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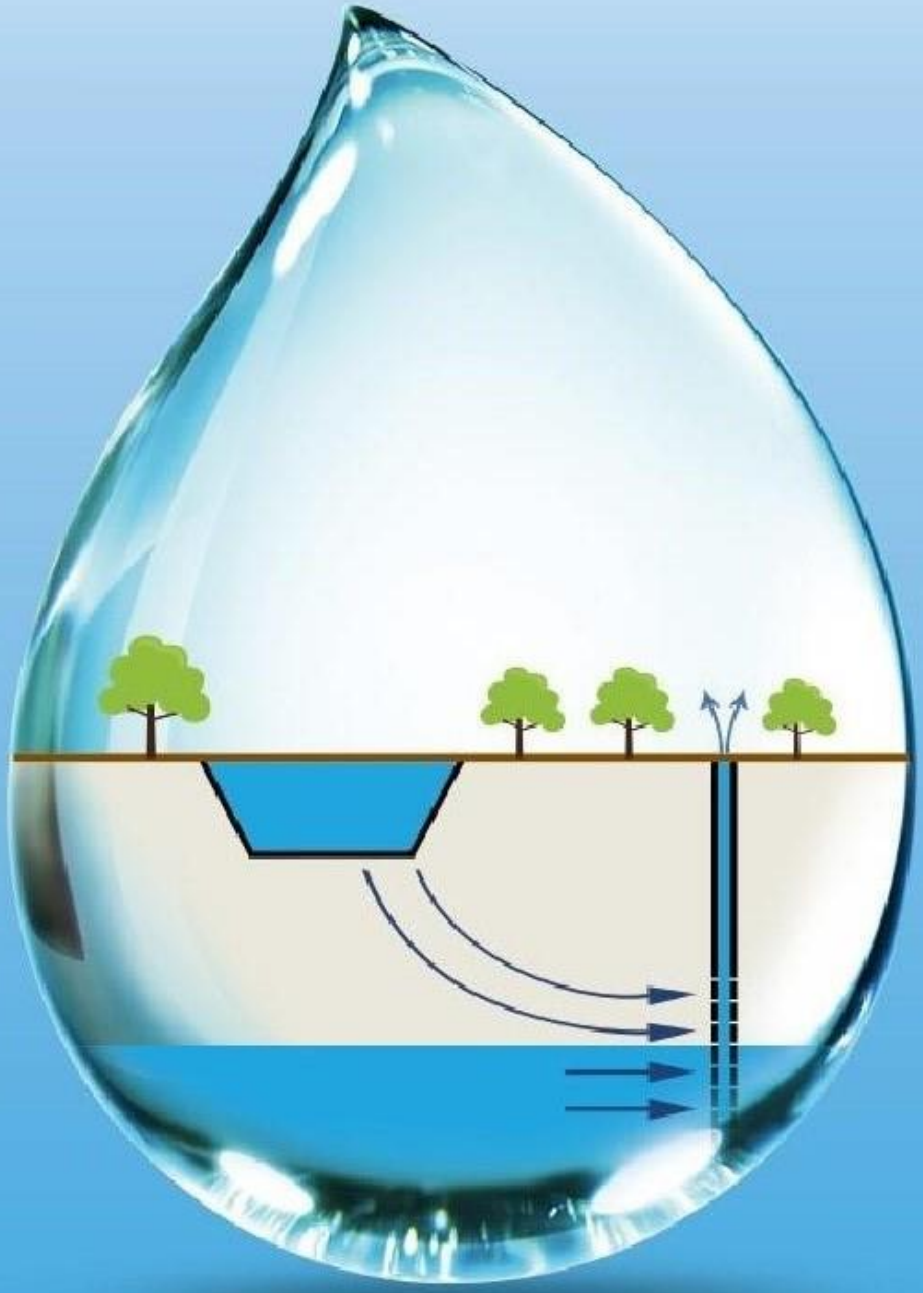

Feifei Wang

# Managed aquifer recharge as a barrier for ozone-based advanced oxidation by-products: $BrO_3^-$ and $H_2O_2$

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof. dr. ir. T.H.J.J. van der Hagen,
voorzitter van het College voor Promoties,
in het openbaar te verdedigen op

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谨以此书献给养育我的父母

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# Summary

Managed Aquifer Recharge (MAR) is a technology that relies on soil passage - after pond infiltration - for water treatment. MAR is a proven technology for the removal of pathogenic micro-organisms, turbidity and a selection of specific organic micro-pollutions (OMPs). Nevertheless, removal of the wide variety of OMPs found in surface waters requires additional treatment. The application of $O_3$-based advanced oxidation processes (AOPs) before MAR has been proposed as a smart solution, because previous studies have documented complementary and synergetic benefits for the removal of OMPs. However, the effect of the installation of $O_3$-based AOP as a chemical process on the subsequent MAR as a biological process is not known yet. Especially the behaviour and fate of $O_3$-based AOP by-products and residuals on MAR raise many questions. This thesis focused on the behaviour and fate of $BrO_3^-$ as an $O_3$-based AOP by-product and $H_2O_2$ as an AOP residual during MAR.

In chapter 2, the $BrO_3^-$ removal in $NO_3^-$-reducing anoxic zones of MAR systems and the potential mechanisms behind this removal was investigated. Batch reactors and columns were used to explore the influence of $NO_3^-$ and increased assimilable organic carbon (AOC) due to ozonation pre-treatment on $BrO_3^-$ removal. 8 m column experiments were carried out for 10 months to investigate $BrO_3^-$ behaviour in anoxic $NO_3^-$-reducing zones of MAR systems. The presence of $NO_3^-$ was found to be a precondition for $BrO_3^-$ reduction in anoxic zones of MAR systems, which indicates that denitrifying bacteria is a main contributor for $BrO_3^-$ reduction. The results also indicated simultaneous and competitive reduction of $BrO_3^-$ and $NO_3^-$ by denitrifying bacteria in the simulated MAR. Denitrifying bacteria prefer $NO_3^-$ to $BrO_3^-$ as an electron acceptor, but usually $BrO_3^-$ is present in trace amounts and the $NO_3^-$ concentration is several orders of magnitudes higher than $BrO_3^-$ in MAR infiltration waters. This may explain why relative $BrO_3^-$ removal (%) was observed greater than relative $NO_3^-$ removal. An increase of AOC as a result of AOPs pre-treatment promoted microbial activity and correspondingly $BrO_3^-$ removal in subsequent MAR systems. Taken together, $BrO_3^-$ removal is likely to occur in $NO_3^-$-reducing anoxic zones, so MAR systems following ozonation are potentially effective to remove $BrO_3^-$.

In chapter 3, $BrO_3^-$ reduction in Fe-reducing anoxic zones of MAR systems and the potential mechanisms behind it were investigated. Anoxic batch experiments were performed to explore the feasibility of $BrO_3^-$ reduction in Fe-reducing zones of MAR

systems and to estimate potential inhibition by $NO_3^-$. The results showed that the reaction rate was affected by initial $Fe^{2+}/BrO_3^-$ ratios and by initial pH. Also, the pH dropped significantly due to the hydrolysis of $Fe^{3+}$ to hydrous ferric oxides (HFO) flocs. These HFO flocs were found to adsorb $Fe^{2+}$, especially at high $Fe^{2+}/BrO_3^-$ ratios, whereas at low $Fe^{2+}/BrO_3^-$ ratios, the mass sum of $BrO_3^-$ and $Br^-$ indicated the formation of intermediate species. Under MAR conditions with relatively low $BrO_3^-$ and $Fe^{2+}$ concentrations, $BrO_3^-$ can be reduced by naturally occurring $Fe^{2+}$ as the extensive retention time in MAR systems will compensate for the slow reaction kinetics at low $BrO_3^-$ and $Fe^{2+}$ concentrations. Under specific flow conditions, $Fe^{2+}$ and $NO_3^-$ may co-occur during MAR but $NO_3^-$ will not compete with $BrO_3^-$ for reduction by $Fe^{2+}$ since $Fe^{2+}$ prefers $BrO_3^-$ over $NO_3^-$. However, it was found that when $NO_3^-$ concentrations exceed $BrO_3^-$ concentrations in multiple orders of magnitude, the presence of $NO_3^-$ may slightly inhibit $BrO_3^-$ reduction by $Fe^{2+}$.

The biodegradation of $BrO_3^-$ was quite apparent, 98% in simulated $NO_3^-$-reducing zones with a residence time of 8 days, while the chemical reduction of $BrO_3^-$ by $Fe^{2+}$ in Fe-reducing zones within 5 days was only 7%-36% at an initial $BrO_3^-$ concentration of 60 μg/L. Therefore, $NO_3^-$-reducing zones seem to be the predominant contributor to $BrO_3^-$ removal and trace amounts of $BrO_3^-$ residuals can be further reduced in Fe-reducing zones. The removal degree of $BrO_3^-$ will greatly depend on the specific retention time, infiltration water matrix and microbial activity and quantity of a specific MAR system. The observed effective removal of $BrO_3^-$ in MAR systems implies a new barrier of $BrO_3^-$ and a broaden applicability of AOPs.

Chapter 4 assessed the impact of five factors on $H_2O_2$ decomposition in MAR systems: pure sand, MAR infiltration water, soil organic matter (SOM), naturally inorganics on the surface of sand grains and living biomass. Batch reactor experiments were conducted to determine the reactions of $H_2O_2$ with biotic (microbial community in water) and abiotic constituents (pure sand, inorganic ions in infiltration water, soil organic matter (SOM) in MAR sand and naturally occurring inorganic substances coating on sand). Results showed that pure sand, MAR infiltration water constituents and SOM do not impact $H_2O_2$ decomposition. Naturally occurring inorganic substances on the surface of sand grains and living biomass are the two main contributors for $H_2O_2$ decomposition in MAR systems. Low concentration (<3 mg/L) of $H_2O_2$ in MAR influent water may decompose below 0.25 mg/L in the first centimeters of MAR systems when the water contains high microbial biomass (such as 38 ng ATP/mL). In most cases the the ATP concentration is

one order of magnitute lower than 38 ng/mL, where 3 mg/L $H_2O_2$ would infiltrate into a deeper zones.

Chapter 5 evaluated how $H_2O_2$ residuals influence sand systems with an emphasis on dissolved organic carbon (DOC) removal, microbial activity change and bacterial community evolution. A low $H_2O_2$ concentration (0.25 mg/L) limited DOC biodegradation by 10%, whereas high $H_2O_2$ concentrations (3 and 5 mg/L) promoted DOC biodegradation by 8% and 28% respectively. Low $H_2O_2$ concentrations (0.25 mg/L) did not influence microbial activity (measured as ATP) while high $H_2O_2$ concentrations (1, 3 and 5 mg/L) decreased microbial activity by 23%, 37% and 37%, respectively. The bacterial communities in sand were dominated by *proteobacteria*, more specifically, *Betaproteobacteria* (33%-39%). Both 0.25 and 5 mg/L $H_2O_2$ residuals influenced bacterial community structure. The bacterial community became more diverse at a concentration of 0.25 mg/L $H_2O_2$ but conversely became less diverse when the $H_2O_2$ concentration increased to 5 mg/L. Aerobic bacteria showed different responses to $H_2O_2$, either sensitive or tolerant. Anaerobic bacteria were found to be sensitive to $H_2O_2$, and their activity was limited by both 0.25 and 5 mg/L $H_2O_2$ (17-88% reduction). The increased DOC removal at higher $H_2O_2$ concentrations could potentially be explained by the aerobic bacteria *rhodocyclaceae* and *comamonadaceae*. *Zoogloea* deserves further consideration as an explanation for DOC removal change. Special attention should be given to the effect of $H_2O_2$ on microbial ecology before introducing AOPs as pre-treatment to biological (sand) processes.

During drinking water treatment, organic micropollutants (OMPs) removal by a multiple barrier system consisting of AOP and MAR has previously shown to be a complimentary and synergistic system for OMPs removal. This thesis underlines their synergistic effect with respect to by-products $H_2O_2$ and $BrO_3^-$. MAR can successfully decompose $BrO_3^-$ as a by-product of $O_3$-based AOP pretreatment, either microbiologically or chemically. $NO_3^-$-reducing zones are likely to be the predominant contributor to $BrO_3^-$ removal and trace amounts of $BrO_3^-$ residuals can be further reduced in Fe-reducing zones. At high microbial biomass concentrations, the trace amounts of $H_2O_2$ residuals (<3 mg/L) from AOPs do not pose a threat to the purification function of subsequent MAR during drinking water treatment. Therefore, the combination of AOP and MAR is a synergistic hybrid system on the aspect of inorganic by-products $BrO_3^-$ and $H_2O_2$. The findings in this thesis mean a new application of MAR and may broaden the applicability of ozone-based AOPs in drinking water treatment.

For drinking water companies which apply or consider to apply $O_3$-based AOP in their treatment scheme prior to a MAR system, this research provides valuable reference. AOP-MAR is a safe hybrid system for drinking water companies, but before the $O_3$-based AOP application, pilot studies need to be done for accurately predicting $BrO_3^-$ removal and $H_2O_2$ decomposition, as many variables affect the behavior and fate of both $BrO_3^-$ and $H_2O_2$. Also a hydrological analysis of the MAR infiltration system and MAR abstraction system is necessary as anoxic zones are a prerequisite for $BrO_3^-$ removal.

# Samenvatting

Duinfiltratie (MAR) is een technologie voor drinkwaterbehandeling en afhankelijk van bodempassage na infiltratie. MAR is een bewezen technologie voor het verwijderen van pathogene micro-organismen, troebelheid en een selectie van specifieke organische microverontreinigingen (OMVs). Er zijn echter veel verschillende OMVs aanwezig in oppervlaktewater waar een aanvullende behandeling voor nodig is. De toepassing van op ozon ($O_3$)-gebaseerde geavanceerde oxidatieprocessen (AOP) vóór toepassing van MAR blijkt een goede oplossing te zijn: uit eerdere studies is gebleken dat dit complementaire en synergetische voordelen heeft voor de verwijdering van OMVs. Echter, het effect van op $O_3$-gebaseerde AOP (als chemisch proces) voorafgaand aan MAR (als biologisch proces) is nog niet bekend. Vooral het gedrag en verloop van op $O_3$-gebaseerde AOP-nevenproducten en residuen tijdens MAR roepen veel vragen op. Dit proefschrift richtte zich op het gedrag en verloop van $BrO_3^-$, een bijproduct van op $O_3$-gebaseerd AOP en van $H_2O_2$, een AOP-residu van de combinatie van $O_3$ en UV met $H_2O_2$.

In hoofdstuk 2 werd $BrO_3^-$ verwijdering en de mechanismen onderzocht in $NO_3^-$ reducerende zuurstofloze zones van MAR-systemen. Met batchreactoren en kolommen werd de invloed van $NO_3^-$ en verhoogde assimileerbare organische koolstof (AOC), een gevolg van $O_3$-gebaseerde AOP voorbehandeling, op $BrO_3^-$ verwijdering onderzocht. Kolomproeven met een kolomlengte van 8 meter werden uitgevoerd gedurende 10 maanden om het $BrO_3^-$gedrag in zuurstofloze zones van MAR-systemen te onderzoeken. De aanwezigheid van $NO_3^-$ bleek een voorwaarde te zijn voor $BrO_3^-$reductie. Dit suggereert dat denitrificerende bacteriën een belangrijke bijdrage leveren aan $BrO_3^-$ reductie. Verder tonen de resultaten een gelijktijdige en competitieve vermindering van $BrO_3^-$ en $NO_3^-$ door denitrificerende bacteriën in de gesimuleerde MAR aan. Denitrificerende bacteriën geven de voorkeur aan $NO_3^-$ en niet aan $BrO_3^-$ als elektronenacceptor. Echter, in MAR infiltratiewater Is $BrO_3^-$ meestal aanwezig in sporenhoeveelheden terwijl $NO_3^-$ concentraties verscheidene orden van grootte hoger zijn. Dit zou kunnen verklaren waarom een grotere relatieve $BrO_3^-$ verwijdering (%) werd waargenomen dan relatieve $NO_3^-$verwijdering. Een toename van AOC als gevolg van de voorbehandeling van AOP's bevorderde de microbiële activiteit en de corresponderende $BrO_3^-$ verwijdering. Samengevat zal $BrO_3^-$ verwijdering waarschijnlijk plaatsvinden in

$NO_3^-$-reducerende zuurstofloze zones, met als gevolg dat MAR-systemen na ozonisatie mogelijk effectief zijn om het nevenproduct $BrO_3^-$ te verwijderen.

In hoofdstuk 3 werd de reductie van $BrO_3^-$ in Fe-reducerende zuurstofloze zones en de potentiële mechanismen daarvan voor MAR systemen onderzocht. Met de uitvoering van anoxische batch experimenten werd de haalbaarheid van het reduceren van $BrO_3^-$ met $Fe^{2+}$ onderzocht en tevens de potentiële remming door $NO_3^-$. De resultaten lieten zien dat de reactiesnelheid afhankelijk was van de $Fe^{2+}/BrO_3^-$ ratio en de pH. Verder was er een significante pH daling vanwege de hydrolyse van $Fe^{3+}$ tot ijzeroxide (HFO) vlokken. Deze HFO vlokken adsorbeerden $Fe^{2+}$ vooral wanneer de $Fe^{2+}/BrO_3^-$ ratio hoog was, maar wanneer de $Fe^{2+}/BrO_3^-$ ratio laag was, wees de totale massa van $BrO_3^-$ en $Br^-$ op de vorming van intermediaire producten. Onder MAR-omstandigheden met relatief lage $BrO_3^-$ en $Fe^{2+}$-concentraties, kan $BrO_3^-$ worden gereduceerd door natuurlijk voorkomend $Fe^{2+}$, omdat de lange retentietijd in MAR-systemen de trage reactiekinetiek bij lage $BrO_3^-$ en $Fe^{2+}$-concentraties zal compenseren. Onder specifieke stromingscondities kunnen $Fe^{2+}$ en $NO_3^-$ gelijktijdig voorkomen tijdens MAR, maar $NO_3^-$ zal niet concurreren met $BrO_3^-$ voor reductie door $Fe^{2+}$, omdat $Fe^{2+}$ voorkeur geeft aan $BrO_3^-$ boven $NO_3^-$. Echtert, zodra de $NO_3^-$ concentratie meerdere orden van grootte hoger is dan de $BrO_3^-$ concentratie, kan de aanwezigheid van $NO_3^-$ de $BrO_3^-$ reductie door $Fe^{2+}$ enigszins remmen.

De biologische afbraak van $BrO_3^-$ was aanzienlijk: 98% in gesimuleerde $NO_3^-$-reducerende zones met een verblijftijd van 8 dagen, terwijl de chemische reductie van $BrO_3^-$ door $Fe^{2+}$ in Fe-reducerende zones binnen 5 dagen slechts 7% -36% was, bij een initiële $BrO_3^-$ concentratie van 60 μg/L. Daarom lijken $NO_3^-$-reducerende zones de belangrijkste bijdrage te leveren aan $BrO_3^-$ verwijdering, en sporen van $BrO_3^-$ kunnen verder worden verminderd in Fe-reducerende zones. De verwijdering van $BrO_3^-$ zal in grote mate afhangen van de specifieke retentietijd, de infiltratiewatermatrix, en microbiële activiteit en biomassa hoeveelheid van een specifiek MAR-systeem. De effectieve verwijdering van $BrO_3^-$ in MAR-systemen impliceert een nieuwe barrière van $BrO_3^-$ en een bredere toepasbaarheid van AOP's.

Hoofdstuk 4 onderzocht de impact van vijf factoren op $H_2O_2$-omzetting in MAR-systemen: zuiver zand, MAR infiltratiewater, bodemorganisch materiaal (SOM), natuurlijk anorganisch materiaal op het oppervlak van zandkorrels, en levende biomassa. Batch-reactor experimenten werden uitgevoerd om de reacties te bepalen, van $H_2O_2$ met biotische bestanddelen (bacteriën in water) en abiotische bestanddelen (puur zand,

anorganische ionen in infiltratie water, SOM in MAR zand en natuurlijk voorkomende anorganische stoffen coating op zand). De resultaten toonden aan dat zuiver zand, MAR infiltratiewaterbestanddelen en SOM geen invloed hebben op de $H_2O_2$-afbraak. Natuurlijk voorkomende anorganische stoffen op het oppervlak van zandkorrels en levende biomassa zijn de twee belangrijkste oorzaken van $H_2O_2$-omzetting in MAR-systemen. Lage concentraties (<3 mg/l) van $H_2O_2$ in MAR infiltratie water kunnen dalen tot minder dan 0.25 mg/L in de eerste centimeters van MAR-systemen, wanneer het water hoge een concentratie aan microbiële biomassa bevat (zoals 38 ng ATP/ml). Echter, in de meeste gevallen is de ATP-concentratie één orde van grootte lager dan 38 ng / ml, waardoor $H_2O_2$ zal infiltreren in een diepere zone.

In hoofdstuk 5 is de invloed van $H_2O_2$-residuen op zandsystemen geëvalueerd, met nadruk op de verwijdering van opgeloste organische koolstof (DOC), de verandering in microbiële activiteit en de evolutie van de bacteriële populatie. Een lage $H_2O_2$-concentratie (0.25 mg/L) beperkte de biologische afbraak van DOC met 10%, terwijl hoge $H_2O_2$-concentraties (3 en 5 mg/L) de biodegradatie van DOC met respectievelijk 8% en 28% bevorderden. Lage $H_2O_2$-concentraties (0.25 mg/L) hadden geen invloed op de microbiële activiteit (gemeten als ATP), terwijl hoge $H_2O_2$-concentraties (1, 3 en 5 mg/L) de microbiële activiteit verminderden met respectievelijk 23%, 37% en 37%. De bacteriële populaties in zand werden gedomineerd door Proteobacteriën, specifiek door Betaproteobacteria (33%-39%). $H_2O_2$ residuen van zowel 0.25 als 5 mg/L beïnvloedden de bacteriële populatiestructuur. De complexiteit van de bacteriële populatie nam toe bij een $H_2O_2$ concentratie van 0.25 mg/L, maar de populatie werd minder divers wanneer de $H_2O_2$-concentratie steeg tot 5 mg/L. Aerobe bacteriën vertoonden verschillende reacties op $H_2O_2$: gevoelig of tolerant. Anaërobe bacteriën bleken gevoelig te zijn voor $H_2O_2$, en hun activiteit werd beperkt door $H_2O_2$-concentraties van zowel 0.25 als 5 mg/L (reductie met 17-88%). De verhoogde DOC-verwijdering bij hogere $H_2O_2$-concentraties kan mogelijk worden verklaard door de aërobe bacteriën Rhodocyclaceae en Comamonadaceae. De verklaring voor de verandering van DOC-verwijdering door Zoogloea verdient nadere aandacht. Speciale aandacht zou gegeven moeten worden aan het effect van $H_2O_2$ op microbiële ecologie voordat AOP's als voorbehandeling voor biologische (zand) processen geïntroduceerd worden.

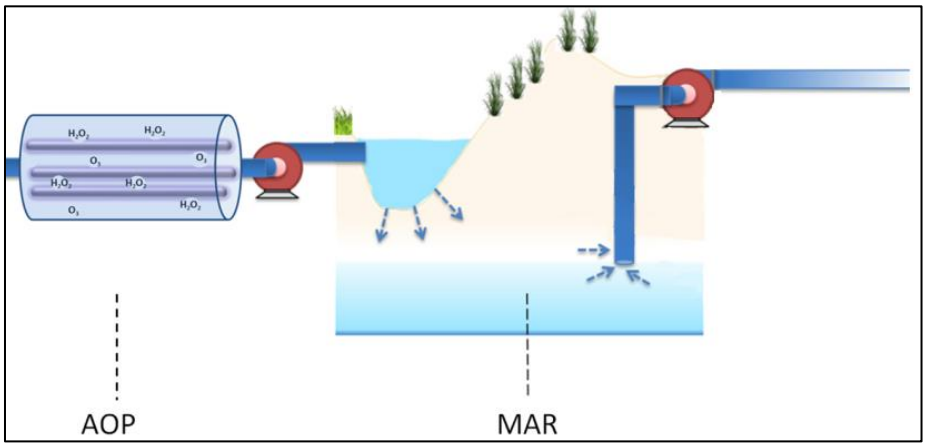
Eerder is gebleken dat de verwijdering van OMVs tijdens de drinkwaterproductie door een systeem met meerdere barrières, bestaand uit AOP en MAR, een complementair en synergistisch systeem is voor de verwijdering van OMVs. Dit proefschrift beschrijft dit

synergetisch effect voor de nevenproducten $H_2O_2$ en $BrO_3^-$. MAR kan op zowel microbiologische als chemische wijze $BrO_3^-$ afbreken, dat is gevormd als bijproduct van AOP voorbehandeling met $O_3$. Een belangrijk deel van de $BrO_3^-$ verwijdering vindt waarschijnlijk plaats in $NO_3^-$-reducerende zones, en kleine hoeveelheden $BrO_3^-$ kunnen verder verminderd worden in Fe-reducerende zones. De kleine hoeveelheden $H_2O_2$ (<3 mg/l) afkomstig van de AOP voorbehandeling is geen bedreiging voor de zuiveringswerking van de er opvolgende MAR bij hoge concentraties microbiële biomassa. Daarom is de combinatie van AOP en MAR een synergistisch hybride systeem voor verwijdering van de anorganische nevenproducten $BrO_3^-$ en $H_2O_2$. De bevindingen in dit proefschrift maken nieuwe toepassingen van MAR mogelijk, en kunnen de toepasbaarheid van ozon-gebaseerde AOP's vergroten in drinkwaterbehandeling.

Dit onderzoek biedt een waardevolle referentie voor drinkwaterbedrijven die $O_3$-gebaseerde AOP toepassen voorafgaand aan een MAR-systeem, of die overwegen om dit te doen. AOP-MAR is een veilig hybride systeem voor drinkwaterbedrijven, maar vóór toepassing van $O_3$-gebaseerde AOP moet verder proefonderzoek worden uitgevoerd om de verwijdering van $BrO_3^-$ en decompositie van $H_2O_2$ nauwkeurig te kunnen voorspellen, aangezien veel variabelen het gedrag en het lot van zowel $BrO_3^-$ als $H_2O_2$ beïnvloeden. Ook is een hydrologische analyse noodzakelijk van het infiltratiesysteem en onttrekkingssysteem van MAR, omdat anoxische zones een voorwaarde zijn voor de verwijdering van $BrO_3^-$.

# 1

# Introduction



# 1 Advanced Oxidation Processes and Managed Aquifer Recharge

## 1.1 Presence of organic micro-pollutants in drinking water resources

Large quantities of organic micro-pollutants (OMPs), such as pesticides, pharmaceutically active compounds, endocrine disrupting compounds, X-ray contrast media and personal care products, are being used all over the world (Bradley et al., 2017). In the past, the problem was not recognized because all compounds were found to be below detection limits. However, with the development of analytical tools and monitoring programs, more and more OMPs have been detected in the raw drinking water resources (Bradley et al., 2017). In recent years, OMPs have been found at ng/L to low μg/L levels in surface waters throughout the world (Hughes et al., 2012; Loos et al., 2009) and questions arise about their effects on the environment and on human health (Houtman et al., 2014; Van der Hoek et al., 2014).

In the Netherlands, the measured concentrations of OMPs in drinking water are very low and the effect on human health for a single compound at these low concentrations is considered negligible (Knol, 2012). However, many substances are still not measured and new emerging compounds can be expected, knowledge about effects of mixtures of OMPs is rare or not available, knowledge about long-term effects of exposure to OMPs is unknown, and from a public perspective these substances do not belong in drinking water. In addition to resource protection, there is a need for robust drinking water technologies that can remove these OMPs.

## 1.2 The need for advanced treatment processes

The current conventional treatment steps do not completely remove these emerging OMPs and advanced treatment is required to achieve a maximum purification. Coagulation, filtration and chlorination as conventional treatment processes can remove about 50% of pharmaceuticals (Van der Hoek et al., 2014). Drinking water utilities are facing the pressure of OMPs in raw water sources. Luckily, advanced treatment such as ozonation, advanced oxidation, activated carbon filtration and membrane filtration can achieve much higher removal rates (WHO, 2012). Effective advanced treatment processes, such as ozone and granular activated carbon filtration (Van der Hoek et al., 2000; Van Der Hoek et al., 1999b), UV/$H_2O_2$ treatment (Kruithof et al., 2002), combination of UV/$H_2O_2$/$O_3$ (Lekkerkerker, 2012), ion exchange in combination with ceramic microfiltration (Galjaard et al., 2011) and nanofiltration (Hofmann et al., 2011), have

been reported. The drinking water quality in the Netherlands meets the requirements of Dutch Drinkingwater Standards (Dutch Human Environment and Transport Inspectorate, 2017). Drinking water utilities have invested in advanced drinking water processes, and may invest further due to the increased pressure of emerging OMPs.

## 1.3 AOP-MAR: a synergetic barrier for OMPs

Managed aquifer recharge (MAR) is a process in which surface water (or wastewater or rain) is infiltrated into the subsurface via infiltration basins and stored in an aquifer to replenish falling groundwater levels (Shan, 2011). After a long residence time (several weeks, months or even years), the stored water can be subsequently abstracted from recovery wells and used as drinking water source. This technology has several advantages over (direct) surface water intake because of its capability to remove biodegradable organic matter, bacteria, viruses, parasites and partial elimination of adsorbing compounds through biodegradation and sorption (Maeng, 2010). In contrast to a high-cost system, MAR is robust and cost-effective for water disinfection. It is frequently applied in Australia, Europe and USA (Dillon et al., 2010; Van der Hoek et al., 2014). For example, in the Netherlands and Germany, water utilities using MAR as a water treatment process supply drinking water without chlorination as disinfection process (Lekkerkerker, 2012; Maeng, 2010). MAR was also reported to be able to remove a range of OMPs during drinking water production, albeit not all OMPs (Bertelkamp et al., 2015; Bertelkamp et al., 2016). Considering the limited OMPs removal capacity of MAR, the application of advanced oxidation processes (AOPs) before MAR has been proposed as a solution that fits into the current treatment train in the Netherlands. For example, the present barriers against OMPs in Dunea drinking water company (The Hague, Netherlands) are MAR by dune passage and the combination of powdered activated carbon (PAC) dosing on the rapid sand filtration (RSF) in the post treatment. This combination has limited OMP removal capacity. Therefore, Dunea drinking water company is planning to install AOP, situated at the pretreatment location in Bergambacht before MAR to limit or remove OMPs and will abandon the PAC dosing (Lekkerkerker, 2012). AOPs, characterized by the generation of highly reactive, non-selective hydroxyl radicals (▪OH), offer a promising alternative to conventional treatment for removing OMPs in contaminated waters (James et al., 2014). Several methods are available for generating ▪OH radicals: Ozone + hydrogen peroxide ($O_3/H_2O_2$), Ozone + catalyst ($O_3$/CAT), Fenton system ($H_2O_2/Fe^{2+}$), $O_3$/UV, $H_2O_2$/UV, $O_3/H_2O_2$/UV, Photo-Fenton/Fenton-like systems and Photocatalytic oxidation (UV/TiO2) (Lekkerkerker, 2012). AOPs have been applied by a

number of drinking water companies to remove OMPs from water to control drinking water contamination (Kim & Zoh, 2016). At Dunea, the combination of $O_3/H_2O_2$/UV was chosen since a lot of studies proved that it is a promising combination for the conversion of OMPs (Knol, 2012; Lekkerkerker, 2012).

It is expected that the combination of AOP and MAR (Figure 1) provides a complementary as well as a synergetic performance for the removal of OMPs. Firstly, AOP and MAR will complement each other, as they degrade OMPs by different mechanisms, oxidation and adsorption/biodegradation respectively. In addition, during the oxidative treatment step macromolecule OMPs can be oxidized into OMPs with lower molecular weights which are more easily biodegraded than the parent compounds (Lekkerkerker, 2012) during the following biological processes in MAR. Non-biodegradable dissolved organic carbon (DOC) and natural organic matter (NOM) can be partly oxidized into biodegradable dissolved organic carbon (BDOC) or assimilable organic carbon (AOC) during AOPs. BDOC and AOC as carbon and energy sources for microorganisms may enhance their growth and activity and therefore the biodegradation of OMPs. Therefore, the increased BDOC and AOC after AOPs will definitely promote the removal efficiency of OMPs during MAR.

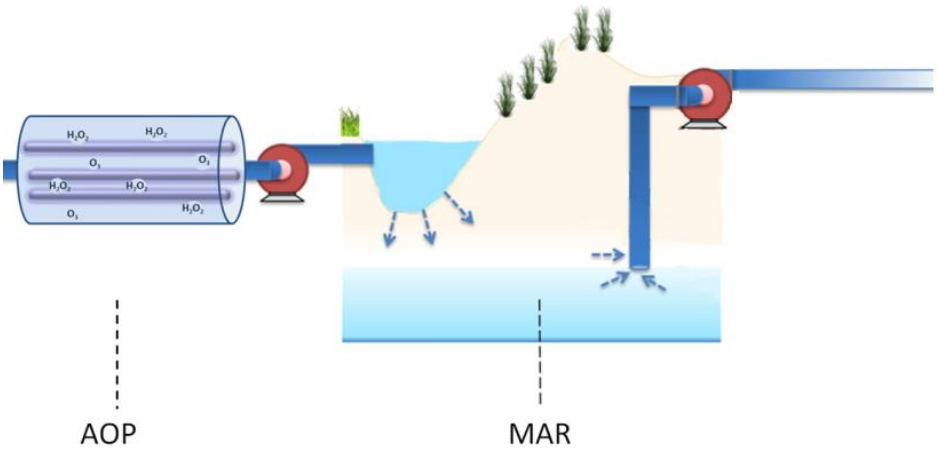



Figure 1 The combination of AOP and MAR during drinking water treatment

AOPs (combinations of $O_3/H_2O_2$/UV) are used at drinking water plants in the United States and in Europe, but the application has some drawbacks. Organic and inorganic byproducts including aldehydes, ketones, ketoaldehydes, carboxylic acids, aldo acids, keto acids, hydroxyl acids, alcohols, esters and alkanes, $BrO_3^-$ and $H_2O_2$ have been

reported (Najm & Krasner, 1995). Among them, the major drawback during $O_3$-based AOPs is that $Br^-$ can be easily oxidized to $BrO_3^-$ (Von Gunten & Hoigné, 1994), a possible human carcinogen (Kurokawa et al., 1990). $BrO_3^-$ formation has historically been the most significant concern related to the use of $O_3$ in water treatment (Pisarenko et al., 2012). In the case of $H_2O_2$ dosage, it is custom to operate at excess levels, leading to residual $H_2O_2$ in the produced water

# 2 AOPs by-products

## 2.1 $BrO_3^-$

### 2.1.1 $BrO_3^-$ formation

$BrO_3^-$ as a carcinogen can be formed during the treatment by $O_3$-based AOPs of potable water containing background $Br^-$. $Br^-$ in drinking water itself has no direct public health effects. However, $Br^-$ is a precursor to the formation of $BrO_3^-$ and other brominated oxidation or disinfection by-products. Worldwide, the occurrence of $Br^-$ in various drinking water sources, rivers, lakes, ground waters and coastal areas, is summarized in Table1. Generally, the investigated $Br^-$ concentration is higher in ground water than in surface water because natural sources of $Br^-$ are seawater, both through meteoric recharge and direct intrusion in coastal areas, and dissolution of evaporitic rocks (D'Alessandro et al., 2008). Human activity has introduced a large number of Br species into aquifers. The oxidation of ethylene dibromide/methyl bromide used to fumigate crops, an antiknock additive to gasoline, constituted a major artificial source of $Br^-$ in the environment (Thomas et al., 1997). $Br^-$ is highly soluble and it is difficult to be economically removed during drinking water treatment.

Table 1 A summary of $Br^-$ occurrence in source waters worldwide

| Location | Source | Number of sources | $Br^-$ range (μg/L) | Average $Br^-$ (μg/L) | Reference |
|---|---|---|---|---|---|
| South Australia | Surface water | 14 | 139-4130 | - | (Magazinovic et al., 2004) |
| | Ground water | 5 | 152-2040 | - | |
| | River Murray | 10 | 30-319 | - | |
| United States | Rivers | 59 | 3-426 | 101 | (Amy et al., 1993) |
| | Lakes | 24 | 3-322 | 38 | |
| | Ground waters | 37 | 2-429 | 96 | |
| | Coastal areas | 11 | 50-400 | 210 | |
| France, UK, Spain | Reservoir | - | 30-70 | - | (Legube 1996) |
| | Other surface water | - | 30-70 | - | |
| | Groundwater | - | 40-140 | - | |
| Tucson Basin, Arizona, United states | Ground water | 24 | 40-320 | 137 | (Stevens, 1990) |
| Occoquan Reservoir in United States | Surface water | >7 | 0-70 | - | (Bonacquisti, 2006) |
| Sicily in Italy | Drinking water utilities | 667 | <25-4760 | - | (D'Alessandro et al., 2008) |

In the presence of ozone, the conversion of $Br^-$ to $BrO_3^-$ occurs via three complicated pathways (Fischbacher et al., 2015; Haag et al., 1984; Song et al., 1996; Von Gunten & Hoigné, 1994; Von Gunten & Oliveras, 1998), since both oxidants, ozone and hydroxyl radical (OH▪), can act simultaneously or in sequence on various oxidation states. Figure 2 shows the $BrO_3^-$ formation pathways throughout the oxidation with $O_3$ and ▪OH.

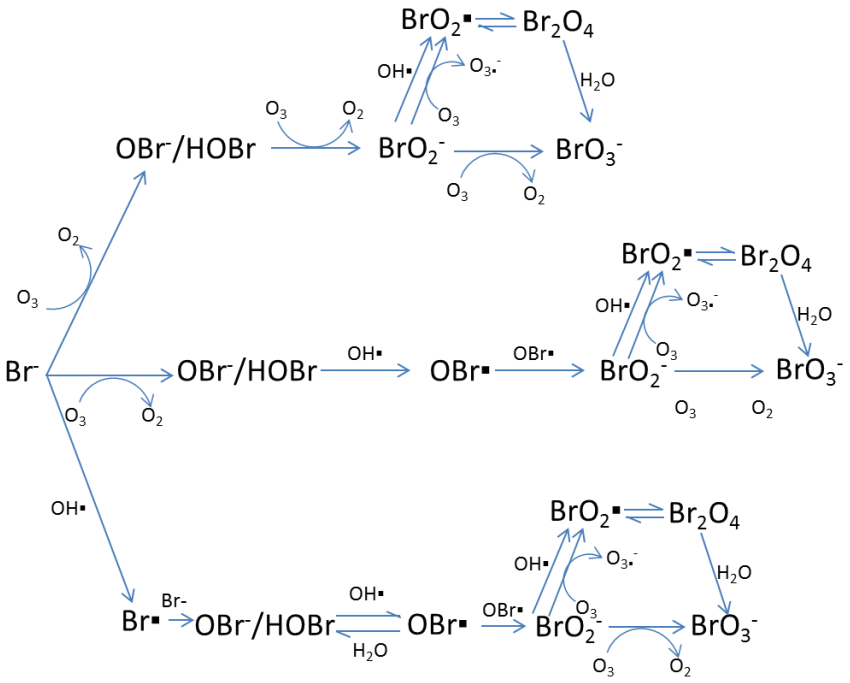



Figure 2 The pathways of $BrO_3^-$ formation from $Br^-$ (adapted from Jarvis et al. (2007); (Von Gunten & Hoigné, 1994) and Fischbacher et al. (2015))

$KBrO_3$ has been classified as a compound belonging to the group 2B, a possible human carcinogen (International Agency for Research on Cancer, 1987). No data demonstrated that $BrO_3^-$ is carcinogenic to humans, but it is plausible to assume that the mechanisms resulting in the formation of tumor in laboratory animals could also occur in humans (Kurokawa et al., 1985; Kurokawa et al., 1984; Murata et al., 2001; Nishimura, 2002; Shiao et al., 2002). A concentration of 0.05-5 µg/L $BrO_3^-$ in drinking water has been calculated to have a lifetime risk of $10^{-6}$-$10^{-4}$ based on a linearized multistage model for a consumption of 2L/day by a 70 kg adult (Ozekin & Amy, 1997). The World Health Organization (WHO) has set a provisional guideline concentration of 10 µg/L $BrO_3^-$ in drinking water (WHO, 2004). The European Drinking Water Directive (1998) specifies that all member states must enforce a maximum $BrO_3^-$ concentration of 10 µg/L. In the USA, regulations also specify a maximum value of 10 µg/L (EPA, 1998) based on a practice limit. In the Netherlands, the $BrO_3^-$ standard is 1 µg/L in case ozone is used for oxidation and 5 µg/L in case ozone is used for disinfection (StateJournal, 2011).

### 2.1.2 $BrO_3^-$ removal

As was stated previously, due to the carcinogenic and genotoxic properties of $BrO_3^-$, many countries have promulgated a 10 µg/L standard of $BrO_3^-$ in drinking water (Butler et al., 2005; Huang et al., 2014). To meet this strict limitation, different methods have been developed to remove $BrO_3^-$, including physical, chemical, electrochemical and biological techniques.

**Physical techniques** With respect to physical techniques, various advanced sorption techniques, such as ion-exchange resins (Chen et al., 2014), layered double hydroxides (Theiss et al., 2014; Zhang & Li, 2014) and nano crystalline akaganeite-coated quartz sand (Xu et al., 2012), have shown the ability to adsorb $BrO_3^-$, but so far these techniques are not applied in drinking water treatment. Granular activated carbon (GAC) as a conventional physical sorption technique is able to reduce $BrO_3^-$ effectively (Du et al., 2014), but the regenerated GAC cannot remove $BrO_3^-$ anymore after a certain running time (Xie & Shang, 2006). Considering the high cost as a result of low membrane fluxes and high operation pressure, reverse osmosis is not a good option either. Only a limited $BrO_3^-$ removal by electrodialysis reversal occurred: 64% in a two stage EDR system and 78% removal in a three stage EDR system (Van Der Hoek et al., 1998).

**Chemical techniques** Coagulating agents are unable to significantly reduce $BrO_3^-$ in natural waters. The rate of $BrO_3^-$ removal by alum and ferric chloride were quite low, 5 % and 20 % respectively. $BrO_3^-$ removal with catalysts, including zero valent iron (Fe) (Wang et al., 2009) and $Pd/Al_2O_3$ (Chen et al., 2010), has been found to be limited in the presence of coexisting anions. Different reducing agents, such as ferrous iron ($FeSO_4$), are too sensitive to dissolved oxygen (DO) and therefore the practical application during water treatment is quite difficult (Siddiqui et al., 1994). UV irradiation reduces $BrO_3^-$ effectively, but it has a high energy demand (Xie & Shang, 2006).

**Electrochemical techniques** Electrochemical methods (Kishimoto & Matsuda, 2009; Mao et al., 2014) have a high energy demand, and could thus far not remove $BrO_3^-$ effectively.

**Biological techniques** Microbiological reduction of $BrO_3^-$ has been observed in anaerobic activated sludge columns, biologically active carbon (BAC) filters and denitrifying bioreactors (Hijnen et al., 1999; Kirisits et al., 2001; Van Ginkel et al., 2005). BAC filters are capable to reduce $BrO_3^-$ effectively, but competitive DO remains a critical factor (Kirisits et al., 2001), because it is a challenge to construct a BAC filter with restricted

oxygen transfer within the biofilm (Liu et al., 2012). Hijnen et al. (1999) showed that $BrO_3^-$ was removed in a denitrifying bioreactor fed with methanol. However, they demonstrated that $BrO_3^-$ removal in a denitrifying bioreactor did not seem to be a realistic option in drinking water treatment due to the long contact times required for $BrO_3^-$ removal and extensive post treatment necessary to remove excessive methanol and released biomass. Altogether, there are only few effective options to remove the highly soluble and stable $BrO_3^-$ in practice till now.

### 2.2 $H_2O_2$

An approach for reducing $BrO_3^-$ formation is to combine $O_3$ with $H_2O_2$ and UV in AOP applications (Lekkerkerker, 2012; Scheideler et al., 2011). On the one hand, combining $O_3$ with $H_2O_2$ accelerates the production of OH▪ radicals, which oxidizes $Br^-$ as the first step in the indirect/direct pathway in Figure 2. On the other hand, $H_2O_2$ can scavenge HOBr which is an important intermediate production of $BrO_3^-$ formation in Figure 2 (Von Gunten & Oliveras, 1998). Dunea carried out several studies with varying $H_2O_2/O_3$ ratios to effectively limit $BrO_3^-$ formation (Knol, 2012; Lekkerkerker et al., 2009b; Scheideler et al., 2011). They found that the optimal full-scale setting concerning the $BrO_3^-$ formation is 6 mg/L $H_2O_2$ / 1.5 mg/L $O_3$ for Dunea. However, this dosage ratio of $H_2O_2/O_3$ results in 5.75 mg/L residual $H_2O_2$ in the AOP effluent (Knol, 2012). $H_2O_2$ can function as a disinfectant with the ability to inactivate microorganisms by oxidising proteins and DNA (Apel & Hirt, 2004; Latifi et al., 2009). It was thought that even quite low concentrations of $H_2O_2$ would damage bacterial cells (Knol, 2012), and might thus have negative effects on the microbial ecology of MAR.

## 3 Knowledge gaps: $BrO_3^-$ and $H_2O_2$ during MAR

### 3.1 $BrO_3^-$ removal during MAR

Both biological processes and chemical processes may offer potential $BrO_3^-$ removal pathways during MAR. During MAR, the water flows from infiltration ponds through an oxic zone, via an $NO_3^-$-reducing zone and then Mn-reducing zone, to the Fe-reducing anoxic zone as shown in Figure 3 (Stuyfzand, 1989). So far there have been only few studies concerning the removal of $BrO_3^-$ during soil passage, including MAR. Only recently, Hübner et al. (2016) studied $BrO_3^-$ removal, with a focus on treatment of secondary effluent (wastewater) instead of drinking water treatment. They observed that

$BrO_3^-$ was effectively reduced under anoxic conditions instead of oxic conditions and that $NO_3^-$ and $BrO_3^-$ were consumed as electron acceptors simultaneously in small-scale columns. However, because microbial biodegradation in secondary effluent differs given high dissolved organic carbon (DOC) and $NO_3^-$ concentrations, these findings cannot be directly translated to surface water infiltration sites for drinking water production. Water composition (e.g. $NO_3^-$, $SO_4^{2-}$, $ClO_3^-$ and $ClO_4^-$) is known to affect $BrO_3^-$ reduction in reactors (Demirel et al., 2014; Fan et al., 2006; Kirisits et al., 2001; Xu et al., 2015b), so it is likely to affect biological $BrO_3^-$ reduction during MAR as well. Downing and Nerenberg (2007) reported that $BrO_3^-$ was reduced to $Br^-$ by denitrifying and $ClO_3^-$-reducing enrichments, possibly via co-metabolic action of $NO_3^-$ reductase and $ClO_3^-$ reductase enzymes. Another study suggested the existence of a specific $BrO_3^-$ reduction pathway not related to $NO_3^-$ reduction (Davidson et al., 2011).

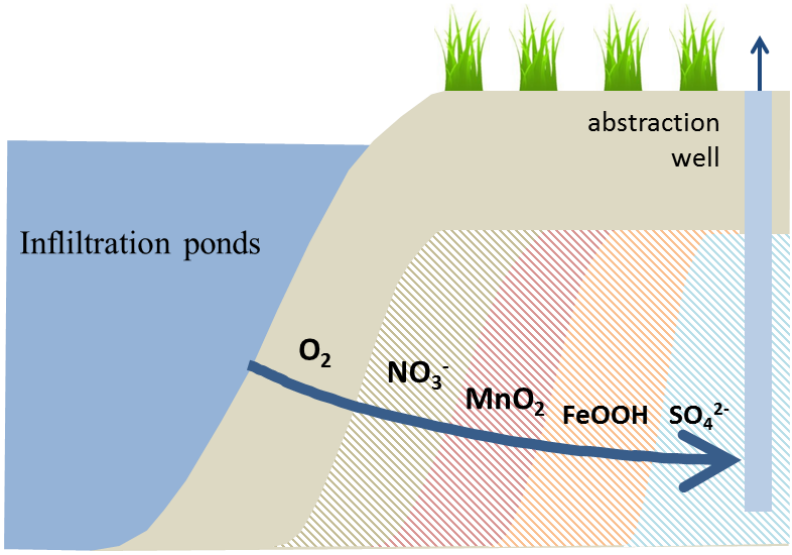



Figure 3 The sequence of terminal electron acceptor in MAR systems

Taken together, little has been known about $BrO_3^-$ biodegradation in $NO_3^-$-reducing zones of MAR systems during drinking water treatment. It is hyphothesized that $BrO_3^-$ can be potentially biodegraded in $NO_3^-$-reducing zones of MAR as denitrifying bacteria are present which can reduce $BrO_3^-$ (Hijnen et al., 1999) and MAR systems offer long retention times.

With respect to chemical processes, $Fe^{2+}$ is a well-known reductant and has been found to be able to reduce $BrO_3^-$ under certain conditions (Dong et al., 2009; Stefánsson, 2007). The reduction of $BrO_3^-$ by $Fe^{2+}$ occurs as the equation (1).

$$BrO_3^- + 6\ Fe^{2+} + 6\ H+ \rightarrow Br^- + 6\ Fe^{2+} + H_2O \quad (1)$$

Redox reactions using $Fe^{2+}$ and zero valent iron ($Fe^0$) have been investigated in the reduction of $BrO_3^-$ to $Br^-$ in the presence of oxygen (Baldwin & Van Weert, 1996; Dong et al., 2009; Siddiqui et al., 1994; Westerhoff, 2003; Zhang et al., 2015a). Some researchers who studied $BrO_3^-$ reduction with $Fe^{2+}$ used low concentration of $BrO_3^-$ (0.2 µM and 0.4 µM) and the low concentration of $BrO_3^-$ was removed partially. For example, a study by Siddiqui et al. (1994) with oxic water found that an initial $BrO_3^-$ concentration of 0.4 µM was lowered to 0.08 µM in 30 minutes after dosing 0.27 mM $Fe^{2+}$. Dong et al. (2009) worked with 0.2 µM $BrO_3^-$, 0.54 mM $Fe^{2+}$ dosage and 0.07 mM DO, reaching a $BrO_3^-$ reduction of 65%. However, from the above examples, it can be seen that the dosage of $Fe^{2+}$ used in these studies are usually much higher than the naturally occurring $Fe^{2+}$ in MAR systems, where Fe concentrations below 0.03 mM are to be expected. For example, the MAR site of Dunea shows concentrations ranging from 0.0015 to 0.029 mM Fe. It is unknown if the low concentrations of naturally occurring $Fe^{2+}$ in MAR systems can reduce $BrO_3^-$.

$BrO_3^-$ may be reduced in the two zones, $NO_3^-$-reducing zones and Fe-reducing zones, either biologically or chemically. An extensive study on the mechanism behind the reduction of $BrO_3^-$ by denitrifying bacteria and $Fe^{2+}$ will definitely provide more insight in the successful removal of $BrO_3^-$ during MAR.

## 3.2 $H_2O_2$ removal during MAR

The fate of $H_2O_2$ in aquatic systems has been investigated comprehensively (Bissey et al., 2006; Miller & Valentine, 1999). $H_2O_2$ is unstable and its decomposition highly depends on the environmental conditions. It was reported that at 30 °C in the absence of catalytic substances only 1 % $H_2O_2$ was decomposed per year, while in the presence of Fe and Mn the decomposition was much faster (Schumb, 1949). The catalytic effects of metal oxides have been confirmed by other studies (Russo et al., 2013; Wilson et al., 2000). Also, the effect of other substances including DOC (Wilson et al., 2000) and activated carbon (Fang et al., 2014) on $H_2O_2$ decay have been investigated. Activated carbon has been proven to be a feasible catalyst in $H_2O_2$ reduction (Fang et al., 2014). Taken together, the main factors impacting $H_2O_2$ decomposition are biotic factors including bacteria (Zappi et

al., 2000) and other microorganisms (Richardson et al., 2007) and abiotic factors, such as catalysts activated carbon, transition metals and lanthanide oxides (Do et al., 2009; Lousada et al., 2013; Wilson et al., 2000).

$H_2O_2$ decomposition has been reported in different surface waters (Cooper & Lean, 1989; Richard et al., 2007; Wilson et al., 2000) while a few studies focused on the reactions of $H_2O_2$ with natural-occurring constituents in soil as well. Also in soil $H_2O_2$ can be fast decomposed due to its interaction with various soil constituents like naturally occurring stabilizers tripolyphosphate, $MnO_4^-$ and $Cu^{2+}$ (Morgan & Watkinson, 1992; Schumb, 1949). The content of soil organic matter does not have an effect on $H_2O_2$ decomposition at pH 3, while it negatively impacts $H_2O_2$ decomposition rate at neutral pH (Bissey et al., 2006). However, among all factors contributing to the decomposition of $H_2O_2$ in water or soil, the strongest one is enzymatic activity of catalases and peroxidases associated with algae and bacteria.

The above mentioned studies, mostly concentrated on the ability of $H_2O_2$ as an oxygen source for bioremediation in soil rather than on quenching $H_2O_2$ after AOP. As stated above, the previous studies demonstrated that several potential interactions of $H_2O_2$ with different soil constituents are present and, therefore, $H_2O_2$ may be degraded fast.

It is hypothesized that $H_2O_2$ in MAR system can be degraded due to the presence of soil constituents, such as Fe oxides, Mn oxides and bacteria as contributors of $H_2O_2$ decomposition. However, since MAR has its own specific environmental conditions different from the studies above, it is hard to conjecture the fate and decomposition mechanism of $H_2O_2$ in MAR systems. The fate of the excessive $H_2O_2$ of AOP in subsequent MAR systems had received very little attention in the past.

### 3.3 $H_2O_2$ effect on MAR's microbial ecology

$H_2O_2$ has two opposite (negative and positive) effects on the growth and the activity of microorganisms. On one hand, $H_2O_2$ can function as a disinfectant with the ability to inactivate microorganisms by oxidising proteins and DNA (Apel & Hirt, 2004; Latifi et al., 2009). The growth of many microbes can be suppressed by 0.34-3.4 mg/L $H_2O_2$, such as *A. nidulans* and *A. variabilis* (Samuilov et al., 1999). However, the ineffectiveness of $H_2O_2$ as a disinfectant, and more specifically the selective impact of $H_2O_2$ on microorganisms, has also been reported. Catalases are known to catalyse the conversion of $H_2O_2$ into water and oxygen, which is part of an adaptive response of bacteria to oxidative stress (Matthijs et al., 2012; Metz et al., 2011; Tusseau-Vuillemin et al., 2002).

Under a certain concentration of $H_2O_2$, the majority of catalase-positive microorganisms instead of catalase-negative strains, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Campylobacter jejuni*, make catalase to deactivate the peroxide radicals, thus allowing them to survive (Rao et al., 2003; Walczak & Swiontek Brzezinska, 2009). On the other hand, $H_2O_2$ as a source of oxygen has been applied successfully in the field of contaminated aquifer remediation (Aggarwal et al., 1991; Tusseau-Vuillemin et al., 2002; Zappi et al., 2000). The oxygen as a product of $H_2O_2$ decomposition stimulates the growth of microbes and thus promotes the degradation of contaminates.

Therefore, $H_2O_2$ is generally used to inactivate microorganisms in aqueous systems, but some microorganisms may favor $H_2O_2$ due to the oxygen benefit and some other microorganisms may be able to tolerate $H_2O_2$ in varying concentrations and situations due to the detoxicity of catalyse existing in their cells. The positive and negative effects of $H_2O_2$ on the growth and activity of microorganisms cause an unclear speculation to $H_2O_2$ impacts on the function of MAR, so another knowledge gap is what the effects of $H_2O_2$ are on MAR systems. Further investigation on the effects of $H_2O_2$ on microbial activity in sand systems is important, scientifically for microbial ecology and practically for surface water purification systems that utilise a combination of AOPs and sand systems, e.g. sand filtration or MAR in a sandy soil. An improved understanding of the fate and effect of $H_2O_2$ in MAR systems would be essential to see whether an extra technique needs to be installed to quench $H_2O_2$ between AOPs and MAR.

## 4 Research questions and thesis outline

MAR as a subsequent water treatment technique after AOP may be a good barrier for the inorganic by-products of AOP and therefore the combination of AOP and MAR could be synergistic also on the aspect of inorganic by-products. In this thesis the focus lies on the fate of inorganic by-products $BrO_3^-$ and the residual $H_2O_2$ in the subsequent MAR. The research questions and the corresponding chapters are described below.

| **Research questions** | **Thesis outline** |
|---|---|
| **1. Is it feasible in $NO_3^-$-reducing zones of MAR systems to biodegrade $BrO_3^-$ and what is the mechanism behind it?**<br>i. what is the effect of AOC due to ozonation pre-treatment on $BrO_3^-$ removal?<br>ii. what is the effect of $NO_3^-$ long-term presence, sudden absence and long-term absence?<br>iii what is the $BrO_3^-$ removal performance in a sand column simulating MAR? | **Chapter 2** presents the results of a one-year data set from oxic and anoxic column experiments, a MAR simulation study, where 1) $BrO_3^-$ removal in the presence, the sudden absence and the long-term absence of $NO_3^-$ was compared, 2) the change of $BrO_3^-$ removal after AOC addition was assessed, and 3) $BrO_3^-$ removal under oxic and anoxic conditions was compared. It also presents the results of three laboratory batch experiments, where 1) $BrO_3^-$ removal in the presence and sudden absence of $NO_3^-$ was compared, and 2) $BrO_3^-$ removal at different AOC concentrations was compared. |
| **2. Is it feasible in Fe-reducing zones of MAR systems to chemically reduce $BrO_3^-$?**<br>i what is the mechanism of the reduction of $BrO_3^-$ by $Fe^{2+}$ ?<br>ii is it possible for $Fe^{2+}$, at concentrations similar to MAR, to reduce trace amounts of $BrO_3^-$?<br>iii what is the potential competition with or inhibition by $NO_3^-$ in a special case, the mix of $NO_3^-$ and $Fe^{2+}$, in MAR? | **Chapter 3** provides a preliminary study about $BrO_3^-$ removal feasibility and mechanism by naturally occurring $Fe^{2+}$ in anoxic Fe-reducing zones of MAR by a series of laboratory batch experiments. |

**3. What is the fate of $H_2O_2$ residual in MAR systems?**

i which factors among the constituents in sand and water impact $H_2O_2$ decomposition?

ii which factors most contribute to $H_2O_2$ decomposition?

ii in how much infiltration depth can $H_2O_2$ fully be removed?

**Chapter 4** presents the results of $H_2O_2$ removal by separate compartments, inorganic ions in infiltration water, soil organic matter in MAR and microbial community in water through a series of laboratory batch experiments.

**4. What is the effect of $H_2O_2$ residual on MAR?**

i what is the effect of $H_2O_2$ on the DOC removal ability of MAR?

ii what is the effect of $H_2O_2$ on microbial community evolution in MAR?

iii what is the effect of $H_2O_2$ on the activity of microorganisms in MAR?

**Chapter 5** shows the results of laboratory batch experiments assessing the change of DOC removal ability of MAR, the evolution of microbial community and the change of microbial activity caused by the involve of $H_2O_2$ residual.

**CHAPTER 6** represents an overall discussion and gives the overall results. Implications for the drinking water practice are described, and recommendations are given for future research and practical applications.

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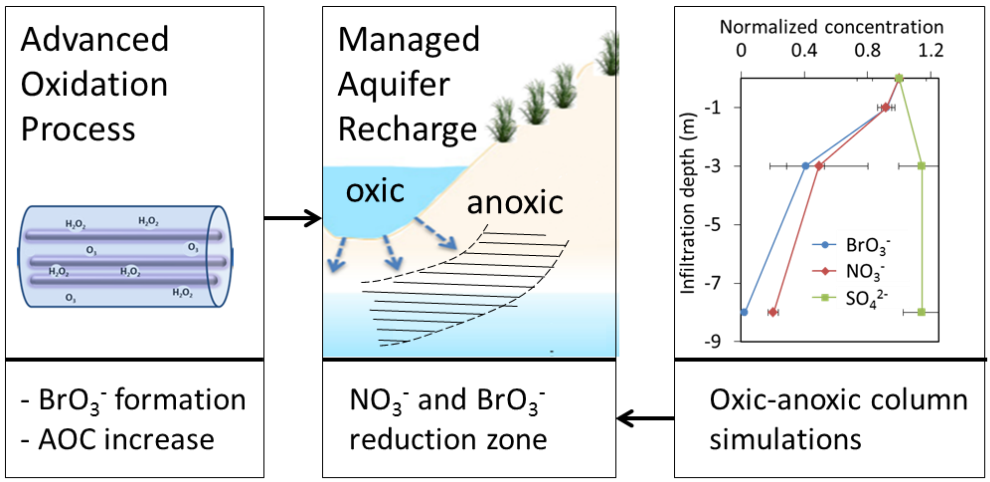
# 2

# Effective removal of bromate in nitrate-reducing anoxic zones during managed aquifer recharge for drinking water treatment: Laboratory-scale simulations

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## Abstract

The removal of bromate ($BrO_3^-$) as a by-product of ozonation in subsequent managed aquifer recharge (MAR) systems, specifically in anoxic nitrate ($NO_3^-$)- reducing zones, has so far gained little attention. In this study, batch reactors and columns were used to explore the influence of $NO_3^-$ and increased assimilable organic carbon due to ozonation pre-treatment on $BrO_3^-$ removal in MAR systems. 8 m column experiments were carried out for 10 months to investigate $BrO_3^-$ behavior in anoxic $NO_3^-$-reducing zones of MAR systems. Anoxic batch experiments showed that an increase of AOC promoted microbial activity and corresponding $BrO_3^-$ removal. A drastic increase of $BrO_3^-$ biodegradation was observed in the sudden absence of $NO_3^-$ in both batch reactors and columns, indicating that $BrO_3^-$ and $NO_3^-$ competed for biodegradation by denitrifying bacteria and $NO_3^-$ was preferred as an electron acceptor under the simultaneous presence of $NO_3^-$ and $BrO_3^-$. However, within 75 days' absence of $NO_3^-$ in the anoxic column, $BrO_3^-$ removal gradually decreased, indicating that the presence of $NO_3^-$ is a precondition for denitrifying bacteria to reduce $BrO_3^-$ in $NO_3^-$-reducing anoxic zones. In the 8 m anoxic column set-up (retention time 6 days), the $BrO_3^-$ removal achieved levels as low as 1.3 µg/L, starting at 60 µg/L (98% removal). Taken together, $BrO_3^-$ removal is likely to occur in vicinity of $NO_3^-$-reducing anoxic zones, so MAR systems following ozonation are potentially effective to remove $BrO_3^-$.

# 1 Introduction

Managed aquifer recharge (MAR), such as artificial recharge and dune filtration, is a natural water treatment process that induces surface water to flow through the soil. After soil passage, the water is abstracted by vertical or horizontal wells (Bouwer, 2002; Tufenkji et al., 2002). In some European countries, water utilities use MAR as a robust and cost-effective water treatment process to supply drinking water without needing to use chlorination as a disinfection process because of its pathogen removal ability (Lekkerkerker, 2012; Maeng, 2010; Van der Hoek et al., 2014). Additionally, MAR has proven to be an effective barrier for multiple organic micro-pollutants (OMPs) present in surface waters during drinking water production due to filtration, sorption, ion-exchange, precipitation and biological degradation (Kim et al., 2015; Laws et al., 2011; Postigo & Barceló, 2015). However, some highly persistent trace organic compounds can still be detected in MAR filtrate (Drewes et al., 2003) and may reach the drinking water supply (Ternes et al., 2002).

Ozonation is a powerful process for the removal of many OMPs, and the combination of MAR with ozonation as a pre-treatment has been suggested as a comprehensive treatment system to effectively remove various OMPs during drinking water production (Lekkerkerker-Teunissen et al., 2012; Lekkerkerker et al., 2009a; Oller et al., 2011b). However, bromate ($BrO_3^-$), a genotoxic carcinogen (Ahmad et al., 2013), may be formed when ozonation is applied in the treatment of bromide-containing water (Assuncao et al., 2011; Haag & Holgne, 1983; Kurokawa et al., 1990). WHO, USEPA, and the European Union have set drinking water regulations for the maximum allowable concentration of $BrO_3^-$ at 10 μg/L (Carney, 1991; EU, 1998; Forum, 2005; WHO, 2011).

$BrO_3^-$ cannot be easily eliminated using conventional treatment technologies due to its high solubility and stability in water (Butler et al., 2005) and its weak sorption characteristics to common soil and sediment components. Several studies involving different chemical, physical and biological techniques have been conducted (Bhatnagar & Sillanpää, 2012; Hijnen et al., 1999; Jia et al., 2015; Wang et al., 2009; Xu et al., 2015a; Zhang et al., 2015b). Microbial $BrO_3^-$ reduction may be an effective treatment strategy because microbiological reduction of $BrO_3^-$ has been observed in anaerobic activated sludge columns, biologically active carbon filters and denitrifying bioreactors (Hijnen et al., 1999; Kirisits et al., 2001; Van Ginkel et al., 2005). The study of Van Ginkel et al. (2005) showed that $BrO_3^-$ reduction was detected only in the absence of $O_2$ in a microbial

culture from activated sludge. However, some other studies found that $BrO_3^-$ reduction could also take place in the presence of $O_2$. For example, a biological activated carbon (BAC) filter almost completely reduced 60 μg/L $BrO_3^-$ to $Br^-$ at both 2 and 8 mg/L influent dissolved oxygen (DO) concentrations (Liu et al., 2012). Therefore, redox condition may be one of the important factors impacting $BrO_3^-$ removal in MAR systems. Hijnen et al. (1995) isolated denitrifying organisms that were able to reduce $BrO_3^-$ with ethanol as the electron donor and carbon source. Hijnen et al. (1999) showed that $BrO_3^-$ was removed in a denitrifying bioreactor fed with methanol. However, they demonstrated that $BrO_3^-$ removal in a denitrifying bioreactor did not seem to be a realistic option in drinking water treatment due to the long contact times required for $BrO_3^-$ removal and extensive post treatment necessary to remove excessive methanol and released biomass. The anoxic zone within MAR systems might be effective in reducing $BrO_3^-$, as retention times in the subsurface are days to months. However, there has been only one study (Hübner et al., 2012) concerning the removal of $BrO_3^-$ in MAR systems since Hijnen et al. (1999) and Kruithof and Meijers (1995) mentioned that soil passage under anoxic conditions, such as artificial recharge and river bank filtration, may enable $BrO_3^-$ removal from ozonated water. Only recently, Hübner et al. (2016) studied $BrO_3^-$ removal in 1 m sand columns, with a focus on treatment of secondary effluent (wastewater) instead of drinking water treatment. They observed that $BrO_3^-$ was effectively reduced under anoxic conditions instead of oxic conditions and that $NO_3^-$ and $BrO_3^-$ were consumed as electron acceptors simultaneously in small-scale columns. However, because microbial biodegradation in secondary effluent differs given high dissolved organic carbon (DOC) and $NO_3^-$ concentrations, these findings cannot be directly translated to surface water infiltration sites for drinking water production. Water composition (e.g. $NO_3^-$, $SO_4^{2-}$, $ClO_3^-$ and $ClO_4^-$) is known to affect $BrO_3^-$ reduction in reactors (Demirel et al., 2014; Fan et al., 2006; Kirisits et al., 2001; Xu et al., 2015b), so it is likely to affect biological $BrO_3^-$ reduction during MAR as well.

Several microbial $BrO_3^-$ conversion pathways have been described in literature. $BrO_3^-$ was reduced to bromide by denitrifying and $ClO_3^-$-reducing enrichments, possibly via co-metabolic action of $NO_3^-$ reductase and $ClO_3^-$ reductase enzymes (Downing & Nerenberg, 2007). Other studies suggested the existence of a specific $BrO_3^-$ reduction pathway (Davidson et al., 2011). Additionally, the aerobically expressed selenate reductase of *Enterobacter cloacae* is capable of low rates of $BrO_3^-$ reduction (Ridley et al., 2006), indicating that oxic bacteria might also be capable of $BrO_3^-$ reduction. Therefore, although

different $BrO_3^-$ removal pathways have been identified, it is unknown whether these pathways exist during MAR soil passage.

The objectives of this study were to explore the $BrO_3^-$ removal in $NO_3^-$-reducing anoxic zones of MAR systems and the potential mechanisms behind this removal. Specifically, the influence of (a) increased assimilable organic carbon (AOC) concentrations (due to ozonation pre-treatment) and (b) $NO_3^-$ long-term presence, sudden absence and long-term absence and (c) $BrO_3^-$ removal performance with infiltration retention time in 8 m anoxic zones were investigated in order to evaluate the feasibility of $BrO_3^-$ removal by MAR systems.

## 2 Materials and methods

### 2.1 Water and sand

The water used in this study was collected every two weeks from the MAR site of Dunea, a drinking water company in the Netherlands. The composition of MAR influent water is shown in Table S1 in Appendix A. The sand used in batch reactors and column reactors was collected from a 1 m depth from the MAR site of Dunea. Chemicals $NaBrO_3$, $NaNO_3$, $CH_3COONa$, $K_2SO_4$ and Purolite A520E resin were purchased from Sigma (St Louis, MO, United States). All chemicals were of AR grade. All solutions used in this study were prepared using water from a Millipore Milli-Q system.

### 2.2 Batch experiments

To investigate the role of increased AOC from ozonation as a pre-treatment for MAR and the influence of $NO_3^-$ on $BrO_3^-$ removal, batch experiments using 15 glass bottles with a volume of 500 mL were performed for approximately 3 months under anoxic conditions. The batch reactors were filled with 100 g sand and 400 mL MAR water. This ratio of MAR water and sand was chosen from previous literature that also focused on MAR studies (Maeng et al. 2010; Wang et al. 2016). Anoxic conditions were provided by stripping the water with nitrogen gas for 15 minutes then sealing the bottles with rubber stoppers and plastic caps. All batch reactors were placed in a dark room with temperature control (11.5 ± 0.5 ℃). A 60 day acclimation period was necessary to stabilize the batch reactors with respect to DOC removal (fill-and-draw mode during the acclimation period, hydraulic retention time (HRT) 7 d). Next, the 15 bottles were divided into 5 groups with different DOC concentrations and different $NO_3^-$ concentrations as shown in Figure 1-a.

Three batch reactors as reference (group 1) to distinguish $BrO_3^-$ adsorption from biological $BrO_3^-$ removal in group 2 were autoclaved at 121 ℃ for 40 minutes to inactivate bacteria. Ozonation can oxidize a part of DOC into biodegradable DOC, so 1 mg/L of additional C-$CH_3COONa$ was dosed in group 3 to investigate the effect of ozonation pre-treatment on $BrO_3^-$ removal. The aim of groups 4 and 5 was to assess the effect of the sudden absence of $NO_3^-$ on $BrO_3^-$ removal. The microbial community may change in the absence of $NO_3^-$ after a certain time. To achieve $BrO_3^-$ removal as early as possible before microbial community change, 4 mg/L C-$CH_3COONa$ was dosed into groups 4 and 5 to promote microbial activity. Also for groups 4 and 5, the concentration of $NO_3^-$ initially present in the MAR water was measured daily until it fell below the detection limit, 0.89 mg/L. Then, 10 mg/L $NO_3^-$ was dosed to group 4 and not to group 5. 60 μg/L $BrO_3^-$ was dosed to all batch reactors after the acclimation period and the above described different treatments. $BrO_3^-$, $NO_3^-$, sulfate ($SO_4^{2-}$), adenosine triphosphate (ATP) and DOC samples were collected from groups 1-3 at day 7 and day 21. For groups 4 and 5, samples were collected after 2.7 hours because of the high microbial activity in these groups caused by the 4 mg/L C-$CH_3COONa$ dose.

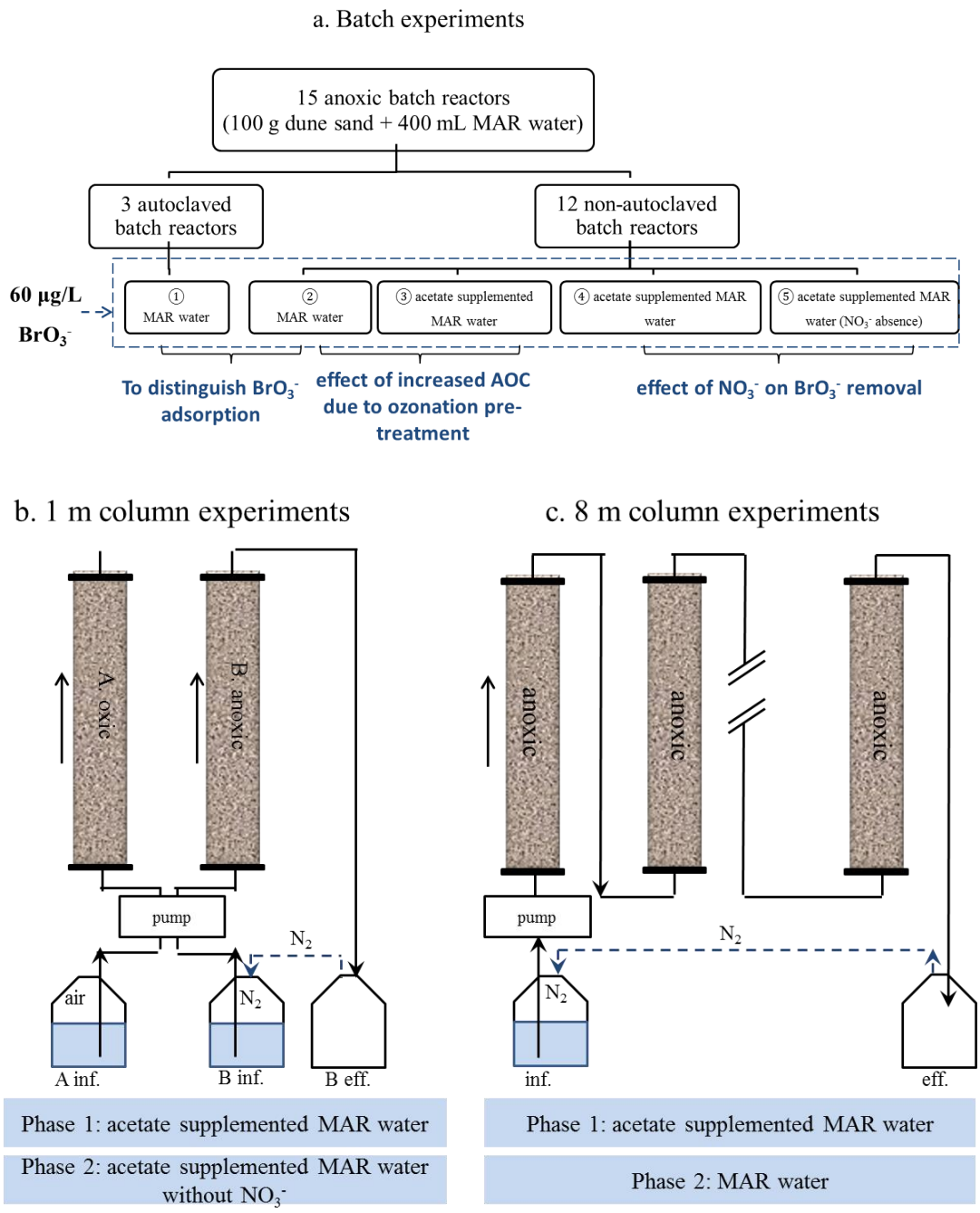


Figure 1 Batch and column experimental designs

## 2.3 Column experiments

All columns (L=1 m, D=36 mm) shown in Figure 1-b and 1-c were constructed from PVC (L = 1 m, D = 36 mm). A peristaltic multichannel pump (205S, Watson Marlow, The Netherlands) using Marprene® pump tubes (d = 0.63 mm, Watson Marlow, The

Netherlands) was connected to the columns by dark polyamide tubing (d = 2.9 mm, Festo, The Netherlands) to feed both columns. The columns were operated in continuous up-flow mode at 11.5±0.5 ℃, corresponding to the natural aquifer temperature, in a dark room to prevent algal growth.

To avoid the leaching of soil/sand grains, both the top and bottom of the column were fitted with perforated PVC plates (30 holes, d = 0.8 mm) that were covered with filter cloth (45 µm, Top7even net & mesh, The Netherlands). The oxic column was fed from a 10 L open glass bottle with Dunea MAR influent water, and the anoxic columns were fed from 10 L sealed glass bottles with $N_2$ flushing as pre-treatment. Feed bottles were washed twice with acetone and flushed several times with demineralized water before refilling to avoid biofilm formation.

Before starting the $BrO_3^-$ experiment, these columns had been acclimated for 3 months until steady state conditions were reached with respect to DOC removal and $NO_3^-$ removal.

### 2.3.1 1 m oxic and anoxic sand columns

To investigate $BrO_3^-$ biodegradation performance in oxic zones and anoxic $NO_3^-$ reducing zones of MAR systems and to study the influence of $NO_3^-$ on $BrO_3^-$ removal, column experiments using a 1 m oxic sand column simulating oxic zones and a 1 m anoxic sand column simulating anoxic zones of MAR systems were carried out in the presence and absence of $NO_3^-$. The hydraulic retention time was 22 hours for both columns, corresponding to a filtration velocity of 1 m/day.

The experiment lasted 13 months in total: a 3 months acclimation period followed by a 10 month period divided into two phases. In the first phase, $NO_3^-$ was present in the influent water, while in the second phase, $NO_3^-$ was absent. During the 13 months experiment, 150 µg/L C-$CH_3COONa$ was dosed to the influent water of both oxic and anoxic columns to simulate the increased AOC from ozonation since, in practice, the ozonation pre-treatment before MAR increases the AOC (Hammes et al., 2006; Orlandini et al., 1997; Sarathy et al., 2011; Van Der Hoek et al., 1998) and as reported by Hammes et al. (2006) 60-90 % of the AOC consists of organic acid carbon. $BrO_3^-$ formation at concentrations ranging from <2 to 293 µg/L has been reported during ozonation of natural waters under normal drinking water treatment conditions (Amy et al., 2000; Glaze et al., 1993; Krasner et al., 1993; Van Der Hoek et al., 1998), but in 100 investigated drinking water utilities $BrO_3^-$ concentration was within the range of <2-60 µg/L after ozonation of water containing 2-429 µg/L $Br^-$ (Butler et al., 2005; Kirisits & Snoeyink, 1999). For this study

it was decided to investigate the upper value of this range, so 60 µg/L $BrO_3^-$ was dosed to the influent of the oxic column and anoxic columns. A summary of $BrO_3^-$ and AOC formed during ozonation based on existing literature (Agbaba et al., 2016; Escobar & Randall, 2001; Huang & Chen, 2004; Orlandini et al., 1997; Van Der Hoek et al., 1998) is presented in Table S2 in Appendix A. The influent water of these columns was $NO_3^-$ containing MAR water in phase 1, while in phase 2 the influent was $NO_3^-$ free MAR water. $NO_3^-$ free water was produced by using a strong base anion exchange resin Purolite A520E (ratio of water and resin: 2 L / 20 g) to remove $NO_3^-$ to below the detection limit (0.89 mg/L). The water was in contact with the resin were for a period of 12 hours. The ion exchange resin, used to remove $NO_3^-$ from the MAR water, was pre-treated as follows. Firstly, A520E resin was soaked in both 1 M NaOH solution followed by 1 M HCl solution or one day each to remove impurities. Afterwards, the resin was washed several times using demineralized water until pH 7 was reached. Finally, the clean resin was dried in an oven at 80 ℃ for 24 hours and kept in a desiccator until use. Since Purolite A520E resin removes not only $NO_3^-$ but also a portion of $SO_4^{2-}$, 50 mg/L $SO_4^{2-}$ was dosed back to the influent water in phase 2. Influent water samples and corresponding effluent water samples were collected every 1-2 weeks during each phase to measure $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ concentrations. DO concentrations in the influent and effluent of oxic and anoxic columns were measured to confirm oxic and anoxic conditions.

### 2.3.2 8 m anoxic columns

A long anoxic column set-up consisting of eight 1 m columns in series was used for 10 months to better simulate anoxic zones of MAR systems since the retention time, 6 days, was much longer than the above 1 m anoxic column in section 2.3.1. The objective of the long anoxic column was to further investigate $BrO_3^-$ biodegradation with respect to retention time in anoxic $NO_3^-$-reducing zones and to further assess the role of AOC formation, as a result of ozonation pre-treatment, on $BrO_3^-$ biodegradation.

The whole experiment consisted of a 4 months acclimation period followed by two phases with and without an extra 150 µg/L C-CH3COONa in the influent water. Each phase was carried out for 3-4 months to establish a stable $BrO_3^-$ removal. Water samples were collected 4-7 times at depth 0 m, 1 m, 3 m and 8 m, that is retention time 0, 0.75, 2.25 and 6 days, during each phase to measure $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ concentrations. DO concentrations in the influent and effluent were measured to confirm anoxic conditions.

## 2.4 Sample analysis

Dissolved oxygen (DO), temperature and pH were measured with a multimeter (SenTix® 940 IDS probe, Multi 340i, WTW, Germany) directly in the feed bottle or in a flow through cell connected to the influent or effluent tubes of the columns.

$BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ samples were analyzed by ion chromatography at Het Waterlaboratorium (Haarlem, The Netherlands). Following ion chromatography, $BrO_3^-$ was also analysed by conductivity detection. 30 mL samples were pre-treated by filtration on barium and silver loaded on guard columns to remove sulphate and chloride respectively, followed by a $H^+$ column for the removal of $Ag^+$ ions leaching from the $Ag^+$ column. 2000 µl of the sample was subsequently concentrated on a positively charged anion exchange column (Dionex IonPac AG9SC). The anions on the ion exchange column were eluted with 1.5 mL/min of a 0.7 mM $NaHCO_3$ solution and separated on an ion exchange analytical column (Dionex IonPac AS9SC). Detection was performed by using suppressed conductivity. The measured $BrO_3^-$ concentration was confirmed using a two point calibrated UV absorption measurement at a wavelength of 200 nm. The $BrO_3^-$ detection limit was 0.5 µg/L. $NO_3^-$ and $SO_4^{2-}$ were analysed with a ProfIC 15 - AnCat ion chromatograph (Metrohm 881 anion (suppressed) and 883 cation system) (Metrohm, Switzerland) after filtering through 0.45 µm filters (Whatman, Germany). A Supp 150/4.0 anion column was used with 3.2 mM $Na_2CO_3$ and 1 mM $NaHCO_3$ eluent for the anions measurement. Regenerant for the suppressor was 50 mM $H_2SO_4$. Detection limits of $NO_3^-$ and $SO_4^{2-}$ were 0.89 mg/L and 0.5 mg/L, respectively. DOC was measured with a Shimadzu TOC analyser according to the protocols described in Wang et al. (2016).

# 3 Results

## 3.1 Batch reactor experiments

### 3.1.1 Effect of increased AOC due to ozonation as pre-treatment

Figure 2 presents $BrO_3^-$ concentrations over 7 days (Figure 2-a) and 21 days (Figure 2-b) in anoxic batch reactors with MAR water and acetate supplemented MAR water and autoclaved batch reactors with MAR water. In the reference experiments with autoclaved batch reactors, $BrO_3^-$ degradation over 7 days and 21 days was not observed, indicating $BrO_3^-$ adsorption did not occur. Therefore, the $BrO_3^-$ removal was caused by biodegradation instead of adsorption, which is in agreement with the studies of Xie and Shang (2006) and Weast (1986). Though differences were small, bromate removal was

found not to be significant ($p>0.05$) in MAR water, while removal was observed in acetate supplemented MAR water. Slightly more $BrO_3^-$ was removed in acetate supplemented MAR water after 21 days (9 µg/L, 16.9%) compared to 7 days (2.4 µg/L, 4.2%).

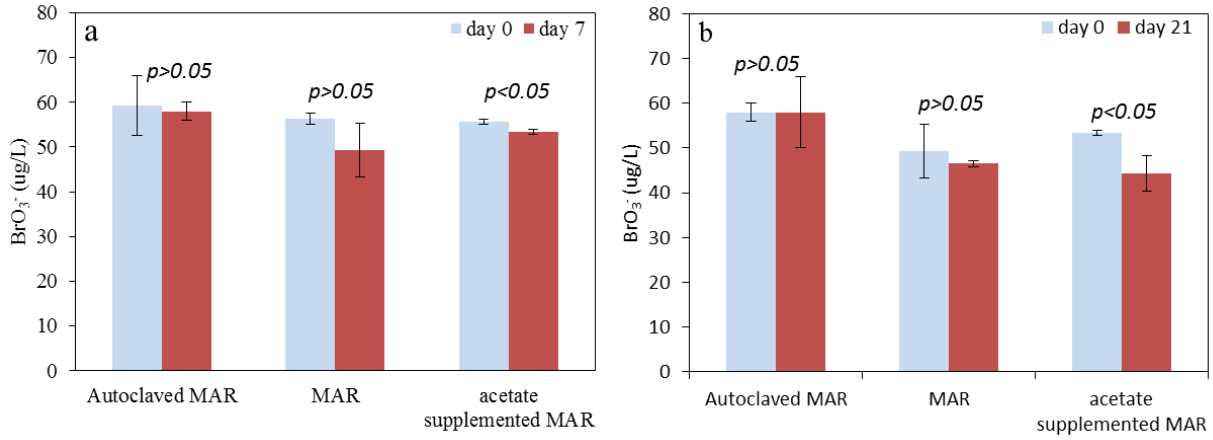


Figure 2 $BrO_3^-$ removal in autoclaved and non-autoclaved batch reactors with MAR water and acetate supplemented MAR water over 7 days (a) and 21 days (b). An additional 1 mg/L AOC from $CH_3COONa$ solution was added to MAR water to create the acetate supplemented MAR water. All batch reactors were in anoxic conditions at 11.5±0.5℃. n=3

Figure 3 presents $NO_3^-$ concentrations over 7 days (Figure 3-a) and 21 days (Figure 3-b) in anoxic batch reactors with MAR water (group 2) and acetate supplemented MAR water (group 3). $NO_3^-$ was not significantly biodegraded in MAR water ($p>0.05$), while $NO_3^-$ was biodegraded in acetate supplemented MAR water over 7 days (2.6 mg/L, 22.7%. $p<0.05$), and at a greater magnitude after 21 days (17.8 mg/L, 87.8%. $p<0.05$). These results demonstrate that the retention time as well as the availability of AOC is an important factor influencing $BrO_3^-$ and $NO_3^-$ biodegradation, with $NO_3^-$ degradation occurring faster than $BrO_3^-$ degradation.

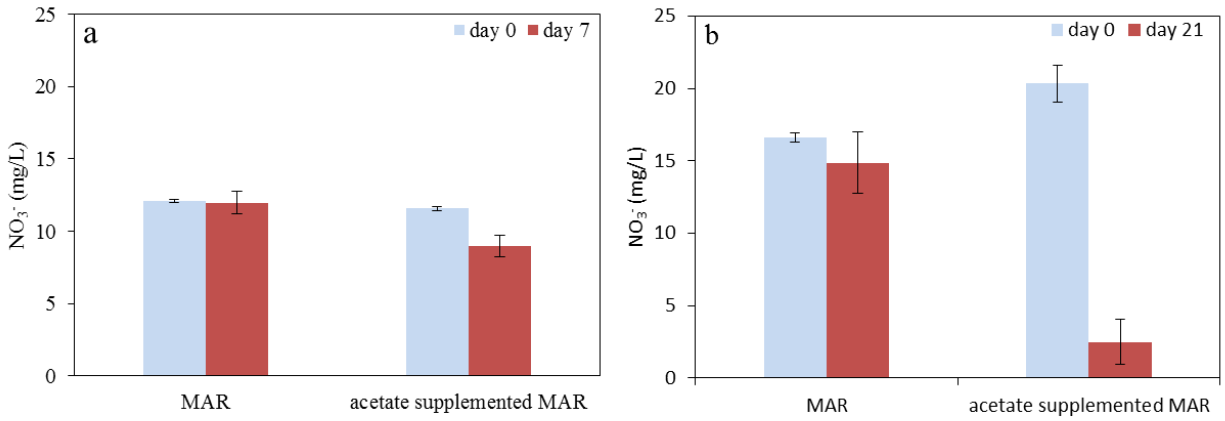


Figure 3 $NO_3^-$ removal in anoxic batch reactors with MAR water and simulated ozonation-MAR water over 7 days (a) and 21 days (b). An additional 1 mg/L AOC from $CH_3COONa$ solution was added to MAR water to create the acetate supplemented MAR water. T=11.5±0.5℃. n=3

### 3.1.2 Presence of $NO_3^-$

The influence of $NO_3^-$ on $BrO_3^-$ removal was investigated in anoxic batch reactors containing acetate supplemented MAR water in the presence and sudden absence of $NO_3^-$ (Figure 4). No $BrO_3^-$ biodegradation (*p>0.05*) was observed in batch reactors with an initial $NO_3^-$ concentration of 6.1 mg/L, while a clear decrease of $NO_3^-$ (*p<0.05*) from 6.1 mg/L to 3.8 mg/L was observed after 2.7 hours. In case of a sudden absence of $NO_3^-$ in the batch reactors (lower than 0.89 mg/L), $BrO_3^-$ was reduced from 47 µg/L to 35 µg/L in 2.7 hours (*p<0.05*), indicating that $NO_3^-$ and $BrO_3^-$ compete for biodegradation.

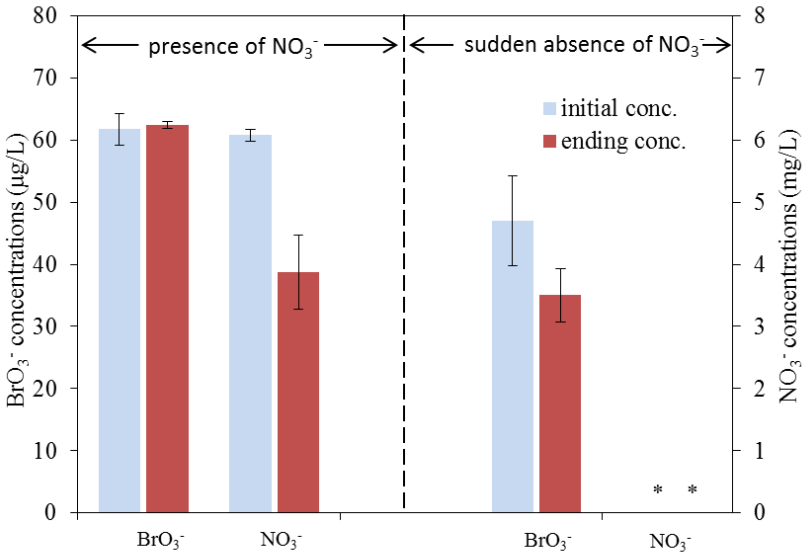


Figure 4 $BrO_3^-$ and $NO_3^-$ removal in batch reactors with acetate supplemented MAR water in the presence and sudden absence of $NO_3^-$ within 2.7 hours. * indicates measurements below the detection limit. T=11.5±0.5℃. n=3

## 3.2 1 m column experiments

### 3.2.1 Oxic and anoxic zones

The removal of $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ in 1 m oxic and anoxic columns (retention time 22 hours) for 98 days are shown in Figure 5. $BrO_3^-$ removal was slightly higher in the anoxic column (8 %) than in the oxic column (5.7 %), although the difference was not significant (*p<0.05*). 10.7 % $NO_3^-$ was removed in the anoxic column, indicating anoxic conditions were indeed reached. In the oxic column, $NO_3^-$ was not converted and passed through the

column. No significant $SO_4^{2-}$ removal in both oxic and anoxic columns was observed, so neither columns reached $SO_4^{2-}$-reducing conditions.

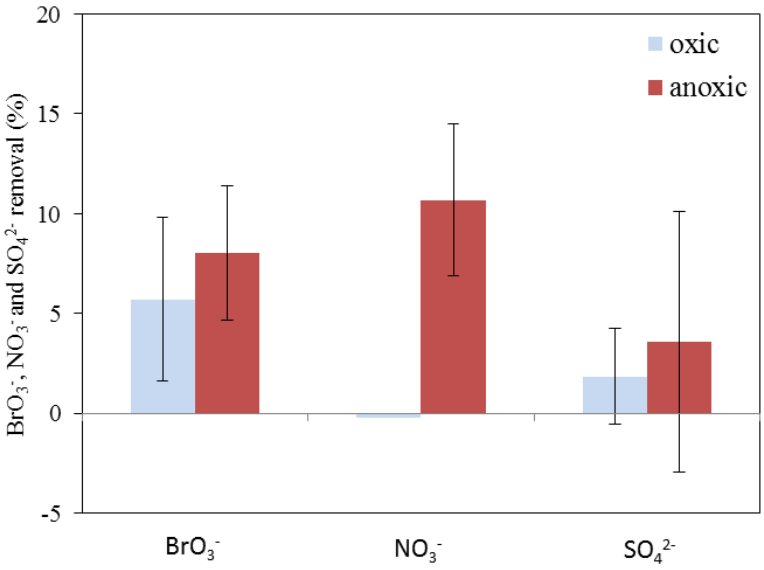


Figure 5 $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ removal in oxic and anoxic columns with acetate supplemented MAR water as the influent. 150 μg/L AOC from $CH_3COONa$ solution was added to MAR water to compose the acetate supplemented MAR water. The concentrations of $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ were 58.9±3.1 μg/L, 10.3±1.8 mg/L and 51.9±10.1 mg/L respectively. T=11.5±0.5 ℃. n=5

### 3.2.2 Effect of $NO_3^-$

The 1 m columns were operated in two subsequent phases: during phase 1 (day 0-98), 10.3±1.8 mg/L $NO_3^-$ was present in the influent, whereas during phase 2 (day 98 to 209), $NO_3^-$ was extracted from the influent until the concentration was lower than 0.89 mg/L. Figure 6 presents $BrO_3^-$ removal in the oxic and anoxic columns with long-term presence and absence of $NO_3^-$. During phase 1, the $BrO_3^-$ removal in the oxic column (1.3-11.2%) and anoxic column (3.9-11.7%), with a 22 hours retention time, was not highly effective. However, during phase 2, the sudden absence of $NO_3^-$ in the influent water at day 98 resulted in sharp initial increases of $BrO_3^-$ reduction (82.5% in anoxic column and 13.6% in oxic column), after which $BrO_3^-$ removal decreased to 61.4% in the anoxic column and 0.32% in the oxic column in day 98-99.5. After that, the oxic column had a very limited $BrO_3^-$ removal of 0-3.3% lower than that in the presence of $NO_3^-$, whereas the $BrO_3^-$ removal in the anoxic column gradually decreased and finally returned to a steady 5.5-12.9% during 99.5-209 days.

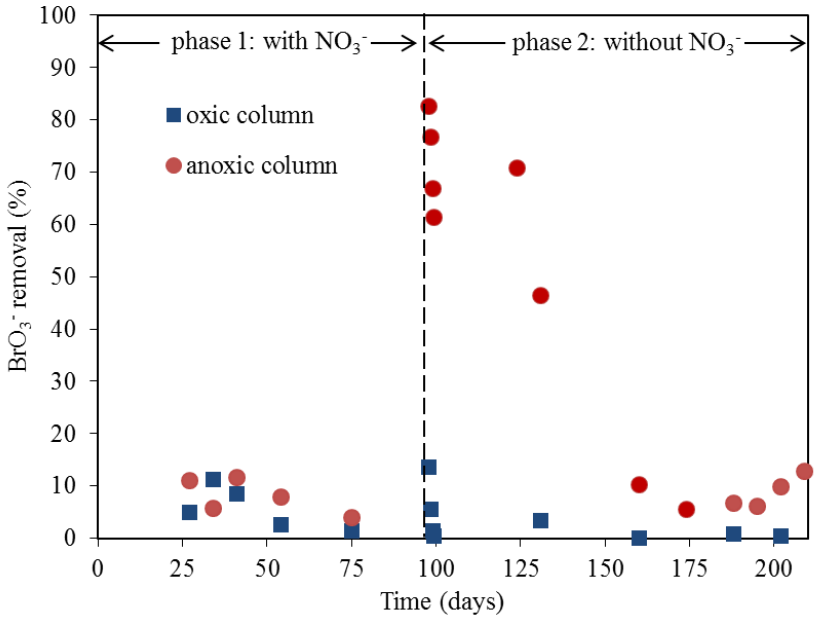


Figure 6 $BrO_3^-$ removal in the 1 m oxic and anoxic columns containing acetate supplemented MAR water as influent with 10.3±1.8 mg/L $NO_3^-$ (phase 1: 0-98 days) and acetate supplemented MAR water as influent with $NO_3^-$ below than detection limit (0.89 mg/L) (phase 2: 98-209 days). 150 µg/L AOC from a $CH_3COONa$ solution was added to the MAR water to compose acetate supplemented MAR water. The dashed line at day 98 separates phase 1 and phase 2. Influent $BrO_3^-$ was 56.6±6.45 µg/L. Influent DO in the oxic column and anoxic column was 8.52-10.74 mg/L and below 0.6 mg/L respectively. T=11.5±0.5℃

## 3.3 8 m column experiments

### 3.3.1 Effect of infiltration retention time

In order to investigate the effect of infiltration retention time during MAR, a series of columns (8 m total, 6 days retention time) was operated with MAR influent water for several months. Figure 7 presents the continuous $BrO_3^-$ removal during the final 2 months for 1, 3 and 8 m infiltration depth. In the first 1 m (corresponding to a retention time of 0.75 day), no clear $BrO_3^-$ and $NO_3^-$ removal was observed. After 3 m infiltration (corresponding to a retention time of 2.25 days), $BrO_3^-$ and $NO_3^-$ remaining concentrations were clearly lower than the influent concentrations with 20.4% $BrO_3^-$ and 15.8% $NO_3^-$ removal. After 8 m of soil passage, 48.2% $BrO_3^-$ and 30.2% $NO_3^-$ were removed and the. Final $BrO_3^-$ concentration reached with a retention time of 6 days was 29.6 µg/L.

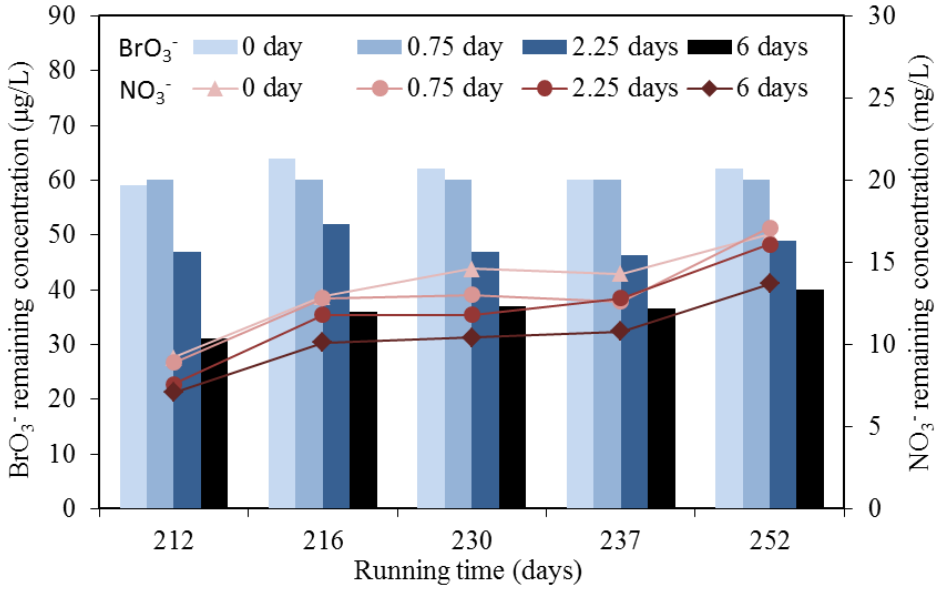


Figure 7 $BrO_3^-$ removal and normalized concentrations of $BrO_3^-$ and $NO_3^-$ in the 8 m anoxic column set-up containing MAR water as the influent. $BrO_3^-$ and $NO_3^-$ in the influent were 63±4 µg/L and 13±3.8 mg/L respectively. Influent DO was below 0.6 mg/L. T=11.5±0.5 ℃

### 3.3.2 Effect of increased AOC due to ozonation pre-treatment

Figure 8 presents concentrations of $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ along the column height of the 8 m anoxic columns in series containing acetate supplemented MAR water (phase 1, Figure 8-a) and MAR water (phase 2, Figure 8-b), respectively. Figure 8-a shows that $BrO_3^-$ was removed by 8%, 59% and 98%, at a depth of 1 m, 3 m and 8 m, respectively. $NO_3^-$ was removed by 8%, 51% and 80% at a depth of 1 m, 3 m and 8 m, respectively. Consequently, at the end of the 8 m column, corresponding to an infiltration retention time of 6 days, the $BrO_3^-$ concentration was as low as 1.3 µg/L and the $NO_3^-$ concentration was 1.1 mg/L. No $SO_4^{2-}$ removal was observed in this column set-up with and without the increased AOC concentration as a result of ozonation pre-treatment, indicating no $SO_4^{2-}$-reducing conditions were reached. Comparison of the $NO_3^-$ and $BrO_3^-$ removal efficiencies of the two phases consistently shows better $BrO_3^-$ and $NO_3^-$ removal over the height of the column, indicating that the addition of 150 µg/L C-CH3COONa resulted in substantially higher $BrO_3^-$ and $NO_3^-$ removals.

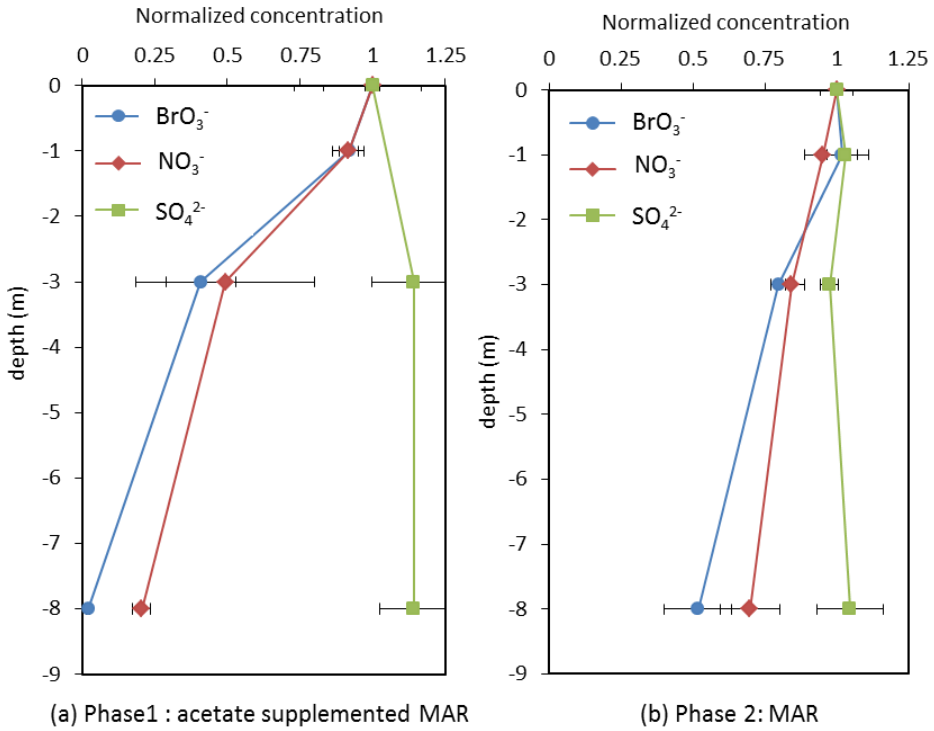


Figure 8 Average concentrations (n=5-8) of $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ with depth in the 8 m anoxic column during phase 1 with acetate supplemented MAR water (a) and during phase 2 with only MAR water (b). 150 µg/L AOC from $CH_3COONa$ solution was added to MAR water to compose the acetate supplemented MAR water. $BrO_3^-$, $NO_3^-$ and $SO_4^{2-}$ concentrations in the influent water were 61.83±5.18 µg/L, 10.7±6 mg/L and 52.5±8.5 mg/L. Mostly, influent DO was below 0.6 mg/L. T=11.5±0.5 ℃

# 4 Discussion

## 4.1 Role of $NO_3^-$ in $BrO_3^-$ removal

As stated in the introduction, it has been reported by other authors that biological $BrO_3^-$ reduction is a side reaction of the $NO_3^-$ reduction pathway (Butler et al., 2005; Korom, 1992), and $BrO_3^-$ can be biodegraded by several other anoxic bacteria instead of denitrifying bacteria (Davidson et al., 2011). Both anoxic batch reactors and 1 m anoxic column experiments showed that $BrO_3^-$ removal in the presence of $NO_3^-$ was low and $NO_3^-$ biodegradation was higher, indicating that $BrO_3^-$ biodegradation can occur in the presence of $NO_3^-$. $BrO_3^-$ removal suddenly increased due to the sudden absence of $NO_3^-$, indicating that $BrO_3^-$ and $NO_3^-$ in MAR systems may compete for biodegradation by denitrifying bacteria, and denitrifying bacteria prefer $NO_3^-$ over $BrO_3^-$ although the biodegradation of

$NO_3^-$ and $BrO_3^-$ occur simultaneously in anoxic $NO_3^-$-reducing zones. In Figure 8, the $BrO_3^-$ biodegradation rate may initially appear higher than $NO_3^-$ biodegradation rate in 1-8 m, but actually the mass of $NO_3^-$ reduction (phase 1: 2.02 mg/L/m in 1-8 m, phase 2: 0.63 mg/L/m in 1-8 m) was much higher than the mass of $BrO_3^-$ biodegradation (phase 1: 20.59 μg/L/m, phase 2: 10.27 μg/L/m in 1-8 m).

Some studies demonstrated the potential role of $NO_3^-$ reductase in $BrO_3^-$ reduction (Davidson et al., 2011; Hijnen et al., 1995). It can be observed from Figure 8 that both $NO_3^-$ and $BrO_3^-$ biodegradation rates in the first 1 m column passage were lower than from 1-3 m. One potential explanation for this result is that even if the anoxic condition were achieved in the first 1 m, DO became lower with increasing retention time and resulted in more active $NO_3^-$ reductase (Bell et al., 1990; Cavigelli & Robertson, 2000), and correspondingly more $NO_3^-$ and $BrO_3^-$ biodegradation. $NO_3^-$ and $BrO_3^-$ biodegradation rates reduced in 3-8 m soil passage than higher up in the column, which can be potentially explained by AOC becoming insufficient as retention time increased and therefore lowered the level of microbial activity.

In the 1 m anoxic column, a rapid decrease of $BrO_3^-$ removal was observed in 1.5 days (running time 98-99.5 days) following an increase due to the sudden absence of $NO_3^-$. Subsequently, a gradual decrease of $BrO_3^-$ biodegradation within 2.5 months (phase 2 in Figure 6) was observed. This study is the first documentation of $BrO_3^-$ removal in the long-term absence of $NO_3^-$. Korner and Zumft (1989) concluded that the presence of nitrogen oxides was a prerequisite to promote the synthesis and the activity of denitrification enzymes. Several other studies (Cove, 1966; Saleh-Lakha et al., 2009; Sun et al., 2016) reported that $NO_3^-$ absence or limited $NO_3^-$ leads to a decrease of denitrification functional genes, and $NO_3^-$ reductase activity decay or denitrification rate decrease in several hours in pure microbial species and mixed microbial strains. Therefore, the rapid decrease of $BrO_3^-$ removal in the 8 m column from 82.5% to 61.4% in 1.5 days (running time 98-99.5 days) can potentially be explained by the limitation of $NO_3^-$ reductase activity of denitrifying bacteria by a $NO_3^-$ concentration below detection limit (0.89 mg/L). The gradual decrease of $BrO_3^-$ biodegradation fits the first-order kinetic model with the first-order decay constant 0.034/day (Figure S1 in Appendix A). The decay of heterotrophic bacteria due to a lack of substrate is a relatively slow process, particularly under anoxic conditions. Lin (2008) showed that when $NO_3^-$ or glucose were limited in a moving-fixed bed biofilm reactor, denitrifying bacterial biomass decayed from 100% to 51.5% in 11 days with a first-order kinetic coefficient of 0.061/day.

Although the decay rate of denitrifying bacteria reported in the previous study (Lin, 2008) is faster (double) than the observed $BrO_3^-$ removal decrease, given that these experiments were performed under different conditions (including higher temperatures; 20-25 ℃ vs 11 ℃), the results of Lin (2008) indicate the hypothesized relationship between denitrifying bacteria biomass and $BrO_3^-$ removal.

## 4.2 Ozonation as MAR pre-treatment

Figures 2, 3 and 7 show that in both the batch experiments and the 8 m column experiment, the addition of extra C-$CH_3COONa$, simulating formation of AOC during ozonation pre-treatment, resulted in slight but significantly higher $NO_3^-$ and $BrO_3^-$ reductions. This observation is similar to the results of Kirisits et al. (2001) who showed that the increase of DOC as an external electron donor resulted in the increase of $BrO_3^-$ reduction in a BAC filter. The addition of extra carbon stimulated microbial growth, which was monitored with ATP measurements. Biomass in the batch reactors with 1 mg/L C-$CH_3COONa$ addition was approximately two times as high as in the reference reactors (3.3 ng/mL and 1.5 ng/mL respectively; Figure S2 in Appendix A). This result suggests that an increase of AOC as a result of the ozonation pre-treatment can promote microbial activity and therefore $BrO_3^-$ removal in subsequent MAR systems.

Inevitably, the ozonation pre-treatment not only affects the AOC concentration but also causes high concentrations of dissolved oxygen (DO) in the MAR influent water. In the column studies, $BrO_3^-$ reduction was much higher in the anoxic column than in the oxic column, indicating that biological reduction of $BrO_3^-$ predominantly occurs in anoxic zones instead of oxic zones in MAR systems. This result is in agreement with previous studies (Hübner et al., 2016; Kirisits et al., 2001; Liu et al., 2012). Hijnen et al. (1995) found that $BrO_3^-$ reduction was inhibited by oxygen. Controlled column studies simulating MAR revealed inefficient $BrO_3^-$ removal under oxic conditions in the study of Hübner et al. (2012). This observation can be potentially explained by DO being preferred over $BrO_3^-$ (and $NO_3^-$) as a competing electron acceptor. It is therefore recommended to design ozonation-MAR systems in such a way that anoxic zones develop, which can generally be achieved by extending the subsurface retention time. Depending on site-specific water quality and hydrogeological conditions, oxic zones are usually found in the first several meters with a retention time of a couple of hours to days (Bertelkamp et al., 2016). Therefore, the ozonation effluent with high oxygen concentrations is not likely to limit biological $BrO_3^-$ reduction in most MAR systems.

### 4.3 Redox conditions in MAR

Figure S3 in the Appendix A shows redox conditions in MAR systems and the theoretical sequence of terminal electron acceptor processes. The initial infiltration phase in MAR systems are usually oxic, followed first by $NO_3^-$-reducing and then Fe/Mn-reducing zones (Bertelkamp et al., 2016; Lekkerkerker-Teunissen et al., 2012; Maeng et al., 2011; Schmidt et al., 2011). This study only focused on $BrO_3^-$ removal in oxic and $NO_3^-$-reducing anoxic zones.

In the oxic column, the observed slight $BrO_3^-$ reduction (Figure 6) is an indication that minor $BrO_3^-$ reduction by oxic bacteria in MAR systems can also take place. Based on the absence of $NO_3^-$ removal in the oxic column, it can be concluded that no denitrifying bacteria or anoxic microniches were present in this column. Therefore, $BrO_3^-$ reduction by denitrifying bacteria in this oxic column can be excluded.

In the current study, the retention time in the 8 m anoxic column was 6 days. 60 µg/L $BrO_3^-$ was biodegraded to 1.3 µg/L and 29.6 µg/L in this long anoxic column set-up with and without increased AOC, respectively. In practice, travel times (weeks, months or even years) for MAR systems are much longer than those used in this study (Baumgarten et al., 2011; Grünheid et al., 2005; Stauder et al., 2012). With a greater retention time of the anoxic $NO_3^-$-reducing zones in MAR systems, more $BrO_3^-$ than in the 8 m anoxic column with 6 days retention time may be biodegraded, as the travel time is longer and thus the reaction time is also longer. In addition, the concentration of $NO_3^-$ as a competitor of $BrO_3^-$ reduction by denitrifying bacteria becomes lower and lower. Therefore, $BrO_3^-$ biodegradation should be more efficient with greater retention time in anoxic zones, especially in the zone immediately after $NO_3^-$ depletion, i.e. at the interface of the anoxic denitrification zone and the Fe/Mn oxide reduction zone. Additional evidence of this inference is illustrated by the study of Hübner et al. (2016), in which it was observed that $BrO_3^-$ removal in the presence of low $NO_3^-$ concentrations was significantly higher than in the presence of high $NO_3^-$ concentrations.

## 5 Conclusions

This study focused on the effect of $NO_3^-$ and the role of increased AOC concentrations on the removal of $BrO_3^-$ in $NO_3^-$-reducing anoxic zones of MAR systems. The following conclusions can be drawn:

- $BrO_3^-$ and $NO_3^-$ compete for reduction by denitrifying bacteria, but $BrO_3^-$ reduction and $NO_3^-$ reduction can occur simultaneously even if denitrifying bacteria prefer $NO_3^-$ to $BrO_3^-$ as an electron acceptor.
- The presence of $NO_3^-$ is a precondition for denitrifying bacteria to reduce $BrO_3^-$ in $NO_3^-$-reducing anoxic zones of MAR systems.
- An increase of AOC as a result of ozonation pre-treatment promotes microbial activity and therefore $BrO_3^-$ removal in subsequent MAR systems.
- In the 8 m long anoxic column (retention time 6 days) simulating anoxic $NO_3^-$-reducing zones of MAR systems, $BrO_3^-$ biodegraded to a concentration of 1.3 µg/L, indicating that $BrO_3^-$ biodegradation by denitrifying bacteria can happen in anoxic $NO_3^-$-reducing zones of MAR systems.
- MAR systems following ozonation are potentially effective to biodegrade $BrO_3^-$, provided that anoxic $NO_3^-$ reducing conditions are reached in MAR systems.

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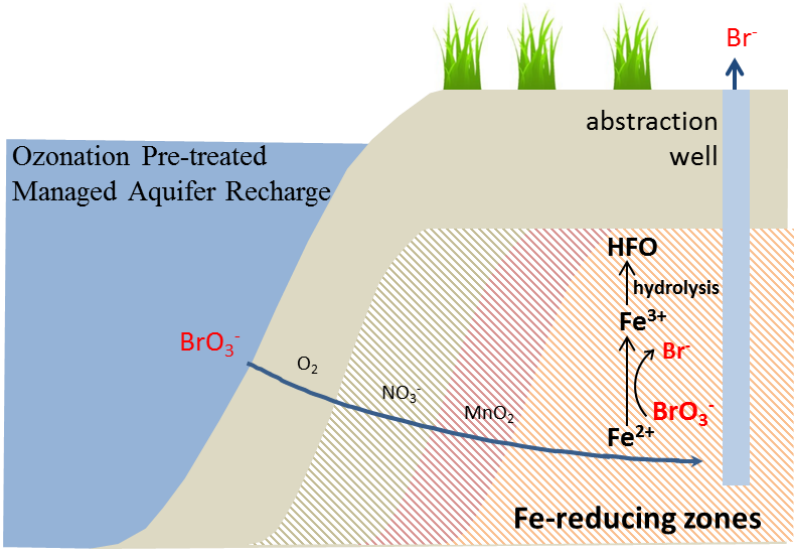
# 3

# Bromate reduction by iron (II) during managed aquifer recharge: A laboratory-scale study

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## Abstract

The removal of bromate ($BrO_3^-$) as a by-product of ozonation in subsequent managed aquifer recharge (MAR) systems, specifically in anoxic iron (Fe) - reducing zones, has so far gained little attention. This preliminary study through laboratory anoxic batch experiments was executed to explore the feasibility of chemical $BrO_3^-$ reduction in Fe-reducing zones of MAR systems and to estimate potential inhibition by $NO_3^-$. The results showed that the reaction rate was affected by initial $Fe^{2+}/BrO_3^-$ ratios and by initial pH. Also, the pH dropped significantly due to the hydrolysis of $Fe^{3+}$ to hydrous ferric oxides (HFO) flocs. These HFO flocs were found to adsorb $Fe^{2+}$, especially at high $Fe^{2+}/BrO_3^-$ ratios, whereas at low $Fe^{2+}/BrO_3^-$ ratios, the mass sum loss of $BrO_3^-$ and $Br^-$ indicated the formation of intermediate species. Under MAR conditions with relatively low $BrO_3^-$ and $Fe^{2+}$ concentrations, $BrO_3^-$ can be reduced by naturally occurring $Fe^{2+}$ during MAR as the extensive retention time in MAR systems will compensate for the slow reaction kinetics of low $BrO_3^-$ and $Fe^{2+}$ concentrations. Under specific flow conditions, $Fe^{2+}$ and $NO_3^-$ may co-occur during MAR, but $NO_3^-$ hardly compete with $BrO_3^-$ since $Fe^{2+}$ prefers $BrO_3^-$ over $NO_3^-$. However, it was found that when $NO_3^-$ concentrations exceed $BrO_3^-$ concentrations in multiple orders of magnitude, the presence of $NO_3^-$ may slightly inhibit $BrO_3^-$ reduction by $Fe^{2+}$.

# 1 Introduction

Ozone-based advanced oxidation processes (AOPs) are increasingly being considered as effective alternatives for the removal of organic micro-pollutants (OMPs) during drinking water treatment (Hollender et al., 2009; Hübner et al., 2012; Scheideler et al., 2011). However, bromate ($BrO_3^-$) is formed during ozone-based treatment when applied to bromide containing water (Assuncao et al., 2011; Haag & Holgne, 1983; Kurokawa et al., 1990). It has been reported that the $BrO_3^-$ concentrations in drinking water after ozone-based AOPs typically range from 0 to 127 μg/L (Xie & Shang, 2006). $BrO_3^-$ is classified as a Group 2B or possible human carcinogen by the International Agency for Research on Cancer (IARC) according to its major toxic effects (Crofton, 2006; Kurokawa et al., 1986; Xiao et al., 2017). The standard of $BrO_3^-$ in drinking water regulated by WHO, USEPA and European Union is 10 μg/L (Carney, 1991; Forum, 2005; WHO, 2011), demanding water companies to control $BrO_3^-$ concentrations in drinking water.

A number of physical, chemical, electrochemical and biological techniques for $BrO_3^-$ removal have already been proposed. With respect to physical techniques, various advanced sorption techniques, e.g. ion-exchange resins (Chen et al., 2014), nano crystalline akaganeite (β-FeOOH)-coated quartz sand (Xu et al., 2012) and layered double hydroxides (Theiss et al., 2014; Zhang & Li, 2014), have shown the ability to adsorb $BrO_3^-$ from aqueous solutions, but so far, these techniques have not been applied in drinking water treatment. Granular activated carbon (GAC) as a conventional physical sorption technique can successfully reduce $BrO_3^-$ (Du et al., 2014), but the regenerated GAC loses effectiveness for $BrO_3^-$ removal after a certain running time (Xie & Shang, 2006). $BrO_3^-$ can be removed by reverse osmosis (Gyparakis & Diamadopoulos, 2007), but it is an expensive process since membrane fluxes are low and high operating pressures are needed. Electrodialysis Reversal (EDR) has been studied in an integrated membrane system for drinking water treatment (Van Der Hoek et al., 1998), in which EDR showed only limited $BrO_3^-$ removal: 64% in a two stage EDR system and 78% removal in a three stage EDR system. $BrO_3^-$ removal with catalysts, including zero valent iron (Fe) (Wang et al., 2009) and $Pd/Al_2O_3$ (Chen et al., 2010), has been found to be limited in the presence of coexisting anions. Different reducing agents, such as ferrous iron ($FeSO_4$), react with dissolved oxygen (DO) and therefore the practical application during water treatment is quite difficult (Siddiqui et al., 1994). UV irradiation successfully reduces $BrO_3^-$, but has a high energy demand (Xie & Shang, 2006), just like electrochemical methods (Kishimoto & Matsuda, 2009; Mao et al., 2014). With respect to biological techniques, biological

activated carbon (BAC) filters are capable to reduce $BrO_3^-$ effectively, but competitive DO remains a critical factor (Kirisits et al., 2001) because it is a challenge to construct a BAC filter with restricted oxygen transfer within the biofilm (Liu et al., 2012). Hijnen et al. (1999) showed that $BrO_3^-$ was removed in a denitrifying bioreactor fed with methanol. However, they demonstrated that $BrO_3^-$ removal in a denitrifying bioreactor did not seem to be a realistic option in drinking water treatment due to the long contact times required for $BrO_3^-$ removal and extensive post treatment necessary to remove excessive methanol and released biomass. Altogether, there are few effective options to remove the highly soluble and stable $BrO_3^-$ in practice.

In this study, a new approach is being proposed, namely to utilize Fe-reducing zones of managed aquifer recharge (MAR) as a barrier for $BrO_3^-$ after ozonation. This sequence of AOP-MAR has been proposed to effectively remove various OMPs during drinking water production (Lekkerkerker-Teunissen et al., 2012; Lekkerkerker et al., 2009a; Oller et al., 2011b). It is hypothesized that not only the removal of OMPs will improve with this sequence, but also the produced $BrO_3^-$ may be removed by MAR. Recently, it was found that $BrO_3^-$ is partially biodegraded in $NO_3^-$-reducing zones of MAR (Hübner et al., 2016; Wang et al., 2018). However, the potential reduction of $BrO_3^-$ to $Br^-$ in deeper, Fe-reducing zones during soil passage has not yet been investigated.

The reduction of $BrO_3^-$ by $Fe^{2+}$ (Siddiqui et al., 1994; Xie & Shang, 2007), the hydrolysis of its product $Fe^{3+}$ under near-neutral pH proceeds as follows (Appelo & Postma, 2004; Stefánsson, 2007):

$$BrO_3^- + 6\ Fe^{2+} + 6\ H^+ \rightarrow Br^- + 6\ Fe^{3+} + 3\ H_2O \quad (1)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3\ (s)} + 3H^+ \quad (2)$$

$$BrO_3^- + 6\ Fe^{2+} + 15\ H_2O \rightarrow Br^- + 6\ Fe(OH)_{3\ (s)} + 12\ H^+ \quad (3)$$

The reduction rate of $BrO_3^-$ by $Fe^{2+}$ is dependent on $Fe^{2+}$ concentration, contact time, pH and DO (Dong et al., 2009; Siddiqui et al., 1994). In MAR systems, water flows from infiltration ponds through an oxic zone, via an $NO_3^-$-reducing anoxic zone and an Mn-reducing anoxic zone, to the Fe-reducing anoxic zone. So depending on the local geochemical situation of MAR, $Fe^{2+}$ may be released into the groundwater leading to natural $BrO_3^-$ reduction by $Fe^{2+}$ in the Fe-reducing anoxic zone of MAR.

A study by Siddiqui et al. (1994) with oxic water (0.22 mM DO) found that an initial $BrO_3^-$ concentration of 0.4 µM was lowered to 0.08 µM within 30 minutes following a

dose of 0.27 mM $Fe^{2+}$. Dong et al. (2009) worked with 0.2 µM $BrO_3^-$, a 0.54 mM $Fe^{2+}$ dosage and 0.07 mM DO, reaching a $BrO_3^-$ reduction of 65%. In these studies, the $Fe^{2+}$ dosage was extremely high compared to $Fe^{2+}$ concentrations to be expected during MAR, where Fe concentrations below 0.03 mM are to be expected (e.g., the MAR site of Dunea, the Netherlands shows concentrations ranging from 0.0015 to 0.029 mM Fe). To what extent $BrO_3^-$ reduction is possible at such low concentrations of $Fe^{2+}$ is not known, although the extensive residence times in the subsurface do not require fast kinetics for this technology to be effective. Also, competition of $BrO_3^-$ with DO is not a problem in these anoxic zones. $Fe^{2+}$ can be formed only when $NO_3^-$ as an electron acceptor is exhausted in anaerobic zones of MAR systems (Barbieri et al., 2011; Kedziorek et al., 2008). However, water containing $NO_3^-$ and water containing $Fe^{2+}$ from different pathways have been found to mix in specific zones of MAR (Grischek & Paufler, 2017), so $NO_3^-$ and $Fe^{2+}$ can be present simultaneously in anaerobic zones of MAR systems. This is confirmed by Dunea measurements, where $NO_3^-$ and dissolved Fe have been simultaneously detected in the effluent of MAR sites (Scheveningen and Monster, the Netherlands). Therefore, $NO_3^-$ may compete with $BrO_3^-$ for reduction by $Fe^{2+}$ (Buresh & Moraghan, 1976; Huang & Zhang, 2004; Song et al., 2016) during MAR. The investigation of $BrO_3^-$ reduction by $Fe^{2+}$ in the presence of $NO_3^-$ may be an important reference for the feasibility of $BrO_3^-$ removal in Fe-reducing zones of MAR systems. Examples of stoichiometric equations for the reaction of $NO_3^-$ and $Fe^{2+}$ are given below (in which the stable endpoint is nitrogen gas), but less complete reactions may have endpoints anywhere along the reduction pathway (Ottley et al., 1997):

$$10Fe^{2+} + 2NO_3^- + 14H_2O \rightarrow 10FeOOH + N_2 + 18H^+ \quad (4)$$

$$15Fe^{2+} + NO_3^- + 13H_2O \rightarrow 5Fe_3O_4 + N_2 + 28H^+ \quad (5)$$

The focus of this preliminary study was to investigate the mechanism of chemical $BrO_3^-$ reduction by $Fe^{2+}$ and the feasibility of $BrO_3^-$ reduction by naturally occurring $Fe^{2+}$ in the Fe-reducing anoxic zones of MAR systems, with an emphasis on the potential competition with or inhibition by $NO_3^-$. Microbiological reactions and biochemical reactions were not included in this study.

# 2 Materials and Methods

## 2.1 Experimental design

The research was designed with two sets of anoxic batch reactor experiments: (A) high $Fe^{2+}$ and $BrO_3^-$ concentrations to investigate reduction mechanisms, and (B) environmentally relevant concentrations of $Fe^{2+}$ and $BrO_3^-$ to simulate the concentrations during MAR. As the focus was in all experiments on chemical $BrO_3^-$ reduction by $Fe^{2+}$, no soil or sediment was added in the batch reactors. Both sets of experiments were executed in absence and presence of $NO_3^-$. An overview of all experiments is provided in Figure 1.

For the experiments with high $Fe^{2+}$ and $BrO_3^-$ concentrations, anoxic batch experiments were performed with 0.03 mM $BrO_3^-$ and 0.26 or 1 mM $Fe^{2+}$. 0.26 mM $Fe^{2+}$ is close to the required concentration to reduce 0.03 mM $BrO_3^-$ according to the stoichiometry of equation (1). The experiments were executed under two pH conditions, pH 7.0 which is a realistic pH for MAR water and pH 5.2 to slow down the reaction in order to identify potential intermediate species.

To investigate the competition between $NO_3^-$ and $BrO_3^-$, the same order of magnitude of $NO_3^-$ (0.07 mM) and $BrO_3^-$ (0.03 mM) were added to anoxic batch reactors, together with the $Fe^{2+}$ (0.26 mM and 1 mM).

To simulate $BrO_3^-$ reduction by $Fe^{2+}$ at concentrations similar to MAR, the concentrations were lowered to 0.5 µM for $BrO_3^-$ and 0.003 - 0.033 mM for $Fe^{2+}$. The concentration of 0.003 mM $Fe^{2+}$ was close to the stoichiometric amount to reduce 0.5 µM $BrO_3^-$ (equation (1)). These experiments were conducted at an initial pH of 7.0. The influence of $NO_3^-$ was investigated by dosing 0.16 mM $NO_3^-$, which was three orders of magnitude greater than the concentration of $BrO_3^-$. All experiments were performed in duplicate.

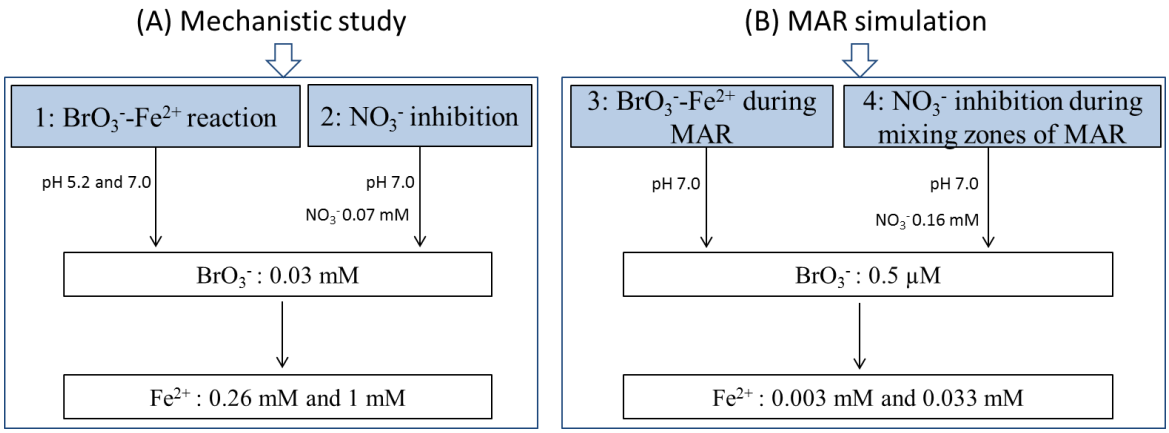


Figure 1 Experimental overview of anoxic batch reactors T=11.5±0.5 ℃. n=2

## 2.2 Anoxic batch reactors

Four series of laboratory-scale batch experiments using 250 mL (for experiments A) and 1 L (for experiments B) glass bottles were carried out under anoxic conditions at a controlled temperature (11.5±0.5 ℃). Anoxic conditions were reached by flushing nitrogen gas until a DO concentration below 0.3 µM (0.01 mg/L) were achieved in the batch reactors. The mouths of the batch reactors were sealed with rubber stoppers to maintain prevent DO intrusion. On the rubber stoppers, there were two needles with valves used as a sampling point and a reagent dosing point.

Water samples were collected 8-10 times within 120 hours contact time to determine the concentrations of $BrO_3^-$, $Br^-$, $NO_3^-$ and $Fe^{2+}$. In the 0.03 mM $BrO_3^-$ experiments (A) and 0.5 µM $BrO_3^-$ experiments (B), 3 mL and 50 mL per sample were collected respectively. After sample collection, several drops of diluted ethylenediamine (EDA) solution (11%) was added to samples to prevent reactions of residual chemicals (Thomas & Rohrer, 2017).

To test the stability of the anoxic system, $Fe^{2+}$ concentrations were monitored in the batch reactors after dosing 0.033, 0.003, 0.26 or 1mM $Fe^{2+}$ to the system. The $Fe^{2+}$ concentrations remained stable during the 120 hours experiment (Figure 2), indicating that the system was well sealed and therefore no $Fe^{2+}$ oxidation by DO was observed.

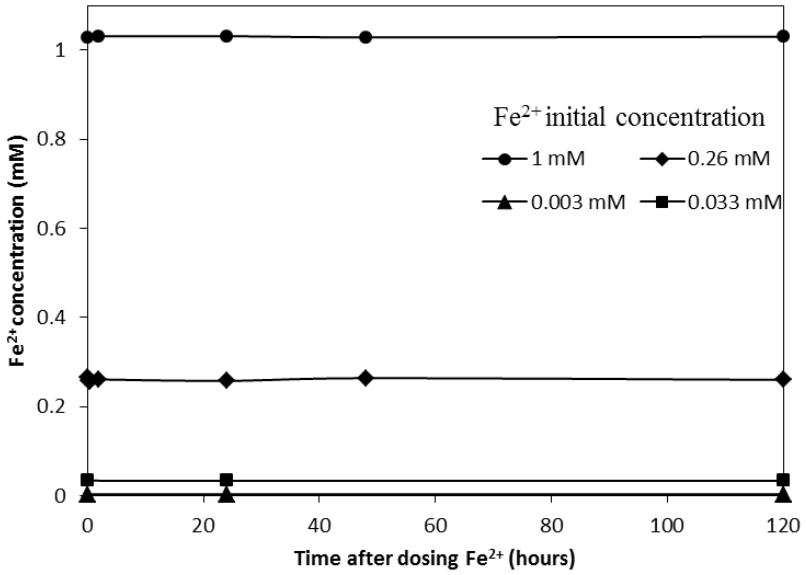


Figure 2 $Fe^{2+}$ concentrations over 120 hours contact time in reactors with $Fe^{2+}$ alone

## 2.3 Water and chemicals

The water used in batch experiments was prepared using chemical reagents and deionized water from a Millipore Milli-Q system. Sodium bromate ($NaBrO_3$), sodium nitrate ($NaNO_3$), ferrous sulphate ($FeSO_4 \cdot 7H_2O$), sodium bicarbonate ($NaHCO_3$) and EDA were purchased from Sigma-Aldrich (St Louis, MO, United States). 2 mM $NaHCO_3$ was prepared for use as a pH buffer, and 0.2 M NaOH was prepared to further adjust the pH. To prevent $Fe^{2+}$ oxidation in $FeSO_4$ solutions, $FeSO_4$ solutions were always prepared immediately before the experiments and concentrated acid (HCl) was used to acidify $FeSO_4$ solutions to pH 2 (Thomas & Rohrer, 2017). All chemicals were of analytical grade.

## 2.4 Analytical methods

DO and temperature were measured with a FDO®925-optical oxygen sensor (WTW) and pH was measured with a SenTix® 940 (WTW) electrode, both using the WTW Multi 3420 meter.

$Fe^{2+}$ was measured by photometry using the Spectroquant ®Iron test (Merck) with a detection range of 0.0002-0.09 mM. Dilution factors of 4 and 16 were needed to measure the $Fe^{2+}$ in the experiments with dosages of 0.26 and 1 mM, respectively. For the dosages of 0.003 and 0.033 mM $Fe^{2+}$, no dilution was required.

The $NO_3^-$ concentration in all experiments was determined by an ion chromatograph (Metrohm 881 Compact IC pro-Anion) with an A Supp 16-150/4.0 anion column. For experiments using 0.03 mM $BrO_3^-$ (A), $BrO_3^-$ and $Br^-$ were measured by the same equipment as for $NO_3^-$. The detection limits of $BrO_3^-$, $Br^-$ and $NO_3^-$ were 0.008 mM, 0.001 mM and 0.002 mM, respectively. For experiments using 0.5 µM $BrO_3^-$ (B), water samples were analysed at Het Waterlaboratorium (Haarlem, The Netherlands), where an ion chromatograph (Dionex ICS-300) with IonPac AS9SC column (250mmx 4mmID) was used to measure $BrO_3^-$. An ion chromatograph (Dionex ICS-1100) with IonPac AG22 column (4 x 50 mm) and IonPac AS22SC column (4 x 250 mm) was used to measure $Br^-$. The detection limits for $BrO_3^-$ and $Br^-$ were 0.004 µM and 0.125 µM, respectively.

# 3 Results

## 3.1 $BrO_3^-$ reduction rate and mass balance

Figure 3 presents the kinetics of $BrO_3^-$ reduction and $Br^-$ formation within 120 hours after the addition of 0.26 and 1 mM $Fe^{2+}$. The experiments were executed at pH 5.2 and 7.0, the latter being most representative for MAR water. For $BrO_3^-$ (0.03 mM), 0.26 mM $Fe^{2+}$ dosage was close to the stoichiometric ratio (1 mol $BrO_3^-$ : 6 mol $Fe^{2+}$) according to equation (1). For this particular setting, >90% of initial $BrO_3^-$ reduced into $Br^-$ within 120 hours (Figure 3-a and 3-c). The $BrO_3^-$ reduction fits second order reaction kinetics well. Moreover, the kinetic constant for the pH 5.2 and 7.0 was 2.5 and 3.3 respectively, indicating pH 7 promoted $BrO_3^-$ reduction compared to pH 5.2. In the case of the 1 mM $Fe^{2+}$ dosage, $BrO_3^-$ reduction was accelerated, with almost 100% $BrO_3^-$ reduction to $Br^-$ within 1 hour at pH 5.2 (Figure 3-b) and at pH 7.0 (Figure 3-d). The above results indicate that the higher the $Fe^{2+}$ dosage, the higher the $BrO_3^-$ reduction rate, which is in line with existing literature (Dong et al., 2009; Siddiqui et al., 1994).

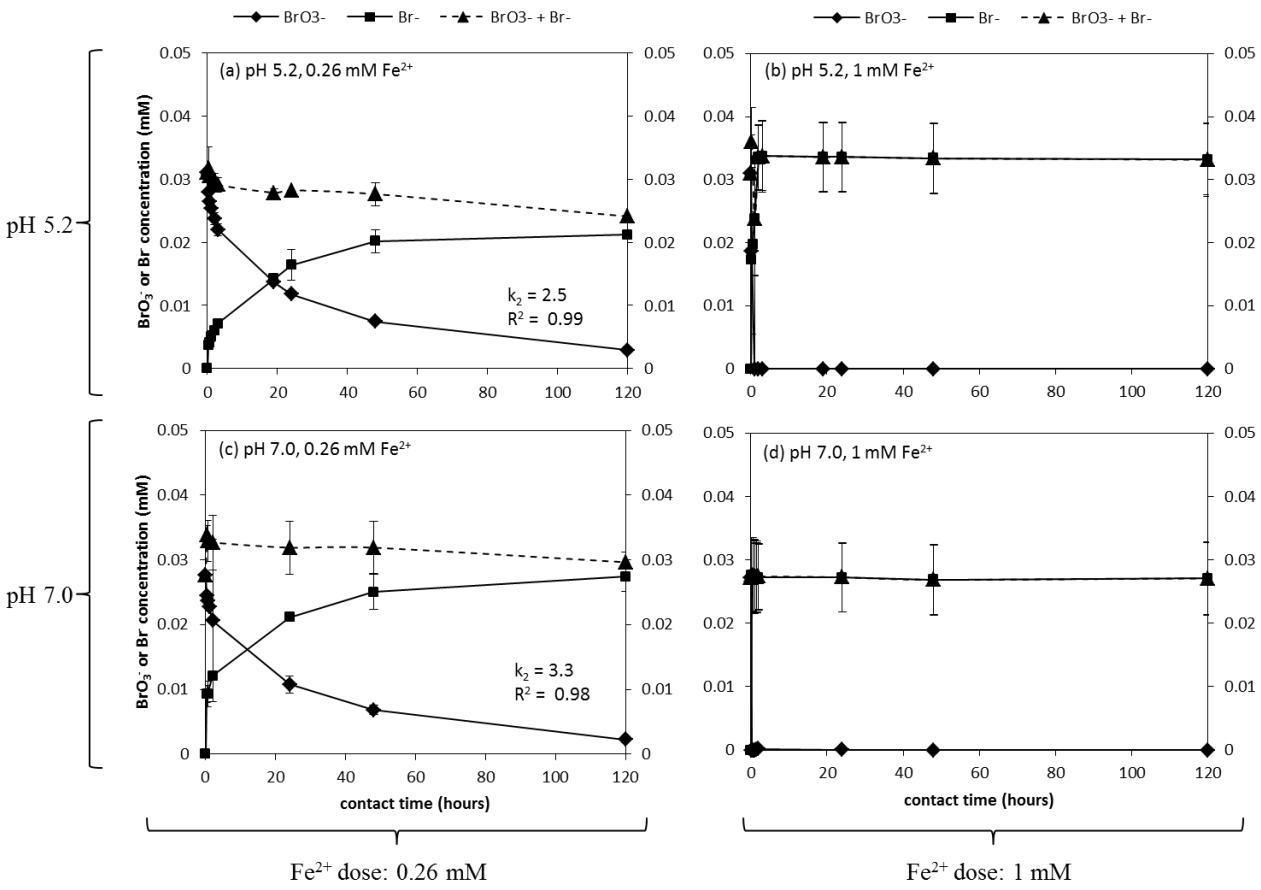


Figure 3 $BrO_3^-$ reduction after dosing 0.26 mM (a, c) or 1 mM (b, d) $Fe^{2+}$. Initial $BrO_3^-$ concentration was 0.03 mM, initial pH levels were 5.2 and 7.0

Figure 4 shows the consumed $Fe^{2+}/BrO_3^-$ ratios after 24, 48 and 120 hours. In the case of the 1 mM $Fe^{2+}$ dosage (corresponding to an initial ratio of $Fe^{2+}/BrO_3^- = 33$), consumed $Fe^{2+}/BrO_3^-$ ratios were higher than the theoretical ratio of 6 according to equation (1) which assumes a total reduction of $BrO_3^-$ to $Br^-$. After 24 hours, the ratios were 8.0 and 9.2 for pH 5.2 and pH 7.0, respectively, with $BrO_3^-$ reduced to below the detection limit. Between 24-120 hours, $Fe^{2+}$ continued to be consumed, and correspondingly $Fe^{2+}/BrO_3^-$ ratios increased. This may be explained by $Fe^{2+}$ adsorption onto hydrolysed $Fe^{3+}$ flocs of hydrous ferric oxides (HFO). Interestingly, there was a consumed $Fe^{2+}/BrO_3^-$ ratio below the stoichiometric ratio of 6 in the case of the 0.26 mM $Fe^{2+}$ dosage (corresponding initial $Fe^{2+}/BrO_3^- = 8$). The ratios below 6 could indicate the production of intermediate Br species during the reduction of $BrO_3^-$, requiring less $Fe^{2+}$ compared to the total reduction of $BrO_3^-$ to $Br^-$ as in equation (1). Additionally, the molar mass sum of $BrO_3^-$ and $Br^-$

slightly decreased during the experiment with 10% - 20%, indicating that intermediate products may have formed.

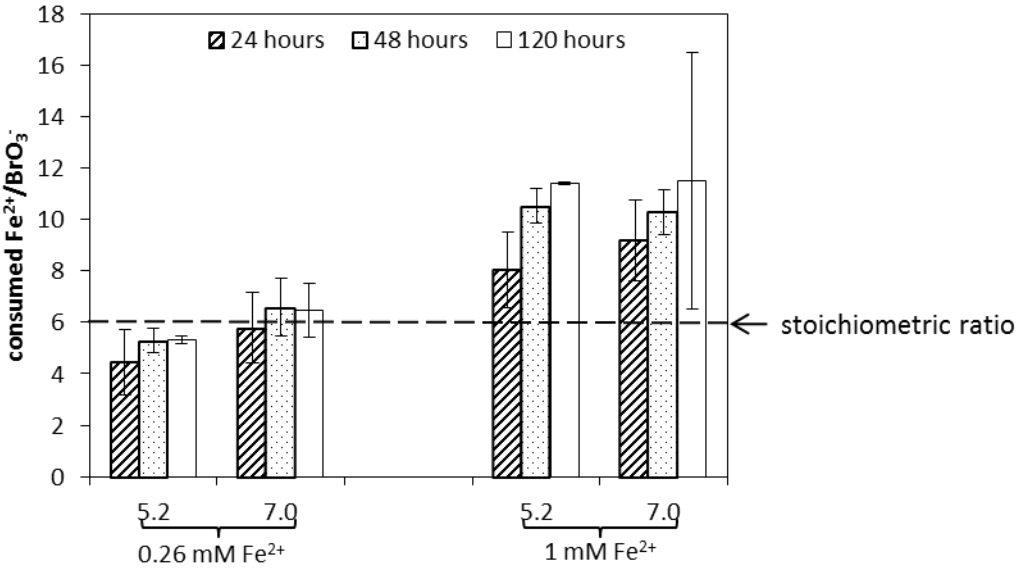


Figure 4 The consumed $Fe^{2+}/BrO_3^-$ ratios after dosing 0.26 mM or 1 mM $Fe^{2+}$ to a solution containing 0.03mM $BrO_3^-$ at two initial pH levels, 5.2 and 7.0

## 3.2 $NO_3^-$, a competing electron acceptor?

$NO_3^-$ is known to act as a competitive electron acceptor in the reaction with $Fe^{2+}$ (Buresh & Moraghan, 1976). Figure 5 depicts $BrO_3^-$ reduction by $Fe^{2+}$ in the presence of $NO_3^-$ at a concentration at the same order of magnitude as $BrO_3^-$ (0.07 mM). The rate of $BrO_3^-$ reduction in the presence of $NO_3^-$ was slightly lower, compared to the absence of $NO_3^-$ (Figure 3-c). $NO_3^-$ concentrations in these experiments were steady during the 120 hours for both $Fe^{2+}$ dosages (Figure 5-a and 5-b), indicating $Fe^{2+}$ did not reduce $NO_3^-$ when $BrO_3^-$ and $NO_3^-$ were simultaneously present.

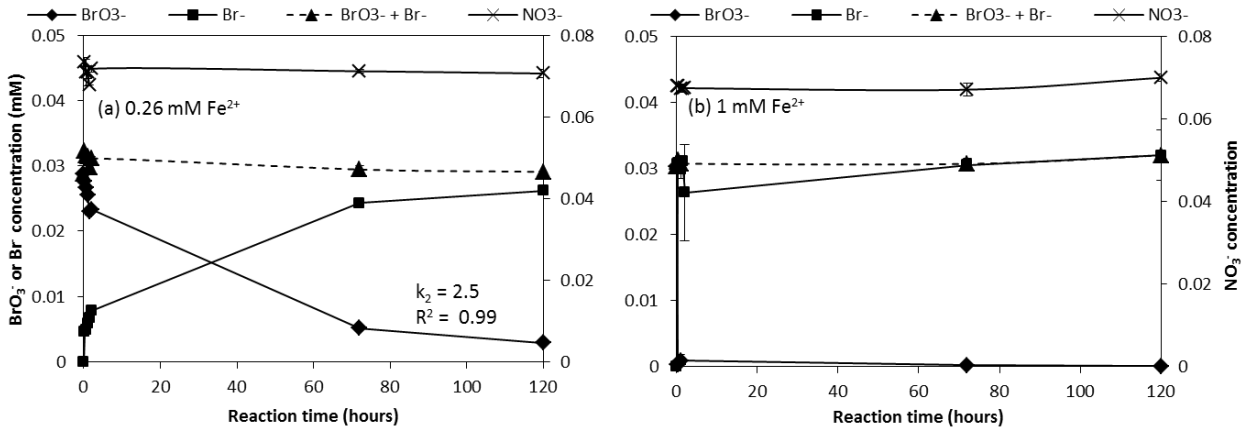


Figure 5 $BrO_3^-$ reduction after dosing 0.26 mM (a) or 1 mM (b) $Fe^{2+}$ in the presence of $NO_3^-$. Initial $BrO_3^-$ and $NO_3^-$ concentrations were 0.03 mM and 0.07mM respectively, initial pH was 7.0

Figure 6 shows the $BrO_3^-$ and $Fe^{2+}$ consumption in the presence and absence of 0.07 mM $NO_3^-$. $BrO_3^-$ removal in the presence and the absence of $NO_3^-$ was the same for both $Fe^{2+}$ dosages, while the presence of $NO_3^-$ lead to a higher $Fe^{2+}$ consumption in the case of the 0.26 mM $Fe^{2+}$ dosage. The additional $Fe^{2+}$ removal (62%→71%), 0.02 mM, might have reacted with $NO_3^-$, but the change would have remained undetected given that it would have resulted in a calculated reduction of <0.005 mM $NO_3^-$ ($NO_3^-/Fe^{2+}$ ratio, equations (4)-(5)). This would not have been noted with our $NO_3^-$ analytical methods. Nevertheless, based on the above results it can be concluded that $BrO_3^-$ reduction was hardly affected by $NO_3^-$ presence and that $Fe^{2+}$ preferred $BrO_3^-$ to $NO_3^-$ as an electron acceptor. This was also observed by Westerhoff (2003), who suggested that the difference in structure (atomic radii and O-bonds) makes it relatively easier to remove an O atom from a $BrO_3^-$ ion compared to a $NO_3^-$ ion.

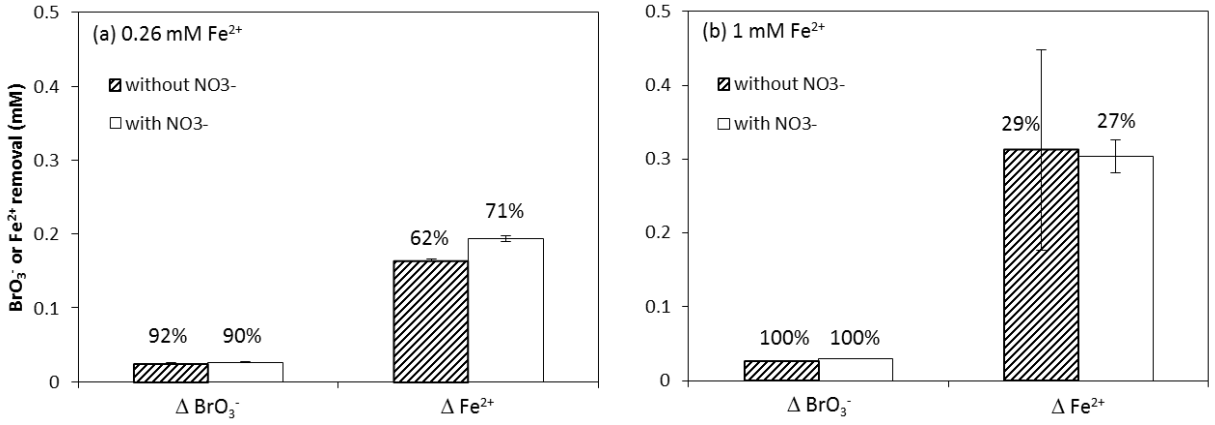

Figure 6 The effect of 0.07 mM $NO_3^-$ on the reduction of 0.03mM $BrO_3^-$ in 120 hours after dosing 0.26 mM (a) or 1 mM (b) $Fe^{2+}$ at initial pH 7.0. % corresponds to the removal percentages

## 3.3 pH change and $Fe^{3+}$ hydrolysis

Although the $BrO_3^-$ reduction equation (1) shows a pH increase, reduction of $BrO_3^-$ by $Fe^{2+}$ consequently means that $Fe^{2+}$ is oxidised to $Fe^{3+}$ and subsequently, $Fe^{3+}$ will hydrolyse to form flocs of hydrous ferric oxides (HFO) (Stefánsson, 2007). Therefore, the pH will drop based on equation (3), the combined $BrO_3^-$ reduction with $Fe^{3+}$ hydrolysis. The pH drop was observed in all of the 0.03 mM $BrO_3^-$ experiments: 1.5-1.6 drop and 2.6-3.0 drop in the case of initial pH 5.2 and 7.0 respectively. The pH drop was an indicator of $Fe^{3+}$ hydrolysis. Moreover, the observed yellow flocs in the batch reactors are also an evidence of HFO formation. The above two phenomena (pH decrease and visible flocs) are strong indications that HFO flocs had been formed in the reactors. The adsorption of $Br^-$ or $BrO_3^-$ onto HFO flocs is not expected to have occurred, as $BrO_3^-$ and $Br^-$ have no affinity for HFO (Shen et al., 2017). However, $Fe^{2+}$ adsorption onto the flocs has been frequently reported (Hiemstra & van Riemsdijk, 2007; Siddiqui et al., 1994; Williams & Scherer, 2004), which may explain the observed $Fe^{2+}/BrO_3^-$ removal ratios beyond the stoichiometric ratio of 6 (in Figure 4).

## 3.4 $BrO_3^-$ reduction under concentrations similar to MAR

To investigate the rate of $BrO_3^-$ reduction by $Fe^{2+}$ in concentrations similar to MAR, the reduction kinetics were monitored for 0.5 µM $BrO_3^-$ after dosing 0.003 and 0.033 mM $Fe^{2+}$. Figure 7-a and 7-b show the $BrO_3^-$ and $Br^-$ kinetics in absence of $NO_3^-$ while Figure 7-c and Figure 7-d show the kinetics of $BrO_3^-$ and $Br^-$ in the presence of 0.16 mM $NO_3^-$. As in the previous experiments with high $BrO_3^-$ concentrations, the reduction rate of 0.5 µM $BrO_3^-$ also depends on the $Fe^{2+}$ concentration, with a higher rate at a higher $Fe^{2+}$ concentration. After 120 hours contact time, Figure 7-a and 7-c show a limited $BrO_3^-$ reduction (7% in the absence of $NO_3^-$ and 12% in the presence of $NO_3^-$) at 0.003 mM $Fe^{2+}$, while Figure 7-b and 7-d show a considerable $BrO_3^-$ reduction at 0.033 mM $Fe^{2+}$ (74% in the absence of $NO_3^-$ and 58% in the presence of $NO_3^-$). Assuming second order reaction kinetics, it was calculated that the rate constant of $BrO_3^-$ reduction at a higher $Fe^{2+}$ dosage (0.033 mM) was 0.049 and 0.023 in the absence and the presence of $NO_3^-$, respectively. Although the $NO_3^-$ concentration was three orders of magnitude higher than the $BrO_3^-$ concentration, the $NO_3^-$ concentration was steady (Figure 7-c and 7-d). It is noteworthy that during these experiments the molar mass sum of $BrO_3^-$ and $Br^-$ also slightly decreased

from 0.50 µM to 0.48 µM and 0.46 µM for 0.003 mM and 0.033 mM $Fe^{2+}$ dosages respectively, indicating the formation of Br intermediate species.

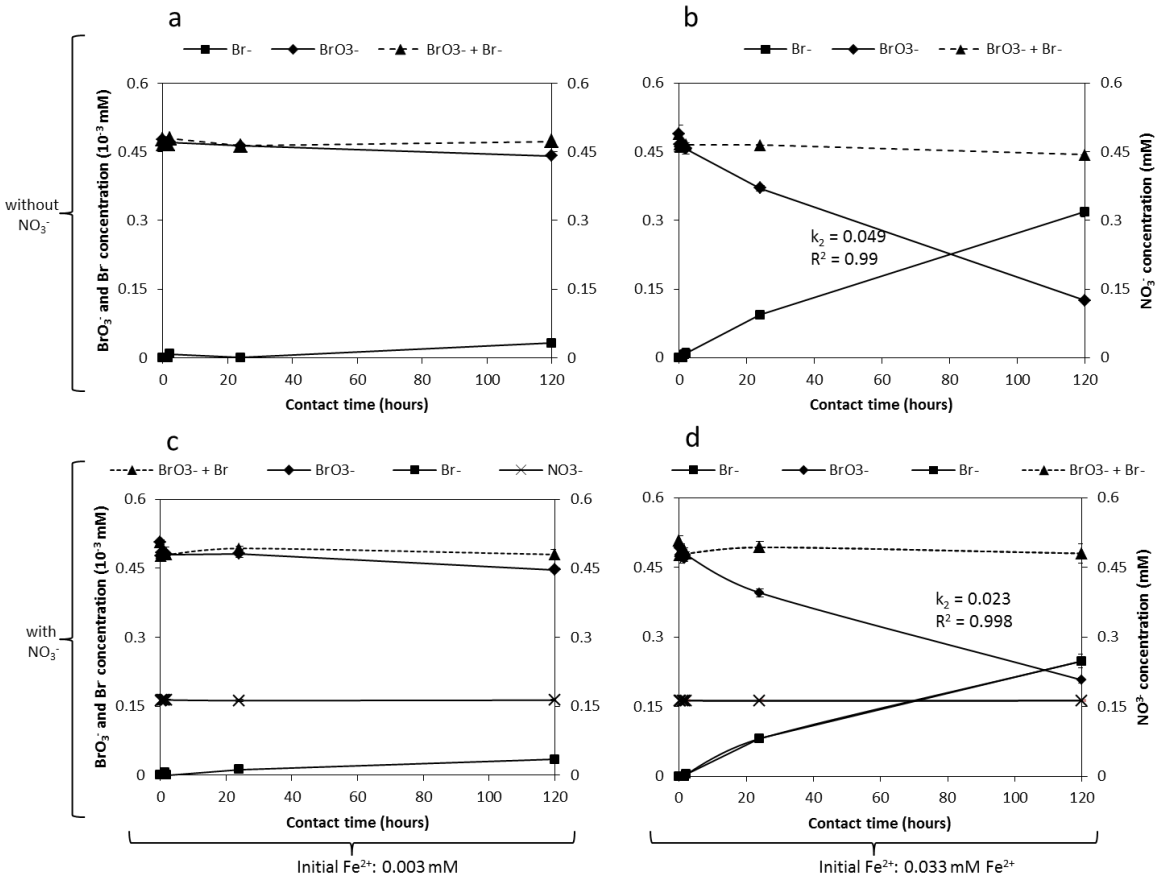


Figure 7 $BrO_3^-$ reduction after dosing 0.003 mM (a, c) or 0.033 mM (b, d) $Fe^{2+}$, simulating MAR concentrations, in the presence (a, b) and the absence (c, d) of $NO_3^-$. Initial $BrO_3^-$ and $NO_3^-$ concentrations were 0.5 µM and 0.16 mM (c, d), repectively. Initial pH was 7.0

Figure 8 shows the reduction of $BrO_3^-$ and the consumption of $Fe^{2+}$ in presence and absence of 0.16 mM $NO_3^-$. In the case of the 0.003 mM $Fe^{2+}$ dosage, it appears that the presence of $NO_3^-$ did not influence $BrO_3^-$ reduction and $Fe^{2+}$ oxidation (Figure 8-a). In the case of the 0.033 mM $Fe^{2+}$ dosage, the presence of $NO_3^-$ led to a lower $BrO_3^-$ reduction and a lower $Fe^{2+}$ oxidation (Figure 8-b). Combining the results in Figure 7 and Figure 8 indicates that $Fe^{2+}$ preferred $BrO_3^-$ to $NO_3^-$ as an electron acceptor but it did inhibit $BrO_3^-$ reduction to some extent. This could possibly be onset by considerably higher $NO_3^-$ concentrations compared to $BrO_3^-$, in combination with the stoichiometric excess of $Fe^{2+}$. One potential reason for explaining the inhibition by $NO_3^-$ is the hypothesized formation

of NO from $NO_3^-$ complexed with $Fe^{2+}$ (Baldwin & Van Weert, 1996) and, therefore, it slowing down the reduction of $BrO_3^-$.

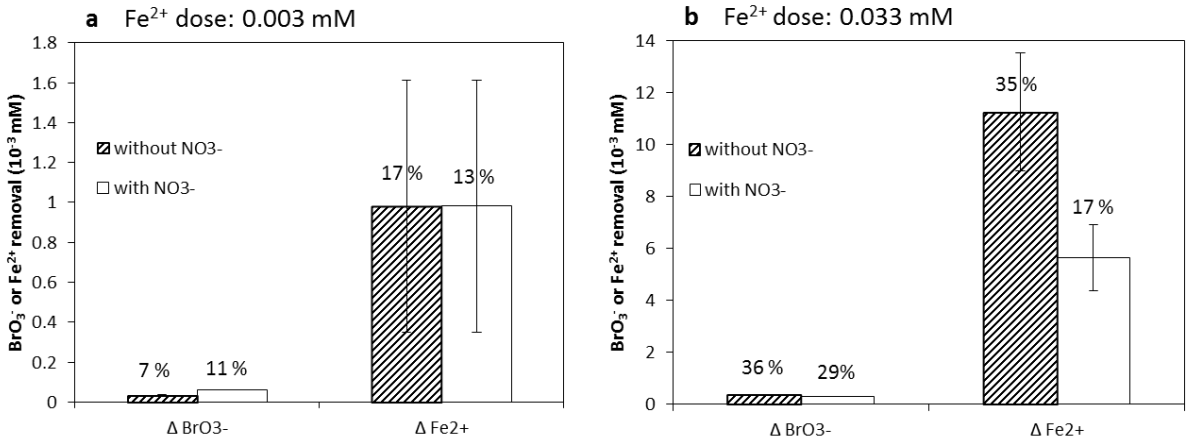


Figure 8 The consumed $BrO_3^-$ and $Fe^{2+}$ in 120 hours after dosing 0.003 mM (a) and 0.033 mM (b) $Fe^{2+}$ in the presence and the absence of $NO_3^-$ at initial pH 7.0. Initial $BrO_3^-$ and $NO_3^-$ concentrations were 0.5 µM and 0.16 mM, respectively. % corresponds to the removal percentages

# 4 Discussion

## 4.1 $BrO_3^-$ reduction mechanism

Figure 9-a shows a summary of the mole sum of $BrO_3^-$ and $Br^-$ in all the experiments with an initial dosage of 0.03 mM $BrO_3^-$. No Br mass loss was observed in the case of a sufficiently high initial $Fe^{2+}/BrO_3^-$ ratio (33) while the molar sum of $BrO_3^-$ and $Br^-$ was 78%-90% of the initial Br in the case of lower stoichiometric $Fe^{2+}/BrO_3^-$ ratios (8). As adsorption of $BrO_3^-$ and $Br^-$ onto HFO flocs is very unlikely (Shen et al., 2017), it is a possibility that Br intermediate species formed during the reduction of $BrO_3^-$. Equations (6)-(8) show the reduction pathways of $BrO_3^-$ to intermediate species requiring less $Fe^{2+}$ as reported by Shen et al. (2017) and Siddiqui et al. (1994):

$$BrO_3^- + 2Fe^{2+} + 2H^+ \rightarrow 2Fe^{3+} + BrO_2^- + H_2O \quad (6)$$

$$BrO_2^- + 2Fe^{2+} + 2H^+ \rightarrow 2Fe^{3+} + BrO^- + H_2O \quad (7)$$

$$BrO^- + 2Fe^{2+} + 2H^+ \rightarrow 2Fe^{3+} + Br^- + H_2O \quad (8)$$

The most frequently reported intermediate species is hypobromous acid ($HOBr/BrO^-$) (Ohura et al., 2004; Siddiqui et al., 1994). Furthermore, the study of Shen et al. (2017)

showed that the sum of $BrO_3^-$, HBrO/$BrO^-$ and $Br^-$ was 98-101% of the initial Br (as $BrO_3^-$) concentration and, therefore, almost no other intermediate species except for HOBr/BrO- existed. Taken together, $BrO_3^-$ was reduced into the end product $Br^-$ most likely via the intermediate species HBrO/BrO during the reaction of $BrO_3^-$ and $Fe^{2+}$ in this study.

Figure 9-b shows the mole sum change of $BrO_3^-$ and $Br^-$ in the case of an initial $Fe^{2+}/BrO_3^-$ ratio of 8 at pH 5.2 and at 7.0. More Br loss was observed at pH 5.2 (22%) than at pH 7.0 (12%). Based on the total reaction (equation 3), pH 5.2 would slowed down the $BrO_3^-$ reduction, providing the intermediate species with a longer lifetime and thus a better chance to be detected in this experiment. Moreover, the intermediate species formation requires less $Fe^{2+}$ as shown in equations (6)-(8). It may be one potential reason for the observation in Figure 4: a relatively low consumed $Fe^{2+}/BrO_3^-$ ratio at pH 5.2 compared to pH 7.0. Although the lower ratio for pH 5.2 can also partially be explained by the decreased sorption of $Fe^{2+}$ onto precipitating HFO in this experiment while the higher $Fe^{2+}/BrO_3^-$ ratio for pH 7.0 correlates with promoted $Fe^{2+}$ sorption at higher pH (Hiemstra & van Riemsdijk, 2007).

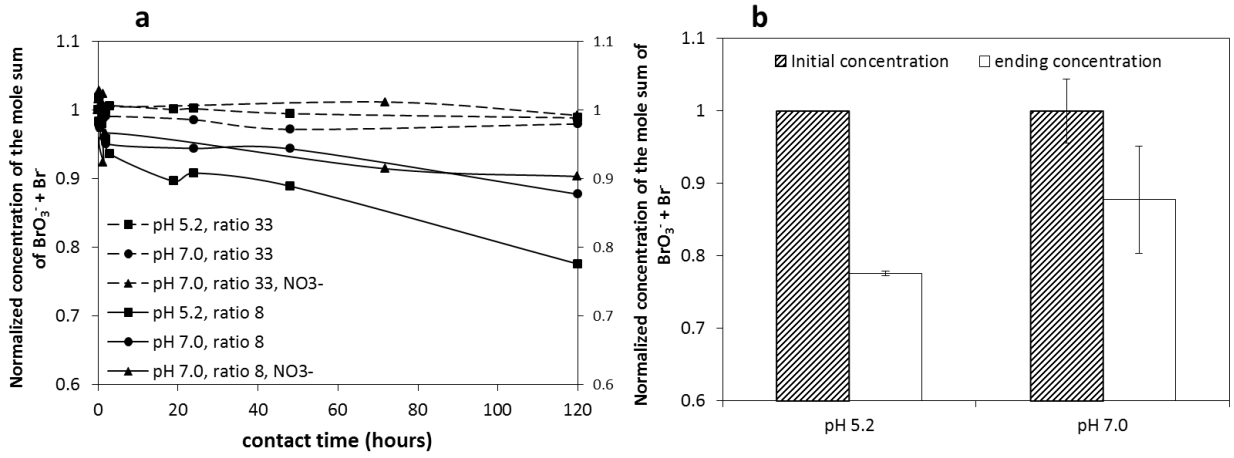


Figure 9 The mole mass sum of $BrO_3^-$ and $Br^-$ in all experiments with initial $BrO_3^-$ concentration 0.03 mM (a) and the comparison of mole mass loss of $BrO_3^-$ and $Br^-$ in 120 hours between pH 5.2 case and pH 7.0 case (b). Ratio means the initial ratio of $Fe^{2+}/BrO_3^-$. n=2

## 4.2 Feasibility of $BrO_3^-$ reduction by $Fe^{2+}$ during MAR

Based on the results in section 3.1 and 3.4, a preliminary conclusion can be drawn that under anoxic conditions and at a sufficiently high $Fe^{2+}/BrO_3^-$ ratio, chemical $BrO_3^-$ reduction can be achieved. In MAR systems, $Fe^{2+}$ concentrations tend to be $10^{-3}$-$10^{-2}$ mM. Fortunately, the same is the case for $BrO_3^-$ production after ozone-based AOPs, where concentrations are generally limited to $10^{-5}$-$10^{-4}$ mM (Wang et al., 2016; Xiao et al., 2017). $Fe^{2+}$ concentrations detected in Dunea MAR effluent range from 0.0015 to 0.029 mM, so the $Fe^{2+}/BrO_3^-$ ratio in MAR systems are sufficiently high (15-2900). From a drinking water production perspective, the extremely slow $BrO_3^-$ reduction shown in section 3.4 might seem to be a very inefficient process since treatment technologies most often have contact times of minutes. However, MAR residence times in the subsurface are weeks to months (Maeng et al., 2011; Wang et al., 2017), making this process a very viable $BrO_3^-$ removal pathway. Assuming that $Fe^{2+}$ and $BrO_3^-$ concentrations in Fe-reducing anoxic zones and the $BrO_3^-$ reduction follows second order kinetics as in Figure 7-b ($k_2$ = 0.049), the required time to reduce $BrO_3^-$ below the drinking water guideline of 10 µg/L (0.08 µM) is on the order of magnitude of 10-20 days.

As stated previously, the theoretical sequence of MAR infiltration zones follows the order of oxic - $NO_3^-$-reducing - Mn-reducing - Fe-reducing - $SO_4^{2-}$-reducing (Stuyfzand, 1989), but the practical possible cross of different flowlines may result in the joint presence of $NO_3^-$ and $Fe^{2+}$. The results in Figure 7 indicate a small negative effect of $NO_3^-$ as an inhibitor for $BrO_3^-$ reduction by $Fe^{2+}$, though at sufficiently high $Fe^{2+}$ concentrations bromate reduction is still not inhibited. Although, $NO_3^-$reduction by $Fe^{2+}$ is thermodynamically not feasible, in the presence of catalysts this reaction may occur (Eckert & Appelo, 2002). A previous study reported that the presence of $Ni^{2+}$, $Cu^{2+}$ and $Ag^{2+}$ promoted the reaction of $Fe^{2+}$ with $NO_3^-$ (Buresh & Moraghan, 1976). Given the presence of these elements in nature, for example the concentration of $Cu^{2+}$ at Dunea's MAR site is $10^{-2}$ mM, these may well onset $NO_3^-$ reduction by $Fe^{2+}$. Moreover, previous studies (Benz et al., 1998; Brons et al., 1991; Oshiki et al., 2013) reported $NO_3^-$-dependent $Fe^{2+}$ oxidation mediated by anaerobic ammonium oxidation bacteria, *Escherichia coli* and $NO_3^-$-reducing bacteria. Therefore, a microbial mediated kinetic reaction of $Fe^{2+}$ and $NO_3^-$ could also occur, leading to competition for $BrO_3^-$ reduction in these mixing flow paths during MAR systems.

Altogether, this study has shown that chemical $BrO_3^-$ reduction by $Fe^{2+}$ is expected to occur in the Fe-reducing anoxic zones during MAR and that $NO_3^-$ on its own is not a

strong inhibitor or competitor; nevertheless the complexity of subsurface processes may still onset conditions where $NO_3^-$ reduction is favoured over $BrO_3^-$. Therfore, a subsequent study to investigate $BrO_3^-$ reduction in simulated Fe-reducing zones, for example a column study, is highly recommended, also to include microbiological and biochemical processes which take place during MAR.

## 5 Conclusions

Based on anoxic batch experiments, it is concluded that $BrO_3^-$ is readily reduced by $Fe^{2+}$. The reaction rate was influenced by the initial $Fe^{2+}/BrO_3^-$ ratio, as well as by the initial pH, i.e. a higher $Fe^{2+}$ concentration and higher pH accelerated the reaction. The pH dropped considerably during the experiments, onset by the hydrolysis of $Fe^{3+}$ to HFO flocs. These HFO flocs were found to adsorb $Fe^{2+}$, particularly at high $Fe^{2+}/BrO_3^-$ ratios, whereas at low $Fe^{2+}/BrO_3^-$ ratios the incomplete $BrO_3^-$-$Br^-$ mass balance indicated formation of intermediate species. Overall it can be concluded that the chemical reduction of $BrO_3^-$ by naturally occurring $Fe^{2+}$ during MAR can occur, as extensive retention times in the subsurface will compensate for the slow reaction kinetics of low $BrO_3^-$ and $Fe^{2+}$ concentrations. In the specific case that $Fe^{2+}$ containing and $NO_3^-$ containing waters cross flow paths during MAR, the presence of $NO_3^-$ will not compete with $BrO_3^-$ as $Fe^{2+}$ is preferred $BrO_3^-$ over $NO_3^-$ as an electron acceptor. However, it was found that the presence of $NO_3^-$ may somewhat inhibit $BrO_3^-$ reduction when $NO_3^-$ concentrations are far higher than $BrO_3^-$ concentrations.

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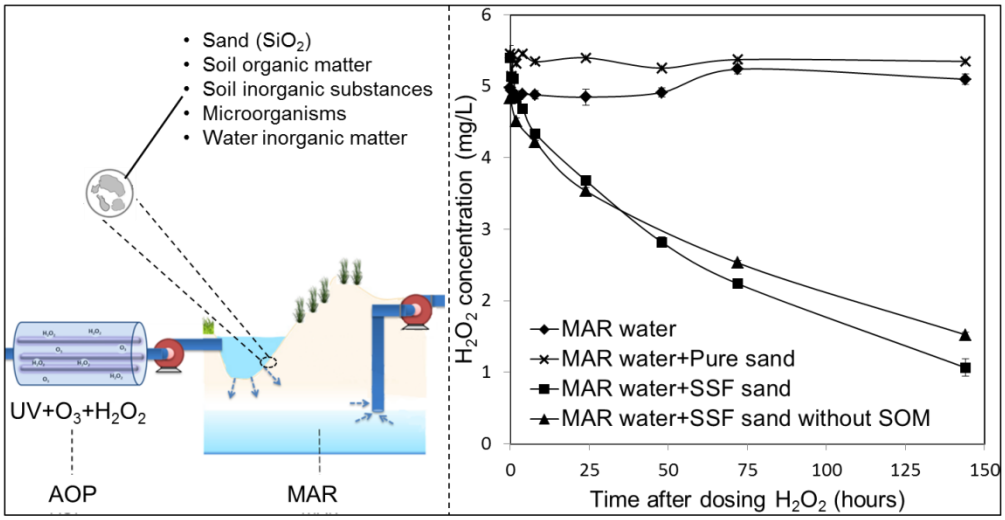
# 4

# The fate of $H_2O_2$ during managed aquifer recharge: A residual from advanced oxidation processes for drinking water production

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Wang F., van Halem D., van der Hoek J.P. 2016. The fate of $H_2O_2$ during managed aquifer recharge: A residual from advanced oxidation processes for drinking water production. *Chemosphere*, 148, 263-269.

## Abstract

The fate of $H_2O_2$ residual from advanced oxidation process (AOP) preceding managed aquifer recharge (MAR) is of concern because $H_2O_2$ could lead to undesired effects on organisms in the MAR aquatic and soil ecosystem. The objective of this study was to distinguish between factors affecting $H_2O_2$ decomposition in MAR systems, simulated in batch reactors with synthetic MAR water and slow sand filter sand. The results showed that pure sand and soil organic matter had no considerable effect on $H_2O_2$ decomposition, whereas naturally occurring inorganic substances on the surface of sand grains and microbial biomass are the two main factors accelerating $H_2O_2$ decomposition in MAR systems. Additionally, the results showed that the $H_2O_2$ decompositions with different initial concentrations fitted first-order kinetics in 2-6 hours in a mixture of slow sand filter sand (as a substitute for sand from a MAR system) and synthetic MAR water with high bacterial population. An estimation indicated that low concentrations of $H_2O_2$ (<3 mg/L) could decompose to the provisional standard of 0.25 mg/L in the first centimeters of MAR systems with the influent water containing high microbial biomass 38 ng ATP/mL.

# 1 Introduction

Managed aquifer recharge (MAR), such as river bank filtration, dune infiltration and artificial recharge, is a natural water treatment process that induces surface water to flow through soil/sediment and into a vertical or horizontal well (Maeng et al., 2011; Tufenkji et al., 2002). This treatment process is robust and cost-effective and is frequently applied in Europe (Van der Hoek et al., 2014). For example, in the Netherlands and Germany, water utilities using MAR as a water treatment process supply drinking water without chlorination as disinfection process (Lekkerkerker, 2012; Maeng, 2010). Previous research demonstrated that the combination of advanced oxidation process (AOP) and subsequent MAR is a potential treatment system to remove various organic micro-pollutants (OMPs) during drinking water production (Lekkerkerker-Teunissen et al., 2012; Lekkerkerker et al., 2009a; Oller et al., 2011b). A disadvantage of applying AOP with $O_3$ is the formation of bromate during oxidation of bromide containing waters. In order to reduce the formation of bromate which has been designated as carcinogenic to humans (Kurokawa et al., 1990), $H_2O_2$ should be dosed excessively (Knol, 2012; Von Gunten & Oliveras, 1998; Wert et al., 2007). Consequently, the MAR infiltration water may contain residual concentrations of $H_2O_2$.

A number of studies about $H_2O_2$ decomposition in aquatic ecosystems and soil ecosystems have focused on biotic factors, such as bacteria (Richard et al., 2007; Zappi et al., 2000) and other microorganisms (Cooper & Lean, 1989; Richard et al., 2007) and abiotic factors, such as iron (Moffett & Zafiriou, 1993; Wilson et al., 2000), manganese (Do et al., 2009; Häkkinen et al., 2004; Russo et al., 2013), transition metals (Lousada & Jonsson, 2010; Moreno et al., 2011), lanthanide oxides (Lousada et al., 2013) and iodide (Wong & Zhang, 2008). $H_2O_2$ decomposition in water also has been reported (Cooper & Lean, 1989; Moffett & Zafiriou, 1993; Richard et al., 2007; Wilson et al., 2000). The results of Schumb (1949) showed that manganese and iron were extremely reactive with concentrated $H_2O_2$ solutions. Also, $H_2O_2$ decomposition studies have been conducted in metal- or DOC-rich waters (Chiritã, 2009; Wilson et al., 2000). Previous research found that a large fraction of $H_2O_2$ loss in both a fresh water system and soil was attributable to biotic mechanisms. Richard et al. (2007) found that biologically based reactions (i.e., catalase) were the primary mechanism for $H_2O_2$ decomposition in a shallow fresh water system in New Zealand. It was observed from the literature of Zappi et al. (2000) that the first-order rate constant of biotic reactions was always much higher than that of abiotic reactions for $H_2O_2$ decomposition in various soils with different calcium, iron, manganese,

TOC and phosphorus contents. It is clear that the fate of $H_2O_2$ in aquatic systems has been investigated comprehensively, and a few studies focused on the reactions of $H_2O_2$ with natural-occurring constituents in soil (Bissey et al., 2006; Miller & Valentine, 1999). These publications investigated the stability of $H_2O_2$ as the oxygen source for bioremediation activities in soil, because of several potential interactions of $H_2O_2$ with various soil constituents and its potentially fast decomposition. Studies of Morgan and Watkinson (1992) and Schumb (1949) reported reaction of $H_2O_2$ with naturally occurring stabilizers, such as tripolyphosphate, $MnO_4^-$ and $Cu^{2+}$ within soils. Bissey et al. (2006) investigated the interactions between catalyzed $H_2O_2$ propagations and soil organic matter (SOM) within surface soil and reported that the $H_2O_2$ decomposition rate decreased with the increase of SOM at neutral pH. Miller and Valentine (1999) examined mechanisms and kinetics of abiotic $H_2O_2$ decomposition in the presence of sand collected from an aquifer and a riverbed. However, more understanding is needed to determine the fate of $H_2O_2$ in MAR systems specifically. High concentrations of $H_2O_2$ can cause damage to cell membranes and have deleterious effects on biological systems (Ananthaswamy & Eisenstark, 1976; Collén & Pedersén, 1996; Wong et al., 2003). Schmidt et al. (2006) concluded that $H_2O_2$ minimum inhibitory concentration (MIC) to the most sensitive bacteria species *Psedomonas aeruginosa* was 5.1 mg/L. The study of Urfer (1998) demonstrated that the continuous presence of around 1 mg/L $H_2O_2$ did not lead to a major inhibition of the biological removal of acetate and formate in a lab-scale sand drinking water biofilter. Knol (2012) stated that even very low concentrations of $H_2O_2$ could lead to undesired destruction of organisms in MAR infiltration ponds and he mentioned a provisional standard of 0.25 mg/L $H_2O_2$ for MAR infiltration water. Consequently, an improved understanding of the fate of $H_2O_2$ in MAR systems would be essential to see whether this provisional standard or lower concentrations can be reached.

The objective of this study was to distinguish between different factors affecting $H_2O_2$ decomposition in MAR systems. The general approach in this study was to divide the aquifer environment into two separate physical compartments (water and sand) that contain naturally existing biological and chemical species that might react with $H_2O_2$. Batch reactor experiments were conducted to determine the reactions of $H_2O_2$ with biotic (microbial community in water) and abiotic constituents (pure sand particles, inorganic ions in infiltration water, SOM in MAR sand and naturally occurring inorganic substances coating on sand).

# 2 Materials and methods

## 2.1. Materials

The top 0.5-2.0 cm (schmutzdecke) of a slow sand filter (SSF) has diverse microbial communities and greatly contributes to the removal of organic matter by biodegradation processes, so this layer is considered to represent aerobic microbial activity of sand filtration systems (Chekol, 2009; Dizer et al., 2004). The SSF sand in the facilities of drinking water utility Dunea (The Hague, the Netherlands) originated from the dune infiltration area. Consequently, schmutzdecke sand (top of SSF) with natural microbial communities was used in batch reactors as a substitute for the sand in the dune infiltration ponds. As a reference, pure sand (silicon dioxide without any impurities; 1.07711.1000, VWR company) was used. The water for batch reactors was prepared with demineralized water (demi-water) and additive chemicals (33 mg $Na_2HPO_4$/L, 7.5 mg $NaH_2PO_4$/L, 22 mg $K_2HPO_4$/L, 140 mg $CaCl_2$/L, 0.031 mg $FeCl_3$/L, 0.032 mg $NH_4Cl$/L, 40.75 mg $MgSO_4$/L, 17.823 mg $NaNO_3$/L, 0.00114 mg $MnCl_2$/L, 82 mg $CH_3COONa$/L) to simulate the water quality at the MAR site of Dunea. The characteristics are presented in Table 1. Based on preliminary experiments, it was found that $CH_3COONa$ (Merck, Germany) was rapidly consumed as the source of DOC in the batch reactors, so 24 mg/L DOC was added in order to have residual DOC in the reactors and avoid bacterial starving conditions. Dosing carbon source to levels exceeding natural MAR systems may lead to higher microbial biomass concentration in batch reactors than in natural MAR systems (Pharand et al., 2014) and enhance the endurance ability to decompose $H_2O_2$. Therefore, a short inventory was performed based on observed adenosine triphosphate (ATP) concentrations in different waters to estimate the effect of carbon dosage on $H_2O_2$ decomposition (§ 3.4). The $H_2O_2$ solutions were prepared from a 30% standard solution (Merck, Germany). All the solutions used in this study were prepared using water from a Millipore Milli-Q system. All chemicals were of AR grade.

Table 1 The quality of MAR influent water in Dunea and synthetic MAR water used in batch reactors

| Parameter | $O_2$ (mg/L) | pH | $NH_4^+$-N (mg/L) | $NO_3^-$-N (mg/L) | $SO_4^{2-}$ (mg/L) | $Fe^{3+}$ (mg/L) | $Mn^{2+}$ (mg/L) | DOC (mg/L) |
|---|---|---|---|---|---|---|---|---|
| MAR influent water | 10.4±1.2 | 7.9±0.2 | 0.00997 | 3.7±0.1 | 48±2 | 0.0106 | 0.001 | 3.9±0.7 |
| Synthetic MAR water | 9±1.0 | 7.8±0.3 | 0.00847 | 2.9±0.1 | 30.6±2 | 0.0106 | 0.0005 | 22±2 |

## 2.2. Batch experimental setup

Batch experiments were performed with 39 glass batch reactors with a volume of 1 L for around 3 months. Batch reactors were filled with 100 g SSF sand and 500 mL synthetic MAR water to simulate MAR systems (Lekkerkerker, 2012; Maeng, 2010). In addition, reference batch reactors were prepared with 100 g pure sand silicon dioxide and 500 mL synthetic MAR water. All batch reactors were placed in a dark room, either temperature controlled (12±0.5 ℃) or ambient temperature (23-27 ℃), depending on the experiment. Batch reactors were uncovered so that air could enter batch reactors to maintain oxic conditions. To avoid anaerobic conditions, the batch reactors were slightly shaken daily without disturbing the biofilm that had developed on the sand.

## 2.3. Experiments

To divide the aquifer environment into two separate physical compartments (water and sand) that contain naturally existing biological and chemical species that might react with $H_2O_2$, this study used an experimental set-up as shown in Figure 1, providing an overview of batch reactors' conditions used in the experiments. All batch reactors were prepared and sampled in triplicate. The performed experiments were divided into:

a) Abiotic: $H_2O_2$ decomposition under autoclaved conditions (with/without sand)
b) Effect of sand: $H_2O_2$ decomposition with 200 g, 100 g, and 50 g autoclaved SSF sand
c) Effect of biomass: $H_2O_2$ decomposition with microbial biomass, 2.74, 1.17, 0.75 and 0 ng ATP/mL
d) Effect of initial $H_2O_2$ concentrations: $H_2O_2$ decomposition with 5.0, 3.0, 1.0 and 0.5 mg/L

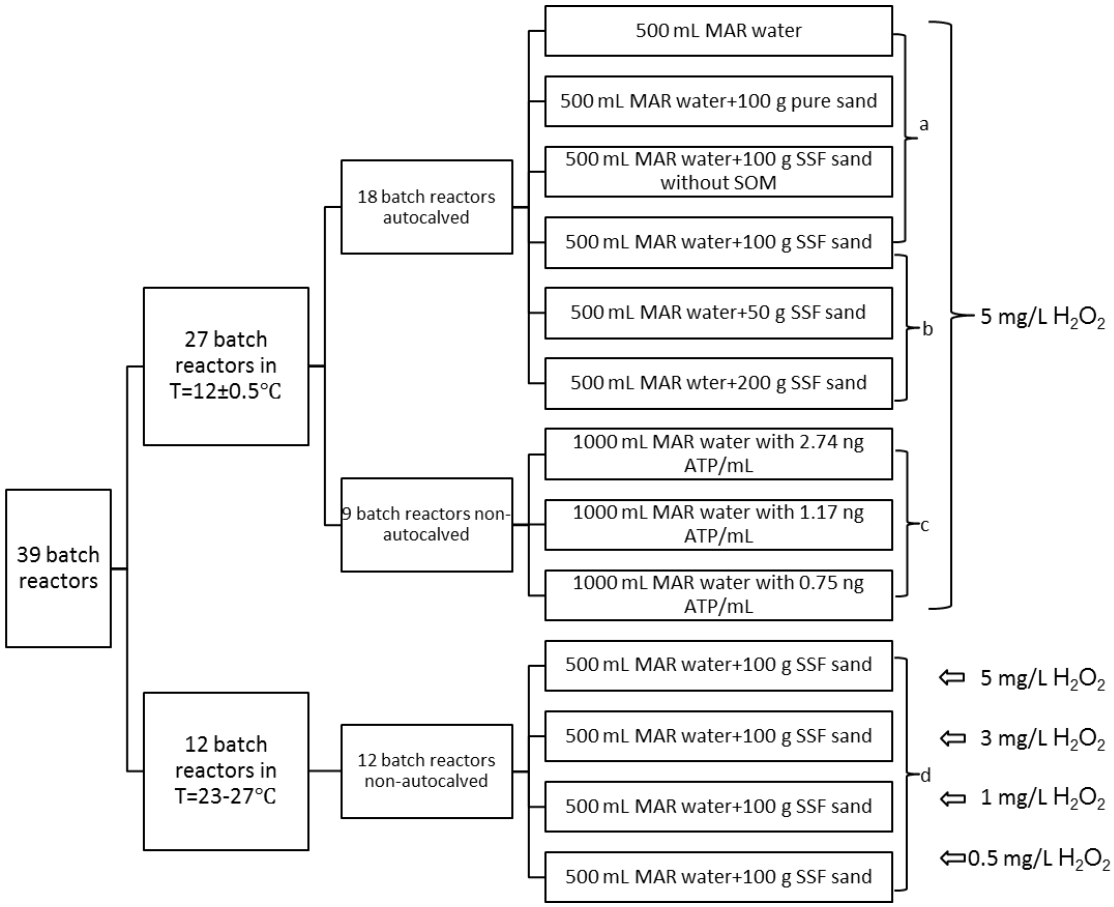


Figure 1 Batch reactors in triplicate with different treatments (non-autoclaved or autoclaved, 23-27 ℃ or 12±0.5 ℃, 5 mg/L, 3 mg/L, 1 mg/L or 0.5 mg/L dosage)

### 2.3.1 Abiotic experiments

To distinguish abiotic reactions from biotic reactions of $H_2O_2$ in MAR, sand (SSF sand, pure sand) and synthetic MAR water were autoclaved at 121 ℃ for 40 minutes to eliminate biological activity. Based on previous study, the enzymatic activity within soil will be completely deactivated by autoclaving (Aggarwal et al., 1991). In this study, ATP was measured in batch autoclaved reactors and was present in the range of 0.04-0.06 ng/mL during the whole experimental process, which indicated that bacteria and enzyme existing in cells and released to water were inactivated by autoclaving. The SOM in SSF sand was removed by heating at 500 ℃ for 2 hours. To further distinguish between the different abiotic decomposition factors of $H_2O_2$, 500 mL MAR water, 500 mL MAR water+100 g pure sand, 500 mL MAR water+100 g SSF sand without SOM and 500 mL MAR water+100 g SSF sand were put in 12 batch reactors respectively (Figure 1 series a). 5 mg/L $H_2O_2$ was dosed into these batch reactors, and $H_2O_2$ concentration was measured

at nine different time points (T=0 h, 1 h, 2 h, 4h, 8 h, 24 h, 48 h, 72 h and 144 h). To further investigate to what extent inorganic content (e.g., metal oxides) on SSF sand impacted $H_2O_2$ decomposition, the experiment was repeated with different amounts of autoclaved SSF sand (50 g, 100 g and 200 g) and 500 mL MAR water (Figure 1 series b). 5 mg/L $H_2O_2$ was dosed into these 9 batch reactors. $H_2O_2$ concentration was measured at six different time points (T=0 h, 2 h, 8 h, 24 h, 72 h, 144 h). All 18 abiotic batch reactors were placed in a temperature controlled room (12±0.5 ℃).

### 2.3.2 Biotic experiments

To investigate the relationship of microbial population and $H_2O_2$ decomposition rate, 5 mg/L $H_2O_2$ was dosed into 9 batch reactors with different initial microbial population (Figure 1, series c). MAR water with microorganisms was collected from effluent water of a batch reactor with 500 mL MAR water and 100 g SSF sand in ambient temperature 23-27 ℃. Batch reactors with 2.74 ng ATP/mL contained the effluent above without dilution. Batch reactors with 1.17 ng ATP/mL and 0.75 ng ATP/mL were prepared by dilution with 500 mL and 725 mL demi-water respectively. $H_2O_2$ concentrations were measured at nine different time points (T=0 h, 4 h, 7 h, 23 h, 30 h, 45 h). The experiments were conducted in a temperature controlled room (12±0.5 ℃).

### 2.3.3 Different concentrations of $H_2O_2$

12 batch reactors filled with 500 mL MAR water and 100 g SSF sand were placed in ambient temperature (23-27 ℃) (Figure 1, series d). Adaptation of the microbial communities on the SSF to the laboratory conditions was achieved by refreshing water every five days until steady state conditions were reached with respect to DOC removal (Lekkerkerker-Teunissen et al., 2012; Maeng, 2010). Steady state conditions (85% DOC removal) were achieved after two months.

After ripening the reactors, $H_2O_2$ spiking experiments started. To evaluate $H_2O_2$ fate, different concentrations of $H_2O_2$ (5 mg/L, 3 mg/L, 1 mg/L, 0.5 mg/L) were dosed to batch reactors one day after water refreshing. The research of Lekkerkerker (2012) and Knol (2012) showed that 6 mg/L $H_2O_2$ dosage was enough to form sufficient OH radicals for oxidation in the AOP, so the residual $H_2O_2$ concentration in effluent water of AOP (being the MAR influent water) will not exceed 6 mg/L. Hence, 0-5 mg/L $H_2O_2$ was dosed into batch reactors in this experiment. $H_2O_2$ concentrations were measured at five different time points (T=0 h, 1 h, 2 h, 4 h and 6 h).

## 2.4. Analysis and measurements

DOC was measured with a Shimadzu TOC analyzer. All samples (30 mL) were measured at constant temperature (20 ℃) after being filtered through 0.45 µm filters (SPARTAN$^{TM}$, Whatman, Germany) which had been flushed twice with demi-water. Samples were acidified by adding 1.6 mL 2 mol/L HCl (Sigma-Aldrich).

ATP is used in all cells as carrier of free energy and phosphate groups to drive many chemical reactions. ATP plays a key role in metabolic processes in the cells and can therefore be used as a measure for biomass. In this study, ATP was measured as total ATP in the supernatant. ATP was measured using a Quench Gone Aqueous test kit and a LB9509 luminometer (both Aqua tools, France).

Hydrogen peroxide test kits (1.18789.0001, VWR company) with a detection range of 0.015-6.00 mg/L were used for water-phase $H_2O_2$ measurements because of ease of operation, the rapid decomposition of $H_2O_2$ and accuracy of results. Since the sand water mixture in this experiment was turbid, 8 mL was pipetted into the reaction cells after being filtered through 0.45 µm filters. After 10 minutes, the sample was transferred to a 10/20 mm rectangular cell and measured in a photometer (Spectroquant NOVA 60).

Based on X-ray diffraction analysis (Department of Materials Science and Engineering, TU Delft), the inorganic constituents of the SSF sand were determined. Table 2 shows the percentages of important metal oxides in SSF sand.

Table 2 The weight percentages of important inorganic constituents other than $SiO_2$ in SSF sand

| Main inorganic constituents | Weight percentage (%) |
|---|---|
| $Al_2O_3$ | 3.532 |
| $Fe_2O_3$ | 0.432 |
| MgO | 0.25 |
| $TiO_2$ | 0.037 |
| MnO | 0.012 |
| ZnO | 0.004 |

# 3 Results and Discussion

## 3.1 Abiotic decomposition of $H_2O_2$ in the presence of SSF sand

Figure 2 shows the abiotic decomposition of $H_2O_2$ in the autoclaved batch reactors with and without SSF or pure sand. $H_2O_2$ in autoclaved MAR water did not decompose in 114 hours (6 days). Also, no $H_2O_2$ decomposition was observed in the presence of autoclaved pure sand, which implies that pure sand (silicon dioxide) does not adsorb or react with $H_2O_2$. However, $H_2O_2$ decomposed by around 64% in both SSF sand groups with and without SOM. There was no significant difference in the $H_2O_2$ decomposition trend in SSF sand with and without SOM, which indicates that SOM in SSF sand has no effect on $H_2O_2$ decomposition. These experiments suggest that the reaction of $H_2O_2$ with naturally occurring inorganic substances on SSF sand (e.g., metal oxides) contributes to $H_2O_2$ decomposition.

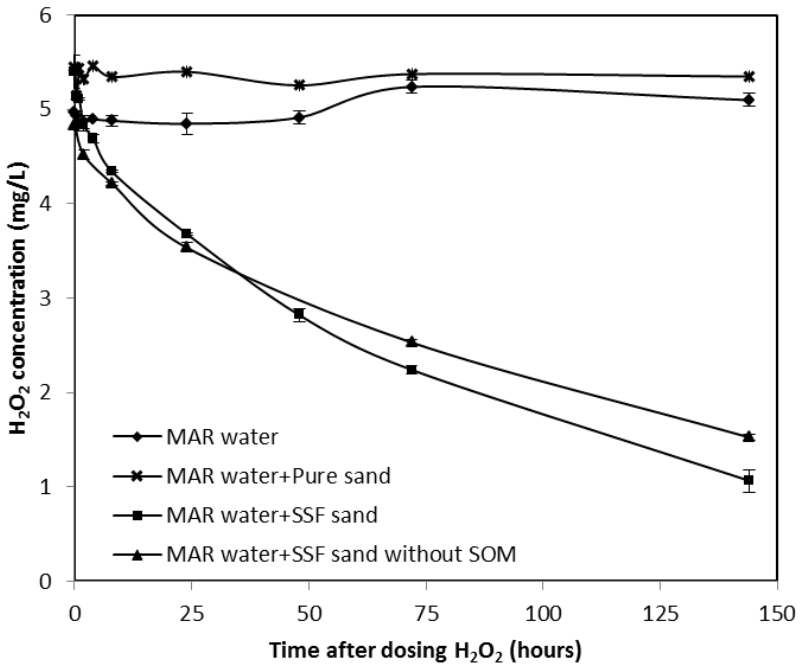


Figure 2 $H_2O_2$ decomposition under autoclaved batch reactors at T=12±0.5 ℃ in triplicate (series a Figure 1)

In contrast to what would be expected, no $H_2O_2$ decomposition was observed in MAR water only. It has long been known that one of the mechanisms of $H_2O_2$ decomposition is due to catalytic species, such as $Cu^{2+}$, $Fe^{3+}$ and $Mn^{2+}$, which initiate radical-chain reactions and cause $H_2O_2$ to decompose more quickly in alkaline solution than in neutral

or acidic media (Galbács & Csányi, 1983). Possible reasons why $H_2O_2$ did not decompose in MAR water could be that the low concentrations of metal ions (0.0106 mg $Fe^{3+}$/L, 0.0005 mg $Mn^{2+}$/L) could not promote $H_2O_2$ decomposition, the pH in this experiment was neutral instead of alkaline, and $Cl^-$ and $SO_4^{2-}$ might have inhibited $H_2O_2$ decomposition (De Laat et al., 2004).

To further investigate to what extent inorganic content (e.g., metal oxides) within SSF sand impacts $H_2O_2$ decomposition, the experiment was repeated with different amounts of autoclaved SSF sand (50 g, 100 g and 200 g). Figure 3 presents the decomposition of $H_2O_2$ in 500 mL MAR water and autoclaved SSF sand, showing an increased removal of $H_2O_2$ (51%, 64% and 69%) at higher SSF content.

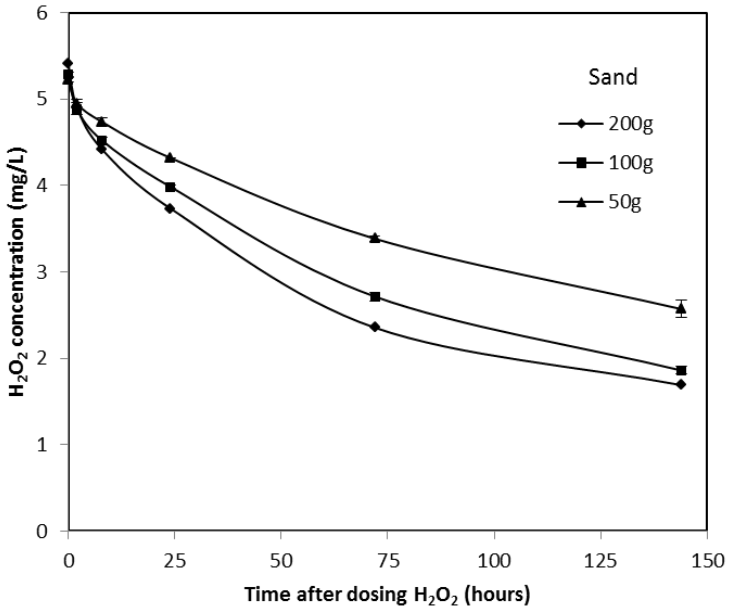


Figure 3 $H_2O_2$ decomposition with 200 g, 100 g, and 50 g autoclaved SSF sand in 500 mL synthetic MAR water at T=12±0.5 ℃. All batch reactors were in triplicate (series b Figure 1)

This supports the finding that inorganic surfaces on the SSF sand effects $H_2O_2$ decomposition. Metal oxides may well be responsible for this observation, as this has also been reported in previous research (Hiroki & LaVerne, 2005; Lousada et al., 2013; Russo et al., 2013) and metal oxides were present in the SSF sand (Table 2). This may also explain why in Figure 2 the $H_2O_2$ decomposition was slightly faster without SOM since inorganic content (e.g., metal oxides) coating on SSF without SOM may have more free surface area. This phenomenon is in agreement with results of Bissey et al. (2006) who

found that $H_2O_2$ decomposition was faster in sand with 0.2% SOM than with 1.6% SOM at pH 7. However, the increase of $H_2O_2$ decomposition with the increase of SSF sand was slow, raising the question whether abiotic $H_2O_2$ decomposition by the natural sand will sufficiently contribute compared to biotic processes.

### 3.2. Biotic decomposition of $H_2O_2$ within MAR water

To investigate the effect of microbial biomass (represented as ATP) on $H_2O_2$ decomposition, 5 mg/L $H_2O_2$ was dosed into four synthetic MAR water groups with various levels of microbial biomass, extracted from SSF sand. Figure 4 shows the $H_2O_2$ decomposition in MAR water with different bacterial populations, without the addition of sand. It was observed that only the group without living biomass did not show $H_2O_2$ decomposition while $H_2O_2$ decomposed in the other groups with biomass. The $H_2O_2$ decomposition rate considerably increased with the increase of microbial biomass.

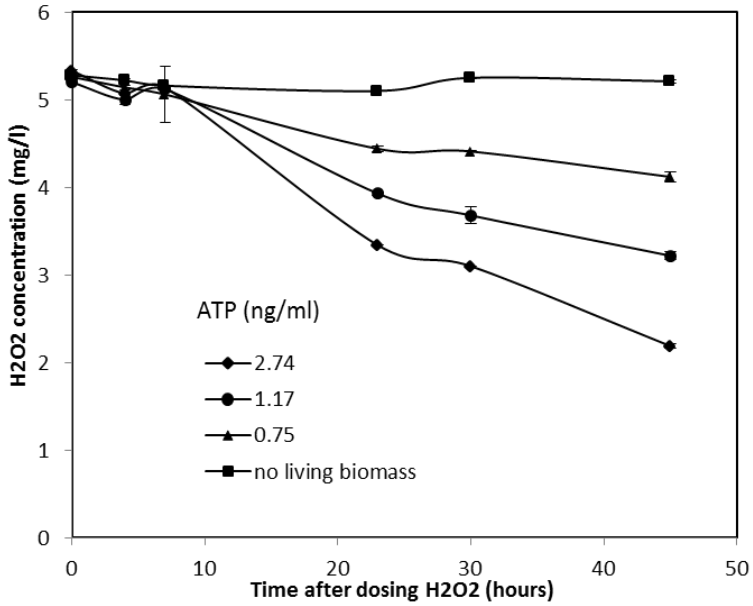


Figure 4 $H_2O_2$ decomposition with microbial biomass, 2.74, 1.17, 0.75 and 0 ng ATP/mL at T=12±0.5 ℃. All batch reactors were in triplicate (series c Figure 1)

Even low microbial biomass (0.75-2.74 ng ATP/mL) resulted in considerable $H_2O_2$ decomposition (22-59%) in synthetic MAR water in only 45 hours. Therefore, microbial biomass is another main factor promoting $H_2O_2$ decomposition in MAR systems. This result is confirmed by previous studies, such as Sarathy et al. (2011) reported that 10 mg/L $H_2O_2$ was removed quickly by biologically activated carbon filters with high

microbial population, Urfer and Huck (1997) reported that the rapid removal of 1 mg/L $H_2O_2$ in a biological filter may be attributed to its reaction with biomass.

### 3.3. Abiotic vs biotic $H_2O_2$ decomposition

The results above indicated that naturally occurring inorganic substances surfacing on sand grains and living biomass would be the two main factors promoting $H_2O_2$ decomposition during MAR. To further compare the effects of these two main factors, Figure 5 shows $H_2O_2$ decomposition trends under abiotic and biotic conditions, with and without SSF sand. The batch reactors with both non-autoclaved SSF sand and MAR water with 38 ng ATP/mL provided the most rapid $H_2O_2$ decomposition by achieving almost complete removal in 6 hours. However, the slowest decomposition occurred in both autoclaved MAR water and SSF sand. Comparing the above results, it indicates that the biotic reactions contributed with a large fraction to $H_2O_2$ decomposition in the reactors with non-autoclaved SSF sand and MAR water with 38 ng ATP/mL. Additionally, $H_2O_2$ decomposition in non-autoclaved MAR water with 2.74 ng ATP/mL decomposed faster than in the reactors with both autoclaved SSF sand and MAR water, illustrating that the contribution of biotic reactions, in the presence of 2.74 ng ATP/mL, to $H_2O_2$ decomposition in SSF sand is more than abiotic reactions. However, at lower ATP concentrations (<1.71 ng ATP/mL), abiotic decomposition is faster and should therefore not be neglected.

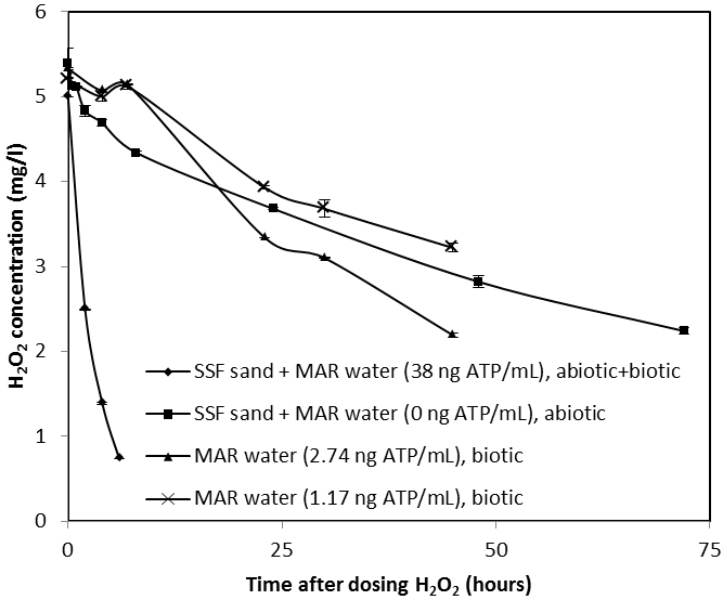


Figure 5 Biotic and abiotic $H_2O_2$ decomposition. All batch reactors were in triplicate

This result is different from previous studies. As was stated in the introduction, the removal of $H_2O_2$ was greatly attributed to biotic factors instead of abiotic factors in most cases investigated, such as biologically active zones in situ (Bajpai et al., 1994) and biologically active filters (Urfer & Huck, 1997) which contain much higher microbial biomass than natural MAR water. Several researchers investigated the microbial biomass in lakes and rivers, as MAR influent water, and found that ATP concentration range of 0.1-2 ng/mL (Cavari, 1976; Hamilton-Galat & Galat, 1983; Kramer, 2012; Noges, 1996; Pridmore et al., 1989). In practice however, especially in the late spring and in the early summer, ATP increases substantially to values of 2.79 ng/mL in Lake Rotorua (Pridmore et at., 1989) and 2.945 ng/mL in Lake Kinneret (Cavari, 1976). This demonstrates that biotic reactions would be the primary mechanism for $H_2O_2$ decomposition in MAR systems only when MAR waters contain much higher ATP concentrations than the range of 0-2.74 ng/mL as used in this study.

### 3.4. $H_2O_2$ decomposition at different initial concentrations

So far, previous research has primarily focused on single $H_2O_2$ concentrations (Häkkinen et al., 2004; Miller & Valentine, 1999; Urfer & Huck, 1997; Zappi et al., 2000), whereas the fate of different $H_2O_2$ concentrations is important for setting the maximum allowable limit to prevent undesired effects on aquatic and soil ecology. Figure 6 presents the $H_2O_2$ decomposition at different initial concentrations in SSF sand and synthetic MAR influent water with a large microorganism content (38 ng ATP/mL). $H_2O_2$ initial concentrations in the range of 0.5-3 mg/L decomposed to below the detection limit 0.015 mg/L in 2-6 hours and 5 mg/L $H_2O_2$ decomposed to 0.73 mg/L in 6 hours.

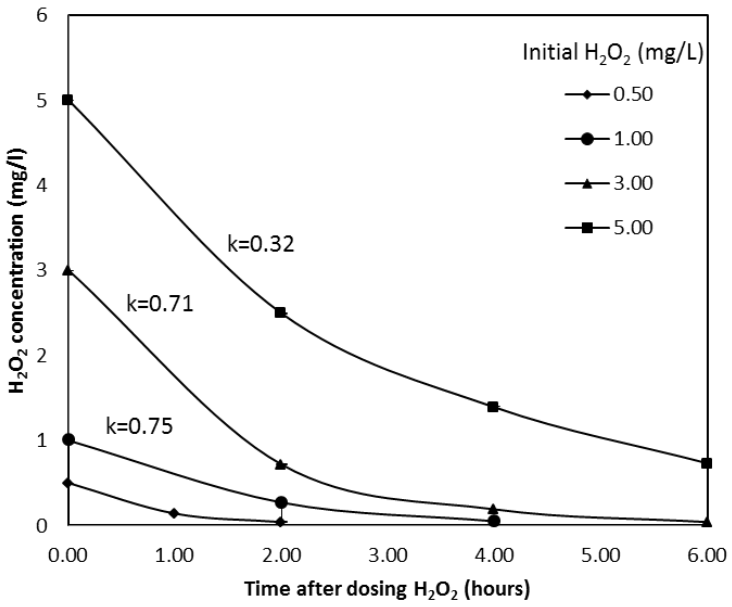


Figure 6 $H_2O_2$ decomposition under different initial concentrations, 0.5, 1.0, 3.0 and 5.0 mg/L, in the presence of SSF sand at T=23-27 ℃. All batch reactors were in triplicate (series d Figure 1)

As is shown in Figure 6, $H_2O_2$ decompositions followed first-order kinetics in the three $H_2O_2$ spiking groups (5, 3 and 1 mg/L) in the presence of SSF sand. It is in agreement with previous studies (Miller & Valentine, 1999; Zappi et al., 2000). Interestingly, first-order rate coefficients k values increased with the decrease of $H_2O_2$ initial concentrations. The same phenomenon was reported in the study of Silhacek and Taake (2005).

It is noteworthy that to maintain the growth of microorganisms in this experiment, DOC was dosed in concentrations higher than in most MAR influent waters, particularly in winter periods. However, the pre-treatment AOP before MAR can increase the degradable organic matter and lead to increased bacterial population in MAR influent water, probably two to three times higher than MAR systems without the pretreatment AOP (Pharand et al., 2014). Also, natural water may contain higher ATP concentrations by themselves, such as 0.07-18 ng/mL in Lake 227 (Canada), 0.07-7.93 ng/mL in St. Lawrence Estuary, 0.03-11.9 ng/mL in Pyramid Lake (NV) (Hamilton-Galat & Galat, 1983). Therefore, microbial biomass in MAR systems after AOPs may reach 38 ng ATP/ml under specific conditions.Assuming a microbial biomass concentration around 38 ng ATP/mL in MAR influent water and $H_2O_2$ decomposition rate is steady in the surface of MAR sand, the first-order kinetics were applied to predict the decomposition of residual $H_2O_2$ in MAR systems. Drinking water utility Dunea operates the MAR with an infiltration velocity of

0.042 m/h (1 m/day). An estimation based on the first-order kinetics is that different initial concentrations (5, 3 and 1 mg/L) of $H_2O_2$ could decompose to the provisional standard, 0.25 mg/L, stated in the introduction within around 9, 4, and 2 hours corresponding to a depth of 36, 17 and 8 cm. However, in practice the microbial activity may not be steady with depths. Previous studies (Das et al., 2013; Haughton et al., 2001) reported that the highest microbial population exists in the top 0-20 cm of soil and the microbial activity decrease a lot below the depth of 20 cm. It could thus be concluded that low concentration of $H_2O_2$ (<3 mg/L) may be decomposed to 0.25 mg/L in the first centimeters of dune sand in the presence microbial biomass of 38 ng ATP/mL in the MAR infiltration water.

## 4 Conclusions

This study investigated the fate of $H_2O_2$ as the residual of AOP during MAR. The main conclusions of this study are:

- No $H_2O_2$ decomposition was observed in batch reactors with synthetic MAR water only, nor in reactors containing pure sand. In MAR systems, pure sand and MAR water have no effect on $H_2O_2$ decomposition.
- $H_2O_2$ decomposed slightly faster in batch reactors with SOM than in batch reactors without SOM, but there was no significant difference in $H_2O_2$ decomposition between the two groups.
- Naturally occurring inorganic substances on the surface of sand grains and living biomass are the two main factors promoting $H_2O_2$ decomposition in MAR systems.
- Low concentration (<3 mg/L) of $H_2O_2$ in MAR influent water may decompose below 0.25 mg/L in the centimeters of MAR systems with water containing high microbial biomass (such as 38 ng ATP/mL).

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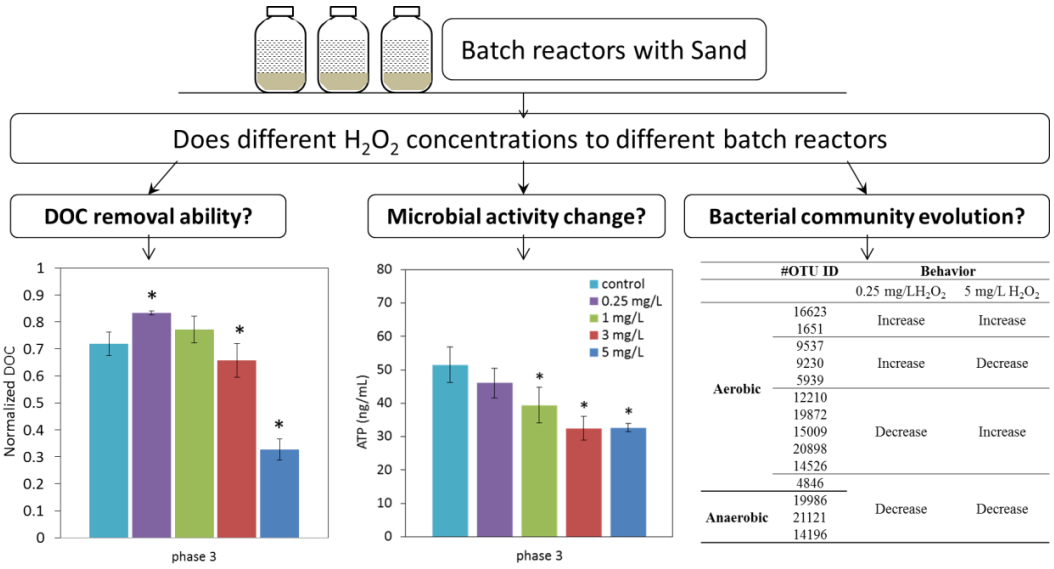
# 5

# Effect of residual $H_2O_2$ from advanced oxidation processes on subsequent biological water treatment: A laboratory batch study




| | #OTU ID | Behavior | |
|---|---|---|---|
| | | 0.25 mg/L $H_2O_2$ | 5 mg/L $H_2O_2$ |
| Aerobic | 16623<br>1651 | Increase | Increase |
| | 9537<br>9230<br>5939 | Increase | Decrease |
| | 12210<br>19872<br>15009<br>20898<br>14526 | Decrease | Increase |
| | 4846 | | |
| Anaerobic | 19986<br>21121<br>14196 | Decrease | Decrease |

This chapter is based on:

Wang F., van Halem D., Liu G., Lekkerkerker-Teunissen K., van der Hoek J.P. 2017. Effect of residual $H_2O_2$ from advanced oxidation processes on subsequent biological water treatment: A laboratory batch study. *Chemosphere*, 185, 637-646.

## Abstract

$H_2O_2$ residuals from advanced oxidation processes (AOPs) may have critical impacts on the microbial ecology and performance of subsequent biological treatment processes, but little is known. The objective of this study was to evaluate how $H_2O_2$ residuals influence sand systems with an emphasis on dissolved organic carbon (DOC) removal, microbial activity change and bacterial community evolution. The results from laboratory batch studies showed that 0.25 mg/L $H_2O_2$ lowered DOC removal by 10% while higher $H_2O_2$ concentrations at 3 and 5 mg/L promoted DOC removal by 8% and 28%. A $H_2O_2$ dosage of 0.25 mg/L did not impact microbial activity (as measured by ATP) while high $H_2O_2$ dosages, 1, 3 and 5 mg/L, resulted in reduced microbial activity of 23%, 37% and 37% respectively. Therefore, DOC removal was promoted by the increase of $H_2O_2$ dosage while microbial activity was reduced. The pyrosequencing results illustrated that bacterial communities were dominated by *Proteobacteria*. The presence of $H_2O_2$ showed clear influence on the diversity and composition of bacterial communities, which became more diverse under 0.25 mg/L $H_2O_2$ but conversely less diverse when the dosage increased to 5 mg/L $H_2O_2$. Anaerobic bacteria were found to be most sensitive to $H_2O_2$ as their growth in batch reactors was limited by both 0.25 and 5 mg/L $H_2O_2$ (17-88% reduction). In conclusion, special attention should be given to effects of AOPs residuals on microbial ecology before introducing AOPs as a pre-treatment to biological (sand) processes. Additionally, the guideline on the maximum allowable $H_2O_2$ concentration should be properly evaluated.

# 1 Introduction

In recent years, organic micro-pollutants (OMPs), such as pesticides, pharmaceutically active compounds, endocrine disrupting compounds, X-ray contrast media and personal care products, have been detected at ng/L to low µg/L concentrations in surface waters throughout the world (Kolpin et al., 2002; Stolker et al., 2004). Surface waters serve vital role to humans such as drinking water, nature, recreation and food production. These functions are susceptible to negative water quality effects from anthropogenic contaminants (Brack et al., 2017; Coppens et al., 2015). However, conventional processes and biological processes do not always provide satisfactory results for drinking water treatment (Bertelkamp et al., 2015; Bertelkamp et al., 2016; Paredes et al., 2016; Ruhl et al., 2014) as many organic pollutants are toxic or resistant to biological treatments. Therefore, an alternative option for such recalcitrant and biologically persistent compounds is the use of advanced oxidation processes (AOPs), widely recognized as highly efficient for water purification (Oller et al., 2011a). In particular, the hydroxyl radicals (▪OH) generated by these methods have the ability to oxidise recalcitrant and non-biodegradable pollutants (Bilińska et al., 2016; Oller et al., 2011a). Previous research demonstrated that the combination of AOPs, e.g. ozonation, UV/$H_2O_2$, ozonation/UV/$H_2O_2$ or photo-Fenton processes, and conventional biological processes offers an optimised treatment system to effectively remove OMPs during water treatment (Lekkerkerker-Teunissen et al., 2012; Oller et al., 2011a). Integrating UV/$H_2O_2$ and subsequent biological activated carbon filtration may also offer a promising approach to eliminate trihalomethanes, haloacetic acids and phenol from raw surface water (Seredyńska-Sobecka et al., 2005; Toor & Mohseni, 2007). In the Netherlands, several water companies utilise intergrated AOPs with subsequent biological treatment processes. For example, Waternet in Amsterdam combines ozonation with biological activated carbon (BAC) filtration to remove OMPs during drinking water production (Bonné et al., 2002; Van Der Hoek et al., 1999a). Another Dutch drinking water company, PWN, uses UV/$H_2O_2$ oxidation and BAC filtration to form a multi barrier approach against OMPs during drinking water production (Martijn & Kruithof, 2012). In The Hague, Dunea water utility company plans to install AOPs before managed aquifer recharge (MAR) in the dunes to form a synergistic system for the removal of OMPs (Lekkerkerker et al., 2009a; Wang et al., 2016). During AOPs with $O_3$, $H_2O_2$ is present in excess to reduce the formation of the by-product bromate (Von Gunten & Oliveras, 1998; Wert et al., 2007). Therefore, $H_2O_2$ residuals are usually present in the effluent of AOPs.

$H_2O_2$ in water can function as a disinfectant with the ability to inactivate microorganisms by oxidising proteins and DNA (Apel & Hirt, 2004; Latifi et al., 2009). The growth of *A. nidulans* and *A. variabilis* was suppressed at concentrations of 0.34-3.4 mg/L $H_2O_2$ in dialysis culture (Samuilov et al., 1999). A study by Knol et al. (2015) suggested that $H_2O_2$, even in concentrations below 2 mg/L, may cause undesired effects on ecosystems in dune ponds. However, the ineffectiveness of $H_2O_2$ as a disinfectant, and more specifically the selective impact of $H_2O_2$ on microorganisms, have also been reported. For example, some phyla types had the potential to detoxify $H_2O_2$ in a humic lake (Glaeser et al., 2014); a concentration below 40 mg/L of $H_2O_2$ did not inactivate *Escherichia coli* bacteria (Labas et al., 2008); 1 mg/L $H_2O_2$ dosage did not decrease acetate removal by biological filters (Urfer & Huck, 1997); and $H_2O_2$ did not affect eukaryotic phytoplankton including green algae, chrysophytes and diatoms, even if 99% of the cyanobacterial population was reduced by $H_2O_2$ (Matthijs et al., 2012). Catalases are known to catalyse the conversion of $H_2O_2$ into water and oxygen, which is part of an adaptive response of bacteria to oxidative stress (Matthijs et al., 2012; Metz et al., 2011; Tusseau-Vuillemin et al., 2002). Some catalase-positive microorganisms, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Campylobacter jejuni*, make catalase to deactivate the peroxide radicals, thus allowing them to survive (Rao et al., 2003). Another study showed additional evidence for catalase-positive bacteria that survived in the presence of $H_2O_2$; concentrations of $H_2O_2$ exceeding 0.034 mg/L were lethal for the majority of catalase-negative strains, but not for catalase-positive strains (Walczak & Swiontek Brzezinska, 2009). Additionally, even strictly anaerobic bacteria could become acclimated to normally lethal doses of $H_2O_2$ (Schmidt et al., 2006). Notably, the assimilable organic carbon removal efficiency slightly increased in a biological filter receiving water with 1 mg/L $H_2O_2$ (Urfer & Huck, 1997). Several reports on the use of $H_2O_2$ injection to supply oxygen into subsurface biologically active zones indicated various degrees of success when applied to contaminated aquifer remediation, but the bacterial damage by $H_2O_2$ has never been reported (Aggarwal et al., 1991; Tusseau-Vuillemin et al., 2002; Zappi et al., 2000), indicating the damage may be negligible. Therefore, although $H_2O_2$ is generally used to inactivate microorganisms in aqueous systems, some microorganisms may be able to tolerate $H_2O_2$ in varying concentrations and situations. In particular, the effect of $H_2O_2$ as a residual of AOPs on microbial activity in subsequent biological water treatment processes, such as BAC filtration and sand filtration, is not yet well understood.

Further investigation into the effects of $H_2O_2$ on microbial activity in sand systems is important, scientifically for microbial ecology and practically for surface water purification systems that utilise a combination of AOPs and sand systems, e.g. sand filtration or MAR in a sandy soil. The objective of this study was to evaluate in batch experiments how different concentrations of residual $H_2O_2$ influence sand systems with an emphasis on dissolved organic carbon (DOC) removal, microbial activity change and bacterial community evolution.

## 2 Materials and Methods

### 2.1 Experimental set-up

Batch reactors with sand and water have been widely used to assess substances degradations, impact factors or influences on microbial communities (Abel et al., 2013; Lekkerkerker, 2012; Maeng, 2010; Maeng et al., 2012; Wang et al., 2016). In the present study, batch reactors (1 L glass bottles) filled with 200 g sand (grain size 0.8-1.25 mm) and 800 mL water were used to investigate the influence of $H_2O_2$ on microbial activity in sand systems.

Sand used in this study was collected from the top 0.5-2.0 cm of a slow sand filter used by the water utility Dunea. The top 0.5-2.0 cm (schmutzdecke) of a slow sand filter has diverse microbial communities and greatly contributes to the removal of organic matter by biodegradation processes, so this layer is considered to represent the microbial activity of sand filtration systems (Chekol, 2009; Dizer et al., 2004).

The water used in batch reactors was prepared with demineralised water and chemical additives (33 mg $Na_2HPO_4$/L, 7.5 mg $NaH_2PO_4$/L, 22 mg $K_2HPO_4$/L, 140 mg $CaCl_2$/L, 0.031 mg $FeCl_3$/L, 0.032 mg $NH_4Cl$/L, 40.75 mg $MgSO_4$/L, 17.823 mg $NaNO_3$/L, 0.00114 mg $MnCl_2$/L, 82 mg $CH_3COONa$/L) and simulated the pre-treated surface water (after AOPs) of Dunea as used in drinking water production. Additionally, in order to have residual DOC and avoid bacterial starvation conditions, the carbon source (as sodium acetate) in the batch reactors was 22 mg/L DOC which was around 5 times higher than that found in pre-treated surface waters. However, in practice, the pre-treatment by AOPs will increase the amount of biodegradable organic matter and may lead to increased microbial activity in the influent water of the subsequent biological process, probably two to three times higher than biological treatment systems without the pre-treatment AOPs (Pharand et al., 2014). Table 1 shows the composition of water in batch reactors. The

$H_2O_2$ solution was prepared from a 30% standard solution (Merck, Germany). All the solutions used in this study were prepared using water from a Millipore Milli-Q system. All chemicals were of analytical grade purity (AR grade ≥ 99% purity or better).

Table 1 The composition of water in batch reactors

| $O_2$ (mg/L) | pH | $NH_4^+$-N (mg/L) | $NO_3^-$-N (mg/L) | $SO_4^{2-}$ (mg/L) | $Fe^{3+}$ (mg/L) | $Mn^{2+}$ (mg/L) | DOC (mg/L) |
|---|---|---|---|---|---|---|---|
| 9±1.0 | 7.8±0.3 | 0.00847 | 2.9±0.1 | 30.6±2 | 0.0106 | 0.0005 | 22 |

## 2.2 Experimental processes

The experimental processes are presented in Figure 1. 18 batch reactors with 200 g sand and 800 mL water were used. The adaptation of microbial communities found on the sand to laboratory conditions was achieved by refreshing water every 5-7 days until steady state conditions were reached with respect to DOC removal calculated as $DOC_{ending}/DOC_{initial}$ (Lekkerkerker-Teunissen et al., 2012; Maeng, 2010). $DOC_{initial}$ was measured at the beginning just after refreshing water and $DOC_{ending}$ was the DOC concentration in the batch reactor just before refreshing water. Figure S1 in Appendix B shows the results for normalised DOC removal during the ripening period. DOC data show that steady state conditions were achieved after around two months.

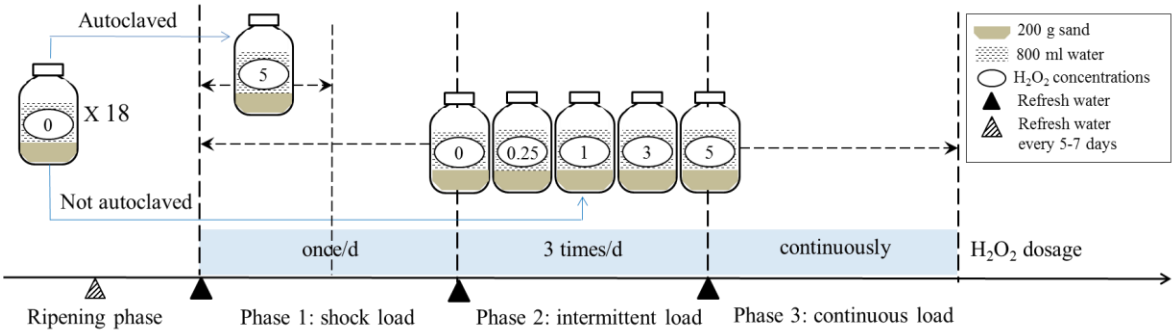



Figure 1 Batch reactors with different operation conditions n=3. The ripening phase lasted for 2 months, then three batch reactors were autoclaved while the other fifteen batch reactors were not autoclaved. 5 mg/L $H_2O_2$ was dosed to the autoclaved reactors, and different concentrations of $H_2O_2$ (0, 0.25, 1, 3, 5 mg/L) were dosed to non-autoclaved reactors. Each $H_2O_2$ dosage phase was 6 days

After ripening the reactors, $H_2O_2$ spiking experiments started. The research of Lekkerkerker (2012) and Knol (2012) showed that a 6 mg/L $H_2O_2$ dosage was adequate to form sufficient ▪OH for oxidation in AOPs so that the residual $H_2O_2$ concentration in effluent water of AOPs will not exceed 6 mg/L. Therefore, different dosages of $H_2O_2$ were added to reactors to result in final concentrations of 0.25, 1, 3, 5 mg/L in 15 non-autoclaved batch reactors after water refreshing. To distinguish DOC oxidised by $H_2O_2$ directly from DOC biodegradation, 3 additional reference batch reactors were autoclaved at 121 ℃ for 40 minutes to inactivate microbes and then dosed with 5 mg/L $H_2O_2$.

To avoid heavy damage to microbial communities from a high $H_2O_2$ load and also to facilitate the gradual adaptation of the microorganisms to the spiked $H_2O_2$, $H_2O_2$ was dosed into the 15 non-autoclaved batch reactors once per day during the initial shock load phase (phase 1, 6 days), 3 times per day during the intermediate phase (phase 2, 6 days), and finally as a continuous load using a pump (phase 3, 6 days). For phase 3, $H_2O_2$ concentrations of 0.25 mg/L, 1 mg/L, 3 mg/L and 5 mg/L groups were realised in the reactors by pumping 9 mL of feed solutions of 133.4, 530, 1590 and 2650 mg/L into these reactors respectively. DOC in each batch reactor was returned to 22 mg/L every 5-7 days by refreshing the reactor with water containing sodium acetate during the ripening phase, while during the $H_2O_2$ spiking period (phase 1, 2 and 3) the same DOC concentration, 22 mg/L, was reached every 2 days by dosing appropriate amounts of sodium acetate to each batch reactor to avoid the impact of DOC concentration differences among batch reactors on microbial community structure. Considering the accumulation of bacterial metabolites with time, the water in the batch reactors was refreshed at the end of each phase. 15 mL water samples for DOC analysis were collected 9-11 times to investigate the potentially different DOC removal responses to $H_2O_2$ over time. To estimate the $H_2O_2$ decomposition, 8 mL $H_2O_2$ water samples were collected on the first day after $H_2O_2$ was added. Adenosine triphosphate (ATP) samples were collected from the water instead of the sand to prevent disturbance and heavy loss of sand in our reactors. A previous study, described in detail in supplemental information 2, showed a positive correlation between ATP in the water and in the sand (Figure S2 in Appendix B), so ATP in the water can be positively correlated with ATP in the sand. 1 mL water samples for adenosine triphosphate (ATP) analysis were taken 4-10 times in each phase to assess the microbial population responses to $H_2O_2$ over time. At the beginning of the spiking experiment, both DOC and ATP sampling frequencies were high in order to determine the optimal sampling time. To investigate the effect of low (0.25 mg/L) and high (5 mg/L) $H_2O_2$ concentrations on

microbial composition and diversity in sand systems, sand samples were taken from the control (0 mg/L $H_2O_2$), 0.25 mg/L and 5 mg/L groups at the end of the experiment for 16-S pyrosequencing measurement (Huang & Chen, 2004).

To distinguish DOC abiotic removal by directly oxidation by $H_2O_2$ from biotic removal in sand systems, 5 mg/L $H_2O_2$ was dosed to 3 autoclaved batch reactors as references at the beginning. DOC and $H_2O_2$ concentrations were measured at 5 different time points (T=0 h, 8 h, 24 h, 48 h, 72 h). ATP was measured at t=0 h, 24 h, 48 h and 72 h to confirm the elimination of biological activity in the autoclaved batch reactors. ATP was present in the autoclaved batch reactors in the range of 0.04-0.06 ng/mL during the 72 h testing period, which indicated bacterial inactivation. The experiment was finished in 3 days in order to minimize growth of bacteria from the surrounding environment inside the batch reactors, which were in contact with air. DOC and $H_2O_2$ results in autoclaved batch reactors within 3 days were sufficient to distinguish DOC abiotic removal from biotic removal.

All batch reactors were placed in a dark, temperature (12 ± 0.5 ℃) controlled room and left uncovered so that the air could enter the batch reactors. All batch reactors were prepared and sampled in triplicate.

## 2.3 Analysis

### 2.3.1 DOC

DOC was measured with a Shimadzu TOC-VCPH/CPN analyser with a standard deviation of 0.1 mg/L immediately or within one day after sampling. First, all samples were diluted one time using deionised water, then 30 mL of the diluted mixture was measured at constant temperature (20 ℃) after being filtered through 0.45 μm filters (SPARTAN$^{TM}$, Whatman, Germany) that had been flushed twice with deionised water. To remove the inorganic carbon, samples were acidified by adding 1.6 mL 2 mol/L HCl (Sigma-Aldrich) before measurement.

### 2.3.2 ATP analysis

ATP is used in all cells as a carrier of free energy and phosphate groups to drive many chemical reactions. It plays a key role in metabolic processes in the cells and can therefore be used as an indicator for microbial activity (Liu et al., 2013; Liu et al., 2016). In this study, ATP was measured as total ATP in the supernatant (Liu et al., 2013) using Quench Gone Wastewater (QG21W) test kits (Canada) and a LB9509 luminometer (Aqua

Tools, France) with a standard deviation of <5%. Based on the test kit instructions, a 1 mL water sample was directly dosed into a QG21W extraction tube with 2 mL UltraLyse 30[21] to lyse the bacteria and release ATP. Secondly, the extraction tube and QG21 dilution tube were mixed to dilute it. Next, the luminescence reaction of sample ATP with Luminase was measured as a Relative Luminescence Unit (RLU), and finally the RLU value was compared to that of a check standard (LuminUltra's UltraCheck) and converted to ATP concentration in ng/mL.

### 2.3.3 $H_2O_2$

Hydrogen peroxide test kits (1.18789.0001, VWR company) with a detection range of 0.015-6.00 mg/L were used for water-phase $H_2O_2$ measurements because of ease of operation, the rapid decomposition of $H_2O_2$ and accuracy of results. Since the sand water mixture in this experiment was turbid, 8 mL was pipetted into the reaction cells after filtration through 0.45 µm filters. After 10 minutes, the sample was transferred to a 10/20 mm rectangular cell and measured in a photometer (Spectroquant NOVA 60).

### 2.3.4 Bacterial qualitative analysis-pyrosequencing

At the end of experiments, 5 g sand was sampled from selected groups (0 mg/l, 0.25 mg/l, 5 mg/l) and bottles (duplicates). DNA was extracted using a Power Soil kit according to the manufacturer's instructions, and the 16S rRNA profiling was performed by 454 pyrosequencing (Medisch Moleculair Microbioloog Streeklab, the Netherlands). The primers used were GACACTATAGGATTAGATACCCBRGTAGTC (forward) and CACTATAGGGTCACGRCACGAGCTGACGAC (reverse). Around 3000 readers were obtained. Obtained sequences were trimmed, merged alignments of the sequences were aligned via the infernal aligner from the Ribosomal Database Project (RDP) pyrosequencing pipeline, and the NAST alignment tool from Greengenes was obtained via the software. The RDP Classifier was used for the taxonomical assignments of the aligned 454 pyrosequencing at the 97% confidence level. The bacterial communities from all samples were analysed for the number of operational taxonomic units (OTUs), species richness and biodiversity using the QIIME program.

### 2.3.5 Statistical analysis

Significant difference in individual parameters between water and $H_2O_2$ treatments (n = 6) was analysed with one-way ANOVA tests using SPSS 17.0 (SPSS, Chicago, IL, USA). A difference was considered statistically significant at $p< 0.05$. As described in section 2.2,

to maintain the same DOC concentration in all batch reactors, DOC was recovered to 22 mg/L by dosing different amounts of the carbon source every 2 days, so cumulative DOC in batch reactors was different and may therefore lead to different total DOC removals. The partial correlation analysis between DOC concentrations and DOC accumulations and $H_2O_2$ dosages was applied to explore if DOC removal differences between each $H_2O_2$ dosage groups were caused by different $H_2O_2$ dosages or different carbon source accumulation.

### 2.3.6 Other analyses

Dissolved oxygen, pH and temperature were measured with a multimeter (Sentix 41 probe, Multi 340i, WTW, Germany).

## 3 Results

### 3.1 DOC removal and $H_2O_2$ decomposition

To show the effect of DOC calibration every two days in each phase and refreshing the reactor water at the end of each phase, DOC fluctuations of the control group and 5 mg/L $H_2O_2$ group are presented as an example in Figure 2-a. To illustrate the influence of $H_2O_2$ on DOC removal in greater detail, Figures 2-b, 2-c and 2-d present the DOC removal of each $H_2O_2$ dosage group.

Two phenomena can be observed in Figure 2-a. Firstly, normalised DOC as $DOC_t$ / $DOC_o$ (initial DOC concentration) in the control group decreased to 21-35% at the beginning (the first 2 days) of each phase, 58-73% in the middle (the second 2 days) and the end (the last 2 days) of each phase. Every 5-7 days, the reactor water was refreshed and $DOC_o$ was returned to 22 mg/L in each batch reactor to ensure sufficient growth space and nutrients. DOC removal between the control and 5 mg/L groups had no apparent difference during phase 1 ($H_2O_2$ shock load), while DOC removal in the control group became slightly lower than 5 mg/L group during phase 2 ($H_2O_2$ intermittent load). This phenomenon became more apparent in phase 3 ($H_2O_2$ continuous load). The same pattern was observed for the other $H_2O_2$ dosage groups: no obvious difference of DOC removal, 29%-33%, between the $H_2O_2$ dosage groups was observed at the end of phase 1 (Figure 2-b); interestingly, DOC removal slightly increased with the increase of $H_2O_2$ dosage at the end of phase 2 (Figure 2-c), and this trend became even more apparent at the end of phase 3 (Figure 2-d).

To assure that the above DOC removal differences between each $H_2O_2$ dosage groups were indeed caused by different $H_2O_2$ dosages and not by the cumulative differentiation in DOC dosage between the groups, Table S1 in Appendix B presents partial correlations between the normalised DOC concentration and cumulative DOC dosage and $H_2O_2$ dosage. These correlations clearly indicate that the manner of dosing DOC – returning to 22 mg/L every two days – did not interfere with the objective of the experiment.

Based on the result of variance analysis, 0.25 mg/L $H_2O_2$ significantly limited DOC removal by 11% while 3 and 5 mg/L $H_2O_2$ promoted DOC removal by 6% and 33% respectively in comparison with the control group (Figure 2). The results above suggest that the DOC removal in batch reactors was enhanced under the presence of $H_2O_2$ after an adaptive period of several days.

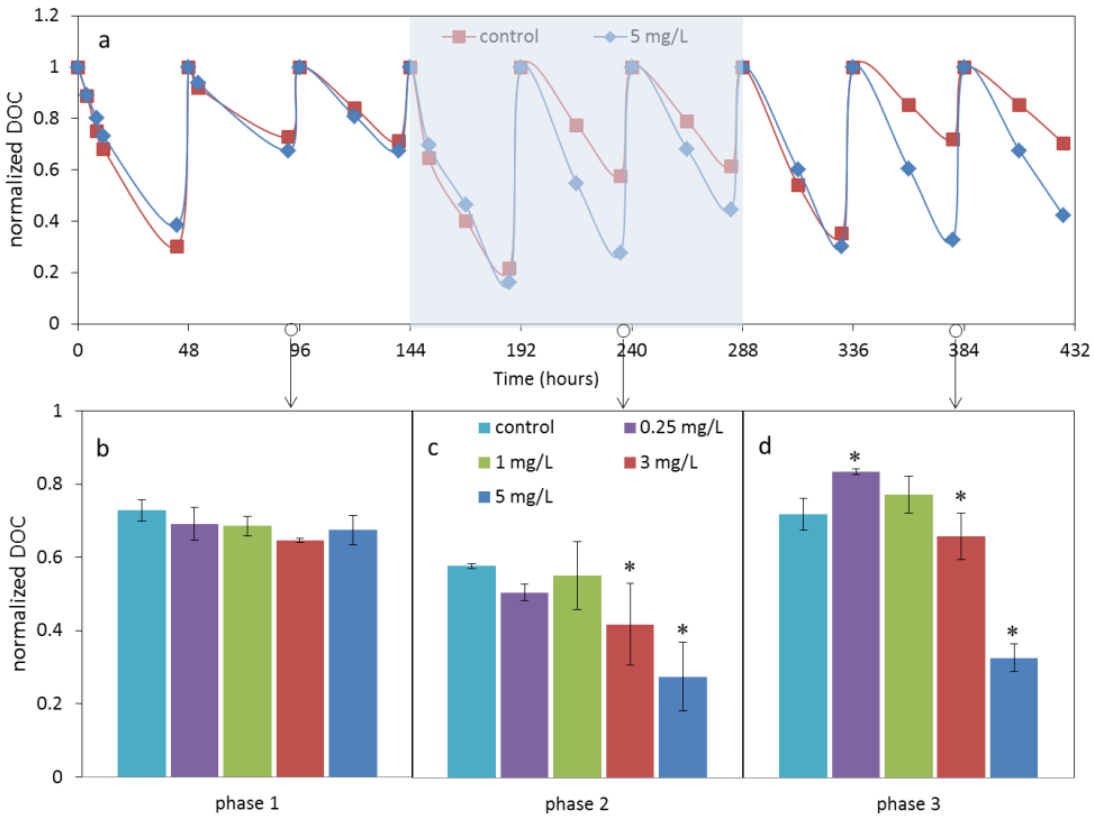



Figure 2 Normalised DOC concentrations in batch reactors n=3 over time (a), at the middle of phase 1 with shock load (b), phase 2 with intermittent load (c) and phase 3 with continuous load (d). The light blue shadow highlights phase 2. $p > 0.05$ for Figure 2-b, $p < 0.05$ for Figure 2-c, and $p < 0.05$ for Figure 2-d. * signifies a significant difference from the control ($p < 0.05$)

In non-autoclaved batch reactors, the $H_2O_2$ decomposition in different $H_2O_2$ dosage groups is presented in Figure 3-a. $H_2O_2$ initial concentrations in the range of 0.25-1 mg/L decomposed to below the detection limit of 0.015 mg/L, and 3-5 mg/L $H_2O_2$ decomposed to 0.08 mg/L in 4 hours. In the autoclaved batch reactors, however, DOC removal over time was not observed, while $H_2O_2$ decreased slowly from 5.4 mg/L to 2.4 mg/L within 3 days after dosing $H_2O_2$ (Figure 3-b). These results illustrate that in this study DOC removal only occurred in non-autoclaved batch reactors and $H_2O_2$ decomposition was strongly accelerated in these reactors.

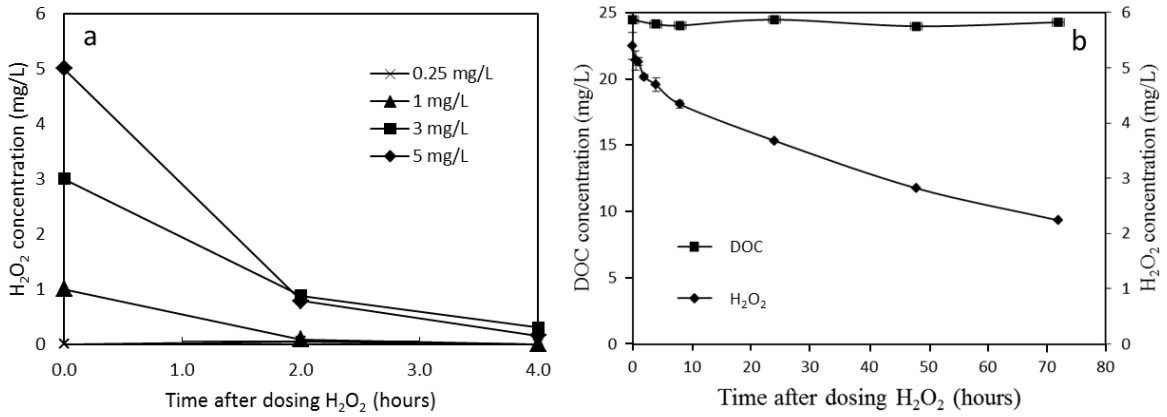



Figure 3 $H_2O_2$ concentrations in non-autoclaved batch reactors in the first day of the experiment (a) and DOC and $H_2O_2$ concentrations n=3 over 3 days after dosing 5 mg/L $H_2O_2$ in autoclaved batch reactors (b). n=3

## 3.2 Microbial activity

ATP concentrations in the supernatant of batch reactors over the three phases are shown in Figure 4. It can be observed that ATP concentrations in each $H_2O_2$ group were comparable ($p > 0.05$) during phase 1 (Figure 4-b) and phase 2 (Figure 4-c), while ATP in the 5 mg/L $H_2O_2$ group became lower than observed in the control group during phase 3 (Figure 4-d), which may be due to the continuous $H_2O_2$ dosing. In phase 3 (Figure 4-d) after the bacterial adaptive period, it appears that ATP values in high $H_2O_2$ concentration groups (1, 3 and 5 mg/L $H_2O_2$) were significantly lower than the control group (by 23%, 37% and 37%) ($p < 0.05$), and the ATP value in low concentration group of 0.25 mg/L had no notable difference compared to the control group. In phase 3, ATP decreased with

the increase of $H_2O_2$ dosage, which indicates that a low concentration of $H_2O_2$ may not impact microbial activity and that only a high concentration of $H_2O_2$ negatively affects the microbial activity.

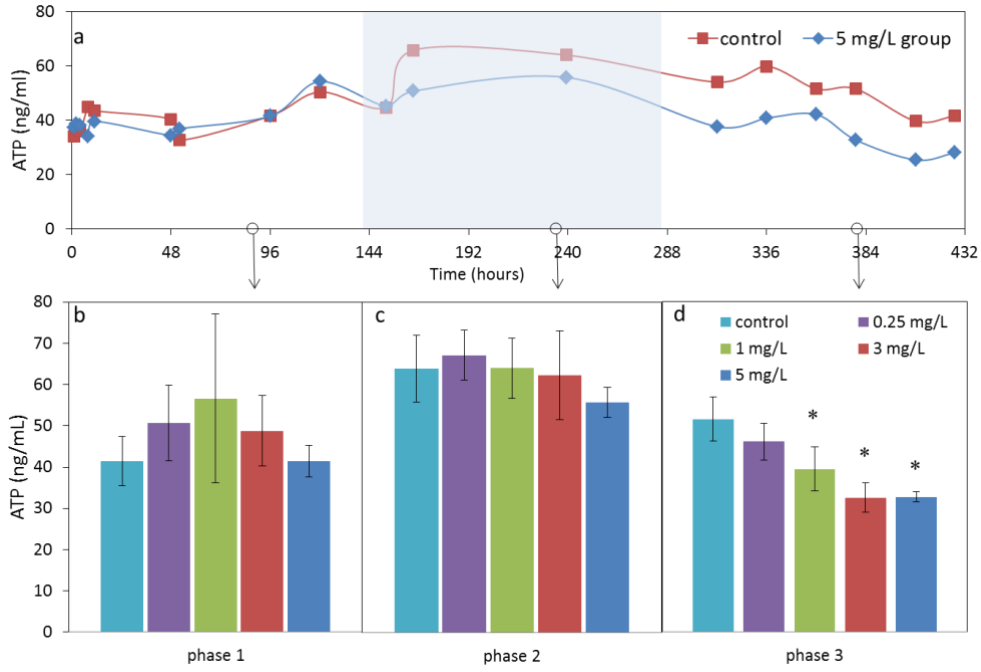



Figure 4 ATP concentrations in the supernatant of batch reactors over time (a), at phase 1 with shock load (b), phase 2 with intermittent load (c) and phase 3 with continuous load (d). $p > 0.05$ for Figure 4-b and Figure 4-c, and $p < 0.05$ for Figure 4-d. * signifies for significant difference from the control ($p < 0.05$). n=3

## 3.3 Microbial structure and composition

Microbial community analysis was conducted on representative sand samples from the control (0 mg/L $H_2O_2$), low concentration (0.25 mg/L $H_2O_2$) and high concentration (5 mg/L $H_2O_2$) groups at the end of this study (after phase 3). A broad microbial community was detected in all samples. Figure 5 shows the phylum level bacterial community composition and their relative abundances. The bacterial communities in all groups were dominated by *Proteobacteria*, more specifically, *Betaproteobacteria* (40%-46%), and around 40% of sequences could not be assigned to any of the known phyla. The results also show that all the percentages of *Alphaproteobacteria* (from 1.45% to 2.94%), *Betaproteobacteria* (from 36.18% to 38.74%) and *Gammaproteobacteria* (from 1.75% to 3.2%) increased with the addition of 5 mg/L $H_2O_2$, but they did not appear to change with the addition of 0.25 mg/L $H_2O_2$, indicating *Proteobacteria* may have a strong resistance to

H2O2. The abundance of *Firmicutes* became lower, from 8.84% via 8.02% to 4.80%, by dosing 0.25 and 5 mg/L $H_2O_2$, indicating that *Firmicutes* may have low resistance to $H_2O_2$. At genera level, 450, 1200, and 870 genera were detected in the control, 0.25 mg/L, and 5 mg/L groups, respectively.

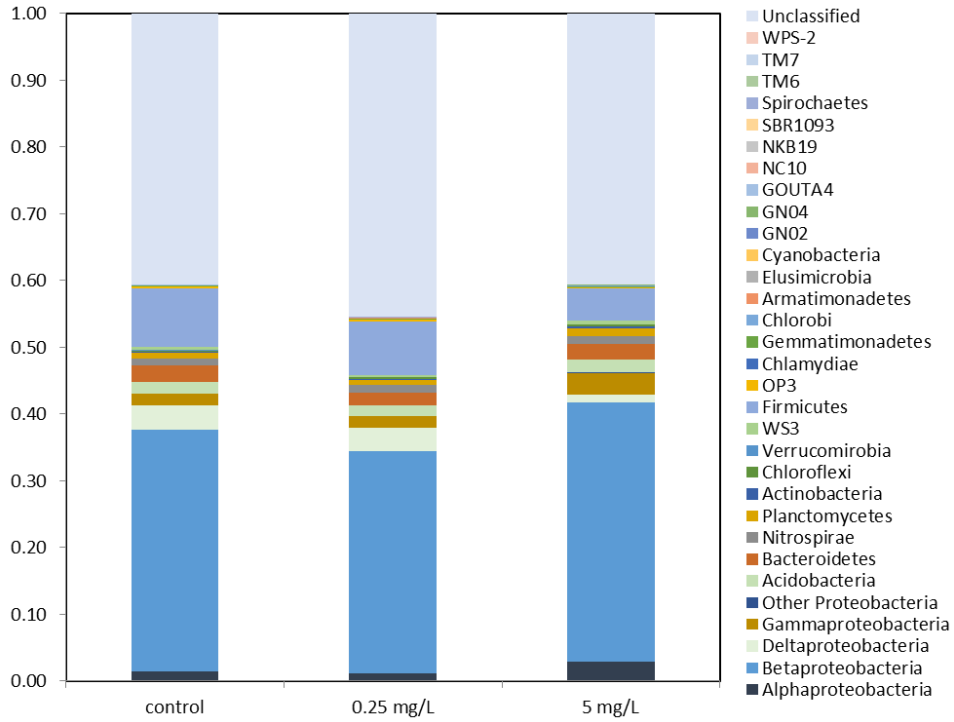



Figure 5 The relative abundance of different phyla and subclasses in *Proteobacteria* with and without the addition of $H_2O_2$. The phylum of *Proteobacteria* is shown in subclasses of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, and *Gamaproteobacteria*. n=2

The abundant genera (>1%) classified into four clusters are present in Table 2. It can be observed that there were not only aerobic bacteria but also anaerobic bacteria in the control group, suggesting that oxygen may have been a limiting factor for aerobic bacteria growth in batch reactors even though all batch reactors were exposed to the atmosphere. Compared with the control group, *Zoogloea* spp. (OTU 16623) and some unknown bacteria (OTU 1651) in cluster 1 increased under the presence of $H_2O_2$, suggesting that these bacteria have a strong tolerance to $H_2O_2$. 0.25 mg/L $H_2O_2$ increased *Zoogloea* spp. (OTU 9537) and *Comamonadaceae* spp. (OTU 9230 and OTU 5939) of cluster 2, but 5 mg/L $H_2O_2$ decreased their percentages, indicating that they may have a weak tolerance. For cluster 3, *Zoogloea* spp. (OTU 12210, 1987 and 15009) *and Comamonadaceae* spp.

(OTU 20898 and 14526) decreased in the 0.25 mg/L $H_2O_2$ group while they increased in the 5 mg/L $H_2O_2$ group. Finally, in cluster 4, percentages of *Rhodocyclaceae* spp. (OTU 4846), *Fusibacter* spp. (OTU 19986 and 21121) *and Geobacter* spp. (OUT 14196) decreased under the presence of 0.25 mg/L $H_2O_2$ and further decreased under the presence of 5 mg/L $H_2O_2$ in comparison with the control group, suggesting sensitivity to $H_2O_2$. Overall, it can be seen that aerobic bacteria showed different responses to $H_2O_2$, either sensitive or tolerant. However, anaerobic bacteria were sensitive to $H_2O_2$ and their growth was limited by both 0.25 and 5 mg/L $H_2O_2$ (17-88% reduction).

Table 2 The genera identified in the control, low $H_2O_2$ concentration (0.25 mg/L) and high $H_2O_2$ concentration (5 mg/L) groups that accounted for >1%

| | | Family | Genus | #OTU ID | Control (0 mg/L) | 0.25 mg/L | 5 mg/L |
|---|---|---|---|---|---|---|---|
| Aerobic | Cluster 1 | Rhodocyclaceae | Zoogloea | denovo16623 | 1.09 | 1.93 | 1.32 |
| | | Unassigned | unknown | denovo1651 | 0.48 | 1.11 | 0.62 |
| | Cluster 2 | Rhodocyclaceae | Zoogloea | denovo9537 | 1.04 | 1.07 | 0.74 |
| | | Comamonadaceae | unknown | denovo9230 | 1.15 | 1.47 | 0.60 |
| | | Comamonadaceae | unknown | denovo5939 | 1.34 | 1.93 | 0.62 |
| | Cluster 3 | Rhodocyclaceae | Zoogloea | denovo12210 | 6.24 | 2.59 | 6.71 |
| | | Rhodocyclaceae | Zoogloea | denovo19872 | 5.43 | 2.62 | 5.21 |
| | | Rhodocyclaceae | unknown | denovo15009 | 0.32 | 0.21 | 0.69 |
| | | Comamonadaceae | unknown | denovo20898 | 1.25 | 0.69 | 2.47 |
| | | Comamonadaceae | unknown | denovo14526 | 1.09 | 0.54 | 1.71 |
| Anaerobic | Cluster 4 | Rhodocyclaceae | unknown | denovo4846 | 1.08 | 0.55 | 0.52 |
| | | Acidaminobacteraceae | Fusibacter | denovo19986 | 4.51 | 3.72 | 2.75 |
| | | Acidaminobacteraceae | Fusibacter | denovo21121 | 3.50 | 2.90 | 1.61 |
| | | Geobacteraceae | Geobacter | denovo14196 | 1.46 | 1.01 | 0.17 |

The changes of their abundances as response to the addition of $H_2O_2$:
Cluster 1 increased at both low and high $H_2O_2$ dosage;
Cluster 2 increased at low $H_2O_2$ dosage but decreased at high $H_2O_2$ dosage;
Cluster 3 decreased at low $H_2O_2$ dosage but increased at high $H_2O_2$ dosage;
Cluster 4 decreased at both low and high $H_2O_2$ dosage.

## 3.4 Microbial diversity

### 3.4.1 Alpha diversity

Selected alpha diversity parameters (Shannon Index, Observed OTUs and Chao1) are presented in Table 3. The results indicate that a low dosage of $H_2O_2$ resulted in a more diverse bacterial community, whereas the high concentration dosage of $H_2O_2$ supressed the diversity of bacterial community.

Table 3 Alpha bacterial diversity in the control, low $H_2O_2$ concentration (0.25 mg/L) and high $H_2O_2$ concentration (5 mg/L) groups

| $H_2O_2$ dosage (mg/L) | Shannon Index | Observed OTUs | Chao1 |
|---|---|---|---|
| 0 (control) | 8.8 (±0.1) | 909 (±10) | 5700 (±300) |
| 0.25 | 9.3 (±0.2) | 975 (±19) | 6700 (±200) |
| 5 | 8.6 (±0.2) | 873 (±2) | 4500 (±10) |

### 3.4.2 Beta diversity

The comparison of the similarity of the bacterial communities was performed by principle coordinates analysis (PCoA) (Figure 6). Results showed that bacterial communities with the same dosage of $H_2O_2$ clustered together while different doses resulted in different clusters, suggesting that the addition of $H_2O_2$ influenced the bacterial community. These changes of bacterial community may explain the different DOC removal efficiency observed based on the DOC results.

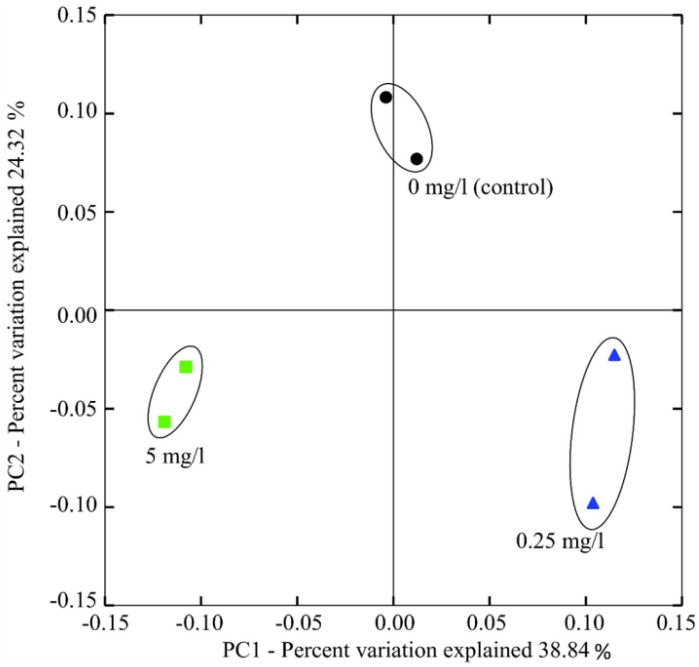



Figure 6 Principle coordinates analysis of bacterial community similarity among different groups of samples. The control group, 0.25 mg/L group and 5 mg/L group are shown in black circles, blue triangles and green squares, respectively. n=2

# 4 Discussion

## 4.1 Increase of DOC biodegradation under $H_2O_2$ presence

Since $H_2O_2$ is thought to disturb natural ecology by inactivating microbes and damaging flora and fauna (Knol, 2012; Kruithof et al., 2007), it is important to quench $H_2O_2$ residuals contained in AOPs effluent water before discharging into subsequent biological systems. This study showed that in the presence of 3 and 5 mg/L $H_2O_2$, the microbial activity in the water phase measured as ATP indeed decreased (Figure 4-d), indicating that microbial activity in the sand also decreased due to the positive correlation as described in section 2.2. However, at the same time DOC removal notably increased instead of decreased (Figure 2-d). A similar phenomenon was also observed by Urfer and Huck (1997), in which acetate removal in a biological filter receiving water with 1 mg/L $H_2O_2$ was slightly higher than in the control column after an adaption period of 28 days. Unfortunately, this phenomenon did not attract enough attention, and an explanation was not provided.

Although $H_2O_2$ may have reacted with DOC, the possibility that $H_2O_2$ removed DOC in this study can be excluded due to the stable DOC concentration in the autoclaved batch

reactors (Figure 3-b). Therefore, DOC removal caused by a high $H_2O_2$ dosage must be related to biological processes. In real sand filtration systems, it is possible that $H_2O_2$ oxidises organic matter into smaller molecules that can be more easily biodegraded (Chelme-Ayala et al., 2011; Metz et al., 2011), but acetate was the only carbon source in this study, and thus this reaction is not relevant. The slow decomposition of $H_2O_2$ in the autoclaved batch reactors can be explained by its reaction with inorganic substances attached to the sand instead of a reaction with DOC (Wang et al., 2016).

During aerobic degradation, free molecular oxygen accepts electrons released by an electron donor (e.g. soil organic carbon), which is reduced to a lower oxidation state (Morgan & Watkinson, 1992). Oxygen, potentially not present in adequate concentrations in the control group as previously described, limited the ability of aerobic microorganisms to actively degrade DOC. Figure 3-a shows that $H_2O_2$ in all groups decomposed within 4 hours, indicating oxygen, the decomposition product of $H_2O_2$, was formed quickly, and more oxygen was released in high $H_2O_2$ dosage groups than in low $H_2O_2$ dosage groups. The low $H_2O_2$ dosage group (0.25 mg/L) inhibited DOC biodegradation while high $H_2O_2$ dosage groups (3 mg/L and 5 mg/L) promoted DOC biodegradation (Figure 2-d). It can be hypothesised that the low concentration of $H_2O_2$ released limited oxygen that was not sufficient to promote aerobic bacterial activity. However, high concentrations of $H_2O_2$ released more oxygen which served as the electron acceptor for DOC biodegradation and therefore promoted aerobic degradation. Alternatively, the increased DOC removal with $H_2O_2$ dosage increase could also be caused by the change in bacterial community composition, which will be discussed in section 4.2.

## 4.2 Effects of $H_2O_2$ residuals on sand bacterial community

In this study, the obtained bacterial community results confirmed that $H_2O_2$ residuals affected sand bacterial community composition and its alpha and beta diversity. The results confirm that the sand bacterial community is sensitive to its surrounding environments, especially to the presence of $H_2O_2$, which can function both as a disinfectant to oxidise proteins and DNA (Apel & Hirt, 2004; Latifi et al., 2009) and as an oxygen source to enhance aerobic bacterial growth (Hinchee et al., 1991; Tusseau-Vuillemin et al., 2002; Zappi et al., 2000). In response, the bacterial community became more diverse after adding 0.25 mg/L $H_2O_2$, whereas the diversity decreased when the $H_2O_2$ dosage increased to 5 mg/L (Table 3). Potential explanations are: 1) $H_2O_2$ can be detoxified by cellular enzymes (e.g. catalases and peroxidases) (Pardieck; et al., 1992) and

2) oxygen from the low concentration of $H_2O_2$ promotes aerobic bacterial growth, although more cells are inactivated when the $H_2O_2$ exceeds the cellular detoxification capacity.

The different responses and resistances of OTUs to $H_2O_2$ dosage (genus results, Table 2) could be a complex result of $H_2O_2$ damage on bacterial cells (Glaeser et al., 2014), the growth promotion of oxygen from $H_2O_2$ decomposition (Aggarwal et al., 1991; Tusseau-Vuillemin et al., 2002) and bacterial catalase-positive property (Pardieck; et al., 1992). As stated previously, cluster 1, Z*oogloea* spp. (OTU 16623) and an unknown bacteria spp. (OTU 1651), has a strong tolerance to $H_2O_2$, which may be explained by their catalase-positive property. Catalase is responsible for the protection, interception and repair of microorganisms against $H_2O_2$/▪OH damage (Pardieck; et al., 1992). To the authors' knowledge, the catalase-positive property of those bacteria has not been reported. However, results without a bacterial cellular catalase in this study cannot test this hypothesis, so further study is necessary. Bacteria in cluster 2 (Table 2) may have a low tolerance to $H_2O_2$, while the damage of $H_2O_2$ on bacterial cells may become a leading role with the increase of $H_2O_2$ concentrations up to 5 mg/L. The change of bacterial percentages in cluster 3 (Table 2) may be explained by the damage of $H_2O_2$ on bacterial cells playing a leading role under the presence of 0.25 mg/L $H_2O_2$ while the growth promotion of oxygen from $H_2O_2$ decomposition became larger/the same level than the control group. A notably large reduction of the bacterial percentage occurred in cluster 4 (Table 2), therefore, those bacteria may be catalase-negative. *Fusibacter* and *Geobacter* are anaerobic bacteria that have been found in anaerobic conditions in soils and aquatic sediment (Lovley et al., 1987). Notably, percentages of all anaerobic bacteria, *Fusibacter* spp. (OTU 19986 and 21121) and *Geobacter* spp. (OTU 14196) were largely lowered under the presence of low and high concentrations $H_2O_2$, which can be explained by oxygen released by $H_2O_2$, inhibiting their growth and/or $H_2O_2$, damaging bacterial cells and DNA.

The observed changes in bacterial community caused by $H_2O_2$ residuals may influence the organic matter removal in sand systems since microbial degradation and assimilation play a dominant role in the attenuation of organic compounds (Amy & Drewes, 2007). This can be confirmed by the above DOC removal efficiencies of different groups: the highest DOC removal was found in the 5 mg/L $H_2O_2$ group, while the lowest removal was found in the 0.25 mg/L $H_2O_2$ group. It is hard to conclude which genus or species

contributed to DOC removal change in low and high $H_2O_2$ dosage groups, but the following hypothesis is provided. Bacteria of cluster 3 had a 34-50% reduction under the low concentration of $H_2O_2$ while they increased by 0% - 116% under the high concentration of $H_2O_2$. The consistent change trend of bacterial percentage and DOC removal indicates that bacteria in cluster 3 might contribute to DOC removal changes between the 0.25 mg/L group and the 0.5 mg/L group (Table 2). In particular, *Zoogloea* spp. (OTU 12210 and 19872) which has a strong ability to degrade different organic materials and has an important function in biological water treatment (Xia et al., 2014) was dominant in the control group, 0.25 $H_2O_2$ mg/L group and 5 $H_2O_2$ mg/L group, therefore deserving further consideration as an explanation for DOC removal change.

## 5 Conclusions

- The increase of DOC degradation with increasing $H_2O_2$ dosage was caused by a biological process and not by a direct reaction with $H_2O_2$. The low $H_2O_2$ concentration (0.25 mg/L) limited DOC biodegradation by 10%, whereas the high $H_2O_2$ concentration (3 and 5 mg/L) promoted DOC biodegradation by 8% and 28%.
- Low $H_2O_2$ concentrations (0.25 mg/L) did not influence microbial activity while high $H_2O_2$ concentrations (1, 3 and 5 mg/L) decreased microbial activity by 23%, 37% and 37%, respectively.
- The bacterial communities in sand were dominated by *proteobacteria*, more specifically, *Betaproteobacteria* (33%-39%). Both 0.25 and 5 mg/L $H_2O_2$ residuals were proven to influence bacterial community structure. The bacterial community became more diverse after the addition of 0.25 mg/L $H_2O_2$ but conversely became less diverse when the $H_2O_2$ dosage increased to 5 mg/L.
- Aerobic bacteria showed different responses to $H_2O_2$, either sensitive or tolerant. Anaerobic bacteria were found to be sensitive to $H_2O_2$, and their growth was limited by both 0.25 and 5 mg/L $H_2O_2$ (17-88% reduction).
- The increased DOC removal at higher $H_2O_2$ concentrations could potentially be explained by the aerobic bacteria in cluster 3, since microbial activity decreased at low $H_2O_2$ dosage whereas it increased at high $H_2O_2$ dosage. The dominant species in this cluster were *Zoogloea* (OUT 12210 and 19872) in the control, 0.25 mg $H_2O_2$ /L and 5 mg $H_2O_2$ /L groups; therefore these bacteria deserve further consideration as an explanation for DOC removal change.

- In conclusion, special attention should be given to the effect of AOP residuals on microbial ecology before introducing AOPs as pre-treatment to biological (sand) processes. In addition, the guideline on the maximum allowable $H_2O_2$ concentration should be properly evaluated.

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# 6

# Conclusions and recommendations

# 1 Conclusions

## 1.1 Successful removal of $BrO_3^-$ during MAR

In chapter 2 the focus was on microbiological bromate ($BrO_3^-$) removal. In the oxic column, neither nitrate ($NO_3^-$) nor $BrO_3^-$ removal was observed. The presence of $NO_3^-$ was found to be a precondition for $BrO_3^-$ reduction in $NO_3^-$-reducing anoxic zones of managed aquifer recharge (MAR) systems, which indicates that denitrifying bacteria are a main contributor for $BrO_3^-$ reduction. The results also indicated the simultaneous and competitive reduction of $BrO_3^-$ and $NO_3^-$ by denitrifying bacteria in the simulated MAR. Denitrifying bacteria prefer $NO_3^-$ to $BrO_3^-$ as an electron acceptor, but usually $BrO_3^-$ is present in trace amounts and the $NO_3^-$ concentration is several orders of magnitudes higher than $BrO_3^-$ in MAR infiltration waters. Therefore, it makes sense that $BrO_3^-$ removal percentage was observed greater than $NO_3^-$. An increase of assimilable organic carbon (AOC) as a result of advanced oxidation process (AOP) pre-treatment promotes microbial activity and therefore $BrO_3^-$ removal in MAR systems. Overall, it can be concluded that $BrO_3^-$ as a by-product of $O_3$-based AOPs can be effectively reduced in $NO_3^-$-reducing zones of MAR.

In chapter 3 the focus was on chemical $BrO_3^-$ removal. It was found that $BrO_3^-$ is readily reduced by $Fe^{2+}$. The reaction rate is influenced by the initial $Fe^{2+}/BrO_3^-$ ratio and the initial pH. A higher $Fe^{2+}$ concentration and a higher pH accelerate the reaction. The pH dropped considerably during the reduction of $BrO_3^-$ by $Fe^{2+}$, onset by the hydrolysis of $Fe^{3+}$ to HFO flocs. These HFO flocs were found to adsorb $Fe^{2+}$, particularly at high $Fe^{2+}/BrO_3^-$ ratios, whereas at low $Fe^{2+}/BrO_3^-$ ratios the incomplete $BrO_3^-$-$Br^-$ mass balance indicated formation of intermediate species. Overall, it can be concluded that $BrO_3^-$ can be reduced by naturally occurring $Fe^{2+}$ during MAR, as extensive retention times in the subsurface will compensate for the slow reaction kinetics of low $BrO_3^-$ and $Fe^{2+}$ concentrations. In the specific case that $Fe^{2+}$ containing and $NO_3^-$ containing waters cross flow paths during MAR, the presence of $NO_3^-$ will not compete with $BrO_3^-$ as $BrO_3^-$ is preferred to $NO_3^-$ as an electron acceptor, but it may somewhat inhibit $BrO_3^-$ reduction when $NO_3^-$ concentrations are far higher than $BrO_3^-$ concentrations.

The biodegradation of $BrO_3^-$ was quite apparent, 98%, in simulated $NO_3^-$-reducing zones with a residence time of 8 days. The chemical reduction of $BrO_3^-$ by $Fe^{2+}$ in Fe-reducing

zones within 5 days was only 7%-36% at an initial $BrO_3^-$ concentration of 60 µg/L. Therefore, $NO_3^-$-reducing zones are likely to be the predominant contributor to $BrO_3^-$ removal and trace amounts of $BrO_3^-$ residuals can be further reduced in Fe-reducing zones. The removal degree of $BrO_3^-$ will greatly depend on the specific retention time, infiltration water matrix and microbial activity and quantity of a MAR system.

### 1.2 Residual $H_2O_2$ no hazard to MAR

Regarding the risk of $H_2O_2$ on MAR systems, chapter 4 indicated that $H_2O_2$ residuals were able to decompose quite fast in the first centimeters of infiltration, assuming the AOP has also provided increased AOC levels. Additionally, chapter 5 showed that $H_2O_2$ has a slight effect on aerobic and anaerobic bacteria diversity, resulting in larger microbial diversity at low residual $H_2O_2$ concentrations (0.25 mg/L). Altogether, it may be concluded that the presented research did not give reason to conclude that residual $H_2O_2$ at concentrations below 3 mg/L posed a threat to the microbial stability of MAR systems.

Chapter 4 assessed the impact of five factors on the fate of $H_2O_2$ during MAR: pure sand, MAR infiltration water, soil organic matter (SOM), naturally inorganic substance on the surface of sand grains and living biomass. Pure sand, MAR infiltration water and SOM did not impact $H_2O_2$ decomposition. Naturally occurring inorganic substances on the surface of sand grains and living biomass are the two main contributors for $H_2O_2$ decomposition in MAR systems. Low concentration (<3 mg/L) of $H_2O_2$ in MAR influent water may decompose below 0.25 mg/L in the first centimeters of MAR systems when the water contains high microbial biomass (such as 38 ng ATP/mL).

Chapter 5 showed that an increase of $H_2O_2$ concentration resulted in an increase of dissolved organic carbon (DOC) biodegradation. The low $H_2O_2$ concentration (0.25 mg/L) limited DOC biodegradation by 10%, whereas the high $H_2O_2$ concentration (3 and 5 mg/L) promoted DOC biodegradation by 8% and 28%. Low $H_2O_2$ concentrations (0.25 mg/L) did not influence microbial activity (measured as ATP) while high $H_2O_2$ concentrations (1, 3 and 5 mg/L) decreased microbial activity by 23%, 37% and 37%, respectively. The bacterial communities in sand were dominated by *proteobacteria,* more specifically *Betaproteobacteria* (33%-39%). At residual $H_2O_2$ concentrations of both 0.25 and 5 mg/L, the bacterial community structure was influenced. The bacterial community became more diverse at a concentration of 0.25 mg/L $H_2O_2$ but conversely became less diverse when the $H_2O_2$ concentration increased to 5 mg/L. Aerobic bacteria showed different responses

to $H_2O_2$, either sensitive or tolerant. Anaerobic bacteria were found to be sensitive to $H_2O_2$, and their activity was limited by both 0.25 and 5 mg/L $H_2O_2$ (17-88% reduction). The increased DOC removal at higher $H_2O_2$ concentrations could potentially be explained by the aerobic bacteria, *rhodocyclaceae* and *comamonadaceae*. *Zoogloea* deserves further consideration as an explanation for DOC removal change. Special attention should be given to the effect of $H_2O_2$ on microbial ecology before introducing AOPs as pre-treatment to biological (sand) processes.

## 1.3 Overall conclusion

The combination of AOP and MAR has already been proven to be an effective barrier for organic microorganisms previously. In this thesis, batch reactor experiments and sand column simulation experiments have gained knowledge on the hybrid system of AOP and MAR on the aspect of inorganic by-products as summarized in this overall conclusion:

***MAR can successfully decompose $BrO_3^-$ as a by-product of $O_3$-based AOP pretreatment, either microbiologically or chemically. At high microbial biomass concentrations, the trace amounts of $H_2O_2$ residuals (<3 mg/L) from AOPs do not pose a threat to the purification function of subsequent MAR during drinking water treatment. Therefore, the combination of AOP and MAR is a synergistic hybrid system on the aspect of inorganic by-products $BrO_3^-$ and $H_2O_2$. The findings in this thesis mean a new application of MAR and may broaden the applicability of ozone-based AOPs in drinking water treatment.***

This thesis found the successful $BrO_3^-$ removal in MAR systems, which implies a new barrier for $BrO_3^-$ removal and broadens the applicability of $O_3$-based AOPs. In MAR systems, oxic zones have no significant $BrO_3^-$ removal ability. With the infiltration of water containing $BrO_3^-$, $NO_3^-$-reducing anoxic zones present an effective $BrO_3^-$ biodegradation capacity. Then the residual $BrO_3^-$ is further reduced by $Fe^{2+}$ with negligible levels of by-products, intermediate Br species, in Fe-reducing zones. The long retention time, from weeks to years, of MAR is quite helpful for the biodegradation and chemical reduction of $BrO_3^-$ in the low concentration range of μg/L. 3 mg/L $H_2O_2$ does not pose a threat to MAR systems at high microbial biomass concentrations. At low

microbial biomass concentrations, the quantity of microorganisms as a main contributor for $H_2O_2$ decomposition is lower, so $H_2O_2$ will not decompose that fast and may infiltrate to deeper areas. In that case, a quenching technology for $H_2O_2$ before infiltration may be necessary. Overall, the hybrid system of AOP and MAR is quite synergistic on the aspect of inorganic by-products.

# 2 Recommendations

## 2.1 Future research

Anoxic zones were found to be able to remove $BrO_3^-$ and two key factors, $NO_3^-$ and AOC, impacted $BrO_3^-$ removal. It has been found that once $NO_3^-$ decreased, $BrO_3^-$ removal increased, which indicates that denitrification functional gene may contribute to $BrO_3^-$ biodegradation. A future study exploring $BrO_3^-$-reducing functional gene is necessary to better understand the $BrO_3^-$ biodegradation mechanism. In addition, till now only the contribution of denitrifying bacteria to $BrO_3^-$ degradation in anoxic zones was observed. In order to optimize $BrO_3^-$ biodegradation, the specific identification of bacteria responsible for $BrO_3^-$ removal needs to be done in the near future.

$BrO_3^-$ reduction by $Fe^{2+}$ in concentrations similar as found in MAR has been proven to be feasible in ultrapure water without the interference of other ions and sediment. Besides $BrO_3^-$ and $Fe^{2+}$ concentrations, water matrix, naturally occurring inorganic compounds on the surface of sand particles and microorganisms will influence $BrO_3^-$ reduction rate as well. $Ni^{2+}$, $Cu^{2+}$ and $Ag^{2+}$ were reported to promote the reaction of $Fe^{2+}$ with $NO_3^-$ (Buresh & Moraghan, 1976). Given the presence of these elements in nature, for example the concentration of $Cu^{2+}$ at Dunea's MAR site is $10^{-2}$ mM, these may well onset $NO_3^-$ reduction by $Fe^{2+}$ and therefore limit $BrO_3^-$ reduction by $Fe^{2+}$. Moreover, previous studies (Benz et al., 1998; Brons et al., 1991; Oshiki et al., 2013) reported $NO_3^-$-dependent $Fe^{2+}$ oxidation mediated by anaerobic $NH_4^+$ oxidation bacteria, *Escherichia coli* and $NO_3^-$-reducing bacteria. Therefore, a microbial mediated kinetic reaction of $Fe^{2+}$ and $NO_3^-$ could also occur, leading to competition for $BrO_3^-$ reduction in these mixing flow paths during MAR systems. Therefore, future work should focus on other interference factors for $BrO_3^-$ reduction and the assessment of $BrO_3^-$ reduction in simulated MAR conditions using MAR sand columns and real MAR water. Additionally, the presence of intermediate species during chemical $BrO_3^-$ reduction is only an inference. The intermediate species need to be identified and their toxicity should also be concluded in a future study.

The successful $BrO_3^-$ reduction during MAR, as found in this thesis, may imply broader applications of $O_3$-based AOPs. As future research, the $BrO_3^-$-reducing bacteria isolation from MAR systems followed by bioaugmentation to biological reactors seems attractive to develop reactor-based technologies for $BrO_3^-$ removal. So far, only around twenty $BrO_3^-$-reducing bacteria have been recognized and isolated. Only a limited number of researchers have tried to build biofilm reactors to reduce $BrO_3^-$, and $BrO_3^-$-reducing rates were not as high as expected. For example, the study of Davidson et al. (2011) reported that bioaugmentation of activated carbon filters with eight of the $BrO_3^-$-reducing isolates did not significantly decrease start-up time or increase $BrO_3^-$ removal as compared to control filters. The unsuccessful application can be explained potentially by two reasons: 1) the current isolated $BrO_3^-$-reducing bacteria are not efficient enough and 2) the optimal conditions for $BrO_3^-$-reducing bacteria to remove $BrO_3^-$ have not been found. Considering the effective removal of $BrO_3^-$ in anoxic $NO_3^-$-reducing zones of MAR systems and the above two reasons, future research should focus on the isolation of new $BrO_3^-$-reducing bacteria from anoxic zones of MAR systems and then looking for the optimal reduction conditions in reactors (for example through response surface methodology). It is an important research direction, not only for drinking water production but also for wastewater treatment where ozonation or $O_3$-based AOP is considered for removal of residuals of pharmaceuticals. $BrO_3^-$ formation is also here a limitation for the applicability of these processes.

In this thesis $H_2O_2$ decomposition in MAR systems was studied under a high biomass concentration (38 ng/mL ATP). Microbial population was observed to be a dominant factor controlling $H_2O_2$ decomposition. Therefore, it is necessary to study $H_2O_2$ decomposition under a low biomass condition in the near future as well to comprehensively understand $H_2O_2$ fate in MAR systems. Additionally, the $O_2$ bubbles formed during $H_2O_2$ decomposition may block the pores and decrease the sand's permeability, so it is hard to maintain the continuity of the flow through sand columns. Batch reactor experiments as performed in this thesis, to avoid this phenomenon, can well evaluate $H_2O_2$ decomposition kinetics under different conditions and decomposition mechanism in MAR systems. However, column experiments as a dynamic system with influent and effluent is closer to MAR systems and therefore sand column experiments will simulate MAR more precisely. In the future, further sand column experiments should be done to accurately estimate the infiltration depth of $H_2O_2$ in the soil.

With respect to the effect of $H_2O_2$ residuals on MAR, the $H_2O_2$ concentration in this study was only 0-5 mg/L. The addition of 5 mg/L $H_2O_2$ in our batch reactors was found to increase the abundance of aerobic bacteria and make a positive change of DOC removal during MAR. The concentration of $H_2O_2$ applied in in-situ bioremediation is usually in a range of several hundred mg/L, but surprisingly the toxicity of these high levels of $H_2O_2$ does not impair the biodegradation process of pollutants and subsurface bioremediation supplemented with $H_2O_2$ (Fiorenza & Ward, 1997; Norris & Dowd, 1993). Considering the successful application of $H_2O_2$ as an $O_2$ source in in-situ bioremediation, probably the addition of $H_2O_2$ in MAR can also improve its biodegradation ability and therefore its water purification ability, such as organic matter and $BrO_3^-$ removal. $H_2O_2$-supplemented MAR may be an option. However, such an approach may decrease the anoxic zone and thus reduce the $BrO_3^-$ removal capacity of a MAR system. Future research should focus on finding the optimum $H_2O_2$ concentration.

## 2.2 AOP-MAR application in practice

This research provides valuable reference for drinking water companies which apply or consider to apply AOPs in their treatment scheme prior to a MAR system. This thesis showed the synergistic effects of implementing $O_3$-based AOP before MAR on the aspect of inorganic by-products ($H_2O_2$ and $BrO_3^-$). AOP-MAR is a safe hybrid system for drinking water companies. The limited $H_2O_2$ residuals seem not to affect the microbial activity and thus seem not to be a problem for MAR systems with high biomass. Instead, microbial activity and the DOC removal ability will be enhanced. However, anoxic zones are a prerequisite for $BrO_3^-$ removal. $O_3$-based AOP will definitely increase the DO so that the redox conditions in MAR systems will change. To make full benefits of the $BrO_3^-$ removal capacity of MAR, a drinking water company should analyse and model the hydrological behavior of the MAR system in order to be able to manipulate the infiltration regime and abstraction regime in such a way that anoxic zones indeed are present in the MAR system. Additionally, before applying AOP-MAR in practice, pilot studies need to be done by drinking water companies for accurately predicting $BrO_3^-$ removal and $H_2O_2$ decomposition, as many variables affect the behavior and fate of both $BrO_3^-$ and $H_2O_2$.

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# Appendix A - Supplementary material Chapter 2

Table S1 Water quality of MAR influent used in this study

| Parameter | DO (mg/L) | pH | EC (µS/cm) | $NH_4^+$ (mg/L) | $NO_3^-$ (mg/L) | $SO_4^{2-}$ (mg/L) | DOC (mg/L) |
|---|---|---|---|---|---|---|---|
| MAR influent | 10.25±0.5 | 8.06±0.2 | 511±8 | <0.1 | 11.37±2.83 | 51±9 | 4.39±1.46 |

Table S2 The formation of AOC and $BrO_3^-$ during ozonation

| $Br^-$ concentration before ozonation (µg/L) | $BrO_3^-$ formation from ozonation (µg/L) | AOC formation from ozonation (µg/L) | Sample | References |
|---|---|---|---|---|
| - | - | 0-120 | Ozonation in a drinking water treatment plant in the USA | (Escobar & Randall, 2001) |
| 810 | 0-65 | 0-190 | Ozonation of ground water in Taiwan under batch conditions | (Huang & Chen, 2004) |
| 92 | 0-10 | 0-200 | Ozonation in a drinking water treatment plant in the Netherlands | (Ross et al., 2016) |
| 115-258 | 0-250 | 0-200 | Ozonation of pretreated Rhine River water in a column experiment | (Orlandini et al., 1997) |
| 602-644 | 0-56 | - | Photolysis and oxidation treatment of groundwater in Sebria in a column experiment | (Agbaba et al., 2016) |
| - | 9.9-75 | - | Ozonation of Rhine River water at Amsterdam Water Supply | (Van Der Hoek et al., 1998) |

- Not reported

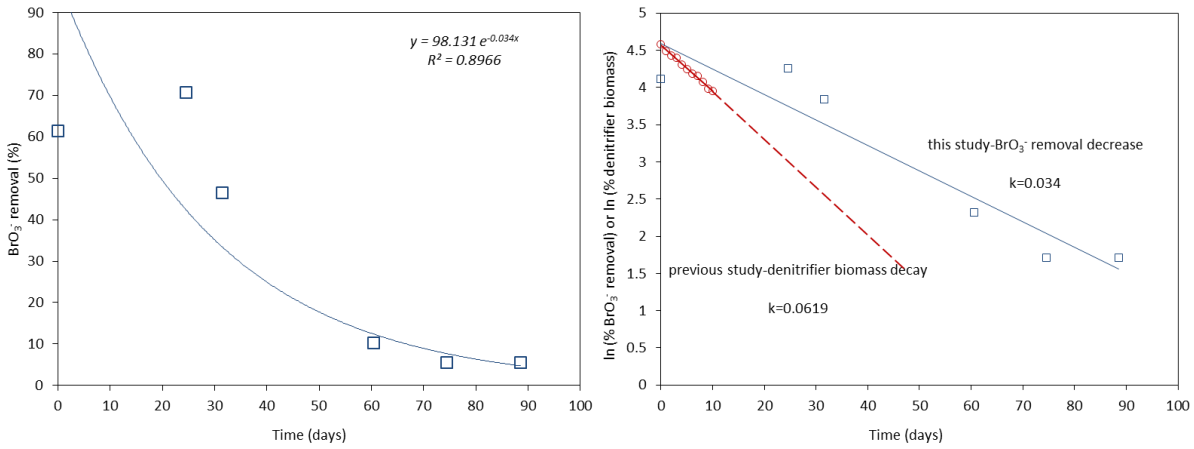


Figure S1 Comparison of the gradual decrease of $BrO_3^-$ removal following a sudden absence of $NO_3^-$ in the 1 m anoxic column in this study (blue squares in a and b) and the decay of denitrifying biomass as a result of exhausted substrates in a previous study (red dots in b). T=11.5±0.5 ℃

Figure S2 presents ATP and DOC concentrations in anoxic batch reactors with and without extra 1 mg/L C-$CH_3COONa$ dose. It shows clearly that the ATP concentration in autoclaved reactors as a reference was almost 0 and DOC removal was not observed, indicating no microbial activity in autoclaved reactors. The ATP concentration in reactors with extra carbon dose was twice of that in reactors without extra carbon dose (1.5 ng/mL and 3.3 ng/mL respectively) and DOC removal in the presence of extra carbon was higher than that in the absence of extra carbon (18.4% and 7.1% respectively). It demonstrates that extra 1 mg/L carbon increases biomass and DOC biodegradation in sand systems.

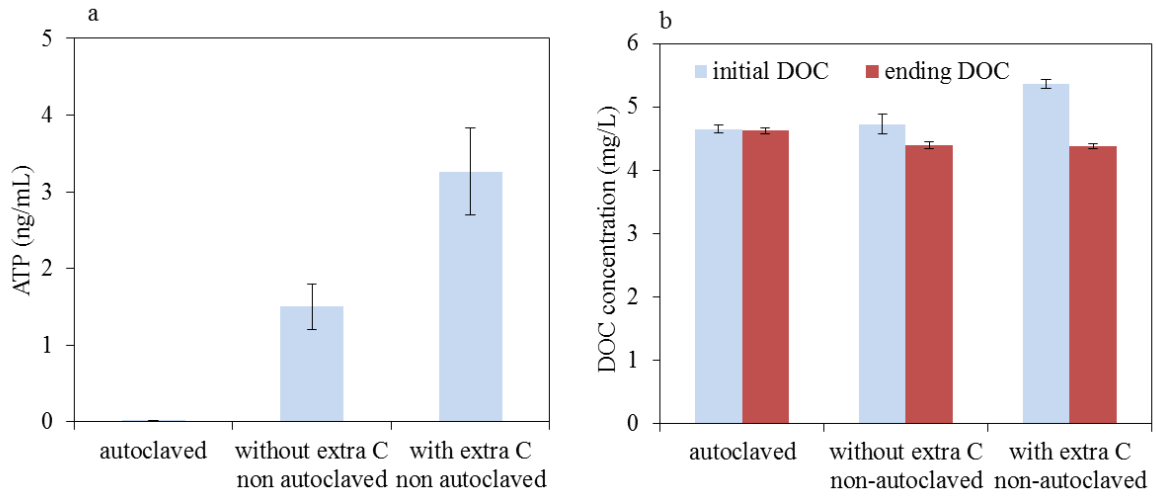


Figure S2 ATP (a) and DOC (b) concentrations in autoclaved and non-autoclaved batch reactors with and without extra carbon dose (1 mg/L C-$CH_3COONa$) at day 21 (a) and over 7 days (b). n=3

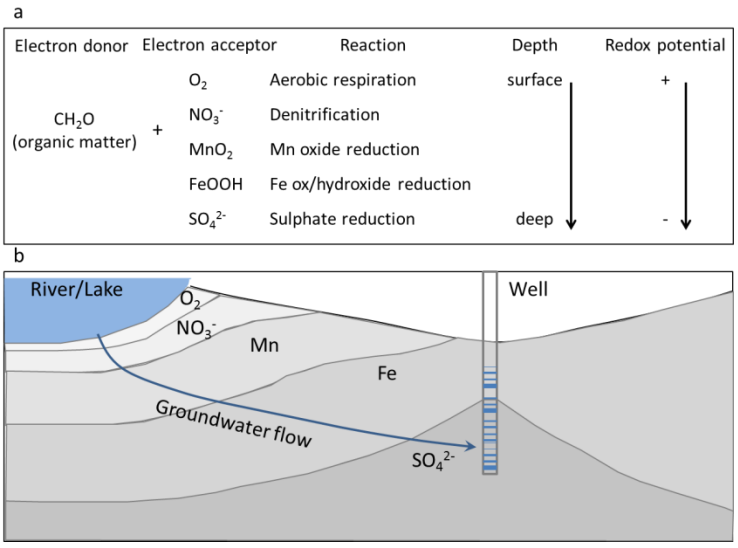


Figure S3 Redox conditions and sequence of terminal electron acceptor processes in MAR systems

# Appendix B - Supplementary material Chapter 5

## Supplemental Information 1: DOC removal in batch reactors during the ripening phase

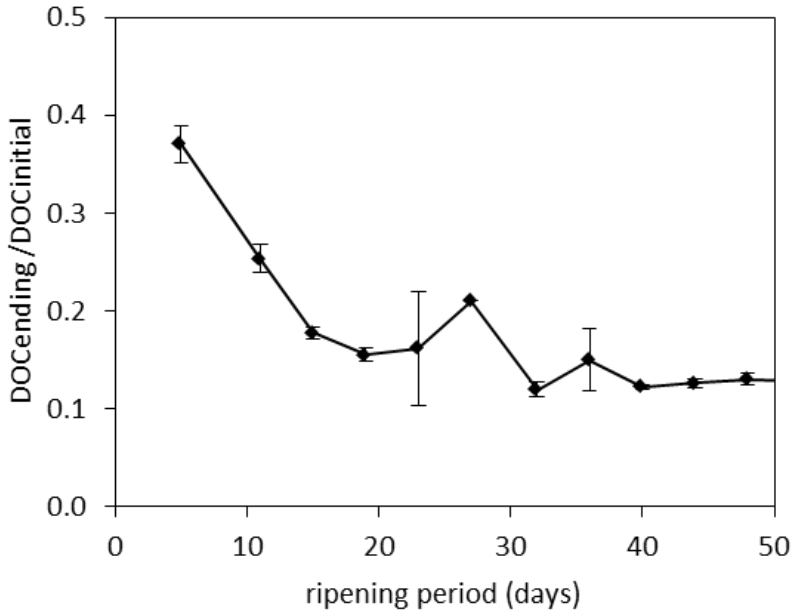


Figure S1 DOC removal in batch reactors during the ripening phase

## Supplemental Information 2: Correlation between ATP in the water phase and ATP in the sand

### S2.1 Materials and methods

The water, the sand, the experimental set-up and the ripening phase were the same as those in the manuscript. After ripening the reactors, $H_2O_2$ spiking experiments started. Different dosages of $H_2O_2$ were added to reactors to result in final concentrations of 0, 0.25 and 5 mg/L in 15 batch reactors after water refreshing. To investigate the response of ATP in the water and in the sand to $H_2O_2$ addition, ATP samples were collected from the supernatant in batch reactors, and a 5 g sand sample was collected from the bottom of 8 batch reactors in 8 hours and the other 7 batch reactors after dosing $H_2O_2$.

ATP concentrations of the supernatant (1 mL) and sand (5 g) samples were determined. The analysis of ATP in the water was the same as that in the manuscript. ATP in the sand was measured using deposit & surface analysis test kits (Canada) and a LB9509 luminometer (Aqua Tools, France). Based on the test kit instructions, a 5 g sand sample was directly dosed into an UltraLyse$^{TM}$ 7 extraction tube with 5 mL ATP extraction reagent to lyse the bacteria and release ATP. Secondly, 1 mL from the extraction tube was transferred to a 9 mL UltraLute dilution tube. Next, the luminescence reaction of sample ATP with Luminase was measured as a Relative Luminescence Unit (RLU), and finally the RLU value was compared to that of a check standard (LuminUltra's UltraCheck) and converted to ATP concentration in pg/g.

## S2.2 Results

Figure S2 showed that ATP in the water decreased and simultaneously ATP in the sand also decreased with increasing $H_2O_2$ doses from 0 to 5 mg/L at both 8 hours (Figure S2-a) and 12 hours (Figure S2-b), indicating the positive correlations between ATP in the water and in the sand.

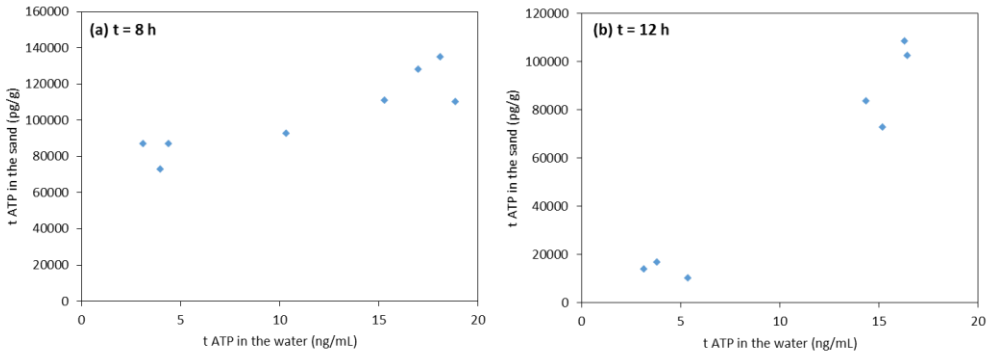


Figure S2 Correlations between ATP in the water and in the sand of the same batch reactor in 8 hours (a) and 12 hours (b) after dosing 0, 0.25 mg/L and 5 mg/L $H_2O_2$. T=11.5±0.5 ℃

## Supplemental Information 3: Partial correlation between DOC concentration, DOC accumulation and $H_2O_2$ dosage

Table S1 the partial correlation between DOC concentration and DOC accumulation and $H_2O_2$ dosage

| | DOC accumulation | $H_2O_2$ dosage |
|---|---|---|
| Normalized DOC in phase 1 | 0.248 | -0.559 |
| Normalized DOC in phase 2 | -0.606 | -0.973* |
| Normalized DOC in phase 3 | -0.839 | -0.925* |

* Correlation is significant at 0.05 level. Normalized DOC is $DOC_t$ / $DOC_o$, standing for the remaining DOC in batch reactors

# List of publications

**Wang F.**, van Halem D., Ding L., Bai Y., Lekkerkerker-Teunissen K., van der Hoek J.P. 2018. Effective removal of bromate in nitrate-reducing anoxic zones during managed aquifer recharge for drinking water treatment. *Water Research*, 130, 88-97.

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Ding S., Chu W., **Wang F.**, Cao Z., Xu B., Gao N. 2018. Degradation kinetics and mechanisms of chlorinated, brominated, and iodinated haloacetamides by sulfite in drinking water. Submitted to *Water Research* (Under Review).

Chen S., Chu W., **Wang F.**, Li X, Wei H., Gao N. 2018. Effect of weak magnetic field on haloacetamides removal from water by zero-valent iron in drinking water (To be submitted).

**Wang, F.**, van Halem, D., van der Hoek, J.P. Bromate removal and denitrification functional genes in nitrate-reducing anoxic zones during managed aquifer recharge (In Preparation).

# Presentations at international conferences

Wang F., van Halem D., Lekkerkerker-Teunissen K., van der Hoek J.P. 2013. The fate of $H_2O_2$ as the residual from AOP within managed aquifer recharge. Young Water Professionals Benelux Conference, Luxemburg.

Wang F., van Halem D., Liu G., van der Hoek J.P. 2014. The effect of $H_2O_2$ as the residual from AOP within managed aquifer recharge. Ninth International Symposium on Subsurface Microbiology, Pacific Grove, California, USA.

Wang F., van Halem D., Liu G., van der Hoek J.P. 2017. Effect of residual $H_2O_2$ from AOPs on subsequent biological water treatment. First Symposium on Microbiological Methods for Waste and Water Resource Recovery, Delft, The Netherlands.

Wang F., van Halem D., Ding L., Bai Y., Lekkerkerker-Teunissen K., van der Hoek J.P. 2018. Effective removal of bromate, as a by-product of advanced oxidation, during managed aquifer recharge for drinking water treatment. The 2nd Disinfection and Disinfection By-Products Conference, Beijing, China.

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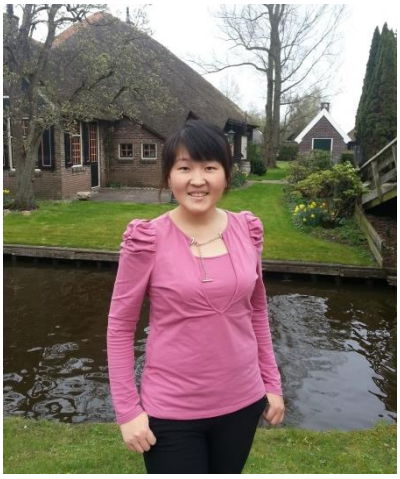
# Curriculum Vitae



Feifei Wang (王菲菲) was born in Weifang City, Shandong Province, China in June, 1986. She obtained her BSc degree in Geography at Jinan University, China in 2009. After that, she started her master study focusing on Ecological Restoration Engineering at State Key Laboratory of Estuarine and Coastal Research, East China Normal University, China. During her master study period from 2009 to 2012, she joined two projects, the National Water Pollution Control and Treatment Science and Technology Major Project and the Improvement of Saline-alkali Land from Chinese Ministry of Agriculture. Her study mainly focused on 1) the corresponding relationship between nutrients nitrogen and phosphorus input and algae growth, and 2) developing a lake nutrient bioassay method named Nutrient Enrichment Bioassay for assessing the relationship mentioned above.

After her master graduation, she started her PhD research under the supervision of Professor Jan Peter van der Hoek and Assistant Professor Doris van Halem at Water Management Department, Delft University of Technology, Netherlands. Her PhD thesis focused on the removal of by-products of $O_3$-based AOPs in the subsequent managed aquifer recharge and was a part of the Topsector Water TKI Watertechnologie project and Dunea drinking water company's project. She presented/published her research outcome at several international workshops, international conferences and in peer reviewed journals.