	k(4095-93900 sec.):	$5.0 \cdot 10^{-5}$
	0 - 20 sec. (µM):	2.35	0 - 14 sec. (µM):	3.79			oxidant consumption within	
							0 - 16 sec. (µM):	5.87
Ferrate	initial dose (µM):	42	initial dose (µM):	70	initial dose (µM):	71	initial dose (µM):	76
	k(15-60 sec.):	$6.5 \cdot 10^{-4}$	k(13-2400 sec.):	$4.0 \cdot 10^{-4}$	k(20-587 sec.):	$6.3 \cdot 10^{-4}$	k(25-480 sec.):	$9.5 \cdot 10^{-4}$
	k(60-3600 sec.):	$4.1 \cdot 10^{-4}$	oxidant consumption within		k(587-2085 sec.):	$4.9 \cdot 10^{-4}$	k(480-2085 sec.):	$6.6 \cdot 10^{-4}$
	oxidant consumption within		13 - 2400 sec. (µM):	43.0	k(2085-6036 sec.):	$2.6 \cdot 10^{-4}$	k(3600-7800 sec.):	$1.7 \cdot 10^{-4}$
	15 - 60 sec. (µM):	1.97			oxidant consumption within		oxidant consumption within	
					20 - 587 sec. (µM):	21.1	25 - 480 sec. (µM):	27.3

Chlorine dioxide and ferrate doses were higher in LGj than in LGn water in order to reach higher oxidant exposures.

a) Rate constants were determined empirically (refer to text). No rate constant for the time between the start of the reaction and the first sampling point is given in the cases where the linear fit of the first phase did not go through the origin.

Ozone

For increasing ozone exposures, a clear decrease of humic substances (-260 μg C/L) and the high molecular weight fraction (-70 μg C/L) was observed after integration of the SEC-OCD data while the fraction of the low molecular weight (LMW) acids and humics increased slightly from 41 to 71 μg C/L. The initially negative values in the differencechromatogram for LMW acids and humics could be explained by the strong tailing of the building blocks into the LMW compound fraction. Integration of the signal showed an initial decrease of the building block concentrations followed by a steady increase. This initial decrease influenced the LMW acid and humics signal. However, integration of the LMW acids and humics showed that their concentration increased directly at the first exposure measured.

Although the concentration of the building blocks increased slightly, their UV signal decreased drastically during ozonation indicating a change in quality of this fraction towards less aromatic or conjugated systems. In contrast, the UV signal of the LMW acids and humics remained constant over a wide range of ozone exposures. We suppose firstly, that this fraction contains UV absorbing compounds which, however, are not very susceptible towards ozonation (e.g. small aromatic compounds without activating substituents). Secondly, we assume that the newly formed acids and humics did not absorb UV light at 254 nm, e.g. carboxylic acid functional groups which are a major ozonation product (Leitzke and von Sonntag, 2009; Ramseier and von Gunten, 2009).

Chlorine dioxide and chlorine

The SEC-chromatograms in Figure 3.2 show a strong decrease of the OCD and the UV signals from the untreated water sample to the samples treated with chlorine dioxide or chlorine. This strong decrease in signal in all DOM fractions is assumed to be a methodological artifact due to the quenching of the oxidants with iron(II)chloride. Iron precipitation formed larger flocks in the water containing the oxidants and, hence, we suppose that the co-precipitation of DOM was stronger than in water with iron(II)chloride but without oxidant (Park and Yoon, 2009).

A further increase of the oxidant exposure did not alter the OCD and UV signals. The only exception was a slight increase of the concentration of LMW acids and humics (+20 μg C/L) at a chlorine dioxide exposure of more than 30 mg*min/L as observed in the SEC-
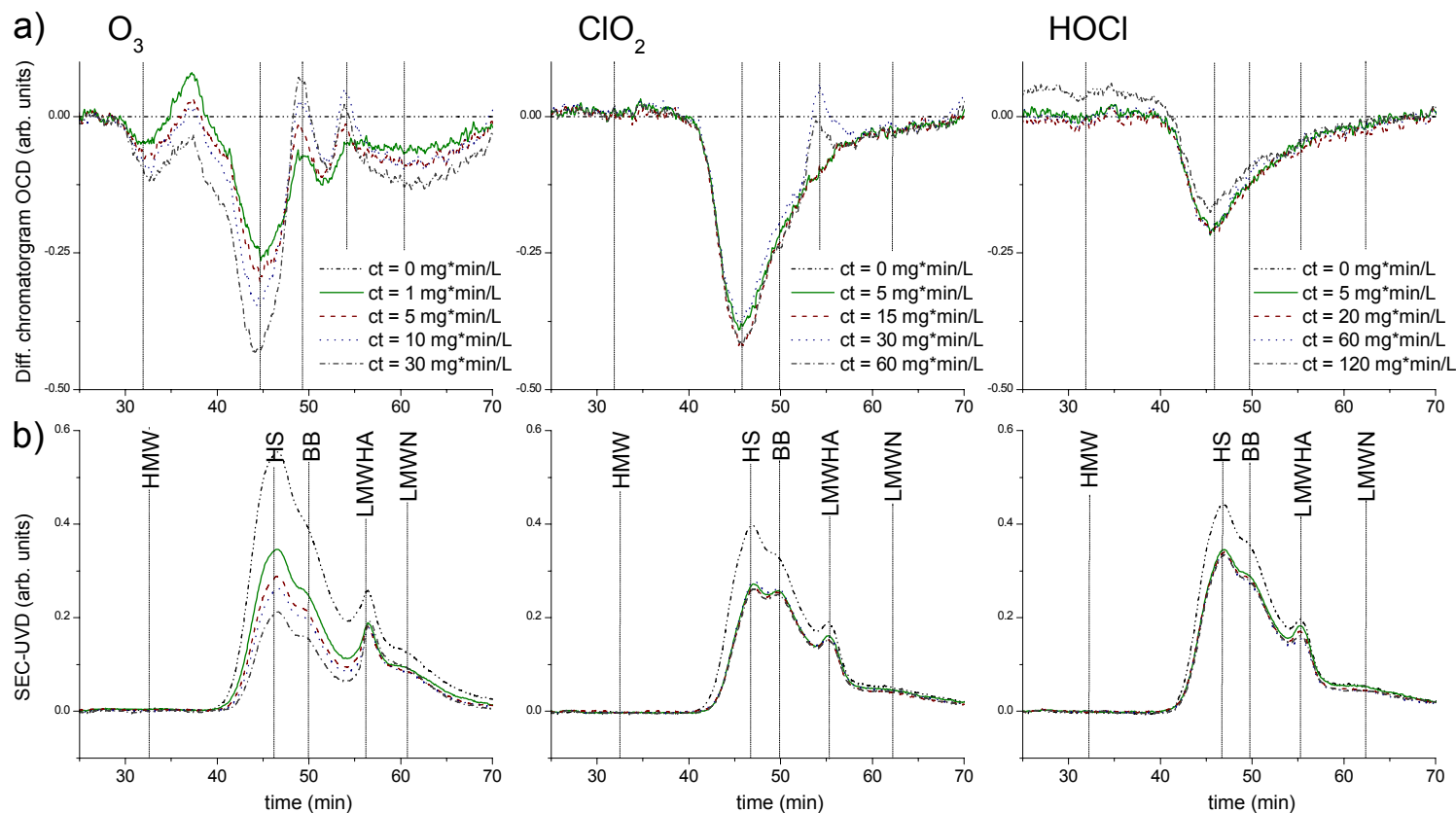
Figure 3.2 *SEC-OCD difference chromatograms (a) and SEC-UV chromatograms (b) of LGj water for varying degrees of oxidation by ozone (4.6 mg/L), chlorine dioxide (3 mg/L), and chlorine (3 mg/L) at pH 8.1 and 22 °C. Initial fraction concentrations and molecular weights were roughly: 70-230 µg C/L high molecular weight fraction (HMW), > 20 000 Da; 850-1150 µg C/L Humic Substances (HS), ≈1000 Da; 700-830 µg C/L Building Blocks (degraded humic substances) (BB), 300-500 Da; 40-50 µg C/L low molecular weight humics and acids (LMWHA), < 350 Da; 260 µg C/L low molecular weight neutrals (LMWN), < 350 Da.*

OCD chromatogram. This confirms the lower reactivity of chlorine and chlorine dioxide compared to ozone. Furthermore, it indicates that the chlorine and chlorine dioxide exposures applied were not sufficient to lead to a major cleavage of UV-absorbing organic molecules. This is in line with data shown by Swietlik and co-autors (2009), where chlorine, although applied in higher doses, had no significant influence on the SEC-UV chromatogram. However, in their study chlorine dioxide decreased the UV signal of larger molecules and subsequently formed oxidation by-products of molecular weights below 300 Da. Our study cannot confirm the decrease of the UV signal of larger molecules due to chlorine dioxide treatment. However, the SEC-OCD data confirm the formation of compounds with molecular weights below 350 Da and, hence, basically the ability of chlorine dioxide to break down organic macromolecules.

It has to be kept in mind that the effects of the oxidants on the DOM fractions are compared in terms of oxidant exposure: to alter the organic matrix, lower ozone than chlorine dioxide exposures were required indeed. However, at the moment where these exposures were reached, more ozone than chlorine dioxide had been consumed, hence, more ozone had reacted with the water matrix components (see Lee and von Gunten (2010)). This demonstrates the difficulty when comparing the effect of oxidants with different reactivity and stability.

3.3.3 AOC formation by ozone, chlorine dioxide, chlorine, permanganate, and ferrate

The SEC-OCD/UV data showed a significant transformation of DOM after oxidation with ozone but not with chlorine and chlorine dioxide. Accordingly, a significant increase of the AOC concentration is only expected for ozonation. Previous investigations support this expectation: while ozone is known to produce high amounts of AOC (e.g. Hammes et al. (2006)), chlorine was found to sometimes produce moderate amounts of AOC (e.g. Polanska et al. (2005)). Less is known about the extent of AOC formation from chlorine dioxide, permanganate, or ferrate.

Figure 3.3 shows the AOC formation for various oxidants in LGj water. The data confirm the prediction made from the SEC-OCD/UV measurements. Significant concentrations of

AOC are only formed during ozonation. An exposure of 1 mg*min/L was achieved after 16 seconds for an ozone dose of 4.6 mg/L (1.3 mg O_3/mg DOC) and resulted in an AOC formation of 60 μg/L. The fast formation kinetics can be explained with the high reactivity of ozone towards several organic functional groups as outlined in the introduction. Similar findings were reported earlier (Hammes et al., 2006) and could also be observed for the production of small organic acids during ozonation of phenol, a surrogate compound for DOM (Ramseier and von Gunten, 2009). Consequently, a significant AOC production cannot be avoided when ozone is applied for disinfection and a suitable technique to control this parameter after ozonation is indispensable.
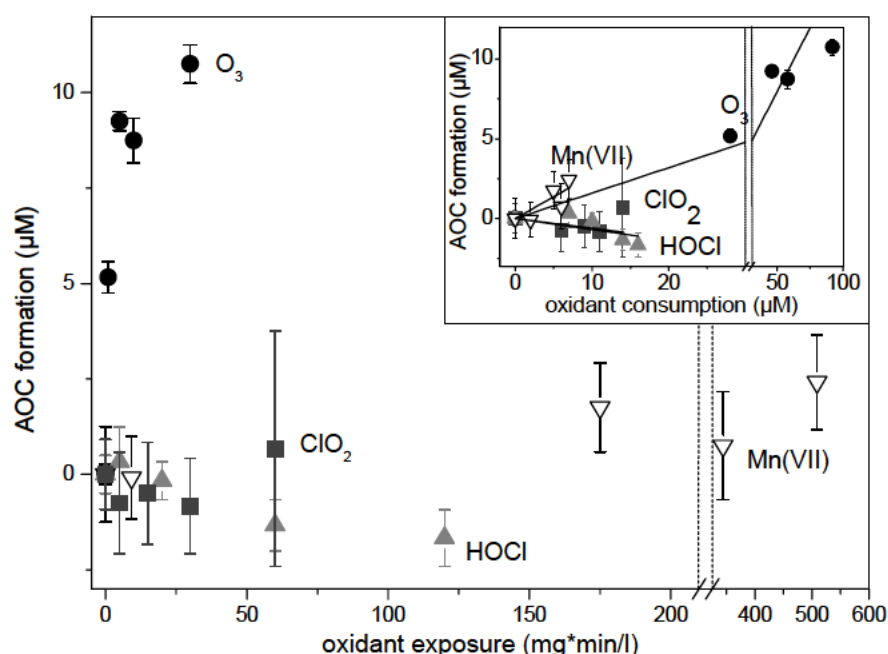
Figure 3.3 *AOC formation in LGj water upon treatment with different oxidants. Inset: same data plotted versus oxidant consumption. The slopes of the linear fits forced through the origin indicate the yield factor for AOC formation as the ratio of μM AOC formed over μM oxidant consumed: ozone 0.16, chlorine dioxide -0.06, chlorine -0.07, permanganate 0.27.*

For chlorine dioxide, the AOC concentration remained constant within the standard deviation of triplicate experiments for the investigated water matrix, despite the formation of LMW compounds observed by SEC measurements in one experiment. The negligible

formation of AOC as well as the low initial consumption and the slow kinetics of consumption of chlorine dioxide are remarkable since from known rate constants a higher reactivity of chlorine dioxide would have been expected (cf. introduction). This indicates that the content of activated aromatic systems (e.g. phenols) in the water was relatively low. Furthermore, mechanistic considerations might explain the low AOC formation in a second way: For phenol oxidation with chlorine dioxide an electron transfer reaction followed by dimerization can be expected. This increases the molecular weight of the products possibly leading to a decrease of the bioavailability. Benzoquinone is another major reaction product of chlorine dioxide with phenol and it has been shown to inhibit bacterial growth. Hypochlorous acid might be produced from chlorine dioxide reactions and hence chlorination of organic molecules could probably occur, as will be mentioned below (Rav-Acha (1984) and references therein). A study where biodegradable dissolved organic carbon (BDOC) was measured after application of chlorine dioxide to natural water samples confirms the low formation of bioavailable compounds by chlorine dioxide (Swietlik et al., 2009).

In an earlier study chlorination of six different water samples led to an increase of the BDOC concentration in only three waters, in one sample no change was observed, and in two water samples the BDOC concentration decreased (Swietlik et al., 2009). Figure 3.3 shows a slight decrease of the AOC concentration in chlorinated LGj water. Chlorination of organic compounds typically renders them less bioavailable, since halogenation of organic compounds is often implicated as a reason for microbial persistence (Rasul Chaudhry and Chapalamadugu, 1991; Dercová et al., 2007). However, additional experiments showed that chlorination (0.1 mg/L Cl_2) of preozonated water decreased the amount of AOC by 30% (data not shown). A similar observation was reported in an earlier study when measuring AOC using strain P17 (Le Chevallier et al., 1992). Considering the low reactivity of chlorine with the oxidized state of the organic compounds after ozonation, it is unlikely that chlorine reacts with large parts of AOC present after ozonation. Therefore, the loss of AOC is probably not attributed to chlorination of former AOC compounds that do not contribute to bacterial proliferation after chlorination anymore. Furthermore, oxalate, a non-chlorinated compound that is known to contribute to AOC (van der Kooij et al., 1989; Hammes et al., 2006), was consumed less in chlorinated than in

ozonated water during AOC measurements (supporting information). These two observations indicate that not only newly chlorinated organic compounds are less assimilable and therefore responsible for the lower carbon bioavailabilty, but chlorination of the water matrix might inhibit consumption of non-chlorinated compounds. Therefore, a lower bacterial regrowth can be expected in chlorinated waters even without residual disinfectant.

However, the AOC decrease upon chlorination presented in this study contradicts former studies that observed an AOC increase (e.g. 31 µg/L (Polanska et al., 2005), 100 µg/L (Miettinen et al., 1998)). As indicated by the data in Figures 3.1, 3.2 and Table 3.3, LGj water did not contain large amounts of moieties that are reactive towards chlorine. This certainly contributes to the absence of AOC increase during chlorination.

Permanganate is the least reactive of the five oxidants investigated and its consumption by the water matrix is only small (Figure 3.1). The small decrease in concentration results in high permanganate exposures after short times. The low reactivity is responsible for the very low AOC formation kinetics shown in Figure 3.3. The inset in Figure 3.3 shows the AOC formation plotted versus the oxidant consumption. The slopes of the linear fits give the AOC formation yield in µM AOC/µM oxidant consumed. Even though the absolute amount of AOC formed is small, permanganate has an even higher AOC formation yield than ozone (note the gap and difference in the scale on the x-axis). Therefore, attention should be paid to AOC formation in cases where the water matrix consumes more permanganate than in this study.

When ferrate was added to water and left to react without quenching, the AOC concentration increased considerably as is shown in Figure 3.4 for LZ water and in the supporting information for LG water (Figure S3.3). This indicates that the reaction of ferrate with DOM increases the AOC concentration. However, when ferrate was quenched with iron(II)chloride immediately upon addition to the water, the AOC concentration increased with increasing ferrate dose (data not shown). This increase cannot be explained by reactions of ferrate(VI) with DOM. The AOC increase was clearly too high to stem from organic contaminations in the ferrate. Further experiments confirmed that (1) the biological growth in the investigated water was not iron limited, hence iron addition itself

does not influence the AOC assay, (2) hydrogen peroxide that can be formed during ferrate application (Lee et al., in preparation), was not responsible for the AOC increase, (3) a Fenton type reaction with iron(II) and H_2O_2 to increase the AOC concentration could be excluded due to the high pH, and (4) iron(II)chloride alone did not lead to increased AOC results when used in water treated with ferrate (supporting information).

Although this study cannot explain the parameters influencing the AOC concentration during ferrate application, it can be concluded that ferrate has the potential to increase the AOC concentration - in some experiments larger yields than during ozonation were measured. This is remarkable because a significant amount of ferrate is consumed by self-decomposition and therefore not available for reaction with the matrix. To our knowledge no systematic study concerning AOC and ferrate has been performed before. To better understand the AOC formation by ferrate further research is needed, especially because after this study it seems plausible that in water treatment with ferrate measures to control the AOC formation might be necessary.

3.3.4 AOC production by oxidants in presence of cyanobacterial cells

The results discussed so far were obtained from experiments performed in 0.2 μm filtered lake water. Figure 3.4 shows AOC concentrations of LZ water that was spiked with cells of the cyanobacterium *Aphanizomenon gracile* after oxidation with three doses of the selected oxidants. The black bars give the AOC concentration in the control water without bacterial cells, while the AOC concentration in the water containing cells is represented as the sum of the black and grey bars. Again, chlorine and chlorine dioxide did not result in the formation of a considerable amount of AOC, in presence or absence of *Aphanizomenon gracile*. In contrast, the treatment of water containing cyanobacteria with ozone, ferrate, and permanganate yielded a significantly higher AOC concentration than treatment of water without cells. In all three cases an oxidant dose of 10 μM was sufficient to increase the AOC concentration considerably but only in the case of ozone a further increase of the oxidant dose resulted in a further increase of the AOC concentration.

At least two mechanisms can be suggested to explain the AOC production by oxidation of cells: (i) damage of the cells membranes by the oxidant and subsequent leaching of easily

bioavailable cell constituents and (ii) oxidation of initially non-assimilable cellular material to better assimilable compounds. The leakage of AOC compounds from algal cytoplasm of *Scenedesmus vacuolatus* upon ozonation was proposed by Hammes and co-workers (2007). Tung and co-workers (2004) showed that besides ozone also chlorine and permanganate were able to damage cyanobacteria to such an extent that cellular 2-methylisoborneol leached into the water.

Permanganate, ferrate, and ozone seem to react according to mechanism (i). This mechanism offers an explanation why only small oxidant doses are necessary to produce large AOC concentrations. Apparently, already low oxidant doses are sufficient to damage the cells. However, in the case of ozone AOC increased with increasing ozone doses. This can be explained by reactions with non-assimilable cellular organic compounds according to mechanism (ii). This can be expected, since ozone has generally the highest reactivity with organic compounds.
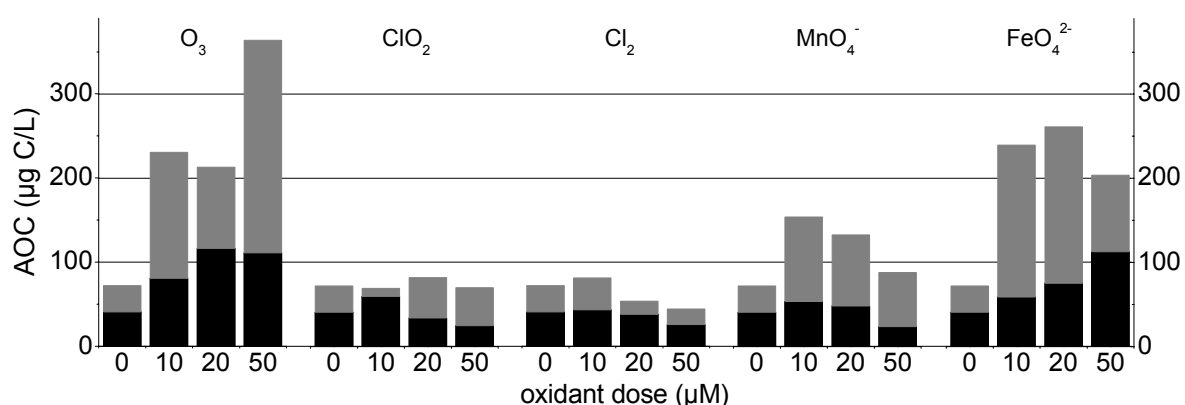
Figure 3.4 *AOC concentration after 24 hours of treatment of LZ water with varying oxidant doses of O_3, ClO_2, Cl_2, MnO_4^-, FeO_4^{2-} in absence and presence of Aphanizomenon gracile. Black bars: AOC in water without Aphanizomenon gracile, grey bars: additional AOC in water containing Aphanizomenon gracile, i.e. the sum (black + grey bars) is the AOC concentration in water samples containing cells. Values are mean values of two AOC measurements.*

Besides AOC, the taste and odor compound geosmin was measured. Geosmin is a compound produced by *Aphanizomenon gracile* (Durrer et al., 1999; Suffet et al., 1999) (intracellular concentration was 2 ng/L). Geosmin is not reactive towards the applied oxidants except OH radicals formed during ozonation (Peter and von Gunten, 2007). When 10 µM chlorine, chlorine dioxide, permanganate, or ferrate were added to the water containing cells, the dissolved geosmin concentration increased by 50% and remained stable at higher oxidant doses (data not shown). This indicates that chlorine and chlorine dioxide are in fact able to damage the cells. However, even though organic compounds are expected to leach from the cells the measured AOC concentration in the solution did not increase (Figure 3.4). This result is in line with the hypothesis discussed in section 3.3 stating that a water matrix treated with chlorine makes organic compounds less bioavailable. Two former studies report similar results: moderate chlorination of picoplankton (Okuda et al., 2006) and of intra- and extracellular (Kim et al., 2006) material increased the AOC concentration, indicating, that chlorine is able to damage the cells and even react with cellular material. However, a further increase of the chlorine dose led to a decrease of the AOC concentration in both cases. Chlorination of extracellular organic material with chlorine doses of > 5 mg/L even decreased the AOC concentration to a lower level than before chlorination (Kim et al., 2006).

3.3.5 Formation of a specific oxidation product: oxalate

Oxalate is a known oxidation product from oxidation of DOM by most oxidants. For example oxidation of phenol-like structures or olefins by ozone following the classical Criegee mechanism and further oxidation can lead to the formation of oxalate, Scheme 3.1A (Gould and Weber, 1976; Ramseier and von Gunten, 2009). Furthermore, it has been shown previously that oxalate concentrations increase in parallel to AOC during ozonation in a full-scale water treatment plant (Ramseier and von Gunten, 2009). Chlorination of diketones is known to yield trihalomethanes and oxalate, Scheme 3.1B (Deborde and von Gunten (2008) and references therein). Substituted phenolic compounds undergo ring opening when reacting with ClO_2 and result in di- and tricarboxylic acids and glyoxal as stable end products (Rav-Acha, 1984). Permanganate forms diols from olefines and is able

to oxidize primary alcohols to aldehydes and subsequently to carboxylic acids, Scheme 3.1C (Smith and March, 2001). Oxalate was also found after treatment of phenolic compounds with permanganate (Mohindra Chawla et al., 1989). Hu and co-workers (2009) propose a reaction mechanism for olefin oxidation with ferrate similar to the permanganate reaction forming a cyclic adduct intermediate. Hence, similar reaction products including oxalate as in the case of permanganate reaction can be expected.
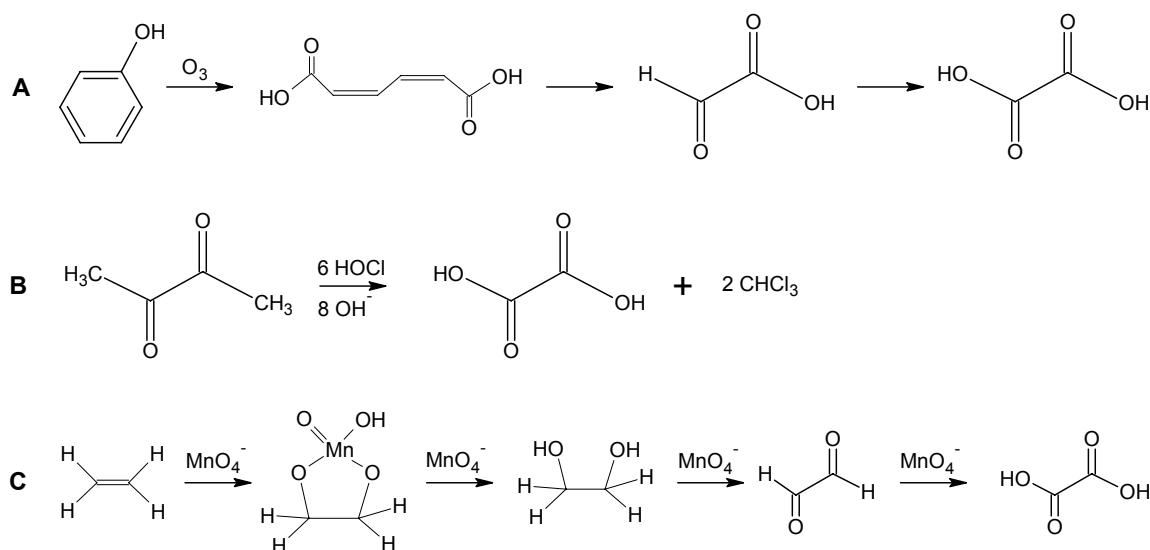
Scheme 3.1 *Oxalate formation mechanisms by different oxidants as proposed in former studies. Formation by (A) ozone (Gould and Weber, 1976; Ramseier and von Gunten, 2009), (B) chlorine (Deborde and von Gunten, 2008), and (C) permanganate (Yan and Schwartz, 2000).*

Figure 3.5 shows oxalate formation as a function of the oxidant consumption in a) CB water for all oxidants and b) in LGj, LGn, CB and LZ waters for chlorine dioxide. A linear increase of oxalate as a function of the consumed oxidants was observed for all conditions. Therefore, the oxalate yield defined by the molar ratio of oxalate produced over oxidant consumed was constant over time. It is also remarkable that the oxalate yields normalized to the DOC concentrations given in Table 3.4 were very similar in the three water samples with a similar DOC concentration (LGn, LGj, and CB water) even though the origin of the LG and CB water is different. In the water sample with a lower DOC concentration (LZ water) more oxalate per DOC was formed. The linearity and independence of oxalate for-
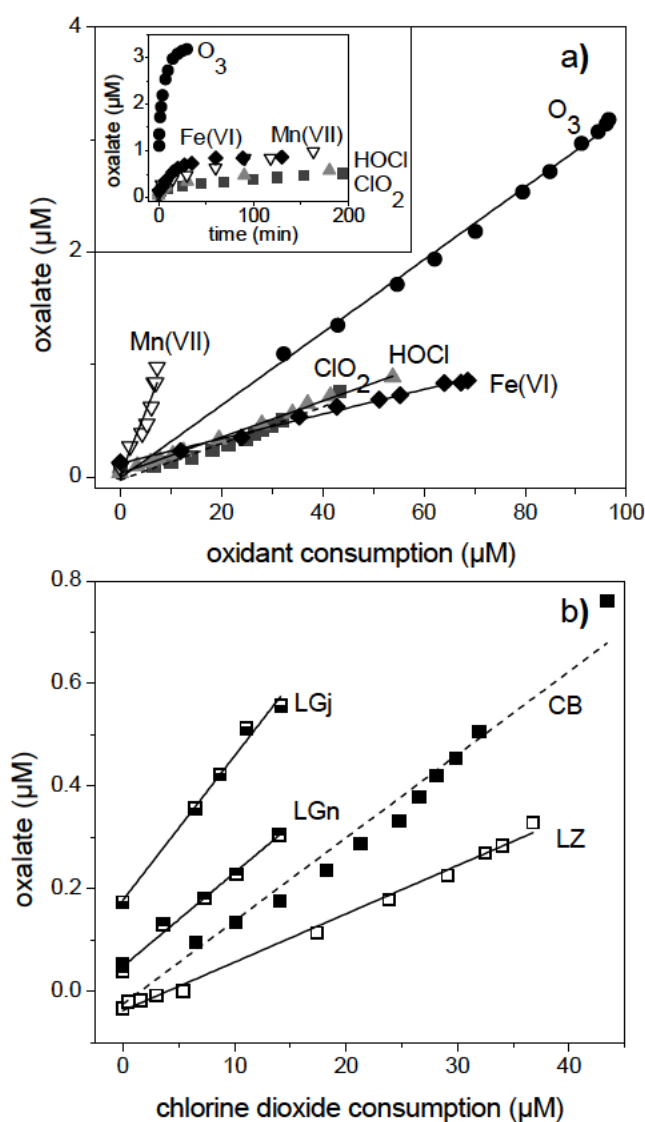
Figure 3.5 *Oxalate formation (a) upon treatment of CB water with five different oxidants (initial doses were 44 - 97 μM cf. Table 3.3), inset: same data plotted vs. time and (b) during oxidation of different water samples treated with chlorine dioxide representative for all five oxidants (for water characteristics cf. Table 3.1). Slopes and R-Square values of the linear fits are (a) ozone: 0.032, 0.997; chlorine dioxide: 0.016, 0.957; chlorine: 0.016, 0.998; permanganate: 0.111, 0.886; ferrate: 0.011, 0.992; (b) LGn: 0.018, 0.990; LGj: 0.028, 0.989; LZ: 0.009, 0.994; CB: 0.016, 0.957.*

mation from the DOM quality suggest that oxalate is a very general oxidation product and not selectively formed from concentration limited precursors.

Table 3.4 Oxalate yields in % (mol oxalate / mol oxidant consumed) normalized to the DOC of the water. For DOC concentrations refer to Table 1.

	LGn	LGj	LZ	CB
ozone	1.0	1.1	1.8	1.0 [a]
chlorine dioxide	0.5[a]	0.7 [a]	0.7 [a]	0.5 [a]
chlorine	0.3	0.7	1.3	0.5 [a]
permanganate		4.2	10.5	3.6 [a]
ferrate			0.9	0.4 [a]

All R^2 values for the determination of the yield were > 0.9
a) Data for the yield determination are shown in Figure 3.6

The inset in Figure 3.5a documents an initial fast increase of the oxalate concentration as a function of time and a subsequent decrease of the formation rate. Similar formation kinetics were also observed in an earlier study for the formation of trihalomethanes (THMs) during chlorination (Gallard and von Gunten, 2002). Due to this similar behavior of production kinetics, the comparison of the formation of THM and oxalate upon chlorination results in a linear correlation (Figure 3.6) with a THM : oxalate formation ratio of roughly 1:1. Scheme 3.2 shows a possible reaction mechanism for oxalate and trihalomethane formation from humic acids. 2,3,3,5,5,5-hexachloro-4-hydroxy-pentanoic acid (compound **1**) was found during chlorination of 3,5-dihydroxybenzoic acid and it was
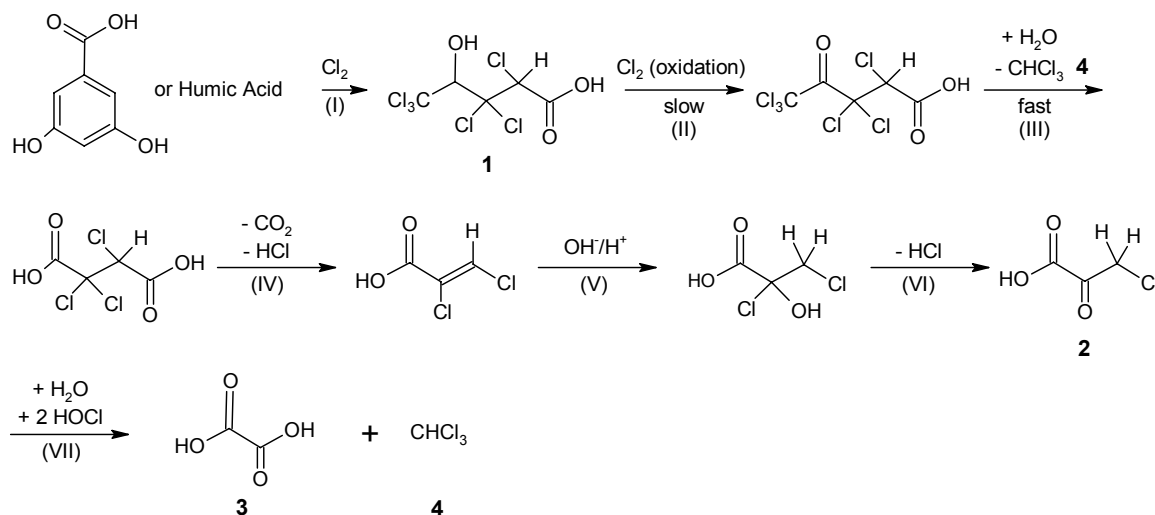
Scheme 3.2 *Proposed reaction mechanism of chlorination of humic acids leading to 2,3,3,5,5,5-hexachloro-4-hydroxy-pentanoic acid **1**, 3-chloro-2-oxo-propionic acid **2**, and oxalate **3** and trichloromethane **4** according to de Leer (1985) and Bartlett (1934).*

the most abundant chloroform precursor after chlorination of humic acids (de Leer et al., 1985). de Leer and co-workers (1985) proposed reactions (I)-(III) leading to the formation of one equivalent of trichloromethane. Reaction (VII) was proposed by Bartlett (1934) and forms a second equivalent of trichloromethane. Hence, the reaction proposed in Scheme 3.2 does not reflect the stoichiometry found experimentally in this study. However, it demonstrates the possibility of simultaneous oxalate and THM formation during chlorination of DOM.

Finally, the similar kinetics of two different oxidation products found in this study and their linear dependency on oxidant consumption shows a potential for further elucidation of disinfection by-product formation during oxidation processes.
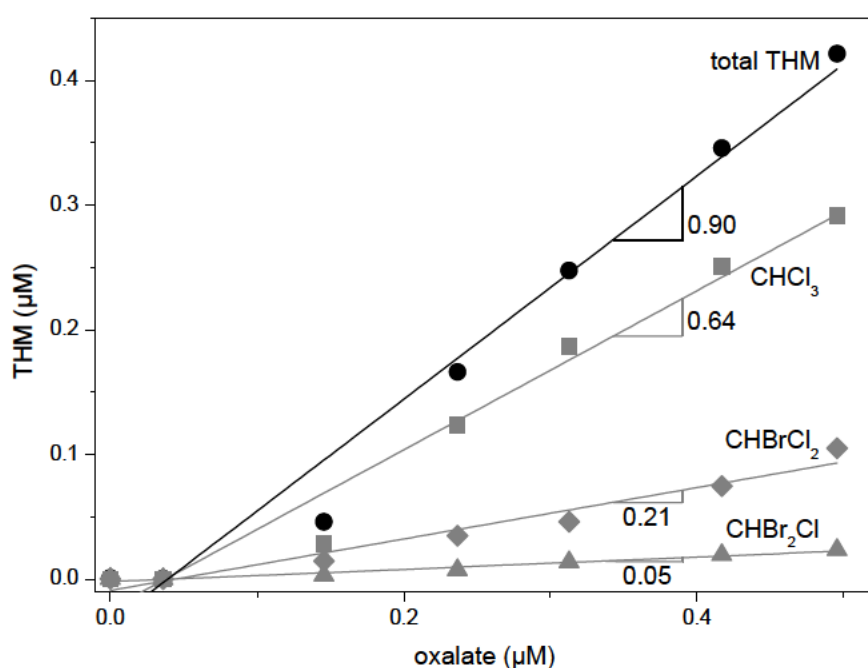
Figure 3.6 *Correlation between the formation of oxalate and THM during chlorination of LGn water. Initial chlorine dose was 4 mg/L. Slopes and R-square values of the linear fits are tTHM: 0.896, 0.972; CHCl₃: 0.638, 0.972; CHBrCl₂: 0.206, 0.958; CHBr₂Cl: 0.049, 0.966.*

In Figure 3.7 the formation of oxalate and AOC are compared for ozone, chlorine, chlorine dioxide, and permanganate. A slight correlation in the case of ozone and permanganate can be observed. However, linearity is rather poor. Oxalate only represents a part of the AOC. The weak correlation suggests that not all parts of newly produced AOC follow the same formation kinetics as oxalate. Furthermore, oxalate does not represent AOC in the cases of chlorine and chlorine dioxide application as discussed in section 3.3. Therefore, oxalate can not be considered as a general AOC surrogate independent of the oxidant used. Nevertheless, the linear formation of oxalate upon oxidation with all oxidants in all water samples tested make it a useful compound as a general tracer for oxidation processes.
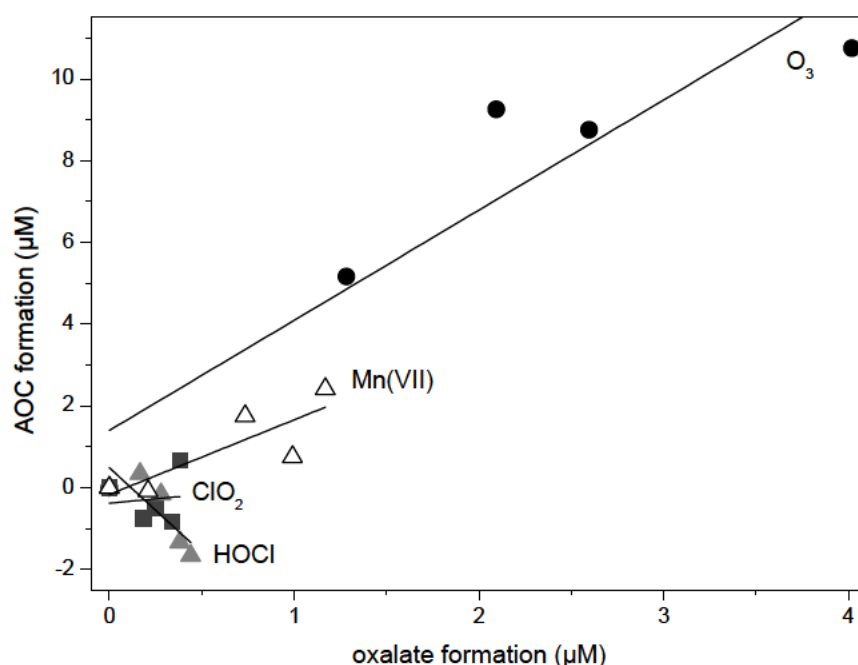
Figure 3.7 Correlation between the formation of oxalate and AOC in LGj water during oxidation with ozone, chlorine dioxide, chlorine, and permanganate. The AOC formation data are the same as in Figure 3.3. Slopes and R-square values of the linear fits are ozone: 2.69, 0.88; chlorine dioxide: 0.42, 0.01; chlorine: -4.15, 0.69; permanganate: 1.83, 0.70.

3.4 Conclusion

- The stability of five oxidants in a natural water sample increased in the following order: ozone << ferrate < chlorine dioxide < chlorine << permanganate. The decrease of the oxidants did not follow pure pseudo first-order kinetics.

- The SEC-OCD and SEC-UV measurements confirmed the reactivity of ozone towards humic substances, high molecular weight compounds and building blocks thereby forming low molecular weight acids and humics. No major changes in the size exclusion chromatograms were observed after chlorine and chlorine dioxide application.

- AOC was formed fast upon ozonation. At an ozone exposure of 1 mg*min/L 60 µg/L of AOC were formed in LGj water. Chlorine dioxide and chlorine produced no AOC. AOC formation from permanganate was slow, however, the AOC yield (AOC formed / permanganate consumed) was higher than for ozone. Ferrate application led to elevated AOC concentrations.

- In water containing *Aphanizomenon gracile*, AOC formation was observed after ozone, permanganate, and ferrate application. No significant AOC formation was found after application of chlorine dioxide and chlorine, despite their ability of damaging the cells, as was shown by an increase of the extracellular geosmin.

- The slight decrease of AOC upon chlorination and the constant AOC concentration during chlorine dioxide application despite the formation of low molecular weight compounds, suggest a negative influence of these two oxidants on bacterial growth in the utilized AOC assay.

- Oxalate was produced linearly as a function of the oxidant consumption from all oxidants in all water matrices, suggesting it to be a general oxidation product and not to be formed from concentration-limited precursors. Furthermore, oxalate was found to be formed in roughly the same concentrations as trihalomethanes during chlorination. Due to the differences in formation kinetics and yields between oxalate and AOC, oxalate can not be considered as a general surrogate parameter for AOC during oxidation processes.

Acknowledgement

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SUPPORTING INFORMATION FOR CHAPTER 3

S-3.1 Determination of the absorption coefficient of permanganate

To directly measure the permanganate concentration spectrophotometrically in the water samples, the absorption coefficient of permanganate was determined: A permanganate stock solution was prepared from solid potassium permanganate in Milli-Q water (nanopure Diamond, Barnstead, Allschwil, Switzerland). A dilution series was prepared and measured at 525 nm in a 5 cm quartz cell and the absorption coefficient was determined according to the Beer-Lambert law:

$$\frac{A}{l} = c \times \varepsilon$$

where A is the absorption, l the length of the cell, c the concentration and ε the absorption coefficient. The slope in Figure S3.1 gives the absorption coefficient as 2 430 $M^{-1}cm^{-1}$.

The stability of the stock solution was checked spectrophotometrically before each use. Furthermore the interference of MnO_2 at 525 nm was measured and was less than 5 % for the reaction times considered in all water matrices.
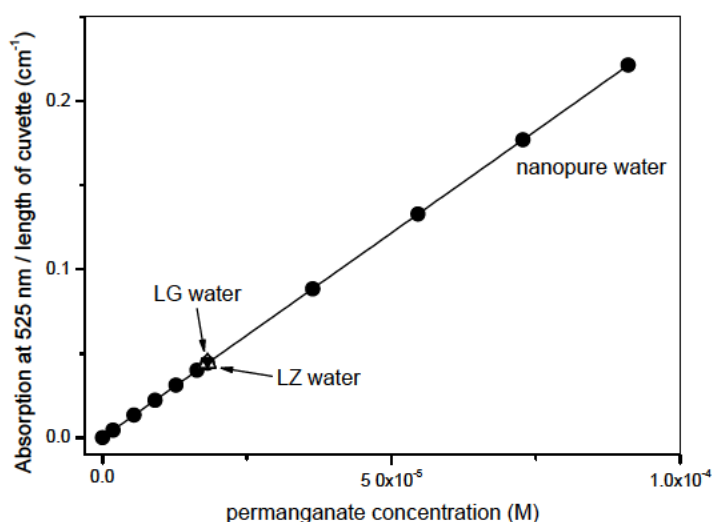

Figure S3.1 *Absorption of a permanganate solution at 525 nm in Milli-Q water (●), in LG (filled triangle), and LZ (empty triangle) water. The slope of the linear fit is 2430 and gives the absorption coefficient of permanganate in water.*

S-3.2 Selection of FeCl₂ as quenching agent

Thiosulfate is a well known quencher for many oxidants. However, in some waters, e.g. LG water, and for the natural bacterial inoculum used for determination of the AOC concentration in the experiments, it could be observed that the bacterial regrowth was stronger when the water sample contained thiosulfate, see Figure S3.2.
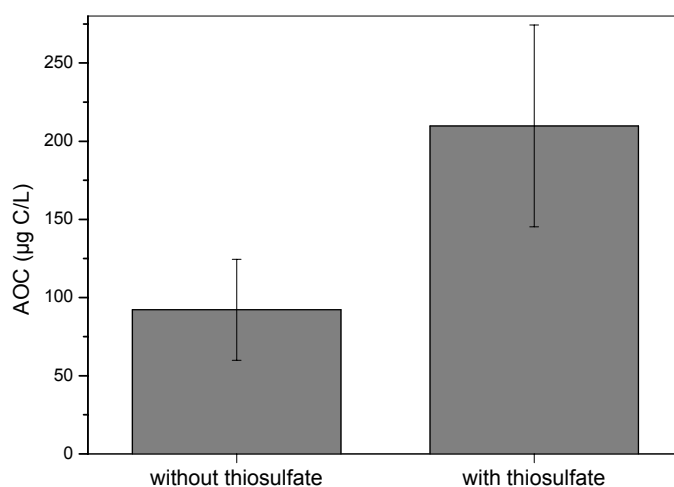
Figure S3.2 *Regrowth of bacterial inoculum represented as AOC concentration in LGn water*

As a consequence, thiosulfate could not be used for quenching the oxidants. Iron(II)chloride was tested instead in 0.2 μm filtered LG water. Oxidants were added to LG water and left to react for three days to generate similar water matrix conditions as in the experiment. Then different doses of iron(II)chloride and the inoculum were added (see Figure S3.3). Varying results were obtained for the differently treated water samples: Iron(II) had no influence on bacterial regrowth in untreated LG water. Therefore, iron(II)chloride was chosen as quencher for the oxidants in the subsequent experiments. However, the bacterial regrowth in water containing iron(II)chloride seemed to depend on the pretreatment of the water. Figure S3.3 shows that water pretreated with ferrate had a high AOC concentration, however, the concentration was lower when iron(II) was spiked. In chlorinated water the AOC concentration increased slightly with increasing iron(II) dose.

Nevertheless iron(II) was used as a quencher. In the experiments investigating the AOC formation over a wide range of oxidant exposures a constant iron(II) dose was added: 0.3 mM FeCl$_2$-solution in the case of chlorine dioxide and ferrate and 0.2 mM in the case of chlorine and permanganate. Different AOC concentrations should therefore result from the influence of the oxidant exposure on the water matrix and are not an artifact from iron(II) addition.
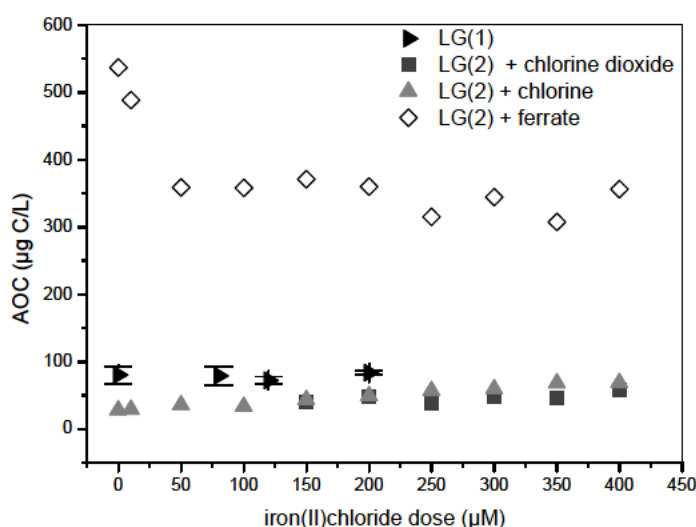
Figure S3.3 *Bacterial regrowth represented as AOC concentrations in LG water from January (1) and March (2) treated with different oxidants and spiked with various doses of iron(II)chloride. For untreated water standard deviations from triplicate experiments are given.*

S-3.3 Simulation of ferrate self-decomposition

To investigate the major reaction pathway leading to ferrate decomposition in natural water samples, the self-decomposition reaction of ferrate was simulated based on the following reaction with a rate constant of k = 3 M^{-1}s^{-1} at pH 8.2 given by Lee et al. (2004).

$$Fe(VI) + Fe(VI) = unknown\ products$$
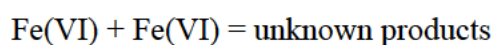
The simulation was performed with the chemical kinetics program Kintecus, version 3.953, www.kintecus.com.

Figure S3.4 shows the simulation of the self-decomposition reaction as well as the concentration decrease over time in three different water matrices. The simulation makes clear that the self-decomposition of ferrate is responsible for a large part of the ferrate concentration decrease in all three matrices.
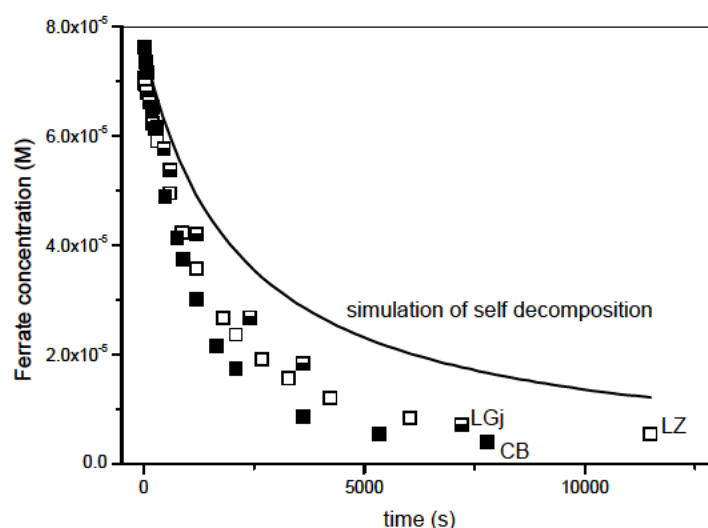
Figure S3.4 *Simulation of self-decomposition reaction and ferrate concentration in LZ, LGj, and CB water. Initial ferrate concentrations were 76 μM for the simulation and 71 μM in LZ, 70 μM in LGj, and 76 μM in CB water.*

S-3.4 Oxalate consumption during bacterial regrowth and influence of chlorination by-products on bacterial regrowth

Oxalate was measured in water samples before and after the bacterial regrowth for AOC determination. From the difference it could be calculated how much oxalate was consumed over the three days of incubation. In ozonated LGj water containing 240 μg/l oxalate, 211 μg/l was consumed by bacteria during the incubation time. In water treated with chlorine dioxide and chlorine only 6 and 1 μg/l oxalate from originally 46 and 50 μg/l oxalate, respectively, were consumed and hence contributed to AOC. Even though the oxalate concentration increased with increasing chlorine or chlorine dioxide exposure, the amount of oxalate being consumed during the incubation time decreased. The contrary happened

during ozonation: the oxalate concentration increased as well as the amount of oxalate being consumed. However, the ratio "AOC / oxalate consumed" decreased with higher ozone exposures, hence oxalate became less and less important for bacterial regrowth, also stating that other products were formed during ozonation that are converted into biomass more efficiently.

The effect of some oxidation by-products produced during oxidation with chlorine (trihalomethanes, THM) and chlorine dioxide (chlorite and chlorate) on the bacterial regrowth was tested. 5 - 100 µg/l of four trihalomethanes, 5 µM chlorate, and 5 µM chlorite were added to preozonated LG water and AOC was measured. Neither the THMs nor the chlorite and chlorate had a significant influence on the AOC result, Figure S3.5.
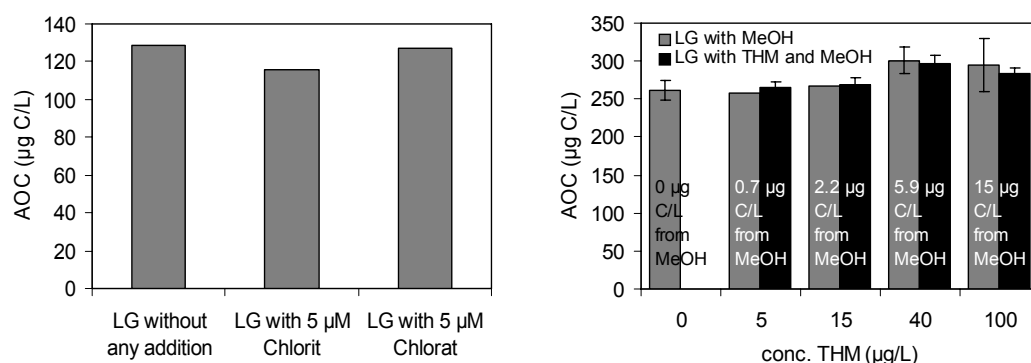
Figure S3.5 *AOC in LG water containing oxidation by-products. THMs were added after dilution in MeOH. The same concentration of MeOH without THMs was added to LG water as a control. Error bars are the standard deviation of triplicate measurements.*

Reference

Lee, Y., Cho, M., Kim, J.Y., Yoon, J., 2004. Chemistry of ferrate (Fe(VI)) in aqueous solution and its applications as a green chemical. Journal of Industrial and Engineering Chemistry 10 (1), 161-171.

4

MECHANISMS OF PHENOL OZONATION - KINETICS OF FORMATION OF PRIMARY AND SECONDARY REACTION PRODUCTS

Abstract
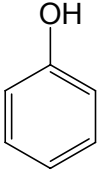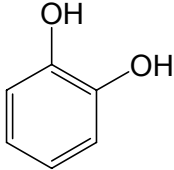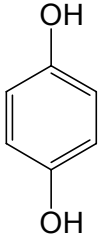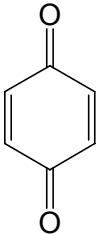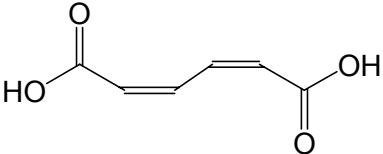
Organic acids are part of assimilable organic carbon (AOC) formed from natural organic matter (NOM) during ozonation for drinking water production. To elucidate the formation of organic acids, phenol as surrogate compound for NOM was ozonated while suppressing reactions of $^{\bullet}$OH radicals by addition of *tert*-butanol. Batch experiments show benzoquinone (40 and 18%), catechol (33 and 1%), *cis,cis*-muconic acid (6 and 3%), and hydroquinone (2 and 7%) to be the most abundant primary products at pH 7 and 3, respectively. The tertiary product oxalic acid was obtained in similar yields from phenol ozonation (0.8%) as during ozonation of lake water (1.6%). Together with other low molecular weight organic acids it was formed upon small ozone exposures, as was shown by time-dependent experiments in time ranges of 0.4 - 23 and 0.07 - 1.0 seconds for pH 3 and 7.25, respectively.
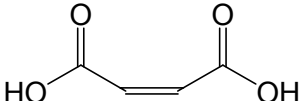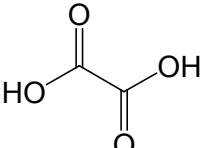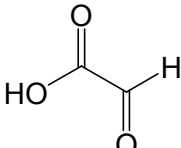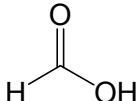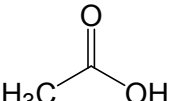
4.1 Introduction

To obtain hygienically safe drinking water, disinfection is an important step during water treatment. Disinfectants are strong oxidizing agents which can engage natural organic matter (NOM) in a series of reactions that generate disinfection by-products (DBPs) (Krasner et al., 2006; Richardson et al., 1999; Weinberg, 1999). One class of mostly oxygen rich DBPs can be summarized as assimilable organic carbon (AOC). AOC is a collective term describing the fraction of labile dissolved organic carbon that is readily assimilated by microorganisms, resulting in their growth (Hammes et al., 2007; Hammes et al., 2006; Hammes and Egli, 2005; van der Kooij, 1992; Zaror et al., 2001).

It was shown that organic acids constitute a large fraction of AOC formed during ozonation of NOM containing waters (Hammes et al., 2006). Their formation occurs nearly instantaneously upon treatment of natural water with ozone. In a previous study with Lake Zurich water (Switzerland) 50% of the total organic acids and 50% of the total AOC was formed with an initial ozone exposure of 0.4 min•mg/l compared to a total exposure of 7 min•mg/l (Hammes et al., 2006). It was hypothesized that the formation of these organic acids results from direct ozone reactions with natural organic matter (NOM). To gain insight into the reaction mechanisms leading to low molecular weight compounds, phenol was selected as a model precursor, representing a small, repeating subunit of NOM (Aitkenhead-Peterson and McDonwell, 2003; Bertilsson and Jones, 2003; Davies and Ghabbour, 1998; Eisenhauer, 1968; Kleinhempel, 1970; Sutton and Sposito, 2005). In previous studies the mechanism of the reaction of ozone with phenol and some of its transient products has been studied and numerous oxidation products and reaction pathways were described (Eisenhauer, 1968; Gilbert, 1980; Gould and Weber, 1976; Komissarov et al., 2006; Leitzke, 2003; Mvula and von Sonntag, 2003; Oh et al., 2006; Singer and Gurol, 1983; Yamamoto et al., 1979). However, most experiments were performed in semi-batch reactors, where an ozone oxygen gas mixture was bubbled through the phenol-containing solution. This experimental setup represents an imitation of realistic treatment conditions, but it has limited chemical and kinetic significance, since the mass transfer of ozone into the solution is often the rate limiting step (Singer and Gurol, 1983). If ozone is added as an aqueous stock solution, shorter reaction

Table 4.1 Phenol and its ozonation products - second order rate constants with ozone

	phenol	catechol	hydroquinone	benzoquinone	muconic acid
analyzed by HPLC-UV					
Nr.	**1**	**2**	**3**	**4**	**5**
pK_a	9.9 [a]	9.3 [b], 9.9 [c]	10.9 [c]		
$k_{O3-prot}$ [M^{-1}s^{-1}]	1.3($\pm$0.2) x 10^{3} [a]	5.2 x 10^{5} [d]	1.8 [e] -2.3 [d] x 10^{6}	2.5 x 10^{3} [f]	see table 4.2
$k_{O3-deprot}$ [M^{-1}s^{-1}]	1.4($\pm$0.4) x 10^{9} [a]				

	maleic acid		oxalic acid	glyoxylic acid	formic acid	acetic acid
analyzed by IC-conductivity detection						
Nr. in this work	**6**		**7**	**8**	**9**	**10**
pK_a [a]	1.8 / 6.1		1.2 / 4.2	3.2	3.75	4.75
$k_{O3-prot}$ [M^{-1}s^{-1}] [a]	ca. 10^{3}-10^{4}			0.17($\pm$0.4)	5($\pm$5)	$\leq$ 3 x 10^{-5}
$k_{O3-deprot}$ [M^{-1}s^{-1}] [a]			$\leq$ 4 x 10^{-2}	1.9($\pm$0.2)	100($\pm$20)	$\leq$ 3 x 10^{-5}

a) Hoigné and Bader (1983), oxalic acid and acetic acid were determined without ˙OH scavenger at pH 5-6 and 2.5, b) Moridani et al. (2003), c) Pearce and Simkins (1968), d) Mvula and von Sonntag (2003) at pH 7, e) Mvula and von Sonntag (2003) at pH 3, f) Mvula and von Sonntag (2003)

times have to be considered. They can be accessed by the use of a continuous quench flow system, where ozone is quenched after the desired reaction time in the range of 0.07 - 23 seconds (Buffle et al., 2006). With this technique time resolved information about the product evolution can be gained.

Furthermore, in many ozonation studies the role of $^{\bullet}$OH radical reactions has not been considered. Only by scavenging the $^{\bullet}$OH radicals, the products can be attributed to direct ozonation reactions. However, while using tert-butanol (*t*BuOH) as a scavenger, H_2O_2 is produced (Acero and von Gunten, 2000; Flyunt et al., 2003; Schuchmann and von Sonntag, 1979), which can possibly act as a reactive compound, too.

Nevertheless, in previous studies, many intermediate and final transformation products were identified: catechol **2**, hydroquinone **3**, muconic acid **5** (Singer and Gurol, 1983), muconic acid aldehyde, maleic acid aldehyde (Yamamoto et al., 1979), oxalic acid **7**, glyoxal, glyoxylic acid **8** (Gould and Weber, 1976), formic acid **9**, H_2O_2, CO_2 (Mvula and von Sonntag, 2003; Yamamoto et al., 1979). Information about these compounds is given in Table 4.1 and 4.2.

Table 4.2 pK_a and second order rate constants for the reaction of muconic acid with ozone

muconic acid	*trans,trans*	*cis,trans*	*cis,cis*
pK_a	2.70, 4.66 [a]	1.90, 4.79 [a]	
k at pH=3 $[M^{-1}s^{-1}]$	1.6×10^{4} [b], 1.5×10^{4} [c]	1.4×10^{4} [c]	2.65×10^{4} [d]
k at pH=7 $[M^{-1}s^{-1}]$	1.4×10^{5} [b], 1.3×10^{5} [c]	2.5×10^{5} [c]	
k $[M^{-1}s^{-1}]$, no pH indicated			4×10^{4} [e]

a) Mei and Wolf (2004), b) Beltran et al. (2006), c) this study, d) Leitzke (2003), e)Mvula and von Sonntag (2003)

The classical direct reaction mechanism between ozone and a double bond is the Criegee mechanism shown in Scheme 4.1, where ozone attacks a double bond as an electrophile resulting in bond cleavage (Bailey, 1982; Criegee, 1953; Smith and March, 2001). In addition, the loss of O_2 after the ozone attack is a possible reaction pathway resulting in a hydroxylation of the substance (Mvula and von Sonntag, 2003). Many follow-up reactions

are possible. Since H_2O_2 is formed as mentioned before, the Baeyer-Villiger reaction of glyoxal and glyoxylic acid **8** is one possibility (Yamamoto et al., 1979).
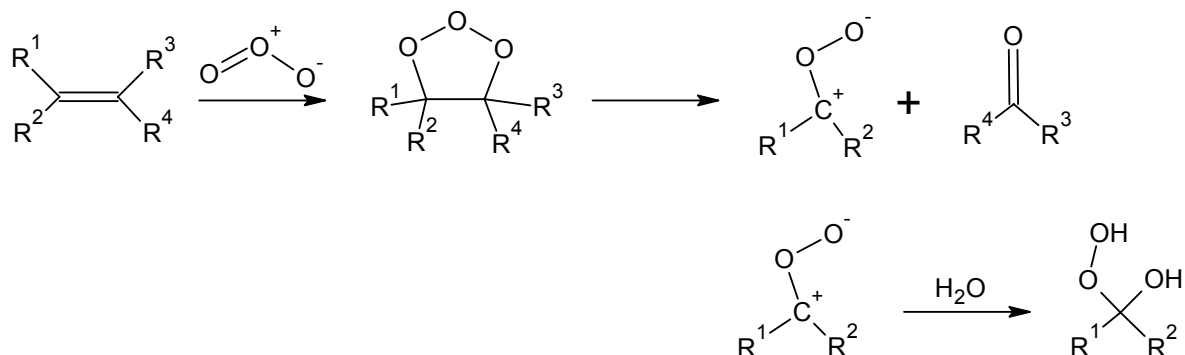
Scheme 4.1 *Criegee mechanism with subsequent water addition to the zwitter-ion.*

The initial phase of phenol ozonation has recently been studied thoroughly by Mvula and von Sonntag (2003). They added ozone by addition of an ozone stock solution and performed experiments both by scavenging ˙OH radicals with *t*BuOH and DMSO and without scavenging ˙OH radicals. They obtained catechol **2**, hydroquinone **3**, benzoquinone **4**, muconic acid **5**, formaldehyde and hydrogen peroxide as major transient products. Some of these results are summarized in Scheme 4.2 and will be discussed below in connection to the results of the present study. A reaction pathway between muconic acid **5** and ozone resulting in glyoxylic acid **8** and H_2O_2, and maleic **6** and fumaric acid has been proposed by Leitzke (Leitzke, 2003). In another study, an intermediate from the reaction of glyoxylic acid with hydrogen peroxide was proposed (Leitzke et al., 2001) that was not the result of the Baeyer-Villiger reaction. It will be discussed in detail.

The objective of this study was to investigate the transformation pathways of phenol during ozonation in absence of ˙OH radicals up to the formation of small molecular weight organic acids. The product formation was investigated both mechanistically as well as kinetically in the range of 0.1 to 23 seconds. Batch experiments were performed to identify the yield of the ozonation products and experiments with a continuous quench flow system were used to gain kinetic information.

Scheme 4.2 *Initial reaction pathway of ozone with phenolate; adapted from Mvula and von Sonntag (2003).*
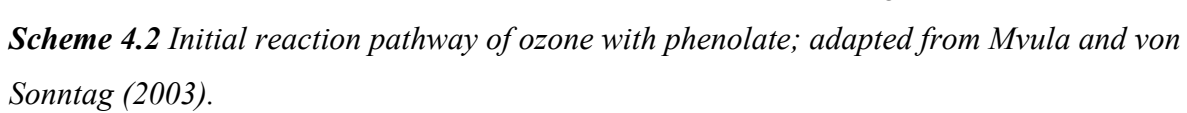
4.2 Materials and Methods

4.2.1 Chemicals and analyses

The following chemicals were used in this study: phenol **1** ($\geq$ 99.5 %, Fluka), catechol **2** ($\geq$ 99+ %, Sigma-Aldrich), hydroquinone **3** ($\geq$ 99 %, Fluka), 1,4-benzoquinone **4** (99 %, Fluka), *cis,cis*-muconic acid **5** (98 %, Acros Organics), *trans,trans*-muconic adic ($\geq$ 97 %, Fluka), *tert*-butanol ($\geq$ 99.7 %, Fluka), di-sodium maleate dihydrate **6** ($\geq$ 98 %, Merck-Schuchardt), di-sodium oxalate **7** ($\geq$ 99.8 %, Merck), glyoxylic acid sodium salt monohydrate **8** ($\geq$ 99 %, Fluka), sodium formate **9** ($\geq$ 99.5 %, Merck), and sodium acetate **10** ($\geq$ 99 %, Merck). The numbers of the substances refer to Table 4.1. Solutions were prepared in Milli-Q-filtered water (NANOpure Diamond^{TM}, Barnstead), from now on referred to as nanopure water. Ozone stock solution was prepared by bubbling ozone from an oxygen-fed ozone generator (CMG 3-3, Apaco AG, Switzerland) through ice-cooled nanopure water. Ozone stock solution concentration was determined spectrophotometrically taking ε(258 nm) = 3000 $M^{-1}cm^{-1}$ and was 1.3 - 1.5 mM. Organic acids were determined by ion chromatography (Dionex ICS-3000) as described elsewhere (Hammes et al., 2006). For separation a Dionex AS11HC column and as mobile phase an OH^- gradient produced by a Dionex EG50 eluent generator ranging from 1 - 60 mM OH^- within 30 minutes were used. A sample volume of 175 µl was injected and measured with a conductivity detector. Limits of quantification were 0.5 µM for formate, acetate, and oxalate, 2 µM for glyoxalate and 5 µM for maleate. Standard deviations of four repetitions of the same sample (20 µM) were 10 - 25%.

The other compounds were determined by HPLC-UV (Ultimate 3000, Dionex, Switzerland, equipped with a photodiode array detector). For separation a 25 cm RP-C18 column (Macherey-Nagel) with a multi-step gradient from 5% methanol and 95% 10 mM phosphoric acid to 45% methanol within 20 minutes was used. Injection volumes were 75 µl for batch experiments and 250 µl for continuous quench flow experiments. The following retention times and detection wavelengths were used: hydroquinone (9.3 min., 290 nm), benzoquinone (12.2 min., 250 nm), catechol (12.5 min., 270 nm), *cis,cis*- and *trans,trans*-muconic acid (13.7 min., 270 nm), *cis,trans*-muconic acid (14.9 min., 270 nm), and phenol (17.0 min., 270 nm). Limits of quantification were 0.2 µM for all compounds.

Five measurements of the same sample (20 µM) gave standard deviations of < 1% except for benzoquinone and hydroquinone, where they were < 5%.

4.2.2 Analysis of muconic acid

Muconic acid is often reported as an intermediate product from the transformation of phenol type compounds (e.g. (Mvula and von Sonntag, 2003; Singer and Gurol, 1983; Yamamoto et al., 1979)). Three species of muconic acid are known: *cis,cis*-, *cis,trans*- and *trans,trans*-muconic acid (Elvidge et al., 1950), where *cis,cis*-muconic acid (ccMA) is the direct product of the reaction of ozone with catechol by the Criegee mechanism (Scheme 4.1). Analytical challenges make a quantification of the three muconic acid species difficult. On the one hand, it is difficult do differentiate analytically between *cis,cis*-muconic acid and *trans,trans*-muconic acid, on the other hand, *cis,cis*-muconic acid is not stable in aqueous solutions and we confirmed that it isomerizes into *cis,trans*-muconic acid (see below). While analyzing the experimental samples, these facts have to be considered. From the moment of the ozonation experiment of catechol or phenol some 50 hours could elapse until the sample was measured. To reduce ccMA isomerization in our ozonation experiments, the samples were neutralized to pH 7 with phosphate buffer immediately after the ozonation reaction. Nevertheless, some ctMA was formed even in the freshly prepared calibration standards. Therefore a larger error has to be considered with the quantitative results of the studies performed with ccMA.

4.2.3 Batch experiments to determine ozonation products of phenol and subsequent products

To determine primary ozonation products - meaning stable products after ozonation of a substrate that have not yet reacted further with ozone - batch experiments were performed as follows: 2 - 12 ml aqueous ozone solution (ozone concentration 0.07 - 1.5 mM) were added to stirred batch reactors with 10 or 15 ml substrate solution containing 0.2 and 0.4 mM substrate and 200 mM tBuOH as $^{\bullet}$OH radical scavenger and 1 or 3 mM phosphate

buffer for pH 3 and 7, respectively. This setup resulted in solutions with an initial molar ratio of ozone to substrate of 0.06 - 0.90 and 0.03 - 0.45 for substrate concentrations of 0.2 and 0.4 mM, respectively. The tBuOH concentration was chosen such that a maximum of 2 % of the $^\bullet$OH could react with the substrate. Exceptions were benzoquinone and muconic acid, where the limited solubility restricted their concentrations to 0.1 and 0.2 mM and 0.2 and 0.25 mM, respectively and glyoxylic acid, where the tBuOH-concentration was 1 mM since otherwise the scavenger would have competed for ozone. Each batch contained only one substrate and for each ozone dose a separate batch was used. Batch experiments were performed at room temperature (21 - 23°C) at an initial pH of 3.1 and 7.0. At the end of the ozonation experiment the pH was 3.4 and 6.9, respectively. Aliquots were taken and analyzed chromatographically within 1 - 60 hours. The aliquots of the experiments at pH ~ 3 were neutralized with 0.4 ml 100 mM phosphate buffer pH 7 (except the maleic acid experiment was not neutralized) before measurement or storage at 4°C.

4.2.4 Samples from lake water treatment plant

Samples were taken in a full-scale water treatment plant (Lengg, Zurich, Switzerland; February 2006) that treats water from Lake Zurich with the following parameters: DOC ~1.4 mg/l, alkalinity ~2.5 mM as HCO_3^-, pH 8. Ozone doses were ~1.2 mg/l for pre-ozonation and ~0.5 mg/l for intermediate ozonation. After taking the samples, ozone was quenched by addition of nitrite and all samples were stabilized by addition of 0.03 g/l benzalkonium chloride (Fluka) and analyzed by ion chromatography as described above.

4.2.5 Time resolved continuous quench flow (CQF) experiments with ozone in excess

Time resolved studies were performed with a continuous quench flow (CQF) system described elsewhere (Buffle et al., 2006; Dodd et al., 2005). In short the two reactants, phenol and ozone in this case, are mixed in a mixing tee and pushed through a reaction loop. After the loop the reaction is quenched with a quenching agent and the mixture is

collected for analysis. The reaction time depends on the length of the loop as well as on the flow rate of the two reactant solutions.

For the experiment at pH 3, the reaction solution initially contained 0.025 mM phenol, 35 mM *t*BuOH and 1 mM phosphate buffer and 0.21 mM ozone were added. After the desired reaction time the reaction was stopped by addition of a five fold excess of sodium nitrite over initial ozone and neutralized by a phosphate buffer to a final pH of 7. The flow rate of each solution was 5 ml/min, reaction times were 0.4 - 23.5 seconds.

At pH 7.25 shorter reaction times were required and a continuous quench flow system with smaller loops was used (Dodd et al., 2005). The initial reaction conditions were 0.24 mM ozone, 0.022 mM phenol, 70 mM *t*BuOH in a 1 mM phosphate buffer and the reaction was stopped with a seven fold excess of nitrite to ozone. Reaction times were 0.07 - 1.04 seconds. The resulting solutions were collected and analyzed chromatographically as described above. The initial ozone concentration was measured by running a blank through the CQF system once with and once without ozone and quenching it with indigo instead of nitrite. The ozone concentration was calculated from the absorption difference of the two quenched samples at 600 nm by the indigo method (Bader and Hoigne, 1981).

4.2.6 Quenching agent

Sodium nitrite was used to quench the residual ozone and hence interrupt the reaction for time resolved monitoring of ozonation products. However, at pH 3, nitrite / nitrous acid reacts with hydroquinone and catechol to a considerable extend within hours. Therefore, after the ozonation reaction was stopped, the samples of the quench flow experiments were buffered to pH 7 and analyzed within 48 hours.

4.2.7 Determination of rate constant of muconic acid with ozone

The rate constant for the reaction of *cis,trans*-muconic acid and *trans,trans*-muconic acid were determined at pH 3.0 and 7.1 with the same quench flow system as above. Ozone was mixed with a phosphate buffer solution in a mixing tee before addition to the muconic acid

solution. The reaction took place in loops with volumes of 16 - 3872 μl, such that reaction times of 0.34 to 20.6 seconds were achieved. Reaction concentrations were 1 μM for muconic acid, 8-10 μM for ozone, 10 mM for *t*BuOH and the phosphate buffer. The reaction was stopped by the addition of cinnamic acid which produces benzaldehyde upon ozonation (Leitzke et al., 2001; Onstad et al., 2007). By measuring the benzaldehyde concentration by HPLC-UV with the method described above the ozone exposure could be determined. Plotting it versus the natural logarithm of the HPLC-peak-area ratio of muconic acid to its initial peak area the slope gave the rate constant.

4.3 Results and Discussion

4.3.1 Primary phenol ozonation products and formation mechanisms

Ozonation of phenol leads partly to stable products, which, however, can react further if ozone is still present in the system. To investigate the formation of these primary products, batch experiments were performed. For varying ozone doses the yield of each product was determined. Since the substrate was used in excess to ozone, it was assumed that, as long as the product formation was linear as a function of ozone dose, the competing reaction of the product with ozone was negligible. Kinetic simulations of different scenarios with the program AQUCHEM (Braun et al., 1988) confirmed that an undesired further reaction would be indicated by a deviation from linearity. Furthermore, the simulations showed that the deviation between the scenarios for no further reaction of the product with ozone and with further reaction of the product was largest when the second order rate constant of formation of a product was similar to its rate constant for the further reaction with ozone.

Figure 4.1 shows the formation of primary phenol transformation products relative to the amount of phenol consumed during ozonation. Initial phenol concentrations of 200 and 400 μM were studied, with only small differences in the results. The main products clearly are benzoquinone and catechol, whereas muconic acid and hydroquinone are formed to a much lower extent (mechanistic discussion to follow). For some samples with a high ozone dose small organic acids were measured, too. An increase of acetate and especially formate could be observed during phenol ozonation. At pH 7 these acids only contribute 5 % to the overall mass balance, however at pH 3 their presence cannot be neglected. From a mechanistic point of view the formation of these low molecular weight organic acids by direct ozonation remains questionable. Indeed, among all the data, there is only one example for an observed non-linearity in product formation versus parent compound consumption and this is the case for benzoquinone production during phenol ozonation at pH 3. The organic acids found could hence be an artifact coming from the reaction of benzoquinone with ozone.
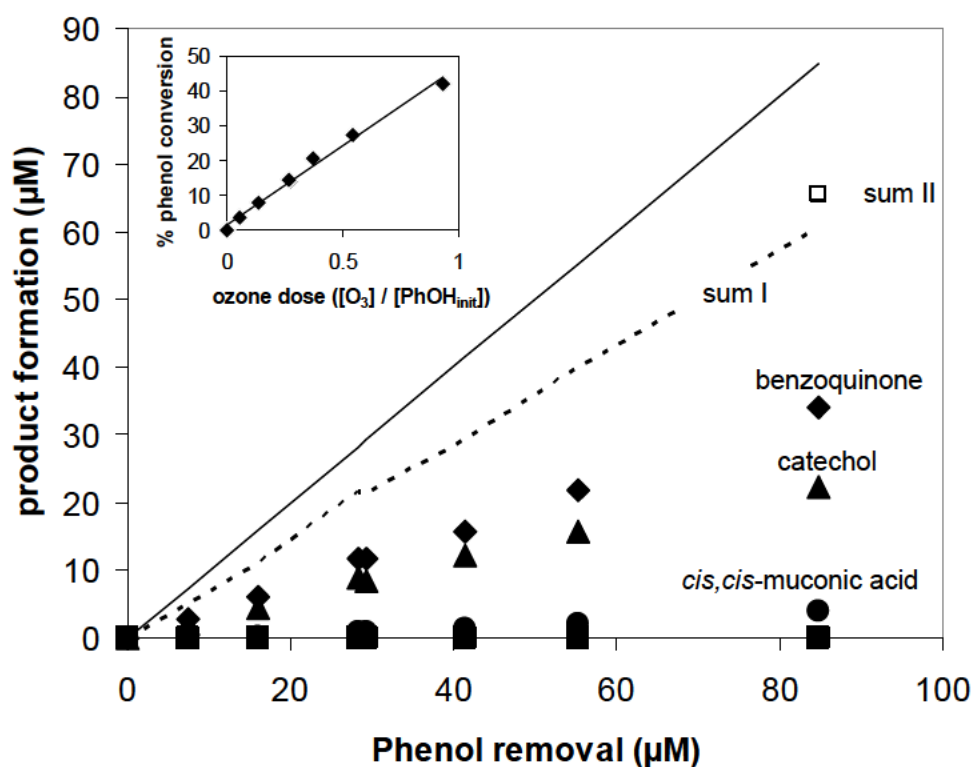
Figure 4.1 *Formation of ozonation products from 200 μM phenol at pH 7. ♦ Benzoquinone, ▲ catechol, ● cis,cis-muconic aicd, ■ hydroquinone, dotted line is the sum of the primary products (sum I), □ is the sum of the primary products including the organic acids (sum II), solid line shows the theoretical 100 % mass-balance. Inset: phenol conversion versus ozone dose expressed as ozone to initial phenol concentration ratio.*

The inset of Figure 4.1 shows the phenol conversion relative to the ozone dose applied. Since phenol was present in excess and the reaction took place at pH 7 where the phenol rate constant is large (see Table 4.1), all ozone was consumed before analysis after 1 - 40 hours (reaction half-live 2 ms). The slope shows that 0.45 and 0.48 moles of phenol are depleted with one mol of ozone, which is a similar result as given by Mvula and von Sonntag (2003). For pH 3 the corresponding values were 0.38 and 0.53 for the experiment with 200 μM and 400 μM phenol, respectively. An explanation for this deviation from a one to one stoichiometry could be an observation made by Mvula and von Sonntag (2003). They showed that part of the ozone can react with phenol through a direct electron transfer resulting in a phenoxyl radical and an ozonide radical. The ozonide radical turns into dioxygen and an ˙OH radical. The phenoxyl radical can react with a hydroquinone anion by

a one-electron-transfer resulting in a phenolate anion and a hydroquinone-radical (dotted lines in Scheme 4.2). By disproportionation, two hydroquinone-radicals transform to benzoquinone and back to a hydroquinone anion (Mvula and von Sonntag, 2003). By reproducing phenolate, ozone can be consumed through this mechanism without decreasing the phenol concentration. Mvula and von Sonntag (2003) indicate that at pH 10 phenoxyl radicals are formed with a yield of roughly 22% based on ozone consumption through the pathway indicated in Scheme 4.2. Reactions of this radical might therefore be important.

However, this mechanism can only account for the relatively high ozone consumption at a high pH. At pH 3 other reactions have to take place that consume ozone. Tentative propositions have been made for other reaction systems but could not be verified nor falsified in this study (Sein et al., 2008).

As mentioned earlier, catechol and benzoquinone are important primary products at pH 7 (see Figure 4.1 and Table 4.3). Benzoquinone **4** can be produced either by ozone attack at the para-position and subsequent loss of H_2O_2 or via the attack of a $HO_2^\bullet$ at the para-position of the phenoxyl radical, with subsequent water loss (Mvula and von Sonntag, 2003), see Scheme 4.2. It is questionable whether the reaction pathway including $HO_2^\bullet$ is important under the conditions used, where $^\bullet OH$ radicals are quenched with tBuOH and hence $HO_2^\bullet$ cannot be formed through the reaction of a OH-adduct phenol radical with dioxygen (Mvula and von Sonntag, 2003). $HO_2^\bullet$ can be produced by the reaction of ozone with OH-: hydroxide ions and ozone form HO2- which reacts with ozone to form $^\bullet OH$, $O_2^{\bullet-}$ and O_2 (von Gunten, 2003). However, at pH 7 under the conditions chosen the reaction time of the phenol ozonation is not longer than 0.013 seconds (for rate constants see Table 4.1). Within this time only an estimated maximum of 10^{-8} mM HO_2^- could be produced over this pathway, since the rate constant of the formation of HO_2^- is only 70 $M^{-1}s^{-1}$ (von Gunten, 2003) and the OH^- concentration is 10^{-7} M. This is not sufficient to explain the higher amount of benzoquinone found at pH 7 compared to pH 3. A second possibility for an explanation could be that at higher pH a larger part of possibly formed hydroquinone is deprotonated, favoring its reaction

Table 4.3 Product yields of ozonation reactions in % (mol product per mol reactant consumed). $^{\bullet}$OH radical reactions were suppressed by tBuOH addition.

parent	product	pH 3 [a]	pH 7 [a]
phenol **1**	benzoquinone	17, 18	40, 35
	hydroquinone	4, 7	0, 2
	catechol	0, 1	27, 33
	cis,cis-muconic acid	2, 3	5, 6
	acetic acid[b]	23	6
	formic acid [b]	90	15
	maleic acid [b]	15	0
recovery of reacted C in %		*24 (54[c]), 28*	*72 (76[c]), 72*
reactant consumption per ozone added [mol/mol]		*0.38, 0.53*	*0.45, 0.48*
catechol **2**	*cis,cis*-muconic acid	26, 24	16, 15
	acetic acid[b]	7	7
	formic acid [b]	24	18
	oxalic acid [b]	4	0
recovery of reacted C in %		*26, 24 (32[c])*	*16, 15 (20[c])*
reactant consumption per ozone added [mol/mol]		*0.45, 0.46*	*0.32, 0.33*
hydroquinone **3**	benzoquinone	61, 52	84, 82
	acetic acid[b]	14	5
	formic acid [b]	10	12
	maleic acid [b]	14	9
recovery of reacted C in %		*61, 52 (67[c])*	*84, 82 (92[c])*
reactant consumption per ozone added [mol/mol]		*0.45, 0.46*	*0.46, 0.42*
benzoquinone **4**	acetic acid	16, 12	55, 23
	formic acid	77, 71	108, 79
	glyoxylic acid	19, 11	16, 10
	maleic acid	0, 0	0, 0
	oxalic acid	1, 1	3, 2
recovery of reacted C in %		*25, 20*	*43, 25*
cis,cis-muconic acid **5**	acetic acid	5, 5	3, 2
	formic acid	124, 162	99, 129
	glyoxylic acid	61, 105	43, 56
	maleic acid	14, 20	14, 17
recovery of reacted C in %		*52, 77*	*41, 52*
reactant consumption per ozone added [mol/mol]		*0.62, 0.57*	*0.77, 0.90*

maleic acid **6**	acetic acid	0, 0	0, 2
	formic acid	100, 90	133, 119
	glyoxylic acid	123, 104	63, 84
	oxalic acid	0, 0	0.3, 0.4
recovery of reacted C in %		*87, 74*	*65, 73*
reactant consumption per ozone added [mol/mol]		*0.88, 1.04*	*0.83, 1.01*
glyoxylic acid **8**	acetic acid		0, 0
	formic acid		6, 0
	oxalic acid	84, 46	47, 27
recovery of reacted C in %		*84, 46*	*50, 27*
reactant consumption per ozone added [mol/mol]		*0.21*	*0.32, 1.19*

a) first number from experiment with 200 μM reactant, second number from experiment with 400 μM reactant

b) only one, two or three samples with different ozone additions measured, whereas normally 8 ozone doses were considered

c) including the organic acids labeled with b)

with the phenoxyl radical and finally resulting in benzoquinone (dotted lines in Scheme 4.2). However, this reaction cannot be very important because already at pH 7 only a minor part of the hydroquinone will be deprotonated. In contrast to pH 7 the rate constant of benzoquinone with ozone at pH 3 is higher than the one of phenol and ozone. As mentioned before, during the formation of benzoquinone from phenol at pH 3 benzoquinone might have reacted with ozone resulting in organic acid formation and benzoquinone yield reduction.

Catechol could not be detected at pH 3 but it was found to be an important primary product of phenol ozonation at pH 7. This is in disagreement with former studies (Table 4.4) (Mvula and von Sonntag, 2003). Since the $^\bullet$OH pathway of catechol formation was suppressed by the addition of *t*BuOH, catechol might be formed over a hydrotrioxide formed by ozone attack on phenol at the *ortho*-position. Mvula and von Sonntag (2003) explained the increased catechol yield at higher pH by an increased stability of the hydrotrioxide at higher pH, allowing a more efficient elimination of O_2. Through equilibrium (1) in Scheme 4.2 a competition between catechol and muconic acid yield is expected. Unfortunately, the low muconic acid yield and the low mass balance at pH 3 do not allow to make any statement about the pH dependence of this equilibrium nor to

confirm higher muconic acid yields at lower pH as was shown in former studies without *t*BuOH-addition (Mvula and von Sonntag, 2003).

Table 4.4 Yields in mol-% of product produced based on the ozone consumed. Comparison with literature data.

parent	product	pH 3 [a]	pH 7 [a]	pH 7 [b]
phenol	benzoquinone	4, 5	20, 20	13
	hydroquinone	2, 4	0, 1	<1
	catechol	0, 0	16, 19	2
	cis,cis-muconic acid	1, 2	2, 2	2
% phenol consumption [mol **1** / mol ozone]		38, 54	50, 48	42
hydroquinone	benzoquinone	32, 30	39, 28	36 / 32
% hydroquinone consumption [mol **3** / mol ozone]		47, 48	46, 43	48

a) first number from experiment with 200 µM reactant, second number from experiment with 400 µM reactant, b) Mvula and von Sonntag (2003)

Hydroquinone is far less abundant than catechol at pH 7. Hydroquinone can be formed by ozone attack at the *para*-position and subsequent loss of O_2 in analogy to catechol formation (Scheme 4.2). However, two mechanisms can lead to a decrease of the hydroquinone yield: firstly the intermediate trioxide needed for hydroquinone formation by O_2 loss can be transformed to benzoquinone, too. It was discussed previously that benzoquinone is formed as a main product and that the $HO_2^{\bullet}$-pathway for benzoquinone formation might not be predominant. Secondly hydroquinone can be transformed to benzoquinone through reaction with the phenoxyl radical (dotted lines in Scheme 4.2), which could explain its even lower yield at higher pH (Table 4.3), as discussed before.

Finally, it has to be mentioned that the mass recovery of reacted phenol is about 70-75 % at pH 7 while it is only 20-30 % at pH 3, indicating that at pH 3 possibly some main reaction pathways are not detectable by the analytical methods utilized in this study.

4.3.2 Ozonation of primary phenol ozonation products and subsequent products

Table 4.3 summarizes the results of the batch experiments that were performed to determine the yields of primary products of ozonation of different compounds. The substrates chosen were the primary products of phenol ozonation or the subsequent products. For some products the yield is also given as per cent of the amount of consumed ozone (Table 4.4). For pH 7, these results can be compared with a previous study (Mvula and von Sonntag, 2003). Good agreements in substrate consumption were achieved for phenol as well as for hydroquinone ozonation. Except for catechol formed during phenol ozonation the yields of the products were similar. Expressing the product yield in terms of ozone consumed can be problematic. With a depletion of the original substrate, i.e. phenol, a shift of the reaction to the intermediate can be expected which leads to a gradual depletion of this intermediate compound as well. The ozone consumption thus depends not only on one specific reaction. Therefore, in Table 4.3 the yields are given as a function of substrate consumption, although in the setup of the current study, reactions of the intermediates should not be important (see above).

Benzoquinone was the main product from hydroquinone ozonation. It is the oxidized form of hydroquinone and was found in high yields of more than 80% at pH 7. Ozonation of benzoquinone resulted in the formation of small organic acids, the reaction mechanism remaining unclear, because no UV-detectable intermediate product was produced. The only ozonation product from catechol was muconic acid. It is formed via the Criegee mechanism (Scheme 4.1). This reaction was known and even under discussion for commercial muconic acid production in earlier years (Wingard and Finn, 1969). Ozonation of muconic acid resulted in the formation of maleic, glyoxylic and formic acid. Reaction of ozone with maleic acid gave glyoxylic and formic acid and ozonation of glyoxylic acid produced oxalic acid as major product. Scheme 4.3 summarizes the results of the batch experiments at pH 7. The ozonation mechanism of muconic acid and its products are discussed in more detail below.
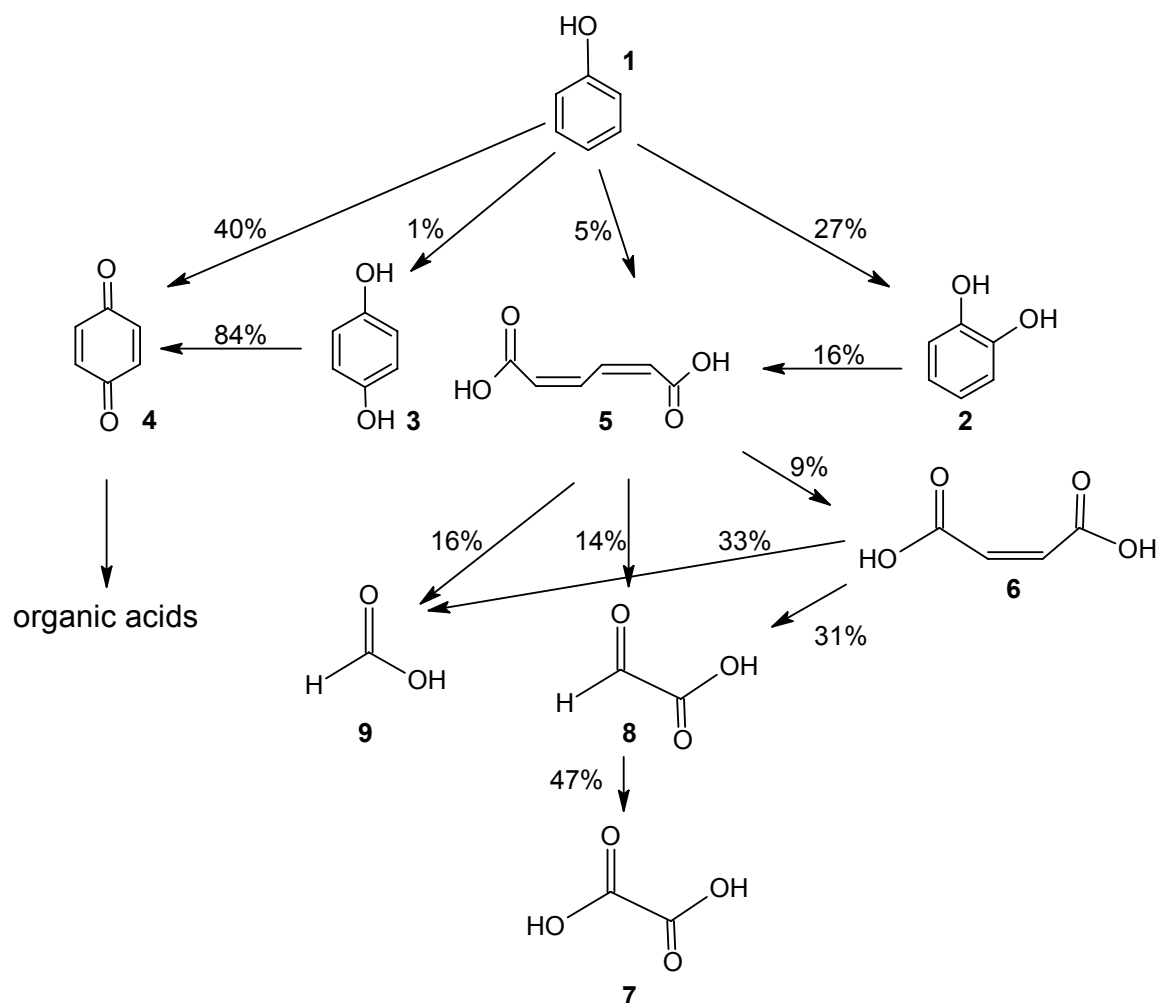
Scheme 4.3 *Simplified reaction pathway of phenol ozonation. The mechanism is based on understoichiometric ozone dose experiments. %-Numbers are the yields at pH 7 based on the carbon atoms of the direct parent compound.*

4.3.3 Transformation of *cis,cis*-muconic acid

Before going into details of the ozonation mechanism of *cis,cis*-muconic acid, its analysis and its transformation into *cis,trans*-muconic acid has to be discussed.

Cis,cis-Muconic acid (ccMA) could not be separated from *trans,trans*-muconic acid (ttMA) on the HPLC column and under the conditions used, but thanks to slightly different absorption maxima (ttMA 262 nm, ccMA 259 nm in MeOH / 10 mM H_3PO_4) ccMA could be identified as being formed during ozonation. However, ccMA is not stable in aqueous

solution at acidic pH. After 12.5 hours it decreased to 36% of its initial concentration at pH 3. However, at pH 7 even after 30 hours only 12% of ccMA was depleted. Neither the phosphate buffer concentration nor the exposure to light had an influence on this result. As ccMA decreased, an additional peak with a higher retention time and an absorption maximum of 261.5 nm emerged in the HPLC chromatogram. ESI-MS-analysis in the negative ionization mode of the emerging HPLC-peak fraction gave a molecular peak of m/z 141. To confirm the assumption that ccMA was transformed into *cis,trans*-muconic acid (ctMA), which was not commercially available, a solution of ccMA was refluxed in water (T ≈ 100°C) which yields ctMA (Matsumoto et al., 2000). The resulting ctMA retention time and absorption maxima matched the ones of the emerged peak. In contrast to Matsumoto et al. (2000) which had to reflux for 6 hours for complete ccMA to ctMA conversion, in our experiment already after one hour 97 % ccMA were transformed. Correspondingly, the ctMA-peak was highest after one hour (first sampling point) and decreased subsequently.

4.3.4 Formation of organic acids upon ozonation

Scheme 4.4 shows possible ozonation mechanisms of muconic acid and its ozonation products, e.g. maleic acid.

Maleic acid **6** can decay - via the Criegee mechanism and subsequent water addition to the zwitter-ion - into glyoxylic acid **8** and the intermediate **11**, a hydroxyhydroperoxide (reactions XII-XIII, Scheme 4.4). No oxalic acid **7** was produced by maleic acid ozonation (Table 4.3), hence water loss of intermediate **11** (reaction X) does not take place. However, intermediate **11** can decay into glyoxylic acid **8** and hydrogen peroxid (reaction IX) with a rate constant of $5 \cdot 10^{-4}$ s^{-1} (Leitzke, 2003) and be reformed with a rate constant of the backward reaction of 16.5 $M^{-1}s^{-1}$ (Leitzke et al., 2001). In its anionic form, intermediate **11** ($pK_a = 2.6$) can also decay into formic acid, water and carbon dioxide with a rate constant of >1 s^{-1} (reaction XI) (Leitzke et al., 2001), which is much faster than reaction IX.

From these considerations, one expects a higher formic acid **9** yield at higher pH, where the anionic form of **11** is predominant. Additionally, at higher pH glyoxylic acid **8** produced in the first step (XII-XIII) will react with deprotonated and hence more reactive

H_2O_2 (pK_a=11.8) (Leitzke et al., 2001) to form formic acid **9** (reactions IX-XI), provided that there is enough H_2O_2 available. Since hydrogen peroxide can also be produced from *t*BuOH during quenching of hydroxyl radicals (Flyunt et al., 2003; Schuchmann and von Sonntag, 1979) this should not be a restricting factor.

The experimental values (Table 4.3) confirm the transformation of glyoxylic acid into formic acid: Theoretically reactions XII-XIII-XI lead to 100% yield in glyoxylic acid (reaction XIII) and 100% yield in formic acid (reaction XI). Within the experimental error these yields are indeed achieved at pH 3 (cf. Table 4.3), confirming that reaction IX does not play a major role. This is in agreement with Leitzke et al. (Leitzke et al., 2001), and implying that intermediate **11** only decays by pathway XI. At pH 7 however, the formic acid yield exceeds 100% by about the same as the glyoxylic acid yield is smaller than 100%, confirming the transformation of **8** (from reaction XIII) into **9** (via **11**). In this case, carbon mass balance is 97 and 112 %, if one mole of CO_2 is accounted for each mol of formic acid, indicating that the glyoxylic acid loss is completely transformed to **9** and CO_2. A further indication that H_2O_2 is a major reagent is given by another experiment: when the reaction of ozone with maleic acid took place at pH 3 but the solution was neutralized one hour after ozonation, the yields were similar to the yields obtained at pH 7 (data not shown). One can assume that all the ozone was consumed by the time of neutralization, thus the production of some formic acid is indeed not caused by ozone itself, but by later reaction of H_2O_2 with **8**.

Pathways of ozonation of *cis,cis*-muconic acid (Scheme 4.4) can be discussed on the basis of the given results (Table 4.3), too. Ozonation products are maleic acid **6**, glyoxylic acid **8**, and formic acid **9**. Maleic acid **6** can be formed over the reaction sequence I-III-IV-VI. Unlike the intermediate **11**, the hydroxyhydroperoxyde formed in reaction IV seems to eliminate water to a certain extent. The maleic acid yield is only 14-20% for both pH 3 and 7 (Table 4.3). Hence maleic aldehyde, which was not measured, or other products have to be formed from 80% of the muconic acid. When maleic acid is produced, glyoxylic acid **8** must be formed, too (reaction III). Indeed, higher yields of glyoxylic acid than maleic acid were measured. From the discussion above it is known that the reaction sequence VII-VIII-IX cannot be responsible for the higher glyoxylic acid yield, hence it is proposed that reaction V takes place to a considerable extent.
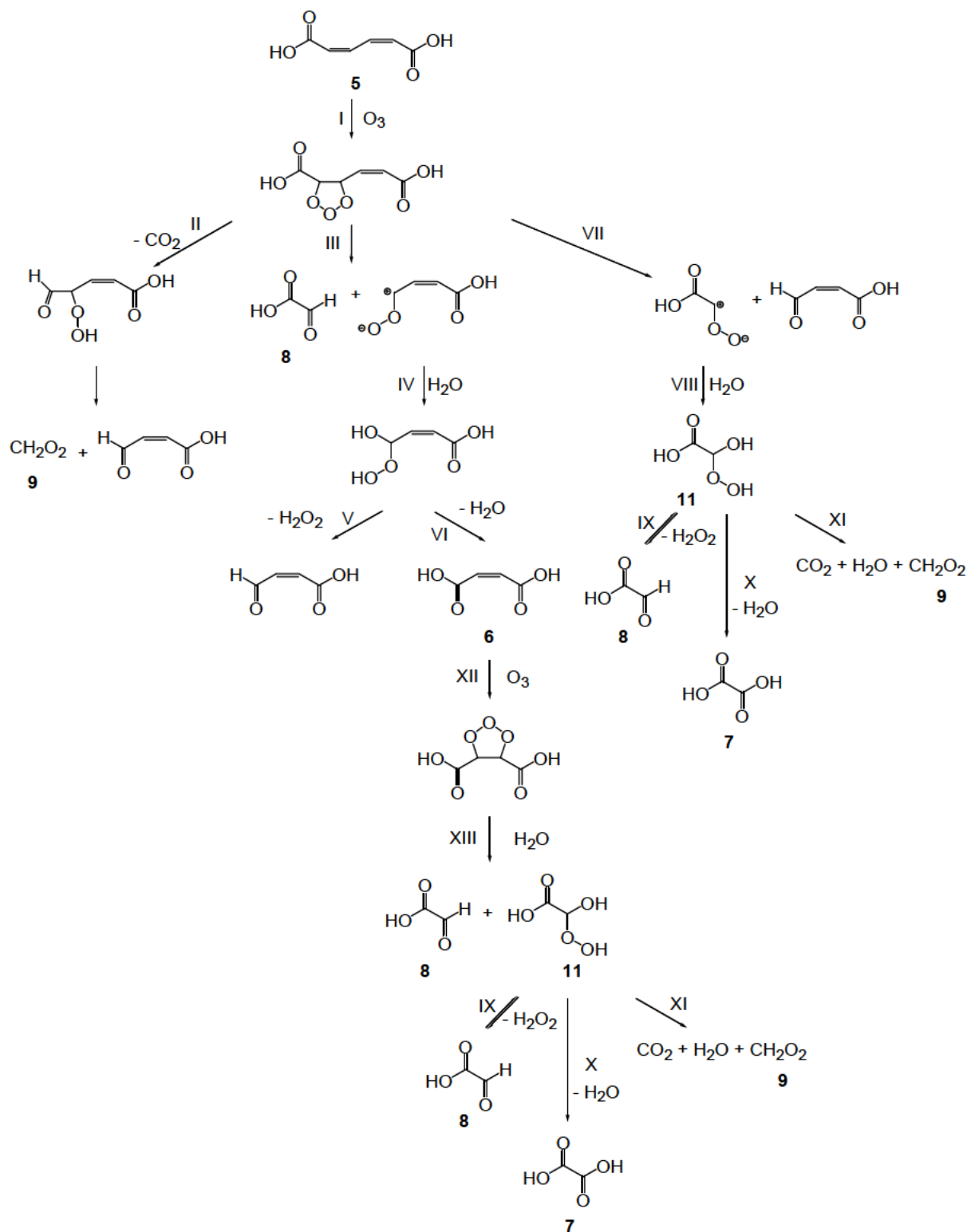
Scheme 4.4 *Product formation during ozonation of muconic **5** and maleic **6** acids. Partly adapted from Leitzke (2003) and Leitzke et al. (2001).*

However, large yields of formic acid were found to be produced at both pH 3 and 7 (Table 4.3). In a former study (Leitzke, 2003), where catalase was added to suppress the degradation of glyoxylic acid to formic acid by H_2O_2, a 98% glyoxylic acid yield was detected in terms of ozone consumption. However, formic acid was detected neither at pH 2 nor at pH 8. Therefore, it was concluded that reaction VII does not take place. Additionally, the decarboxylation pathway (reaction II) was also not observed. This raises the question by which mechanisms formic acid is formed in the present study. The yield of glyoxylic acid in terms of ozone consumption is only 40-60% at pH 3 leaving the possibility open that more glyoxylic acid was produced but consumed before measurement. However, from the discussion above it is assumed that at low pH the reaction of glyoxylic acid with hydrogen peroxide to formic acid (reactions IX-XI) is of minor importance. Hence, this does not explain the high formic acid yields, especially since in this case the sum of the yield of glyoxylic and formic acid should not exceed 100%. Even if one considers the possibility that freshly formed maleic acid **6** might react with ozone - the rate constant of muconic and maleic acid are similar (Table 4.2) - the formic acid yield should not exceed 100%. In conclusion, it can be proposed that reactions that are not outlined in Scheme 4.4 have to be responsible for the high formic acid yield. The presence of H_2O_2 might for instance open a broad field of further reactions.

4.3.5 Comparison of oxalic acid formation from phenol and lake water ozonation

Oxalic acid is a major and quite specific product of ozonation of natural waters, and it contributes considerably to AOC (Hammes et al., 2006). Unlike other organic acids, such as acetate and formate, oxalic acid was not present in measurable concentrations in natural lake water (Lake Zurich, Switzerland), but reached a high concentration in a drinking water treatment plant after pre-ozonation and intermediate ozonation (Figure 4.2). Therefore, oxalate often shows a similar pattern to the AOC behaviour in the treatment plant. Considering a dissolved organic carbon (DOC) concentration of 1.4 mg/l in lake water, roughly 1.6 % of the total DOC is transformed into oxalic acid upon ozonation.
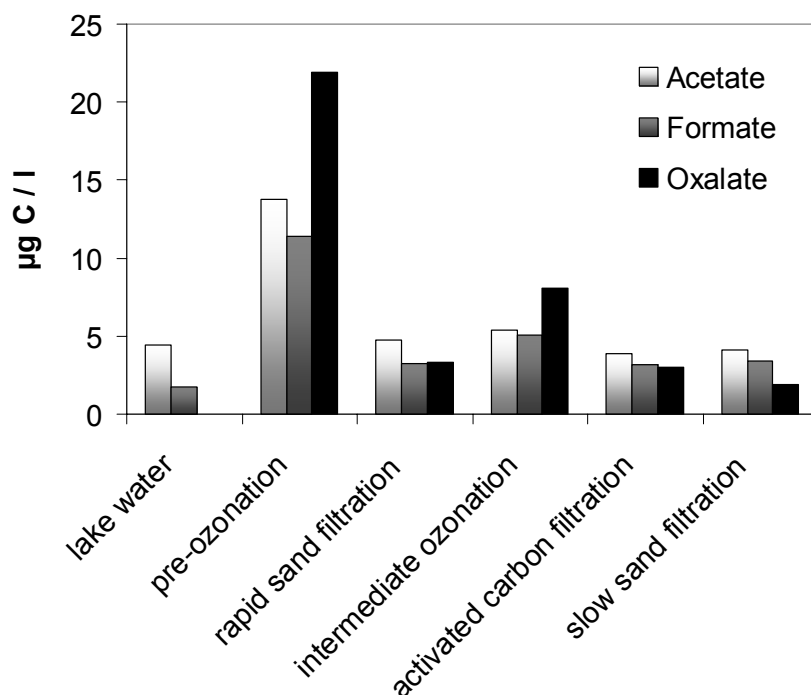
Figure 4.2 *Evolution of concentrations of acetic, formic and oxalic acid during full-scale treatment of Lake Zurich water (Zurich, Switzerland). Raw water parameters were: DOC ~1.4 mg/l, alkalinity ~2.5 mM as HCO_3^-, pH 8, temperature 4°C, ozone doses: pre-ozonation ~1.2 mg/l and intermediate ozonation ~0.5 mg/l.*

Scheme 4.3 summarizes the ozonation pathway of phenol, as surrogate compound for NOM, and its subsequent products. It becomes obvious that oxalic acid **7** is only formed upon glyoxylic acid **8** oxidation. Calculating the yield of each reaction ultimately leading to formation of oxalic acid, it can be said that only roughly 0.8 % of the initial phenolic carbon is transformed into oxalic acid. This value is close to the 1.6 % of oxalic acid formed from ozonation of NOM of Lake Zurich water. Regarding that only direct ozonation reactions have been considered in this study, phenol can be accounted as a reasonable surrogate compound. It can also be assumed that a large part of the oxalic acid produced is derived from phenolic moieties in NOM and direct ozonation reactions are of importance.

4.3.6 Time resolved product formation from phenol ozonation

The experiments and results discussed so far describe the ozonation products at the end of the reaction. However, they do not give any time-resolved information on product formation. Furthermore, in real drinking water treatment systems the ozone concentration is far higher than phenolic moieties and in a similar range as the NOM concentration. Hence a dynamic reaction system with an excess of ozone relative to phenol was investigated in this part of the study by use of a continuous quench flow system.

The ozonation of phenol leads to the formation of the intermediate and final products discussed above with time-dependent variations of the concentrations. In Figure 4.3, the reaction of phenol with ozone and the product formation over time at pHs of 3 and 7.25 are given in % carbon of a certain product based on the initial carbon content given by the phenol concentration. The fast phenol consumption at pH 7.25 and the slower decrease of the phenol concentration at pH 3 is obtained as expected from the pH dependence of the rate constants for the reaction of phenol/phenolate with ozone. At pH 7.25 a half life time of $1.2 \cdot 10^{-3}$ seconds is expected. Since the first sampling point at pH 7.25 is at 66 ms, no residual phenol can be measured in our experiments (Figure 4.3c).

In agreement with the results from the batch experiments at pH 7, benzoquinone is the product with the highest yield at the beginning, followed by catechol. Its immediate concentration increase indicates a fast rate of formation and is an indication that it could be a direct ozonation product of phenol and not only formed via hydroquinone oxidation. The slow decline at pH 7.25 can be explained by the relatively small rate constant of benzoquinone with ozone ($k = 2.5 \cdot 10^3$ $M^{-1}s^{-1}$, Table 4.1). The measured half life time of benzoquinone (49% decrease in 0.76 sec) agrees with its theoretical value of 0.75 seconds.

At pH 3 the rate constant for the reaction of phenol with ozone is smaller than that of benzoquinone with ozone. The formation of primary intermediates might hence become slower than their consumption. That explains the decrease of benzoquinone concentration even in presence of phenol. The concentration decrease of intermediates in presence of phenol is even more pronounced for catechol, Figure 4.3b.
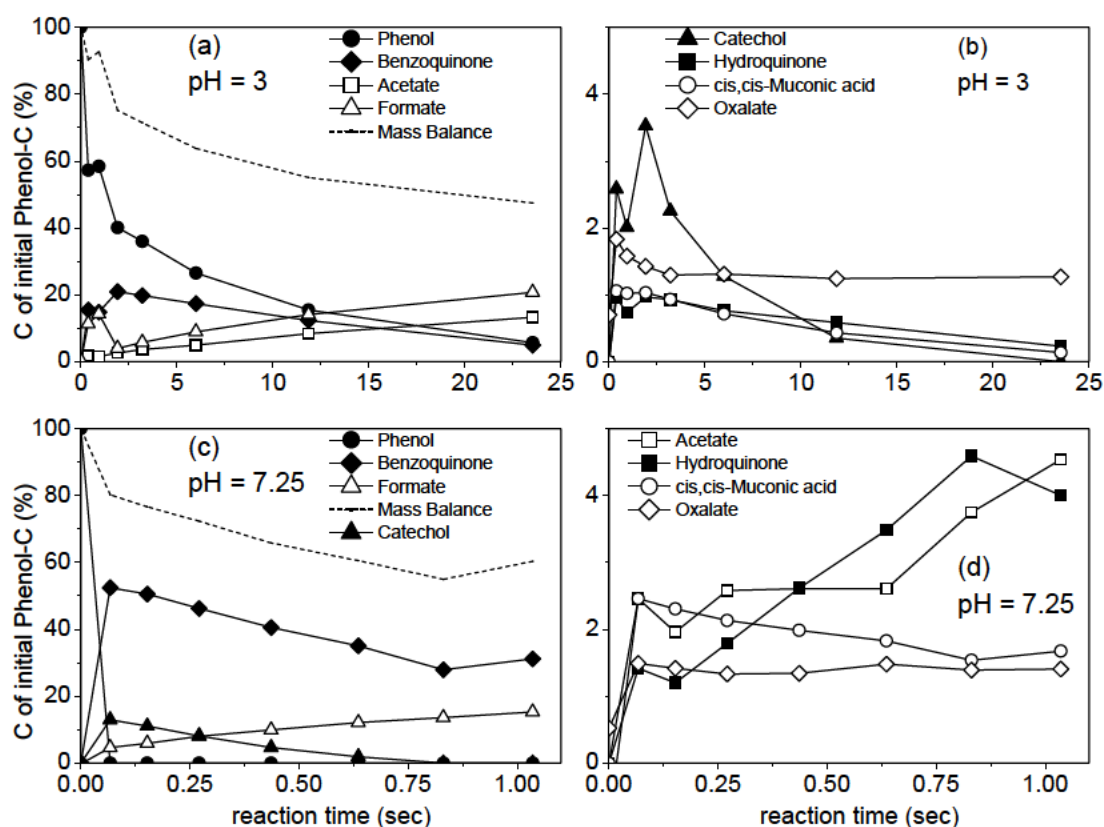
Figure 4.3 *Ozonation of 25 µM phenol in a CQF-system at room temperature with 9 and 11-fold ozone excess at pH 3 and 7.25, respectively. tBuOH was used to scavenge ˙OH radical reactions. Data are given in % C of the initial phenol-C. a) b) pH 3.0, c) d) pH 7.25, b) d) zoomed y-axis of a) c), respectively.*

Similar to the batch experiments, in the continuous quench flow experiments catechol was only found in considerable yields at pH 7.25. Hydroquinone was produced in less than 10 % yield under all conditions. At pH 3, the hydroquinone concentrations were close to the limit of quantification. In a former time-dependent ozonation study in an ozone-gas batch system (Singer and Gurol, 1983) similar catechol and hydroquinone relative concentrations were found. In this study the maximum catechol concentration was 18 % of the initial phenol concentration at pH 6 and 7 % at pH 8.9, in our study 13 % catechol at pH 7.25 could be measured. For hydroquinone 0.2 %, 6 % and 0 % were found at pH 3, 6 and 8.9, respectively (Singer and Gurol, 1983), and < 1 % and 4.5 % at pH 3 and 7.25, respectively, were measured in this study. However, the slight increase in hydroquinone concentration at pH 7.25 (Figure 2.3d) while all the phenol has already been consumed, cannot be explained by our proposed mechanism (Scheme 4.3). Yet, in the discussion of the batch

experiments it became clear that other reactions than the ones shown in Scheme 4.3 take place, especially in presence of H_2O_2.

Also muconic acid was measured in previous studies (Singer and Gurol, 1983). They found 2-3 % and 5-6 % muconic acid at pH 3 and 6, respectively, while in the present study 1 % and 2.5 % at pH 3 and 7.25 were detected, where the concentrations of muconic acid at pH 3 lie just at the limit of quantification. Muconic acid is formed in small yields from phenol and to a more important extent from catechol (Table 4.3). Under the conditions given, phenol is calculated to be consumed within roughly 5-10 ms at pH 7.25 with an 11-fold ozone excess, allowing its primary products to react with ozone for circa 50 ms before the first sample after 66 ms was taken. Hence the concentration of catechol must have been even higher before the first sampling point and it must have generated muconic acid.

The half-life of muconic acid can be estimated to roughly 10-20 ms. This means that there is sufficient time to produce some smaller acids from ozonation of muconic acid within the first 66 ms. Besides the acids shown in Figure 4.3b and 4.3d, maleate could be detected but it's concentration lay below the limit of quantification. However, it seemed to increase and subsequently decrease within the reaction times measured in our system. In terms of their contribution to AOC, the organic acids are interesting. Although the batch experiments showed that organic acids are not primary phenol ozonation products, they are produced within short reaction times and for small ozone exposures. At pH 7.25 for a reaction time of only one second, an ozone exposure of 0.3 mg•min•l^{-1} was achieved. The sharp rise of small organic acids between zero and 0.1 seconds resembles the sudden increase in AOC concentration upon ozonation of lake waters (Hammes et al., 2006).

The contribution of the organic acids to the mass balance might not be very important, but the results show nevertheless that the formation of these compounds and hence the formation of AOC takes place at the very beginning of the ozonation process and is - at least partially - a consequence of the direct ozone reaction. Therefore, AOC production cannot be avoided if ozone is applied for disinfection purpose in water treatment.

4.4 Conclusions

Results have been presented that show the reaction pathway and the time resolved production of primary, intermediate and final products of phenol ozonation.

- Benzoquinone is the major primary product and is rapidly formed during phenol ozonation.

- Small organic acids are readily produced for small ozone exposures, even though they are not primary phenol ozonation products. The results indicate that direct ozonation reactions are important for organic acid formation.

- Oxalic acid was only formed during oxidation of glyoxylic acid, which in turn, is only a tertiary phenol ozonation product. Nevertheless, the relative amount of oxalic acid produced from phenol ozonation is in the same range as its relative amount based on organic carbon after pre-ozonation of Lake Zurich water. Therefore, phenolic moieties seem to be important precursors for organic acid production.

- The study confirms that even when $^\bullet$OH radicals are scavenged, other mechanisms than direct ozonation reactions are important, e.g. the reaction with H_2O_2. These reactions are of special importance for compounds that do not react with ozone with a high rate and that therefore have a longer life time (e.g. glyoxylic acid).

Acknowledgement

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5

GENERAL CONCLUSIONS AND OUTLOOK

Due to limited methodological options, the investigation of chemical inactivation of bacteria has so far mainly been performed with pure cultures, indicator organisms or with cultivable bacteria. As pointed out in Chapter 1, this approach is not sufficient to assess the disinfection efficiency in water treatment. Currently, the selected indicator organisms are mostly more susceptible to disinfectants than pathogens which may lead to an overestimation of the extent of disinfection. In addition, culture techniques only observe a small selection of the bacteria that are present in real water samples. In Chapter 2 a wide range of oxidants was investigated with regard to their inactivation kinetics of a native mixture of bacterial cells present in drinking water. To examine the effect of the oxidants on this mixture of bacteria, plating techniques cannot be applied. Instead, the cells were stained by DNA-stains that allowed the differentiation between cells with intact and damaged cell membranes. The concentration of intact and damaged cells after disinfection was determined by flow-cytometry (FCM). This method has the advantage to measure all, not only cultivable bacteria. However, it does not differentiate between pathogenic and harmless cells.

Ozone was too efficient in damaging the cells for the time resolution of our experiments. Therefore, only a minimum rate constant k of > 30 L/(mg*min) could be estimated. Apart from ozone, chlorine was the most efficient disinfectant with a second-order rate constant of 0.5 L/(mg*min), followed by chlorine dioxide with k = 0.02 L/(mg*min) and ferrate with a similar rate constant of 0.01 L/(mg*min). Permanganate only damaged the cells very slowly (k = 2 x 10^{-3} L/(mg*min)) and chloramine was even slower (k = 4 x 10^{-5} L/(mg*min)). Based on the fact that chlorine damaged the cells faster than chlorine dioxide and ferrate, and that it reacts faster with amines, it was suggested that the reaction of the oxidants with amine-containing moieties in the cell membrane played an important role in causing cell damage.

Although the overall inactivation rate constants of chlorine dioxide and ferrate were similar, their behavior towards two clusters of bacteria, namely low nucleic acid bacteria (LNA) and high nucleic acid bacteria (HNA), was different. These two clusters can be distinguished by FCM. While ferrate reacted with both clusters with the same rate constant, chlorine dioxide reacted ten times faster with HNA cells than with LNA cells. Ferrate reacts with amines as well as with all amino acids. Chlorine dioxide does not react with primary and secondary amines and it only reacts with amino acids containing functional

moieties such as phenyl-, thiol-, or indole-groups. From these differences in reactivity it was hypothesized that the HNA cells, in addition to amines, contain more diverse or more easily accessible functional groups in their membranes than LNA cells.

The combination of fundamental knowledge about the reactivity of the selected oxidants with a method that allowed the detection of membrane damage, permitted the deduction of new information about possible oxidation targets in the cell membranes. This combination opens a broad field for further investigations to elucidate not only reactive sites in membranes but also oxidatively-induced inactivation mechanisms. For further investigations, stains targeting other viability indicators such as efflux pump functioning, esterase activity and bacterial respiration could be utilized.

The results from this study have shown that some inactivation rate constants obtained by FCM were up to four orders of magnitude smaller than rate constants derived from cultivation based methods. This was expected because membrane damage is a conservative parameter for bacterial viability. Using other viability indicators as mentioned above as an inactivation criterion might give other results. Therefore, before this approach can be used for routine testing, more research needs to be done to elucidate the suitability of different viability indicators for the assessment of drinking water disinfection.

When oxidants are applied for disinfection purposes their reaction with water matrix components has to be considered as well. Besides the consumption of the oxidants, which leads to a lower disinfection efficiency, these reactions can lead to the formation of disinfection by-products (DBPs). As described in Chapter 1, small oxygen rich compounds that are assimilable by bacteria are one class of DBPs. Assimilable organic carbon (AOC) which is a sum parameter for such compounds has been measured in drinking water treatment systems for three decades. However, the measurements were restricted by a time-consuming plating method which did not allow high frequency sampling. In the current study, AOC was measured by inoculating the sterilized water sample and determining the number of cells grown on the AOC within a given time by FCM. This method allows to process larger numbers of samples which is necessary for kinetic measurements. In literature, information about the influence of ozone and chlorine on AOC formation is available mainly from investigations in full-scale systems. However, only limited information is available under controlled laboratory conditions and for oxidants such as

chlorine dioxide, permanganate, and the emerging oxidant ferrate. In particular, no systematic study has been performed on AOC formation kinetics and mechanisms comparing these different oxidants. Chapters 3 and 4 focus on AOC formation in homogeneous (filtered water) and heterogeneous (water containing cyanobacterial cells) systems by the selected oxidants.

AOC measurements in filtered lake water for various oxidant exposures gave the following results: ozone formed more than 100 μg/L AOC upon small exposures (5 mg*min/L), chlorine and chlorine dioxide did not lead to AOC formation, permanganate formed small amounts of AOC at a slow rate, and the application of ferrate led to a significant AOC formation. When cyanobacteria were spiked into the water before oxidation, the AOC concentration increased significantly for ozone, ferrate, and permangante, compared to the samples without addition of cells. Chlorine dioxide and chlorine did not form significant amounts of AOC even in presence of cyanobacteria.

The increase of AOC during ozonation in presence of cyanobacteria was further investigated by measuring geosmin which is produced by these cells. The release of geosmin indicated that the cells were damaged by ozone to such an extent that intracellular material could leach into the water. Obviously, such intracellular material contains AOC such as amino acids and oligopeptides. For chlorine and chlorine dioxide application in the heterogeneous system, geosmin was released, but no AOC increase was observed. It was concluded that compounds which might inhibit bacterial growth in the AOC assay are formed by these two oxidants. Permanganate is a stable oxidant and reacts slowly with organic moieties of the water matrix. This explains the slow and, in the time considered, low formation of AOC. However, permanganate was able to increase the AOC and the geosmin concentration in water containing cyanobacteria. Therefore, it was concluded that permanganate can also damage cells. Ferrate produced AOC from cyanobacteria as well as from water matrix components. Ferrate reacts with similar functional groups as ozone but at a much lower rate. Therefore, the formation of AOC was expected. This study suggests that ferrate is the most critical oxidant beside ozone concerning AOC formation. Further research needs to be conducted to elucidate the AOC formation mechanisms for ferrate and strategies to minimize the AOC concentration for ferrate application.

In addition to AOC, oxalate was measured as a specific compound representing part of the AOC. Oxalate was produced by all selected oxidants. For all oxidants a linear increase of oxalate as a function of the consumed oxidant was found. This finding showed that all oxidants underwent oxidative reactions with water matrix components, however for chlorine and chlorine dioxide the operationally defined AOC did not reflect this observation.

In many cases the differences between the AOC formation potential of the various oxidants could be explained based on their reactivity. In Chapter 4 this was further elucidated by the reaction of ozone with phenol, a model compound for natural organic matter (NOM). Benzoquinone and catechol were formed as major primary products from direct ozone reactions. Benzoquione and catechol reacted with ozone to yield considerable amounts of organic acids within a few seconds. These mechanistic considerations correlate remarkably well with the results presented in Chapter 3. Size exclusion chromatography with organic carbon detection showed a change in the composition of the water matrix towards lower molecular weight compounds (i.e. low molecular weight acids) that contribute to AOC. The fast AOC production during ozonation can be partly attributed to direct ozone reactions with phenolic structures. Therefore, it could be concluded that AOC formation by ozonation cannot be avoided during disinfection, and a suitable post treatment for AOC removal is necessary.

During drinking water treatment the two processes, disinfection and AOC formation, have an opposing impact on the water quality. This study showed that disinfection processes can be investigated for natural bacterial communities with a modern microbiological method which is independent of cultivable bacteria. Fluorescent staining coupled with FCM can provide a more comprehensive understanding of disinfection processes and allow some insights into the mechanisms of chemical inactivation of bacteria as has been exemplified in this study. This knowledge can help to optimize disinfection during water treatment. Since this method is relatively fast and easy to use, it has potential for application in routine analysis. The full range of staining methods combined with FCM needs to be investigated to develop new procedures which will be suitable for the assessment of the bacteriological water quality in water supplies after chemical disinfection.

In addition, this study provides insights into the formation of AOC by the selected disinfectants, which is a critical water quality parameter after disinfection. This is especially important for Switzerland, where often no or minimal disinfectant is applied to the distribution system. For ozone, the oxidant with the most significant AOC formation, appropriate post-filtration systems are already in place in water treatment plants. A mechanistic and kinetic study with phenol could illustrate many of the processes which occur during ozonation of natural waters. For the other two main disinfectants applied in Switzerland, chlorine and chlorine dioxide, no AOC increase was measured. It is unclear whether this is a real effect, i.e. whether these oxidants did not form AOC, or caused by the inhibition of bacterial growth in the AOC assay. If the latter was the case it is important to investigate whether natural bacteria in distribution systems would behave similarly to the cells used in the AOC assay. Therefore, a better understanding of the AOC assay with regard to the types of assimilable compounds and possible inhibitions is necessary. AOC formation by permanganate and ferrate has not been investigated before. It appears that especially for the emerging oxidant ferrate biological post-filtration will be necessary to cope with the significant AOC formation. In both cases, however, more mechanistic information will be required to clearly understand the chemistry of the underlying processes.

In agreement with the results obtained for membrane damage in Chapter 2, the experiments in presence of geosmin-producing cyanobacterial cells showed that all selected oxidants were able to release geosmin which was an indicator for cell damage. This shows clearly that application of these oxidants bears the potential for realease of additional AOC but also other intracellular compounds, such as cyanotoxins and taste and odor compounds. Therefore, it is advisable to remove particles (incuding cyanobacterial cells and algae) before oxidative treatment.

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