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DOI
10.4233/uuid:d36194de-1203-4342-bf20-8d03380c5b40

Publication date
2018

Document Version
Final published version

Citation (APA)
Wang, F. (2018). *Managed aquifer recharge as a barrier for ozone-based advanced oxidation by-products: BrO3- and H2O2*. [Dissertation (TU Delft), Delft University of Technology].
https://doi.org/10.4233/uuid:d36194de-1203-4342-bf20-8d03380c5b40

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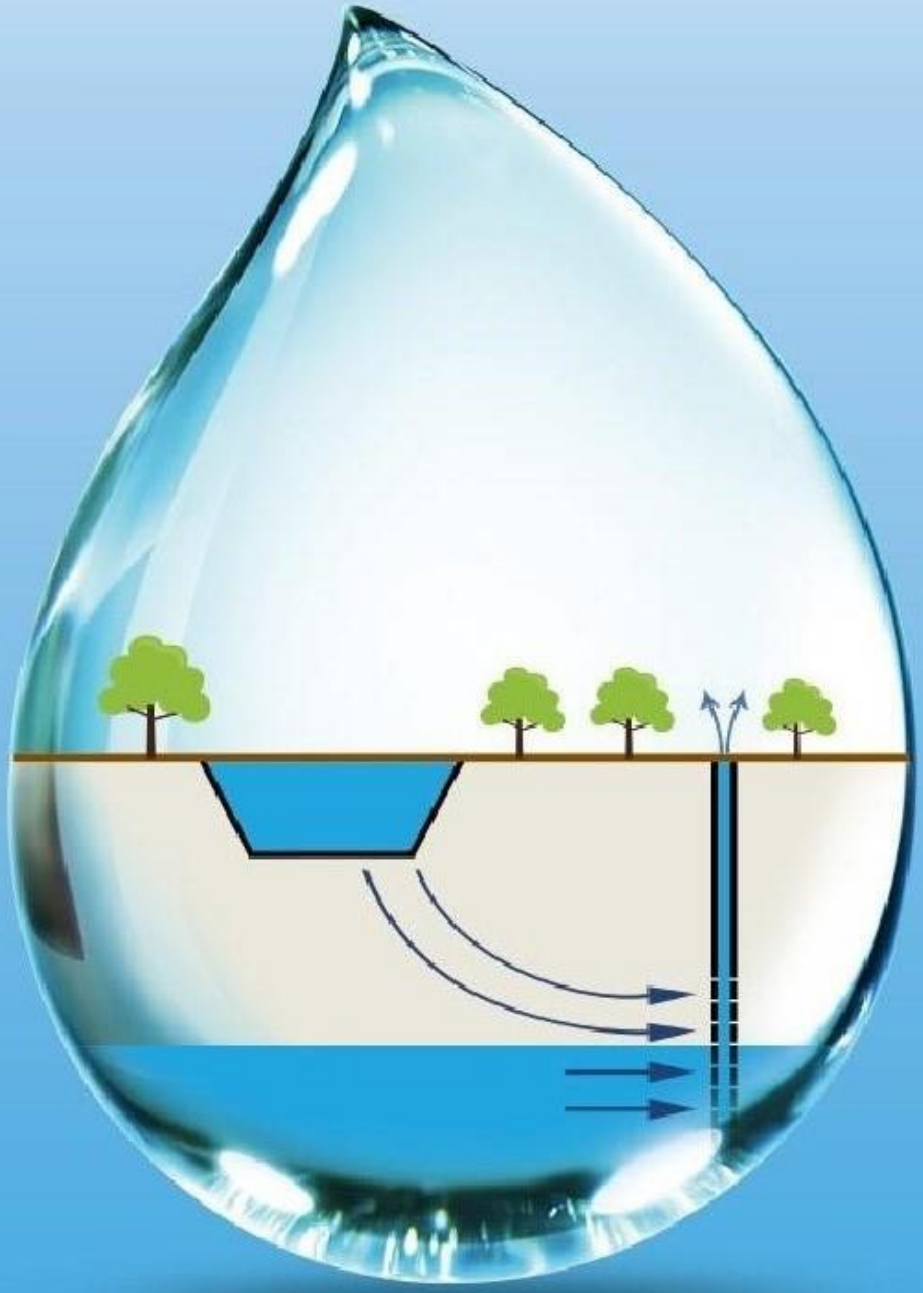

Feifei Wang

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

Woensdag 16 mei 2018 om 12:30 uur

door

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The research was funded by the Dunea drinking water company and by the Topsector Water TKI Watertechnology Program of the Dutch Ministry of Economic Affairs (No. 2013TUD001). The PhD candidate was supported by scholar from China Scholarship Council (201206140009).

Printed by: Delft Academic Press
ISBN: 978-90-6562-422-2
Cover designer: Feifei Wang

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.

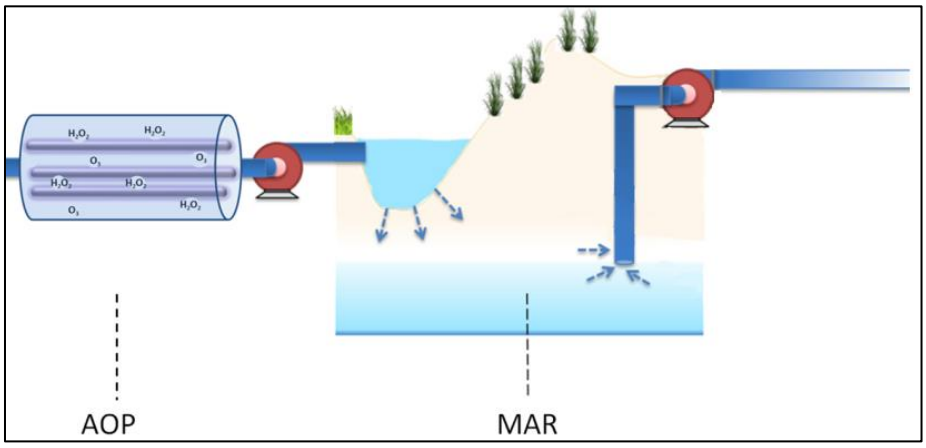
Eerder is gebleken dat de verwijdering van OMVs tijdens de drinkwaterproductie door een systeem met meerdere barrières, bestaand uit AOP en MAR, een complementair en synergistisch systeem is voor de verwijdering van OMVs. Dit proefschrift beschrijft dit

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.

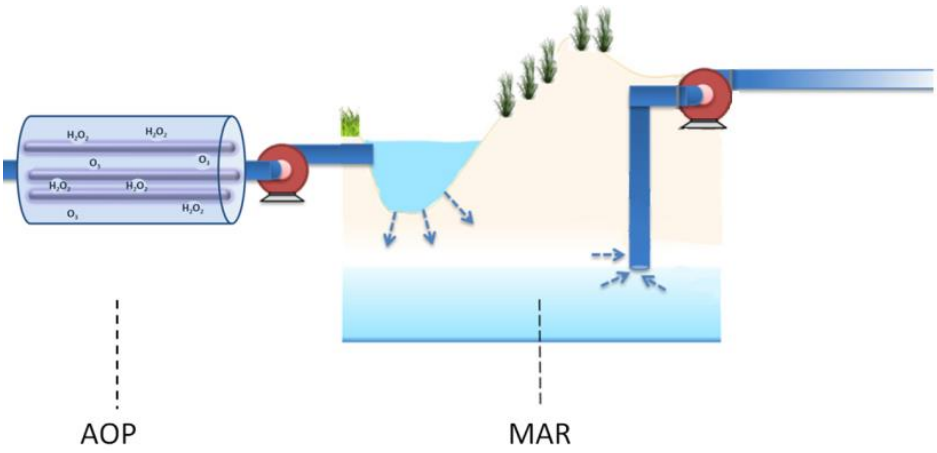


Figure 1 The combination of AOP and MAR during drinking water treatment

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.

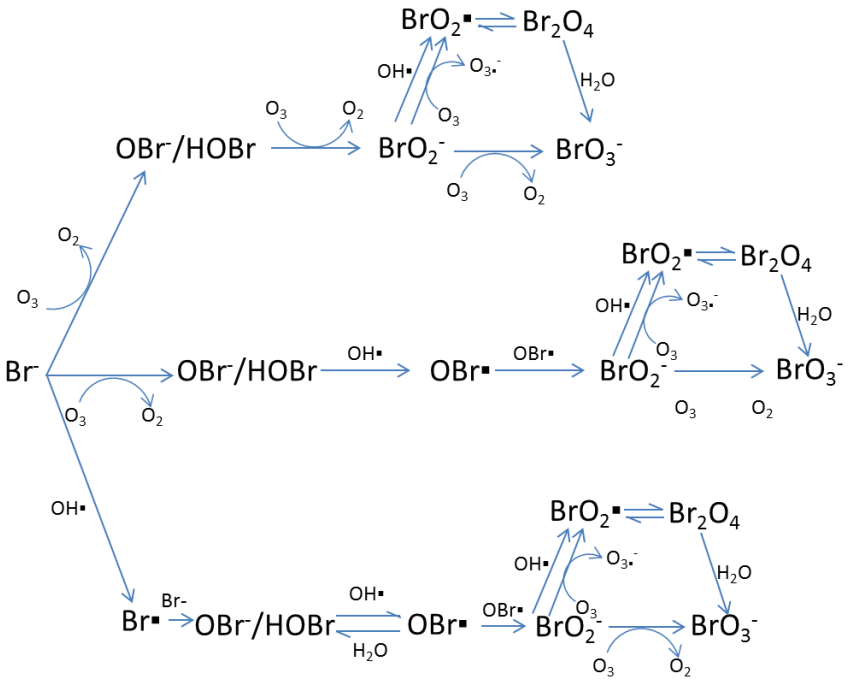


Figure 2 The pathways of BrO_3^- formation from Br^- (adapted from Jarvis et al. (2007); (Von Gunten & Hoigné, 1994) and Fischbacher et al. (2015))

$KBrO_3$ 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).

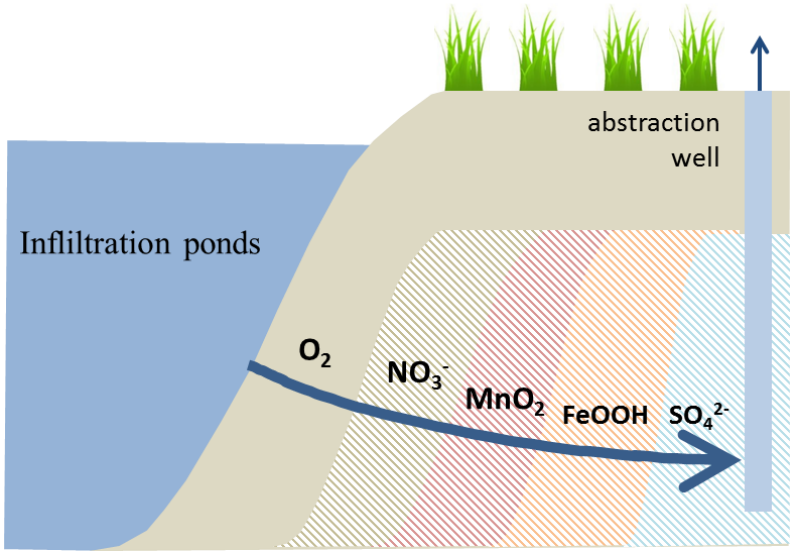


Figure 3 The sequence of terminal electron acceptor in MAR systems

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? i. what is the effect of AOC due to ozonation pre-treatment on BrO_3^- removal? ii. what is the effect of NO_3^- long-term presence, sudden absence and long-term absence? 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^-? i what is the mechanism of the reduction of BrO_3^- by Fe^{2+} ? ii is it possible for Fe^{2+}, at concentrations similar to MAR, to reduce trace amounts of BrO_3^-? 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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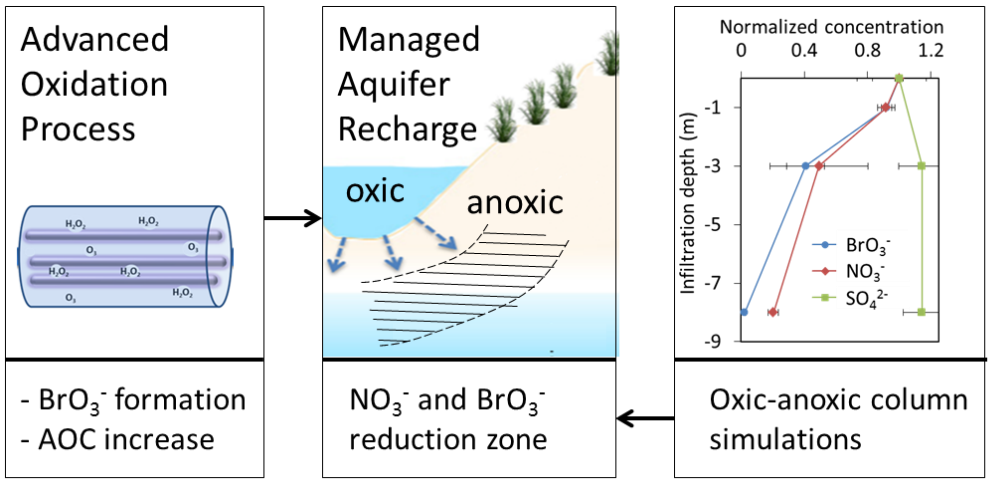
2

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



This chapter is based on:

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.

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.

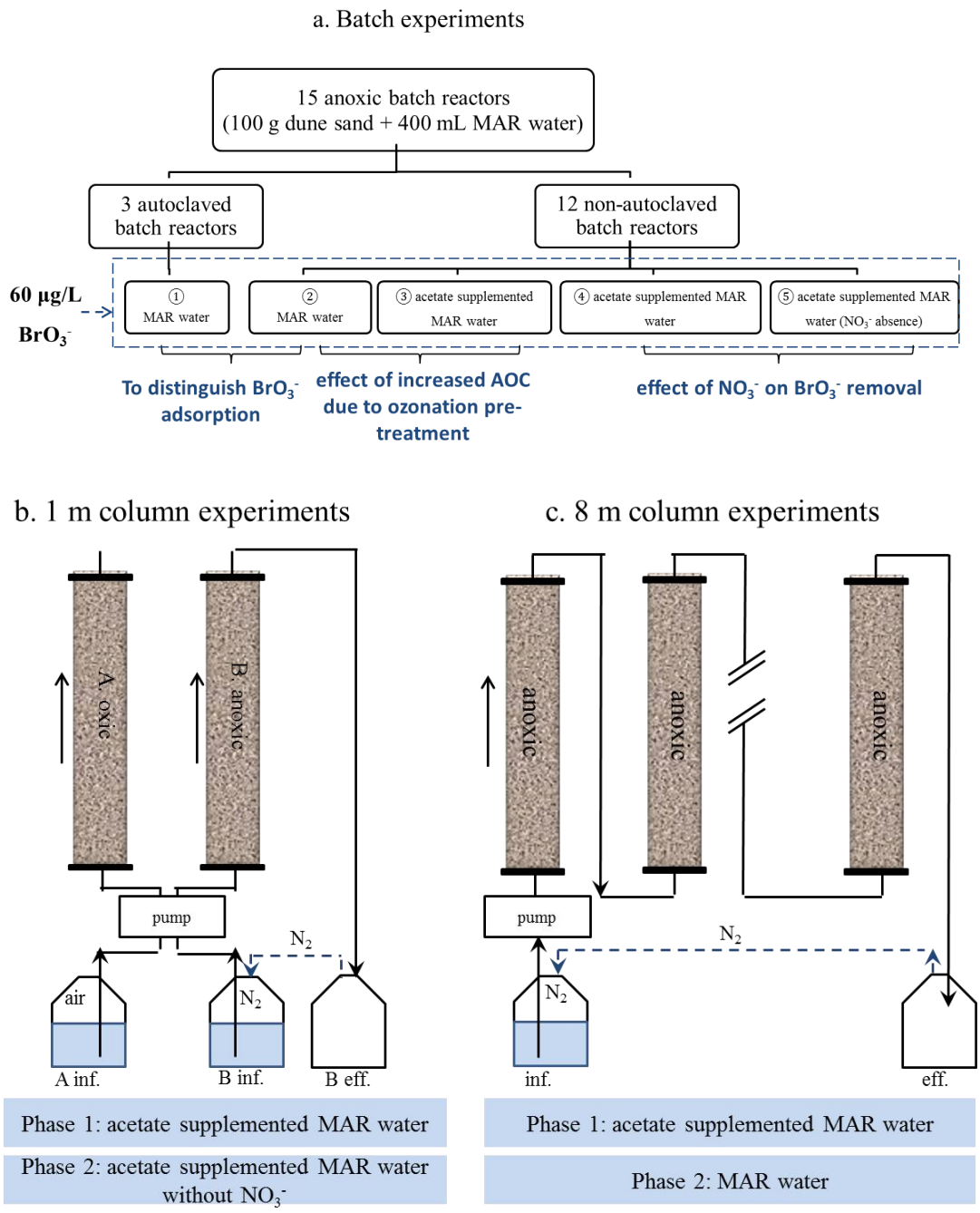


Figure 1 Batch and column experimental designs

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%).

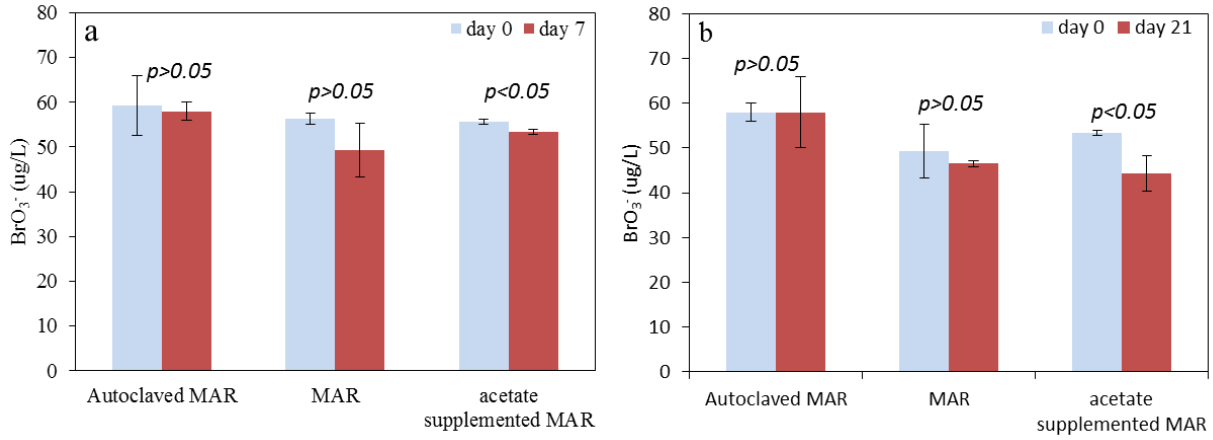


Figure 2 BrO_3^- removal in autoclaved and non-autoclaved batch reactors with MAR water and acetate supplemented MAR water over 7 days (a) and 21 days (b). An additional 1 mg/L AOC from CH_3COONa solution was added to MAR water to create the acetate supplemented MAR water. All batch reactors were in 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.

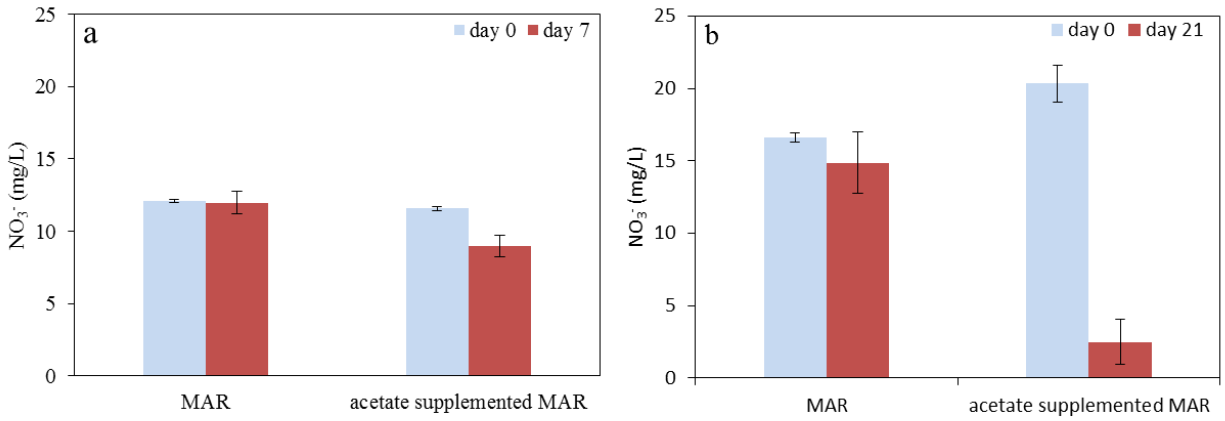


Figure 3 NO_3^- removal in anoxic batch reactors with MAR water and simulated ozonation-MAR water over 7 days (a) and 21 days (b). An additional 1 mg/L AOC from CH_3COONa solution was added to MAR water to create the acetate supplemented MAR water. T=11.5±0.5℃. n=3

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.

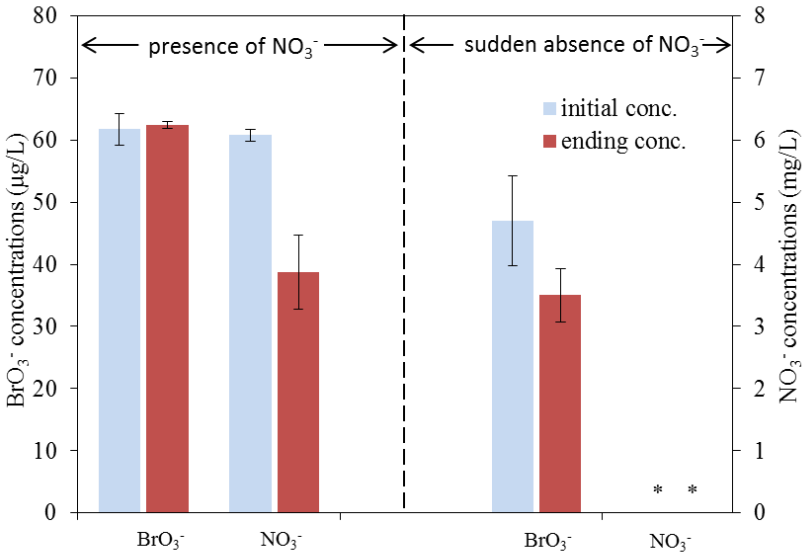


Figure 4 BrO_3^- and NO_3^- removal in batch reactors with acetate supplemented MAR water in the presence and sudden absence of NO_3^- within 2.7 hours. * indicates measurements below the detection limit. T=11.5±0.5℃. n=3

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.

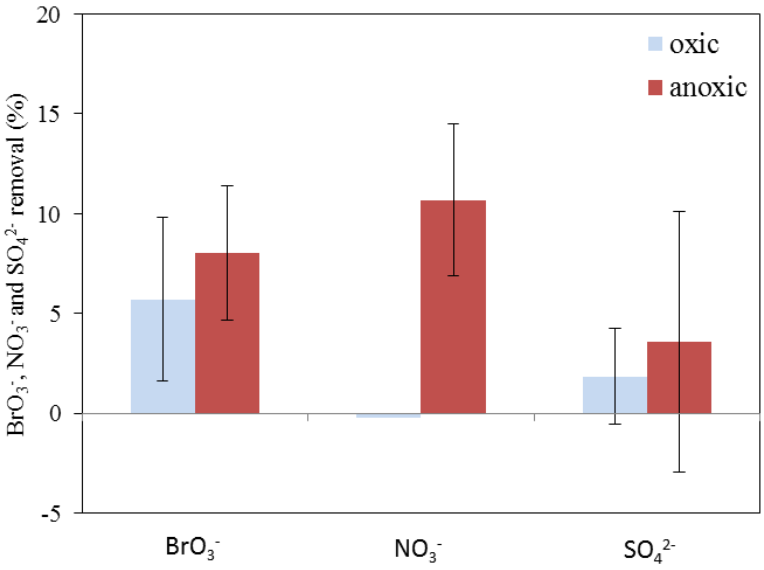


Figure 5 BrO_3^-, NO_3^- and SO_4^{2-} removal in oxic and anoxic columns with acetate supplemented MAR water as the influent. 150 μg/L AOC from CH_3COONa solution was added to MAR water to compose the acetate supplemented MAR water. The concentrations of BrO_3^-, NO_3^- and SO_4^{2-} were 58.9±3.1 μg/L, 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.

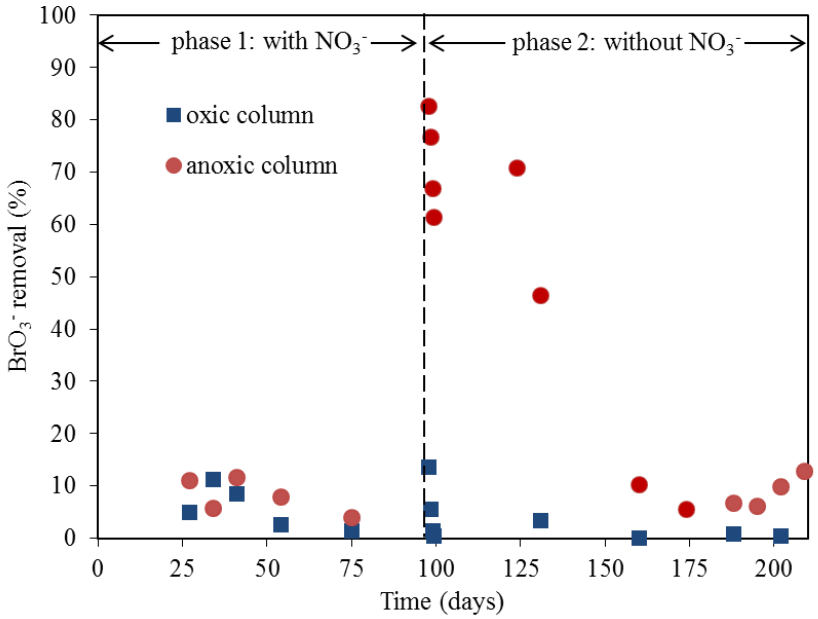


Figure 6 BrO_3^- removal in the 1 m oxic and anoxic columns containing acetate supplemented MAR water as influent with 10.3±1.8 mg/L NO_3^- (phase 1: 0-98 days) and acetate supplemented MAR water as influent with NO_3^- below than detection limit (0.89 mg/L) (phase 2: 98-209 days). 150 µg/L AOC from 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.

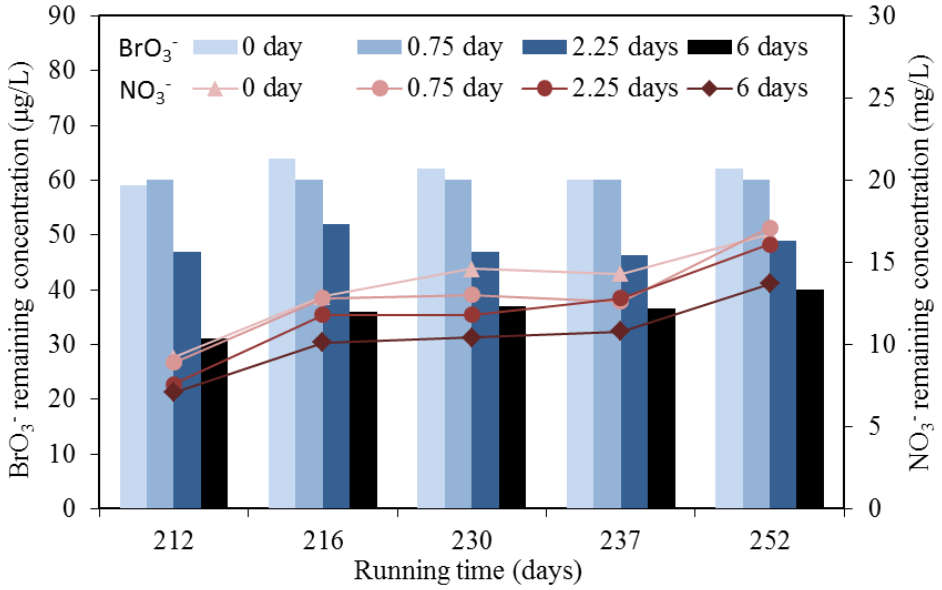


Figure 7 BrO_3^- removal and normalized concentrations of BrO_3^- and NO_3^- in the 8 m anoxic column set-up containing MAR water as the influent. BrO_3^- and NO_3^- in the influent were 63±4 µg/L and 13±3.8 mg/L respectively. Influent DO was below 0.6 mg/L. T=11.5±0.5 ℃

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.

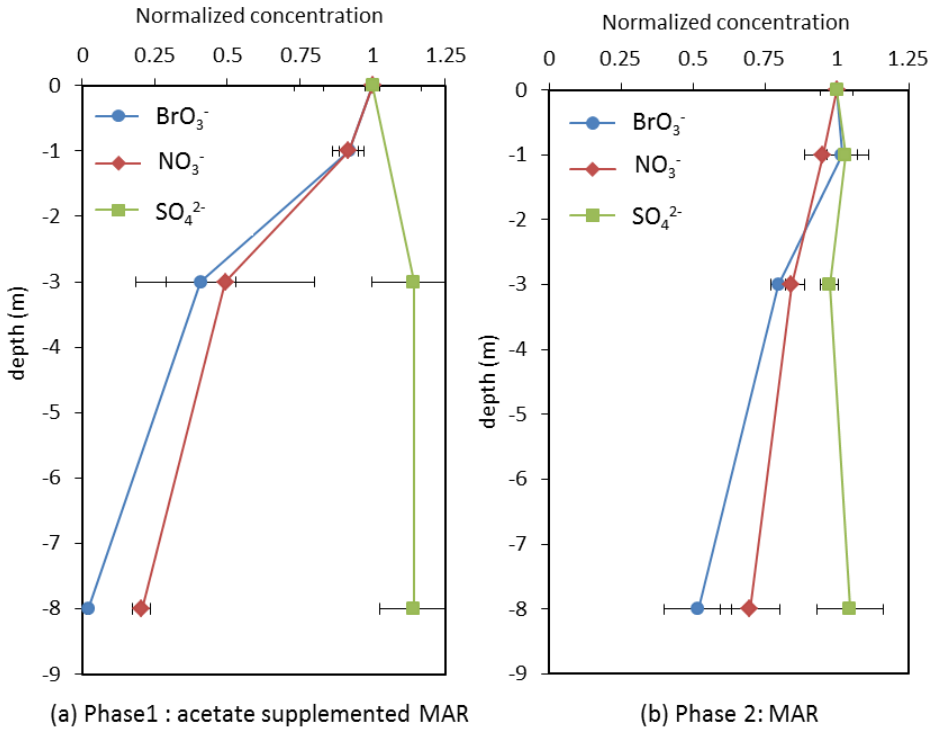


Figure 8 Average concentrations (n=5-8) of BrO_3^-, NO_3^- and SO_4^{2-} with depth in the 8 m anoxic column during phase 1 with acetate supplemented MAR water (a) and during phase 2 with only MAR water (b). 150 µg/L AOC from CH_3COONa solution was added to MAR water to compose the acetate supplemented 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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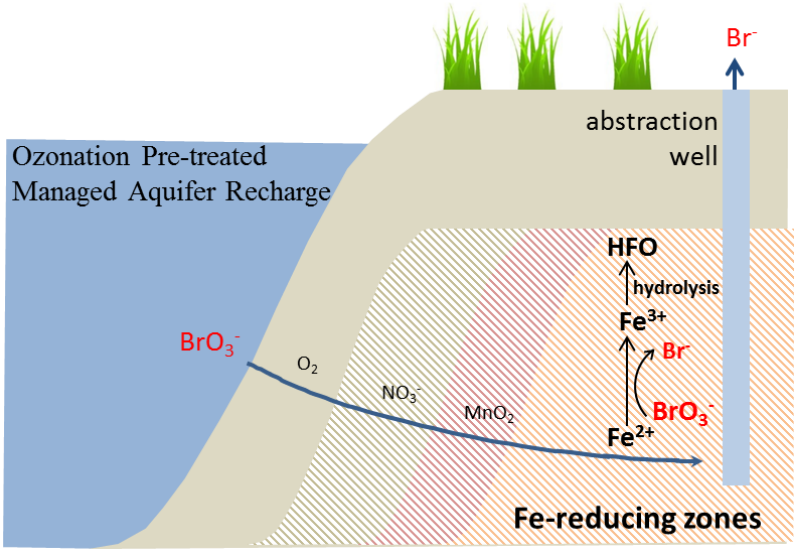
3

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



This chapter is based on:

Wang F., Salgado V., van der Hoek J.P., van Halem D. 2018. Bromate reduction by iron (II) during managed aquifer recharge: A laboratory-scale study. *Water*, 10, 370.

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.

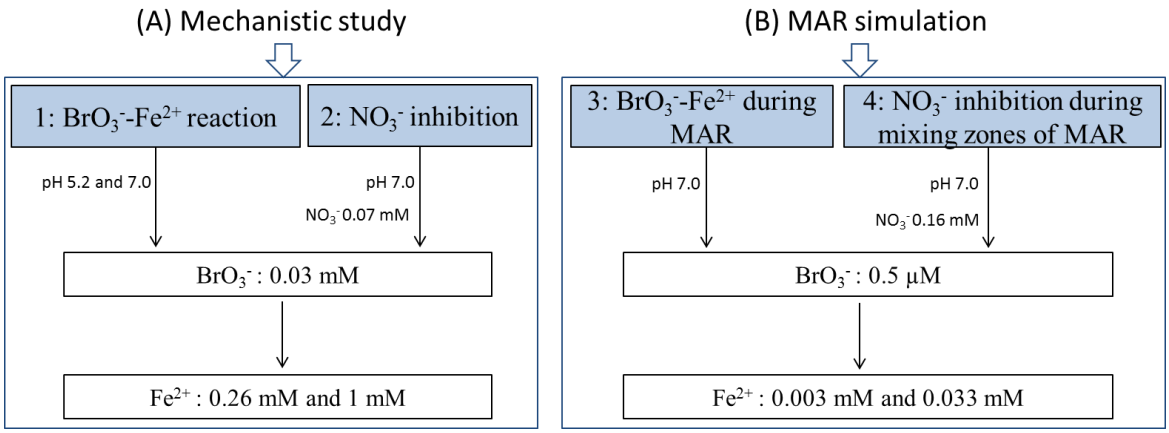


Figure 1 Experimental overview of anoxic batch reactors T=11.5±0.5 ℃. n=2

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.

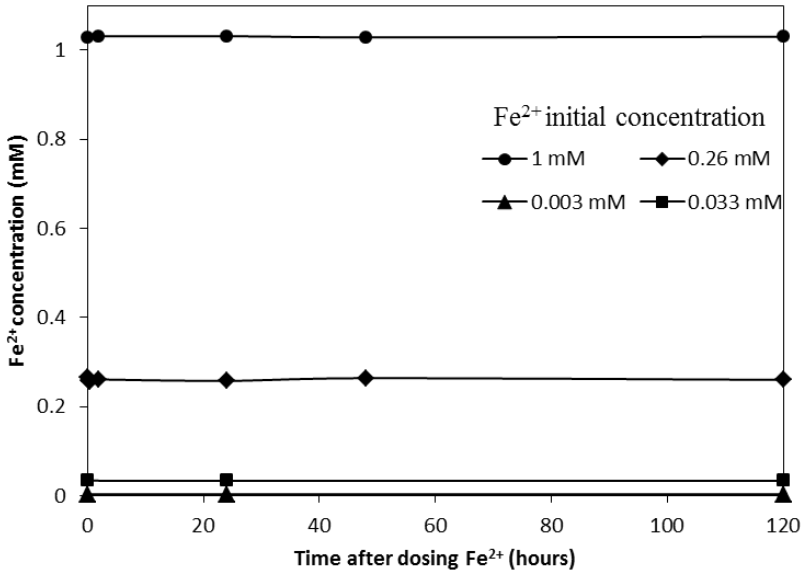


Figure 2 Fe^{2+} concentrations over 120 hours contact time in reactors with Fe^{2+} alone

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).

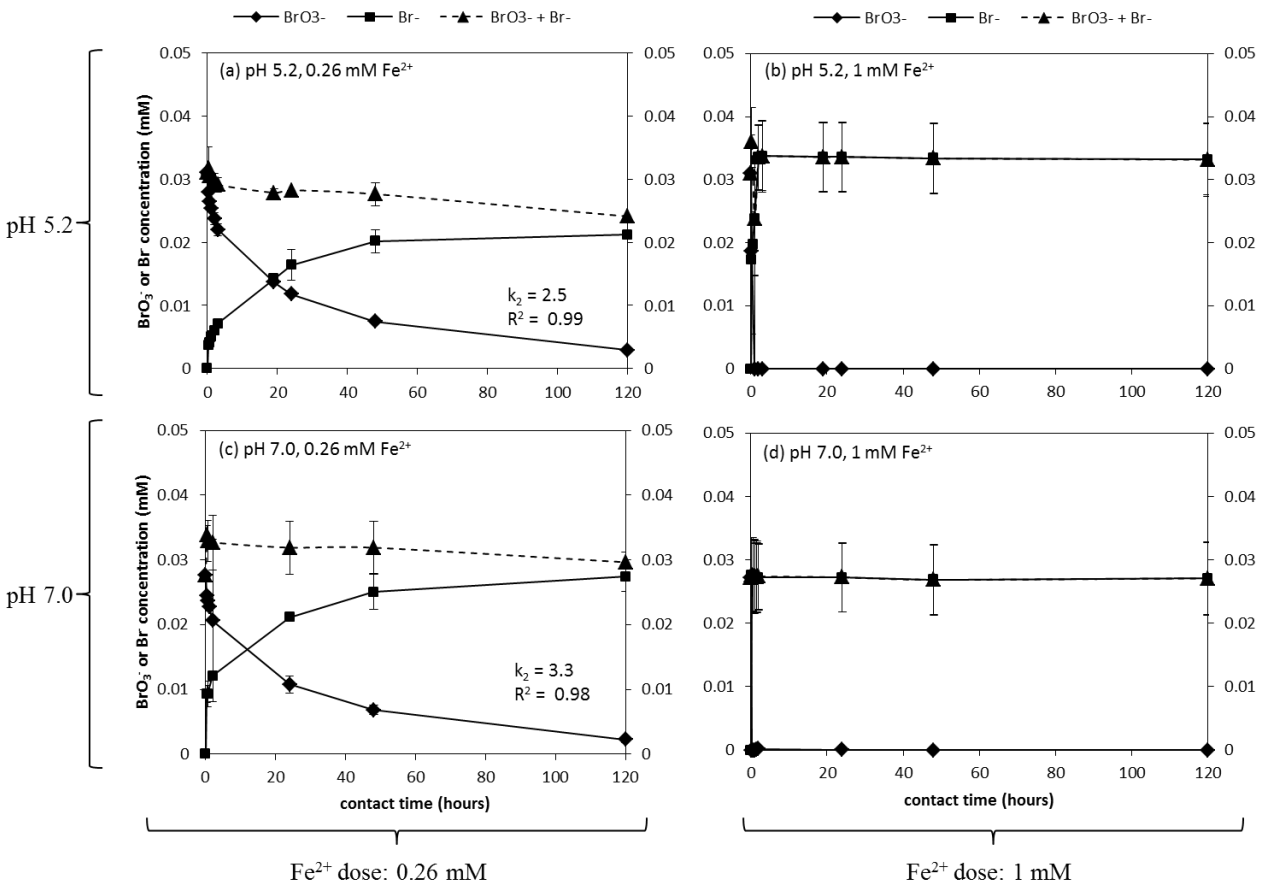


Figure 3 BrO_3^- reduction after dosing 0.26 mM (a, c) or 1 mM (b, d) Fe^{2+}. Initial BrO_3^- concentration was 0.03 mM, initial pH levels were 5.2 and 7.0

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.

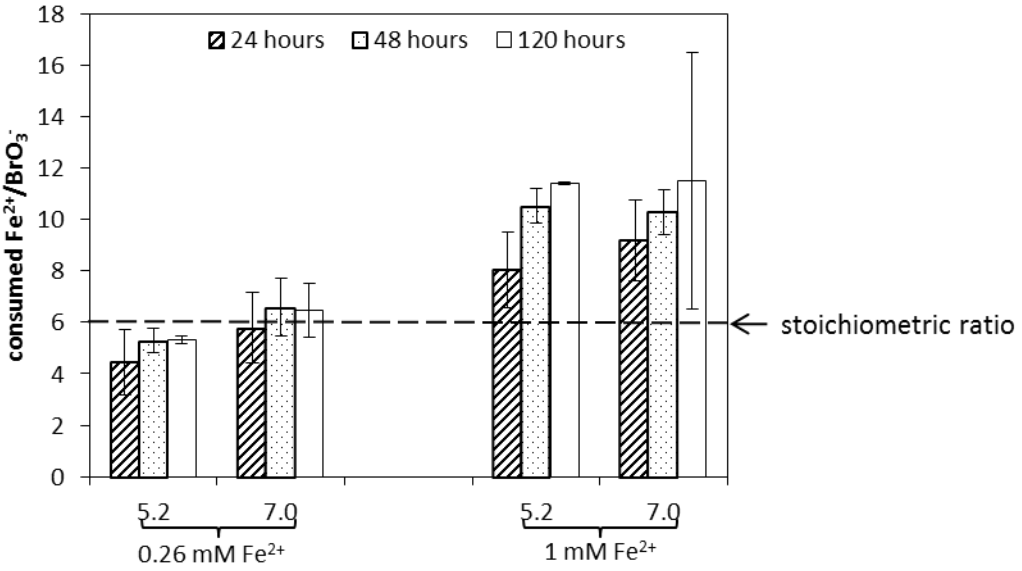


Figure 4 The consumed Fe^{2+}/BrO_3^- ratios after dosing 0.26 mM or 1 mM Fe^{2+} to a solution containing 0.03mM BrO_3^- at two initial pH levels, 5.2 and 7.0

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.

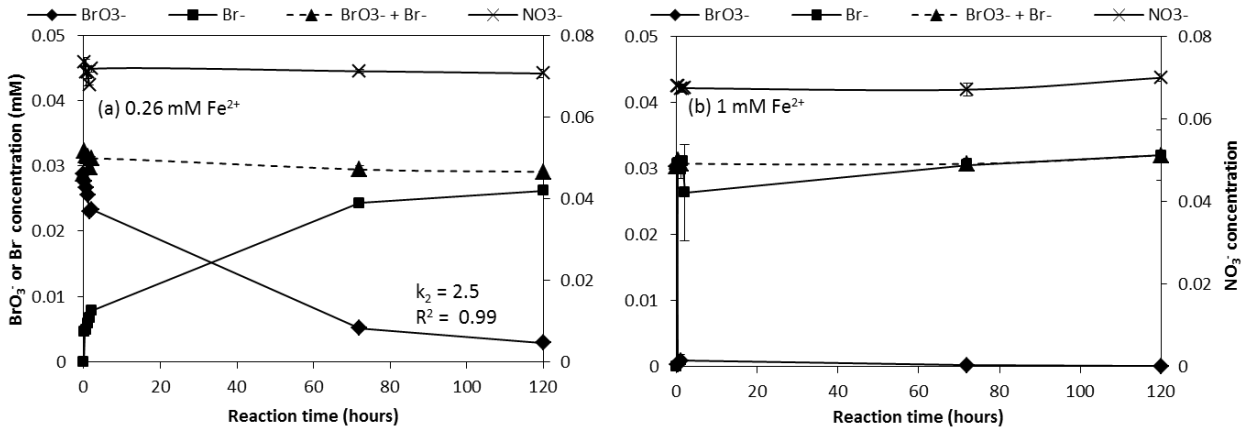


Figure 5 BrO_3^- reduction after dosing 0.26 mM (a) or 1 mM (b) Fe^{2+} in the presence of NO_3^-. Initial BrO_3^- and NO_3^- concentrations were 0.03 mM and 0.07mM respectively, initial pH was 7.0

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.

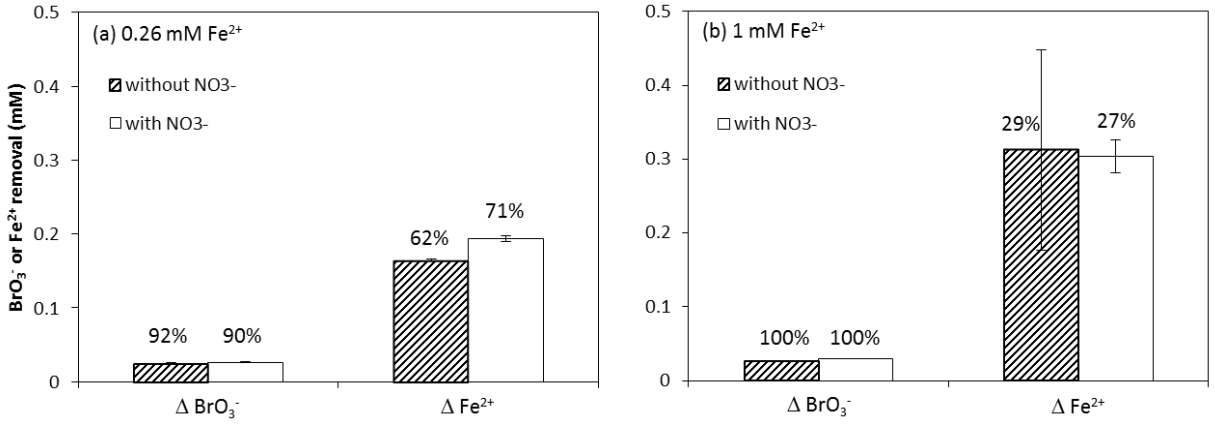


Figure 6 The effect of 0.07 mM NO_3^- on the reduction of 0.03mM BrO_3^- in 120 hours after dosing 0.26 mM (a) or 1 mM (b) Fe^{2+} at initial pH 7.0. % corresponds to the removal percentages

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.

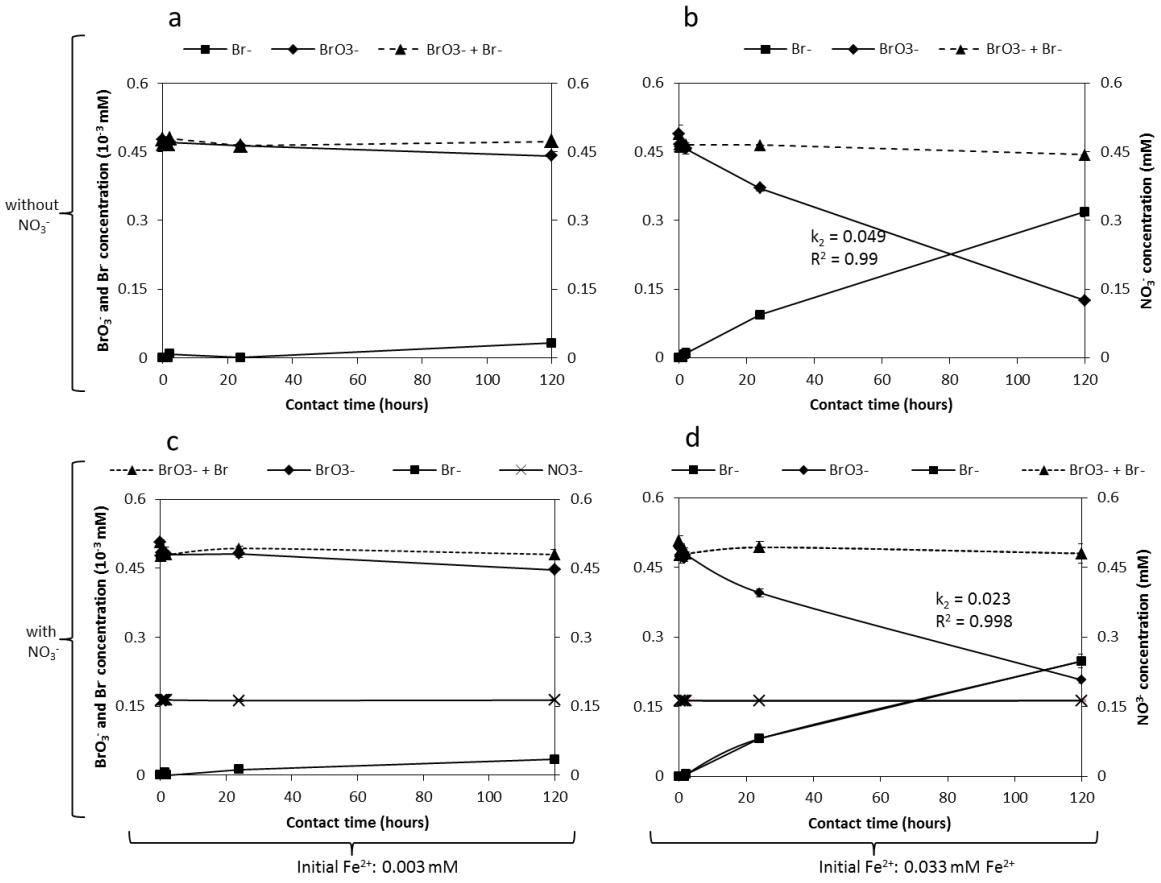


Figure 7 BrO_3^- reduction after dosing 0.003 mM (a, c) or 0.033 mM (b, d) Fe^{2+}, simulating MAR concentrations, in the presence (a, b) and the absence (c, d) of NO_3^-. Initial BrO_3^- and NO_3^- concentrations were 0.5 µM and 0.16 mM (c, d), repectively. Initial pH was 7.0

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^-.

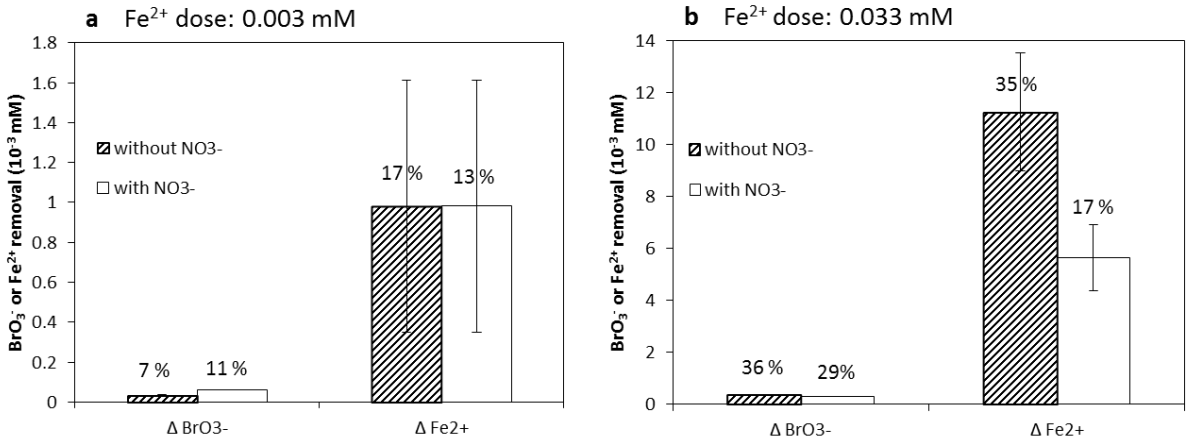


Figure 8 The consumed BrO_3^- and Fe^{2+} in 120 hours after dosing 0.003 mM (a) and 0.033 mM (b) Fe^{2+} in the presence and the absence of NO_3^- at initial pH 7.0. Initial BrO_3^- and NO_3^- concentrations were 0.5 µM and 0.16 mM, respectively. % corresponds to the removal percentages

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).

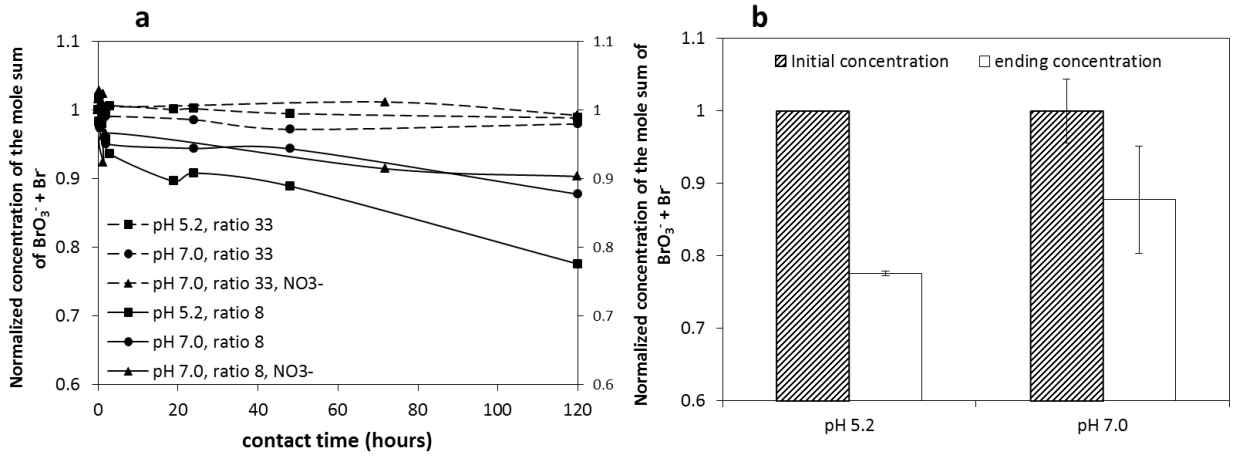


Figure 9 The mole mass sum of BrO_3^- and Br^- in all experiments with initial BrO_3^- concentration 0.03 mM (a) and the comparison of mole mass loss of BrO_3^- and Br^- in 120 hours between pH 5.2 case and pH 7.0 case (b). Ratio means the initial ratio of Fe^{2+}/BrO_3^-. n=2

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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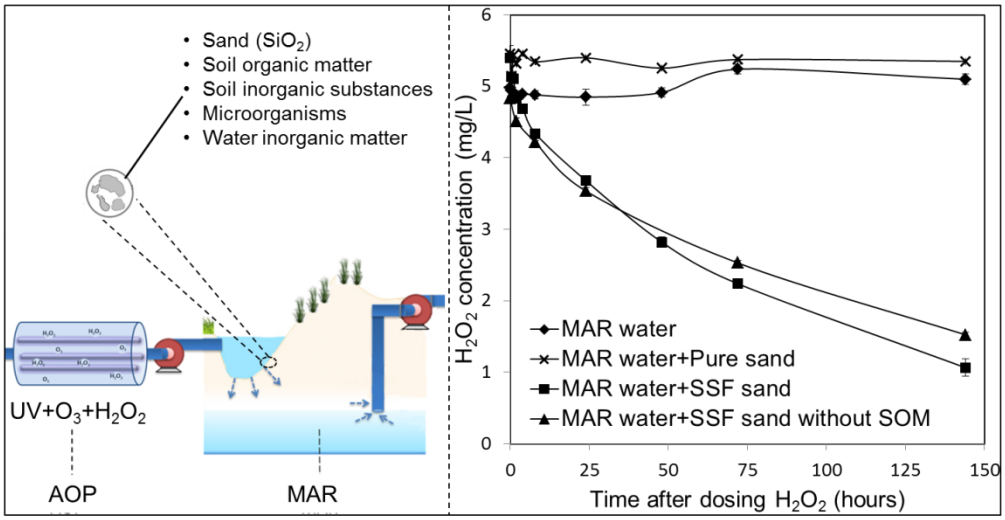
4

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



This chapter is based on:

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

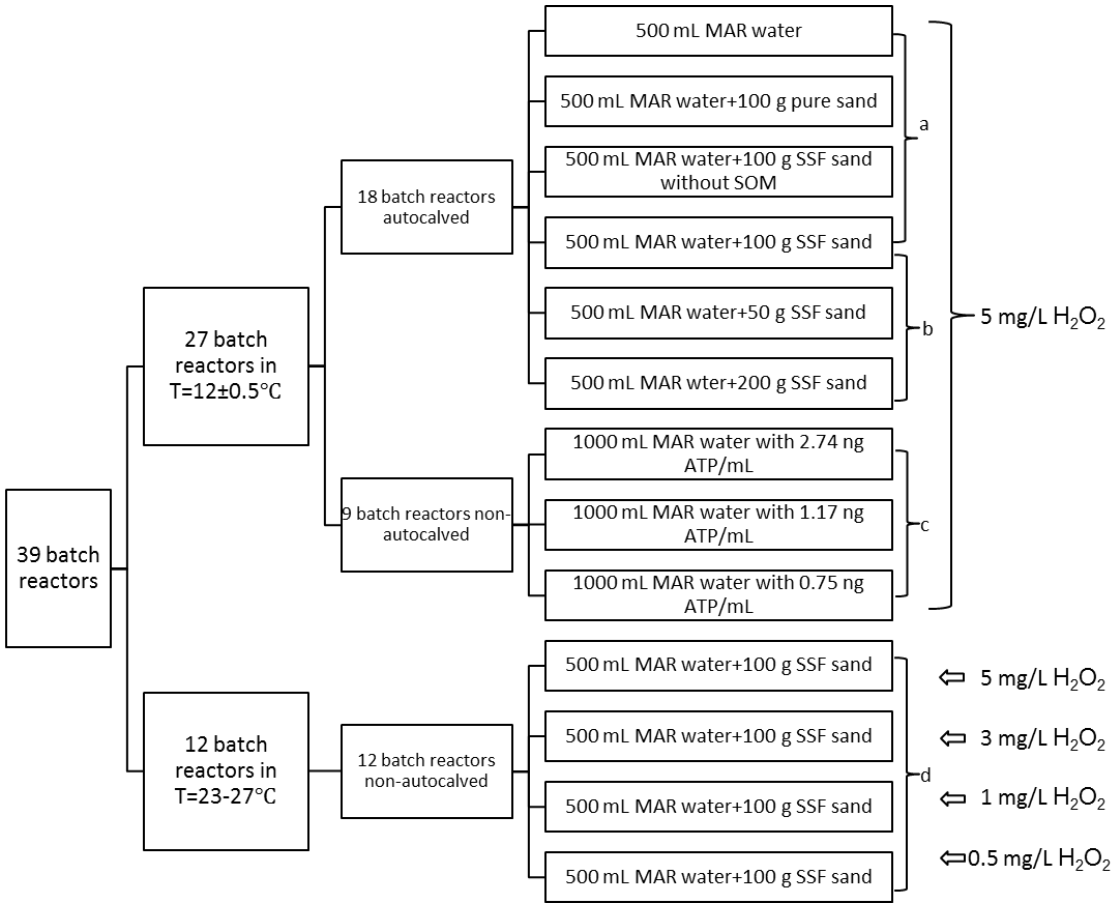


Figure 1 Batch reactors in triplicate with different treatments (non-autoclaved or autoclaved, 23-27 ℃ or 12±0.5 ℃, 5 mg/L, 3 mg/L, 1 mg/L or 0.5 mg/L dosage)

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.

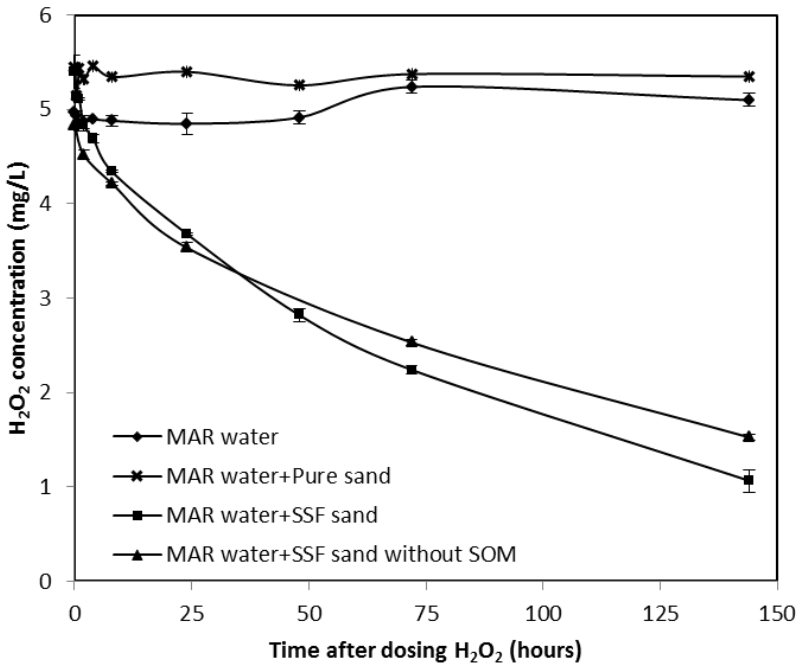


Figure 2 H_2O_2 decomposition under autoclaved batch reactors at T=12±0.5 ℃ in triplicate (series a Figure 1)

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.

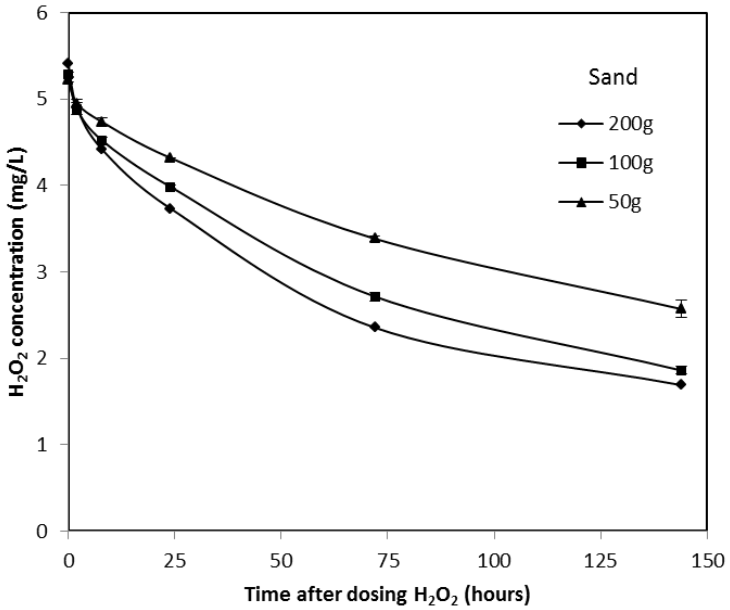


Figure 3 H_2O_2 decomposition with 200 g, 100 g, and 50 g autoclaved SSF sand in 500 mL synthetic MAR water at T=12±0.5 ℃. All batch reactors were in triplicate (series b Figure 1)

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.

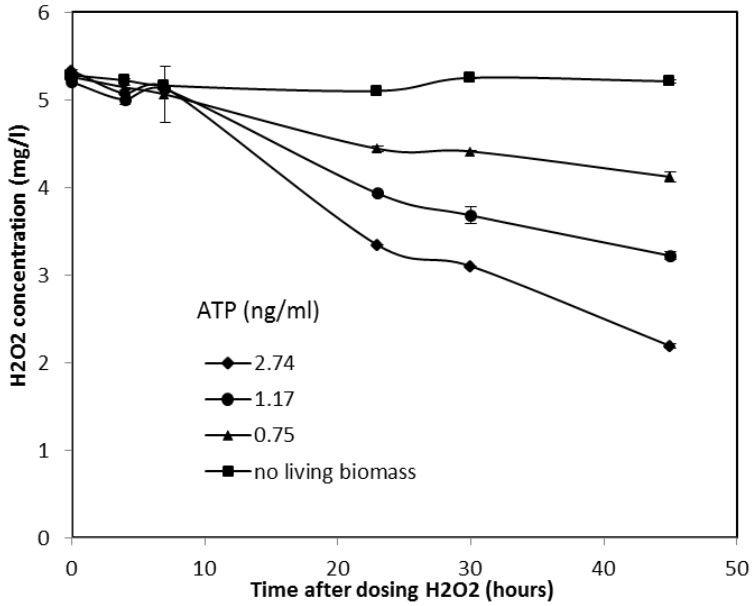


Figure 4 H_2O_2 decomposition with microbial biomass, 2.74, 1.17, 0.75 and 0 ng ATP/mL at T=12±0.5 ℃. All batch reactors were in triplicate (series c Figure 1)

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.

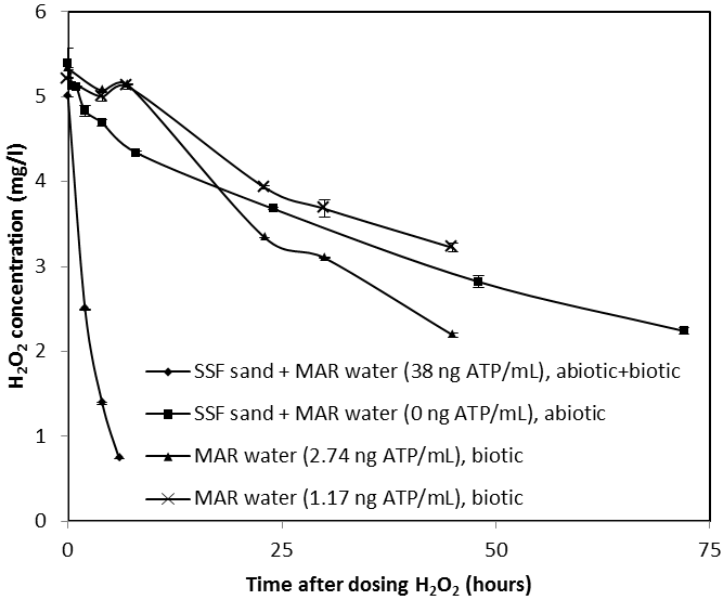


Figure 5 Biotic and abiotic H_2O_2 decomposition. All batch reactors were in triplicate

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.

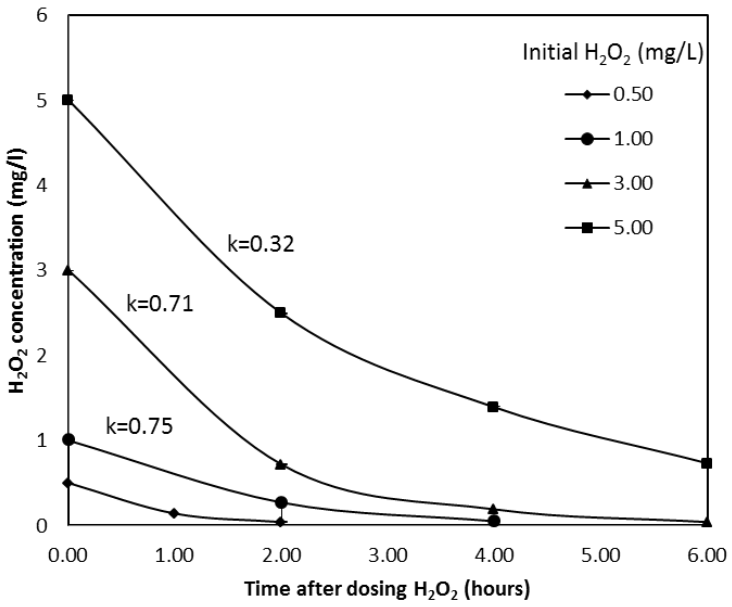


Figure 6 H_2O_2 decomposition under different initial concentrations, 0.5, 1.0, 3.0 and 5.0 mg/L, in the presence of SSF sand at T=23-27 ℃. All batch reactors were in triplicate (series d Figure 1)

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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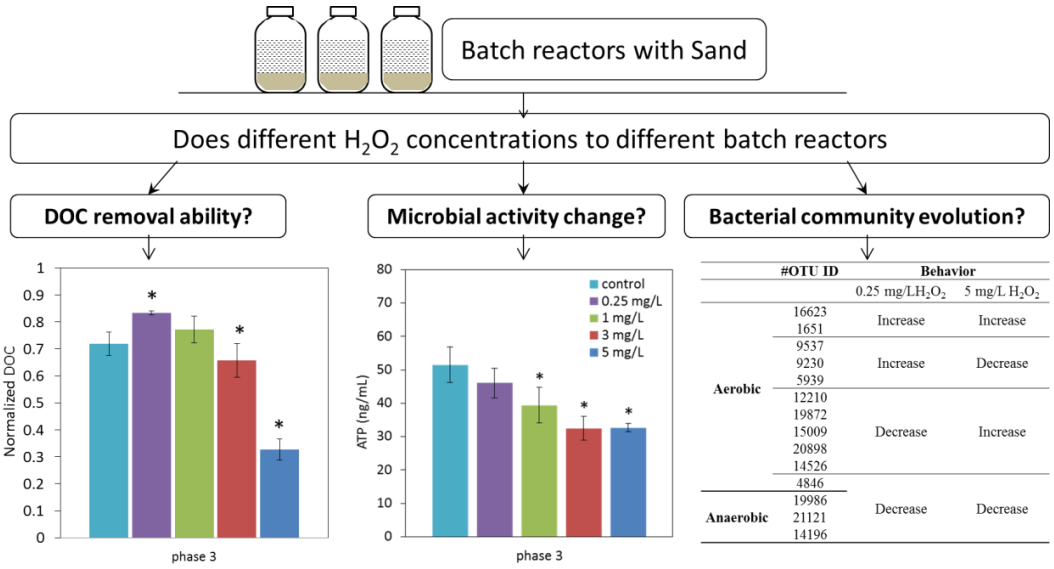
5

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 1651	Increase	Increase
	9537 9230 5939	Increase	Decrease
	12210 19872 15009 20898 14526	Decrease	Increase
	4846		
Anaerobic	19986 21121 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.

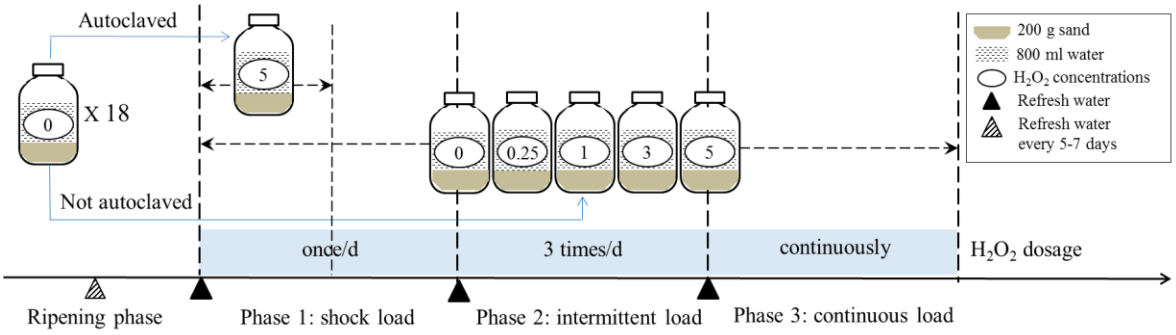


Figure 1 Batch reactors with different operation conditions n=3. The ripening phase lasted for 2 months, then three batch reactors were autoclaved while the other fifteen batch reactors were not autoclaved. 5 mg/L H_2O_2 was dosed to the autoclaved reactors, and different concentrations of H_2O_2 (0,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

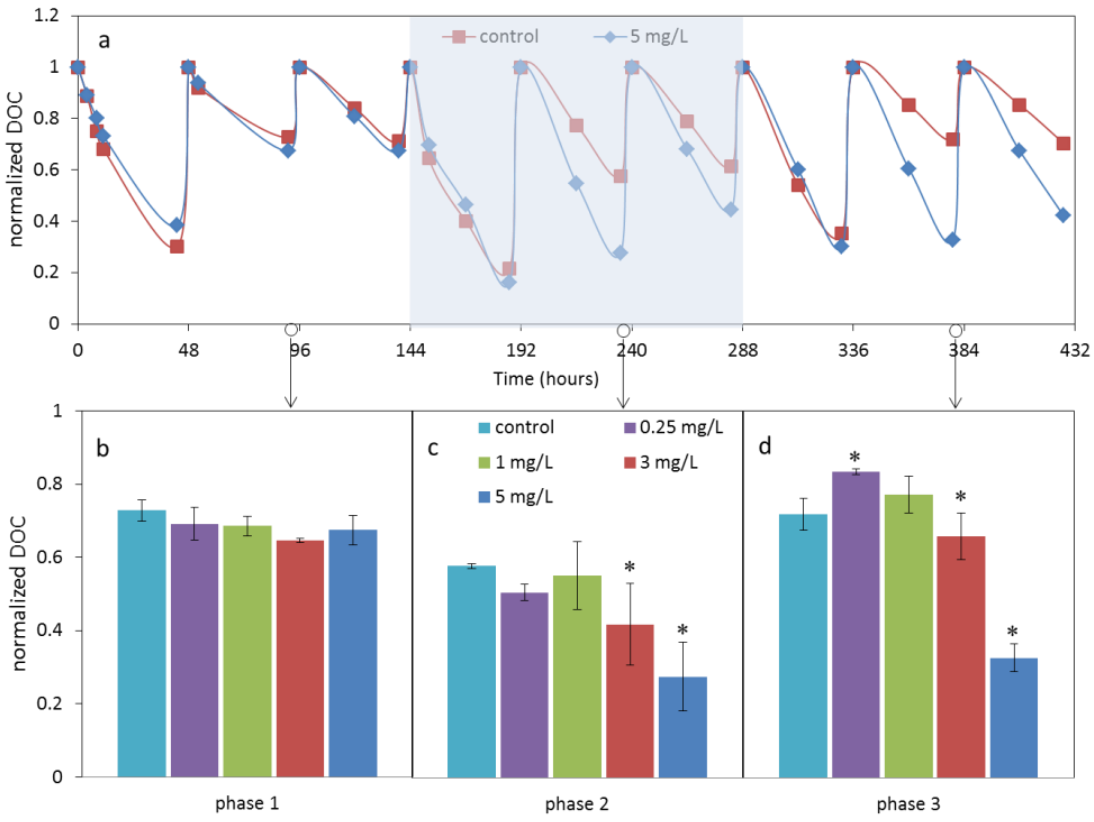


Figure 2 Normalised DOC concentrations in batch reactors n=3 over time (a), at the middle of phase 1 with shock load (b), phase 2 with intermittent load (c) and phase 3 with continuous load (d). The light blue shadow highlights phase 2. $p > 0.05$ for Figure 2-b, $p < 0.05$ for Figure 2-c, and $p < 0.05$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

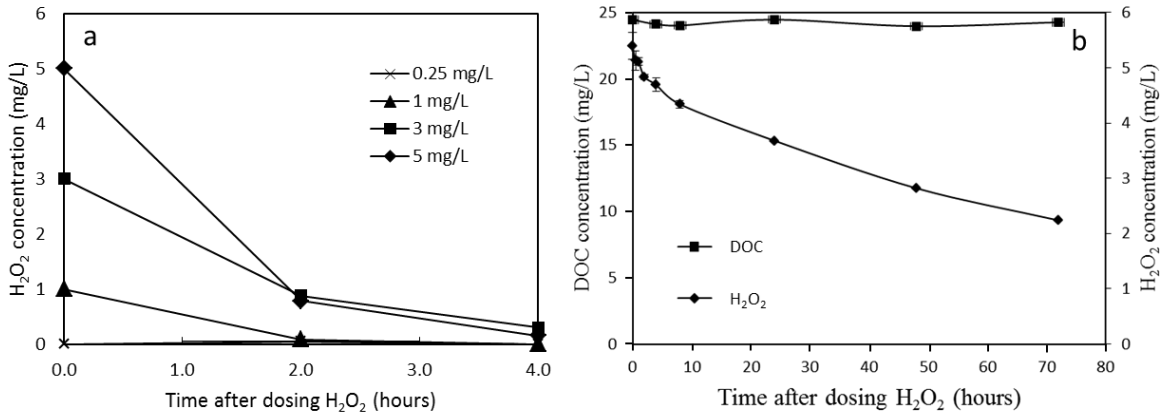


Figure 3 H_2O_2 concentrations in non-autoclaved batch reactors in the first day of the experiment (a) and DOC and H_2O_2 concentrations n=3 over 3 days after dosing 5 mg/L H_2O_2 in autoclaved batch reactors (b). n=3

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.

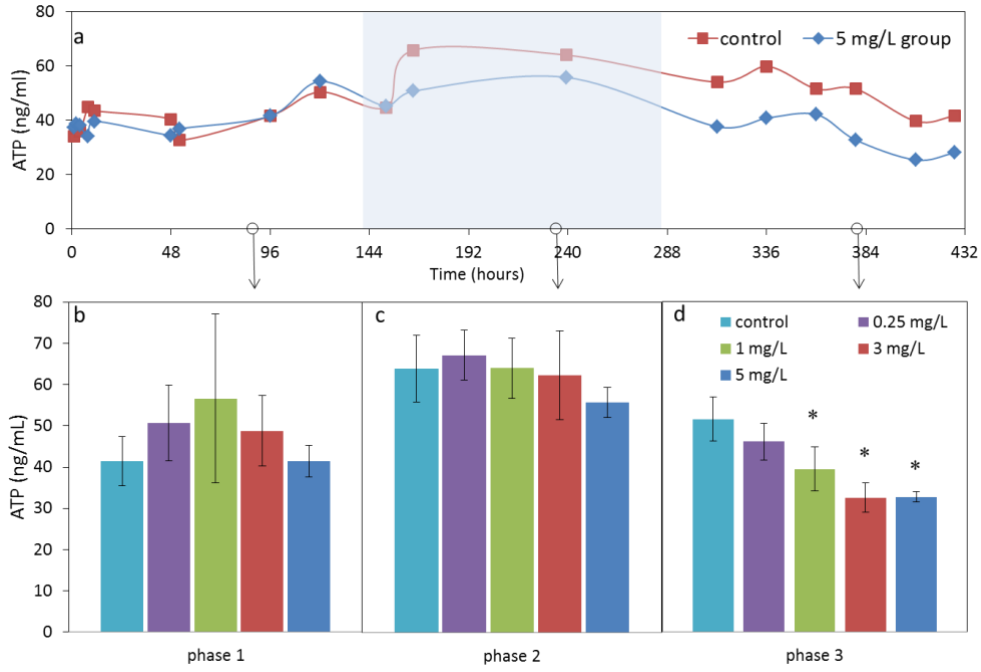


Figure 4 ATP concentrations in the supernatant of batch reactors over time (a), at phase 1 with shock load (b), phase 2 with intermittent load (c) and phase 3 with continuous load (d). $p > 0.05$ for Figure 4-b and Figure 4-c, and $p < 0.05$ for Figure 4-d. * signifies for significant difference from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

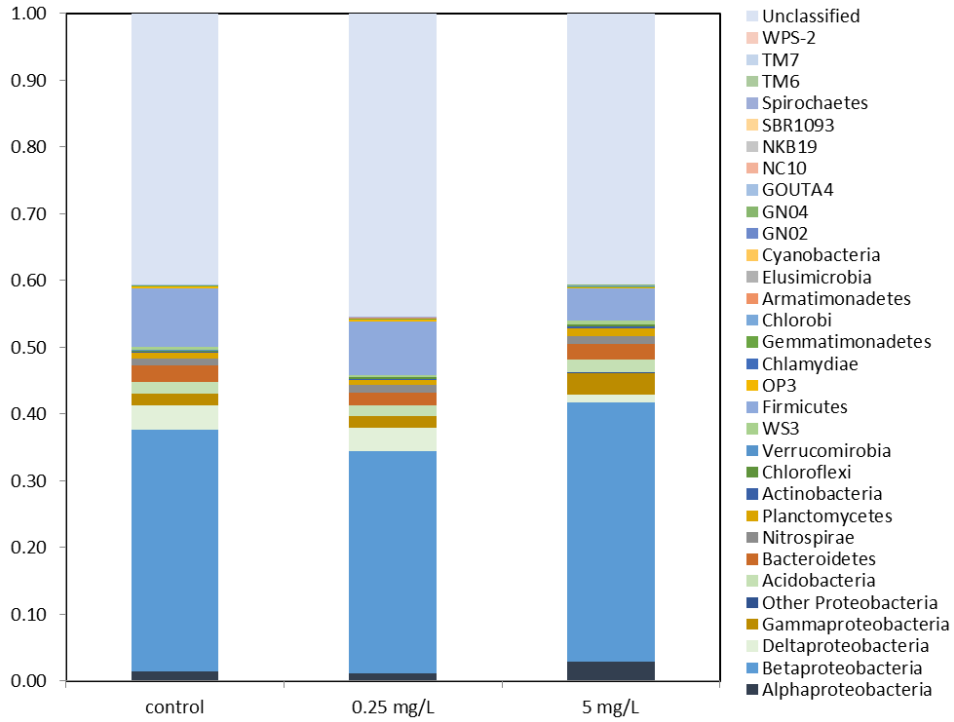


Figure 5 The relative abundance of different phyla and subclasses in *Proteobacteria* with and without the addition of H_2O_2. The phylum of *Proteobacteria* is shown in subclasses of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, and *Gamaproteobacteria*. n=2

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.

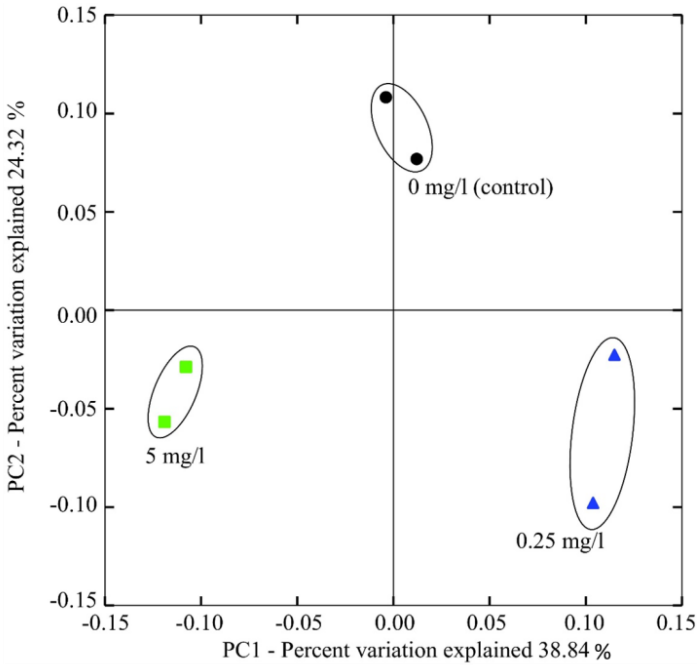


Figure 6 Principle coordinates analysis of bacterial community similarity among different groups of samples. The control group, 0.25 mg/L group and 5 mg/L group are shown in black circles, blue triangles and green squares, respectively. n=2

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

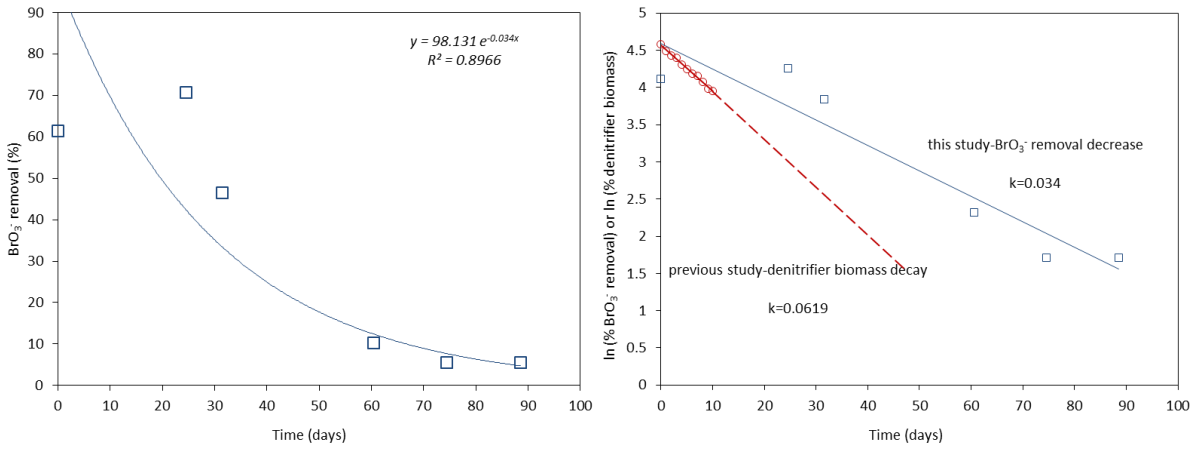

Figure S1 Comparison of the gradual decrease of BrO_3^- removal following a sudden absence of NO_3^- in the 1 m anoxic column in this study (blue squares in a and b) and the decay of denitrifying biomass as a result of exhausted substrates in a previous study (red dots in b). T=11.5±0.5 ℃

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.

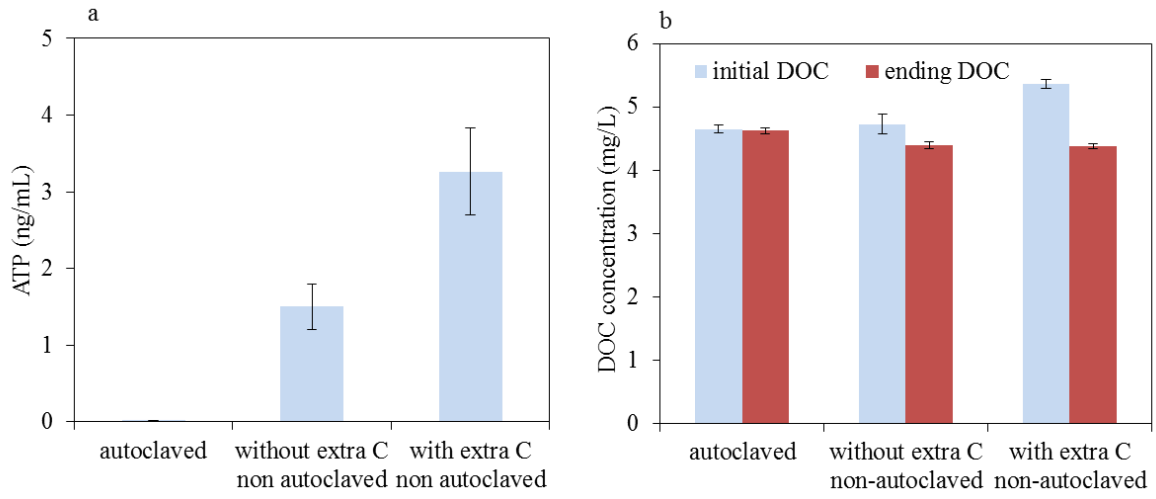

Figure S2 ATP (a) and DOC (b) concentrations in autoclaved and non-autoclaved batch reactors with and without extra carbon dose (1 mg/L C-CH_3COONa) at day 21 (a) and over 7 days (b). n=3

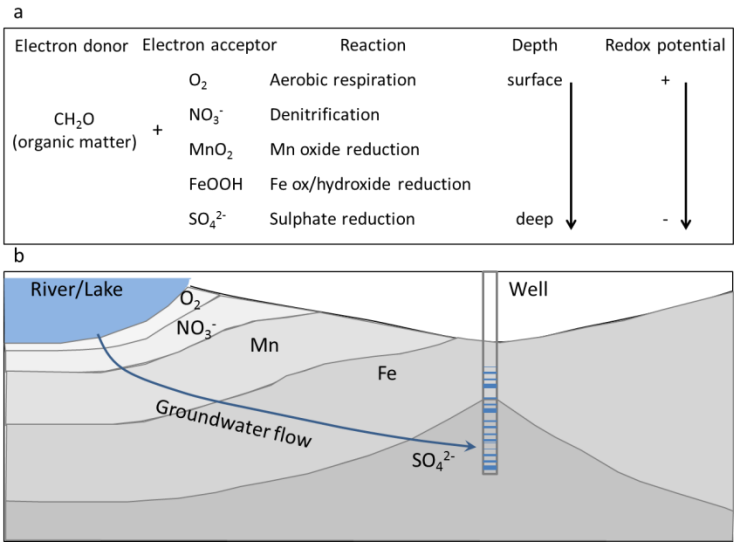

Figure S3 Redox conditions and sequence of terminal electron acceptor processes in MAR systems

Appendix B - Supplementary material Chapter 5

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

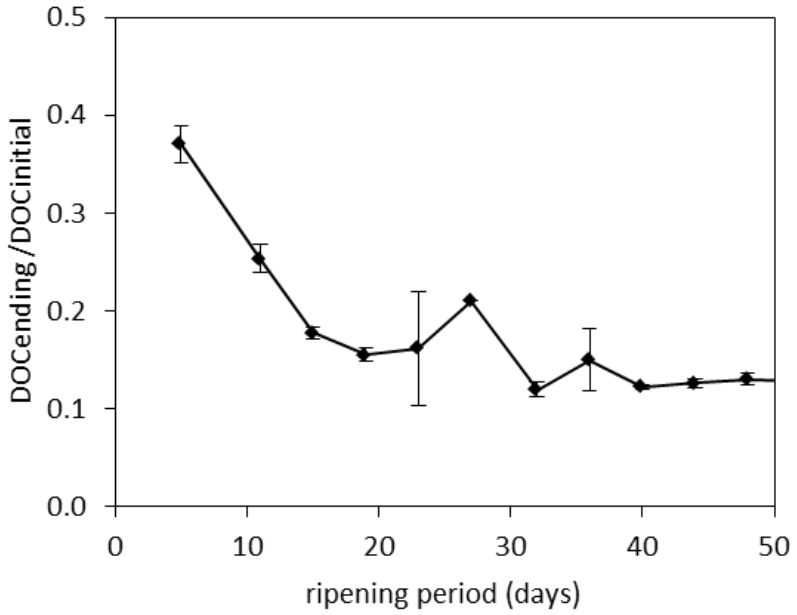

Figure S1 DOC removal in batch reactors during the ripening phase

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.

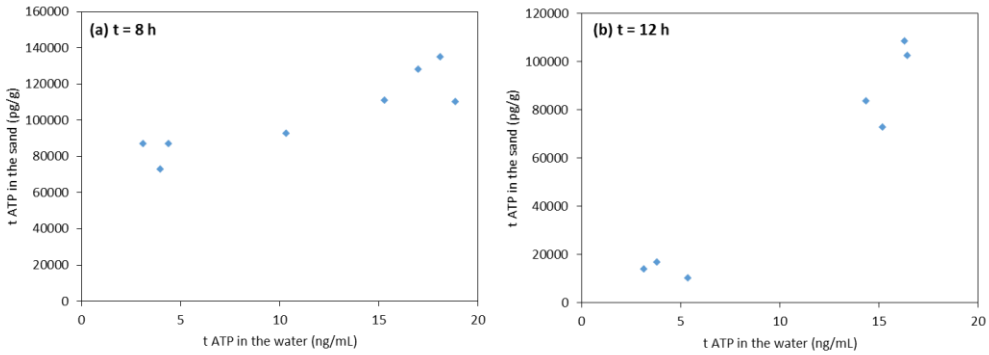

Figure S2 Correlations between ATP in the water and in the sand of the same batch reactor in 8 hours (a) and 12 hours (b) after dosing 0, 0.25 mg/L and 5 mg/L H_2O_2. T=11.5±0.5 ℃

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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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.

Acknowledgements

The five years PhD study is almost done now! It is longer than the time I expected. The past five years is full of challenges on the language, finding research questions, writing the first English paper and modifying papers again and again, but I overcame all the difficulties under your help and learned so much knowledge from you. After the five years' tempering, my abilities have been improved a lot. Without your understanding, support and encouragement, I would not achieve the current achievements and improvement. Thank all of you!

First of all, my promotor Jan Peter. Thank you for giving me the opportunity to do a PhD five years ago. You always respected all ideas/viewpoints which I proposed and always let me free in the things I wanted to go, but you were there to keep me on the right track and give me guide at any time when I need. When I got anxious/sad due to the bad experimental results or slow progress, you never blamed me and always encouraged me. I admired your rigorous attitude on research. When you reviewed my paper draft, you not only corrected the total direction but also never let any minutiae errors or inappropriate words go. After several years PhD study, I recognized that the details make a difference. I learned the earnest manner and the rigorous attitude from you. Thanks for all your advice on experimental design, comments on manuscripts and everything you taught me. I remember all your help and support in my mind forever. I hope that we will keep in touch for research discussion wherever I will be in the future.

Secondly and also importantly, my co-promotor Doris. If Jan Peter was a gentle father, then you would be a strict mother. You are very strict, but I like you very much because you made me improve a lot and always solved the problem for me. Several times I disagreed your viewpoints with tears in my eyes, but undoubtedly you are the one contributing a lot lot lot to my PhD study. Although sometimes I was impatient for modifying my paper draft again and again before submitting it, but indeed your comments were constructive. Without your that much critical comments, these papers would not be accepted by those high quality journals. I admire your flexible thinking and clever insight. Thanks for all your advice on experimental design, comments on manuscripts and everything you taught me. I remember all your help and support in my mind forever. I hope that we will also keep in touch for research discussion wherever I will be in the future.

My PhD research was funded by the Dunea drinking water company and by the Topsector Water TKI Watertechnology Program of the Dutch Ministry of Economic Affairs (No.

2013TUD001). Big thanks to two managers of my project from Dunea, Karin Lekkerkerker-Teunissen and Ton Knol. I enjoyed all our discussions during the project meetings and your valuable feedback on my work. Thank for providing us the basic data of MAR water, the water every two weeks and the sand for my experiments. Thanks to the financial support for my living in the Netherlands from the China Scholarship Council (201206140009).

There were a lot of supports from my colleagues. Thanks Armand for always solve the urgent problems occurring during my experiments. Patrick, Tonny and Thom, thank you for helping me in the lab. Professor Lei Ding, thanks for teaching me how to use the IC machine and discuss with me about BrO_3^- removal in MAR systems. Professor Wei Jin, thanks for your advice on my outline of my PhD research. Cheryl, Xinyue, Ying, Liangfu, Zhongbo, Xuedong, Peng, Jenny, Ran, Gang, Marjet, Hongxiao, Wei Fang, Yu Tao, David R and Marjolein, thank you all for sharing your knowledge and helping me to solve experimental problems. Thank Boris, Henri, Bas and Luuk for answering my questions about my research and providing me good suggestions. Thank Franca for providing me the ATP data. Thank Irene for contributing so much for our team work and helping me pass the drinking water exam which was really difficult for me at that moment I just came to the Netherlands. Julian, Astrid, Mostafa, Roslinda and Shirin, thank you for helping me to improve English. Thank Katie for editing English for my manuscripts. Thank Irene, David R, Marjet, Armand, Marieke, Alex and Tamara for editing Dutch for my summary. Thanks to our secretaries, Mieke, Jennifer, Mariska and Tomaro for making my PhD journey smoother. The communication/chat with Alex, Marieke, Jawairia, Juan Pablo, Annelies, Victor, Steef, Ljiljana, Aditya and Laura brought me a lot of fun and made me relax. Thank all my colleagues' company and communication which made me have a happy PhD journey.

Furthermore, I would like to thank all my students that worked on my PhD project for their BSc, MSc thesis or internship project: Elena Albini, Lukas Lesmeister, Nathalie Pinto Silva, Irene Caltran, Mirjam Pruijssen, Vanida Salgado Ismodes, Bosch Sanz Oriol, Gwenael Danigo, Marta Ballester, Zeinab, Rishi van der Raaf and Matthijs Schaap. I always enjoyed your company in the lab and discussions we had. I learned a lot from all of you.

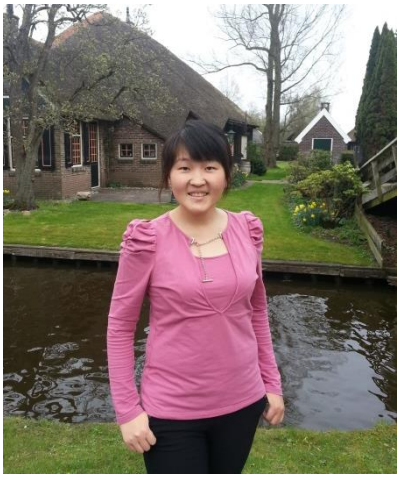
My gratitude goes to my dear friends in Delft. In water management department, Xuedong, Ran, Yu, Gang, Peter, Jenny, Haoyu, Wei Jin, Zhongbo, Peng, Cuijie, Nan, Hongxiao, Liangfu, Ying, Wei Fang, Wei Wang, Xinyue, Xuefeng, Bensheng, Hongbo, Weilai, Zheyi, Xuan, Jingwen and the next generation of Chinese students, Lihua, Mingyan, Bin, Mingliang…, thanks for all the great time being together with you guys

every day. Outside of my department, Zhen, Lin, Ming, Xin Li, Xueming, Jinhu, Cui, Xiaowei, Yuling, Tao Lv, Jingyu, Qujiang, Xin Tian, Jicheng, Wuyuan, Xiangrong, Yang and Mingjuan, I enjoyed the great time with you guys and it made me happy and relax.

Xiaochen, my husband, thanks for your company so that I did not feel lonely in the past 10 years. Thanks for your spuring and correcting my shortcoming. I believe that I am becoming more and more intelligent. Also, I would like to thank my parents in law for your dedication on taking care of my daughter and our business. Yuhe, my lovely daughter, it is nice to see you grow up. I enjoyed the happy moment you brought to me. Your every improvement surprised me. Your immature voice, cute ideas, curiosity, kiss and smile made me happy every day. I can see your strong logical ability and kind hearted now and you will be an excellent person. The only wish for you is that you are healthy, happy and useful socially!

My final thank is to my parents, thanks for your selfless dedication to me. It was hard raising a young baby and at the same time doing experiments. Nobody would give me more help than you. My daughter is 3 years now and you have stayed in the Netherlands with us for 15 months so that my daughter thought one family consisted of father, mother, grandmother and kids. You never say to be tired although you did all the homework and almost all the business work and took care of my daughter day and night. You are the only one who can do this for me without any requests all over the world. The food you cooked is most delicious. Your comfort to me is most powerful for healing my wounds when I was angry, anxious or sad. Without your endless support, I would not achieve the end. 在过去的 30 年，是父母无私的爱给了我前进的动力，让我勇敢无畏的前行，看见都市的喧嚣、世界的斑斓；接下来的 30 年父母将在我的陪伴中尽享天伦之乐！

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.