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Importance of copper for nitrification in biological rapid sand filters for drinking water production

Wagner, Florian Benedikt; Albrechtsen, Hans-Jørgen; Boe-Hansen, Rasmus

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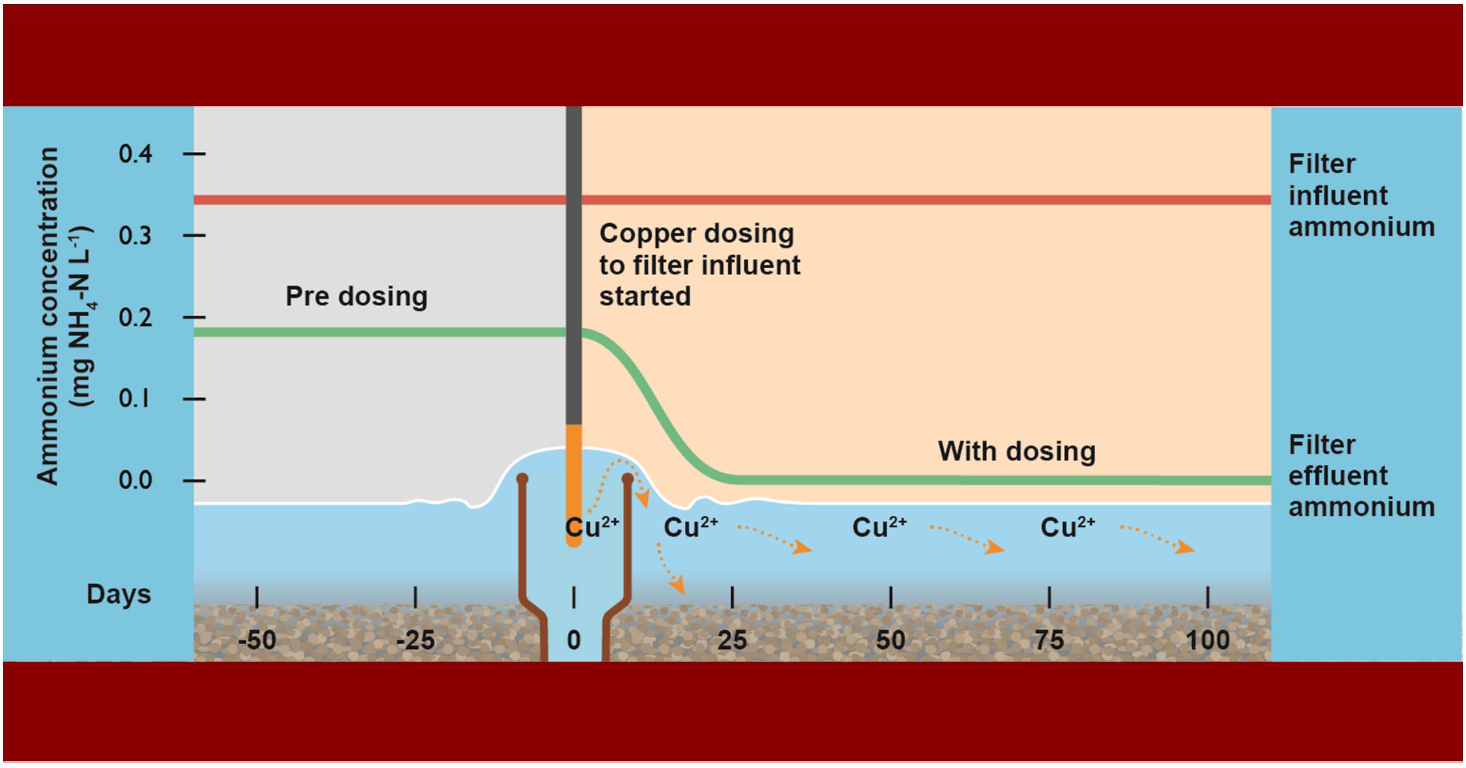
Importance of copper for nitrification in biological rapid sand filters for drinking water production


Ammonium concentration (mg NH_4-N L^{-1})
0.4
0.3
0.2
0.1
0.0
Pre dosing
Copper dosing to filter influent started
With dosing
Filter influent ammonium
Filter effluent ammonium
Cu^{2+}
Cu^{2+}
Cu^{2+}
Cu^{2+}
Days
-50
-25
0
25
50
75
100

Florian B. Wagner

PhD Thesis
September 2017

DTU Environment
Department of Environmental Engineering

Importance of copper for nitrification in biological rapid sand filters for drinking water production

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Florian B. Wagner

PhD Thesis, September 2017

The synopsis part of this thesis is available as a pdf-file for download from the DTU research database ORBIT: http://www.orbit.dtu.dk.

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Preface

This PhD thesis is based on research carried out at the Department of Environmental Engineering at the Technical University of Denmark (DTU). The project was conducted in the period from November 2013 to July 2017 (of which 8½ months were a leave, during which research on other projects was carried out). The PhD project was supervised by Prof. Hans-Jørgen Albrechtsen from DTU Environment (main supervisor) and PhD Rasmus Boe-Hansen from Krüger Veolia A/S (co-supervisor).

The thesis is organized in two parts: the first part (synopsis) puts into context the main findings of the PhD in an introductive review; the second part consists of 3 scientific papers and 1 patent application, listed below. These will be referred to in the text by author names and roman numerals (e.g. Wagner et al., **I**).

I **Wagner, F.B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J., 2016. Copper deficiency can limit nitrification in biological rapid sand filters for drinking water production. *Water Research,* 95:280-288.

II Albrechtsen, H-J., **Wagner F.B.**, Borch Nielsen, P., Boe-Hansen, R., Fischer, E.V., 2015. Apparatus comprising trace element dosage and method for treating raw water in a biofilter. *Patent application published under patent cooperation treaty,* WO2015/132283 A1.

III **Wagner, F.B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J. Remediation of incomplete nitrification and capacity increase of biofilters at different drinking water treatment plants through copper dosing. *Submitted.*

IV **Wagner, F.B.**, Diwan, V., Dechesne, A., Fowler, S. J., Smets, B.F., Albrechtsen, H-J. Copper-induced stimulation of nitrification in biological rapid sand filters for drinking water production by proliferation of *Nitrosomonas* spp. *Manuscript.*

In this online version of the thesis, papers **I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following publications in conference proceedings, not included in this thesis, were also concluded during this PhD study:

- Lee, C. O., **Wagner, F. B.**, Boe-Hansen, R., & Albrechtsen, H-J., 2017. Increasing nitrification in biological rapid sand filters: Diagnosing and supplementing micronutrients needed for proper filter performance. 11th annual meeting Danish Water Forum (pp. 27-27). Frederiksberg, Denmark.

- **Wagner, F. B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J., 2016. Copper dosing stimulates nitrification in full-scale biological drinking water filters. 10th Nordic Drinking Water Conference, Reykjavik, Iceland.

- Lee, C. O., **Wagner, F. B**., Boe-Hansen, R., & Albrechtsen, H-J., 2016. Optimizing nitrification in biological rapid sand filters: Diagnosing and supplementing micronutrients needed for proper filter performance. 10th Nordic Drinking Water Conference, Reykjavik, Iceland.

- **Wagner, F. B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J., 2016. Copper dosing to biological rapid sand filters increases nitrifier activity and abundance. IWA Specialized Conference Microbial ecology and water engineering & biofilms (MEWE2016) (pp. 102-103). Copenhagen, Denmark.

- Albrechtsen, H-J., Smets, B. F., Lee, C. O., Tatari, K., Borch Nielsen, P., Binning, P. J., Boe-Hansen, R., **Wagner, F. B.**, 2016. Optimizing nitrification in biological rapid sand filters for drinking water production. IWA World Water Congress & Exhibition 2016, Queensland, Australia.

- **Wagner, F. B.**, Lee, C. O., Borch Nielsen, P., Albrechtsen, H-J., 2015. Stimulation of Nitrification in Biological Rapid Sand Filters by Addition of Phosphorus and Trace Metals. IWA Specialized Conference biofilms in drinking water systems from treatment to tap (pp. 252-253). Arosa, Switzerland.

- Albrechtsen, H-J., **Wagner, F. B.**, Borch Nielsen, P., Boe-Hansen, R., Fischer, E. V., 2015. Tilsætning af spormetaller stimulerer nitrifikation i sandfiltre. Dansk vand konference 2015 (pp. 21-22). Aarhus, Denmark.

- **Wagner, F. B.**, Lee, C. O., Tatari, K., Borch Nielsen, P., Albrechtsen, H-J., 2014. Phosphorus Limitations in Rapid Sand Filters Studied with Different Packed Column Assays. IWA World Water Congress & Exhibition 2014, Lisbon, Portugal.

- Albrechtsen, H-J., Gülay, A., Smets, B. F., Lee, C. O., **Wagner, F. B.**, Tatari, K., Borch Nielsen, P., Binning, P. J., Boe-Hansen, R., Musovic, S., 2014. Diagnostics in biological rapid sand filters treating groundwater – governing factors for nitrification. AWWA Water Quality Technology Conference & Exposition 2014, New Orleans, LA, United States.

- **Wagner, F. B.**, Borch Nielsen, P., Lee, C. O., Tatari, K., Boe-Hansen, R., Albrechtsen, H-J., 2014. Phosphorus limitations of nitrification in biological sand filters for drinking water treatment – A study at 3 different scales. AWWA Water Quality Technology Conference & Exposition 2014, New Orleans, LA, United States.

- **Wagner, F. B.**, Borch Nielsen, P., Lee, C. O., Tatari, K., Boe-Hansen, R., Albrechtsen, H-J., 2014. Stimulation of nitrification in biological rapid sand filters for drinking water treatment by trace metals. AWWA Water Quality Technology Conference & Exposition 2014, New Orleans, LA, United States.

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I would furthermore like to acknowledge help from Sonsoles Quinzaños and Charlotte Loewe Jensen from Krüger Veolia in full-scale investigations, and help from the lab-techs at DTU Environment, especially Sihn Nguyen, Susanne Kruse, and Lene Kirstejn Jensen, and analytical chemist Mikael Emil Olsson. I am also grateful to all the water utilities which have contributed to this study for their curiosity and willingness to let us do research with their full-scale drinking water filters. Special thanks go to Jimmi Jørgensen from Novafos (formerly Forsyningen Allerød Rudersdal A/S), Anne Esbjørn from VandCenter Syd, Henrik Nicolaisen from Glostrup Forsyning, and Søren Thygesen from DIN Forsyning. It was a pleasure to work with all of you and to obtain so many practical insights from real-life filter and treatment plant operation.

DTU Environment has been a great place to work, and many have contributed to that. It would not have been the same without my (former and current) office mates Sarah, Sune, Mathilde, Jonas, Fynn, and Carson, and friends and colleagues Pauly, Aiga, Danielle, Camilla B., Martin, Ravi, Arnaud, Vaibhav, Carlos, Alex, Jane, Will, Arda, Marta, Dongah, Elena, Fabio, Elham, Pedram, Ioannis, and of course Anne Harsting. Thanks especially to Odell, for being such a good friend.

Finally, I would like thank my family and particularly my mother for continuously supporting me to pursue my goals. Most importantly, I wish to thank my fiancé Mariann and our wonderful little boy Georg for their amazing support and for always caring and being there for me, at all times.

Summary

When anoxic groundwater is treated to produce drinking water, ammonium is commonly removed through nitrification in rapid sand filters. Nitrification is a biological process, and is mediated by chemoautotrophic microorganisms. Ammonia oxidizing bacteria (AOB) and archaea (AOA) oxidize ammonium to nitrite, which is further oxidized by nitrite oxidizing bacteria (NOB) to nitrate. Besides these canonical, strict ammonia and nitrite oxidizers, bacteria capable of complete oxidation of ammonia all the way to nitrate have recently been described (comammox). Although nitrification is commonly applied, the reaction rate is sometimes not high enough. This results in incomplete nitrification, with residual ammonium and nitrite concentrations in the finished water, which are problematic for the biological stability of the drinking water. In Denmark, 11 % of the larger water works (>350,000 m^3/year) fail to remove ammonium to below the national drinking water quality standard of 0.05 mg NH_4^+/L. A better process understanding of nitrifying biofilters is needed to optimize treatment performance, remediate existing filters, and to prevent future nitrification problems.

The frequent incidents of insufficient nitrification during drinking water production provided the motivation to investigate if a lack of copper could be responsible for the problems in nitrifying biofilters. Copper is believed to be an essential cofactor in the enzyme ammonia monooxygenase (AMO), which catalyzes the first essential step in the oxidation of ammonia to hydroxylamine. Thus, slow and incomplete nitrification could be caused by a lack of sufficient amounts of copper.

The overall aim of this PhD project was therefore to determine whether copper supplementation could enhance nitrification in rapid sand filters with incomplete nitrification. Investigations were carried out *in situ* at full-scale drinking water treatment plants (DWTPs) in Denmark, thereby underlining the problem-solving approach, which focuses on process optimization of real systems.

Initial investigations were carried out at Nærum DWTP. The plant has had a long history of incomplete nitrification, with ammonium effluent concentrations of approx. 0.23 mg NH_4^+/L. Copper concentrations in the raw and filter inlet water were <0.01 µg Cu/L. Subsequently, initiated controlled dosing at approx. 0.4 µg Cu/L doubled a filter's ammonium removal rate (ARR) within only 20 days, so that ammonium effluent concentrations

dropped to <0.01 mg NH_4^+-N/L soon after. Water sampling over filter depth with a specially constructed sampling probe revealed that nitrification activity had moved upwards in the filter, with an almost 14-fold increase in ARR in the top 10 cm. The stratified removal meant that less contact time was necessary for complete oxidation of ammonium. In another filter, the stimulation of nitrification lasted for at least 238 days after the dosing was stopped. This, taken together with the stimulatory effect of the dosing, is of great practical importance and shows that continuous dosing is not necessary.

During initial experiments at Nærum DWTP, a novel method for trace metal dosing, employing active release of copper through electrolysis from solid metal electrodes, was developed (patents pending). The method has the benefits that low amounts of copper can be supplied in a controlled fashion, and that little maintenance and no chemicals are required.

Copper dosing through the novel electrolysis method, as well as through passive dosing from solid copper and active dosing of copper solution, was studied at nine more DWTPs, which all shared a long history of incomplete nitrification. The plants represented a wide range of various parameters, with differences in water chemistry, ammonium concentrations and loading rates, filter design and operation, and treatment steps. Ammonium removal was stimulated at all plants and was complete after an average dosing time of only 19 days. This confirmed a generic, broad applicability of copper dosing to remediate nitrification problems in copper deficient biofilters. After ammonium removal was complete, ammonium loading rates (ALRs) twice as high as the normal ALR were imposed on a filter at one plant. Nevertheless, the filter successfully removed increased loads, with a 2.6-fold increase in ammonium removal capacity. The acquired extra capacity makes the filters more robust towards ALR upshifts during dynamic filter operation.

To evaluate bioavailability of copper in rapid sand filters, filter sand extractions and chemical speciation modelling were carried out for Nærum DWTP. Results showed that substantial amounts of copper were bound to iron oxide-hydroxides in the filter and that bicarbonate heavily complexed copper in the water phase. Only <3 % were bioavailable free Cu^{2+}, whereas 91 % of total copper was present as $CuCO_3$. Furthermore, copper availability can be affected by desorption of copper from iron oxides or deposits, biofilm diffusion limitations, (re)cycling of copper within the biofilm, and competition for copper with other microorganisms. Since factors such as iron and bicarbonate concentrations can vary between plants, a site-specific evaluation of copper bioavailability is recommended to adjust dosing accordingly.

State-of-the-art microbial methods (qPCR and Illumina sequencing) revealed that not only nitrification activity was affected by the dosing, but also nitrifier densities. At two investigated sites, genus *Nitrosomonas* (betaproteobacterial AOB) increased from 0.5 to 2.7 % and from 1.1 to 8.5 %, respectively, relative to the total community. With dosing, the ratio within ammonia oxidizing prokaryotes (AOP) was shifted towards *Nitrosomonas* and away from the comammox *Nitrospira*, which were also abundant in the filters. Prior to dosing, cell specific ammonium removal rates of betaproteobacterial AOB were low, at 0.03 to 0.08 x 10^3 fg N/cell/day, possibly due to the copper deficiency. With dosing, rates increased to 0.15 to 0.79 x 10^3 fg N/cell/day, making it plausible that canonical AOB were the main active ammonium oxidizers during the dosing.

This PhD project revealed that copper is of vital importance for efficient nitrification in biological rapid sand filters for drinking water production. The results of this study have important practical implications for biofilters currently in operation and for future filter designs. The developed dosing apparatus is at present successfully applied at a number of Danish DWTPs. Today, all investigated treatment plants have efficient and complete nitrification, and are able to deliver water of better quality to the consumers.

Dansk sammenfatning

Under behandling af anoxisk grundvand i drikkevandsproduktion, fjernes ammonium typisk gennem nitrifikation i hurtige sandfiltre. Nitrifikation er en biologisk proces, som udføres af kemoautotrofe mikroorganismer: Ammoniumoxiderende bakterier (AOB) og archaea (AOA) oxiderer ammonium til nitrit, som oxideres yderligere af nitritoxiderende bakterier (NOB) til nitrat. Udover disse kanoniske mikroorganismer som enten oxiderer ammonium eller nitrit, er der for nylig påvist en gruppe (comammox) der kan foretage fuldstændig oxidation af ammonium hele vejen til nitrat. Selvom nitrifikation er almindeligt anvendt, er processen til tider ikke hurtig nok. Dette resulterer i ufuldstændig nitrifikation med restkoncentrationer af ammonium og nitrit i det færdigbehandlede vand, hvilket er problematisk for den biologiske stabilitet af vandet. I Danmark er 11 % af alle de store vandværker (>350.000 m^3/år) ikke i stand til at fjerne ammonium til under grænseværdien på 0,05 NH_4^+/L. En bedre procesforståelse af biofiltre behøves for at optimere vandbehandling, afhjælpe nuværende samt modvirke fremtidige problemer med nitrifikation.

Hyppige tilfælde af ufuldstændig nitrifikation under vandbehandlingen motiverede os til at undersøge om manglen på kobber kunne være årsag til problemerne i nitrificerende filtre. Kobber menes at være en essentiel co-faktor i enzymet ammonia monooxygenase (AMO), som katalyserer det første vigtige led i oxidation af ammonium til hydroxylamin. Langsom og ufuldstændig nitrifikation kan således skyldes utilstrækkelige mængder af kobber.

Det overordnede formål med dette Ph.d. projekt var derfor at undersøge, hvorvidt tilførsel af kobber kan forbedre nitrifikation i hurtig sandfiltre med ufuldstændig nitrifikation. Undersøgelserne blev udført *in situ* på fuldskala vandværker i Danmark, hvilket understreger en problemløsningstilgang, som fokuserede på procesoptimering af virkelige systemer.

Indledende undersøgelser blev udført på Nærum vandværk. Vandværket har en lang historie med ufuldstændig nitrifikation med ammoniumkoncentrationer i udløbet på omkring 0,23 mg NH_4^+/L. Kobberkoncentrationen i råvandet og indløbsvandet til filtrene var <0,01 µg Cu/L. Da der blev igangsat kontrolleret dosering af ca. 0,4 µg Cu/L, fordobledes filtrenes ammoniumfjernelseshastighed (ARR) på under 20 dage, så ammoniumkoncentrationen i udløbet kort efter faldt til <0,01 mg NH_4^+-N/L. Vandprøver, der blev udtaget

over filtrets dybde med en specialkonstrueret prøveudtager, viste, at nitrifikationsaktiviteten var flyttet længere op i filteret med en omkring 14 gange så høj ARR i de øverste 10 cm. Denne lagdelte fjernelse betød, at en kortere kontakttid var nødvendig for komplet oxidation af ammonium. I et andet filter fortsatte stimuleringen af nitrifikation i mindst 238 dage efter, at doseringen stoppede. Dette, sammenholdt med doseringens stimulerende effekt, har stor praktisk vigtighed og viser, at kontinuert dosering af kobber ikke er nødvendig.

Under de indledende undersøgelser på Nærum vandværk, blev der udviklet en ny patentanmeldt teknologi til spormetaldosering, med aktiv dosering af kobber ved elektrolyse fra metalelektroder. På denne måde er det muligt at dosere lave koncentrationer af kobber under kontrollerede forhold, og teknologien kræver begrænset vedligeholdelse og ingen kemikalier.

Kobberdosering blev undersøgt ved forskellige teknologier: denne nye elektrolysebaserede teknologi, passiv dosering fra kobberplader, og med aktiv dosering af en kobberopløsning. Dette blev undersøgt på yderligere ni grundvandsvandværker, som alle havde haft længerevarende problemer med ufuldstændig nitrifikation. Vandværkerne repræsenterede et bredt spænd i forskellige parametre som vandkemi, ammoniumkoncentrationer og -belastning, filterdesign og drift, samt behandlingstrin. Ammoniumfjernelsen blev stimuleret på alle vandværkerne og var fuldstændig efter en gennemsnitlig doseringsperiode på kun 19 dage. Dette demonstrerer en generel, bred anvendelighed af kobberdosering til at afhjælpe problemer med nitrifikation i biologiske filtre. På et af vandværkerne blev belastningen af ammonium (ALR) forøget til det dobbelte af den normale ALR efter der var opnået fuldstændig ammoniumfjernelse. Filtrene var ikke desto mindre i stand til at fjerne øgede belastninger med en 2,6 gange så stor kapacitet til ammoniumfjernelse. Den forøgede kapacitet gør filtrene mere robuste mod ALR stigninger under dynamisk drift af filtrene.

For at undersøge biotilgængeligheden af kobber i hurtige sandfiltre, blev filtersandet ekstraheret og den kemiske speciering på Nærum vandværk blev modelleret. Resultaterne viste, at en betydelig mængde kobber var bundet til jernoxid-hydroxider i filteret og at bikarbonatet i høj grad dannede komplekser med kobber i vandfasen. 91 % af den totale mængde kobber var til stede som $CuCO_3$, og kun <3 % var biotilgængeligt, frit Cu^{2+}. Derudover kan tilgængeligheden af kobber påvirkes af desorption af kobber fra jernoxider eller aflejringer, begrænsninger i biofilm diffusion, (re)cirkulering af kobber i biofilmen og konkurrence om kobber med andre mikroorganismer. Da faktorer som koncentrationer af jern og bikarbonat varierer mellem vandværker, er en stedsspecifik evaluering af kobbers biotilgængelighed anbefalet for at kunne justere kobberdoseringen tilsvarende.

State-of-the-art mikrobiologiske analysemetoder (qPCR og Illumina sequencing) afslørede, at ikke kun aktiviteten af nitrificerende mikroorganismer, men også deres forekomst, blev påvirket af kobberdoseringen. På to lokaliteter, steg forekomsten af slægten *Nitrosomonas* (betaproteobakterie) fra 0,5 til 2,7 % og fra 1,1 til 8,5 % af det samlede mikrobiologiske samfund. Med doseringen forskød forholdet indenfor ammoniumoxiderende prokaryoter (AOP) sig mod *Nitrosomonas* og væk fra comammox bakterien *Nitrospira*, som også var til stede i alle filtrene. Inden doseringen var de cellespecifikke ammoniumfjernelseshastigheder lave: 0,03 til 0,08 x 10^3 fg N/celle/dag muligvis på grund af kobbermangel. Under doseringen steg hastighederne til 0,15 til 0,79 x 10^3 fg N/celle/dag, hvilket gør det sandsynligt, at kanoniske AOB er de mest aktive ammoniakoxiderende mikroorganismer under doseringen.

Ph.d. projektet demonstrerede, at kobber er af vital vigtighed for effektiv nitrifikation i biologiske hurtige sandfiltre i drikkevandsbehandling. Resultaterne fra projektet har vigtig praktisk betydning for biologiske filtre, der allerede er i drift, og for design af fremtidige filtre. Den udviklede doseringsteknologi anvendes i dag med succes på et antal danske vandværker. I dag har alle de undersøgte vandværker effektiv og fuldstændig nitrifikation, og er i stand til at levere vand af en forbedret kvalitet til forbrugerne.

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1 Introduction

1.1 Basis and motivation

Groundwater is, together with surface water, the most common source of drinking water worldwide (IWA, 2014). The quality of the source water dictates the treatment processes necessary to produce potable water fit for human consumption. When anaerobic groundwater is used, main concerns are reduced compounds such as dissolved iron and manganese, or ammonium.

Ammonium (NH_4^+) is commonly removed through nitrification in biological granular media filters. Nitrification is a microbial mediated process: Ammonia oxidizing bacteria (AOB; Prosser, 1989) or archaea (AOA; Könneke et al., 2005) oxidize ammonia to nitrite (NO_2^-), which is oxidized further to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB; Prosser, 1989). Additionally, some members of the genus *Nitrospira* have been found capable of complete oxidation of ammonia to nitrate (comammox; Daims et al., 2015; van Kessel et al., 2015). These nitrifying microorganisms are organized in a biofilm on the filter media (Gülay et al., 2014).

Unfortunately, nitrification during drinking water production is not always complete, leaving ammonium residues in the finished water. This can lead to nitrification during water distribution, which is problematic, as it compromises drinking water quality. In water systems without disinfection, microbial growth is controlled by removing nutrients to levels as low as possible. Here, nitrification during distribution can lead to microbial regrowth (including possible growth of pathogens, aesthetic problems) and material corrosion (van der Kooij, 2000). In chlorinated systems, nitrification can consume the disinfectant residual and cause formation of hazardous disinfection by-products (Zhang et al., 2009). In Denmark, a substantial number of drinking water treatment plants (11 % of plants treating >350,000 m^3/year, between 2011-2013; Danish Nature Agency, 2014) fails to remove ammonium to concentrations below the national water quality standard of 0.05 mg NH_4^+/L (Ministry of Environment and Food of Denmark, 2016).

Besides energy from the oxidation of natural substrates ammonium and nitrite, nitrifying microorganisms require nutrients for growth. Nutrients not present in sufficient amounts may impede growth, resulting in poor biofilter nitrification performance. Lack of phosphorus for example can limit nitrification in rapid sand filters treating surface water (Kors et al., 1998) or groundwater (de Vet et al., 2012; Lee, 2014), but dosing of the nutrient when limitations are suspected is not always successful in process remediation (Wagner et al., 2014). Besides phosphorus, contained in DNA and RNA, cell membranes, coenzymes, and ATP (Westheimer, 1987), certain trace nutrients are required in comparably smaller amounts. Among those, the metal copper is believed to play a role in catalysis (Ensign et al., 1993) as a component of the enzyme ammonia monooxygenase (AMO), which is responsible for the crucial first step in the oxidation of ammonia to hydroxylamine. Much of what is known about the role of copper in AMO is derived from laboratory studies with pure cultures (e.g. *Nitrosomonas europaea*; Ensign et al., 1993). In the environment, lack of copper has been suggested as potential limitation of nitrification in marine systems (Jacquot et al., 2014).

Despite the importance of copper for the AMO enzyme, it was unknown if problems with incomplete nitrification in biofilters can be caused by a lack of the metal. Here, we hypothesized that if copper is needed for nitrification in biofilters, then copper dosing could stimulate the process in copper limited filters (Fig. 1). A better understanding of possible limitations of nitrification in biofilters, based on scientific principles, is needed to optimize treatment performance. Findings can be applied to remediate existing problems, and also in future filter designs to prevent problems before they even occur.

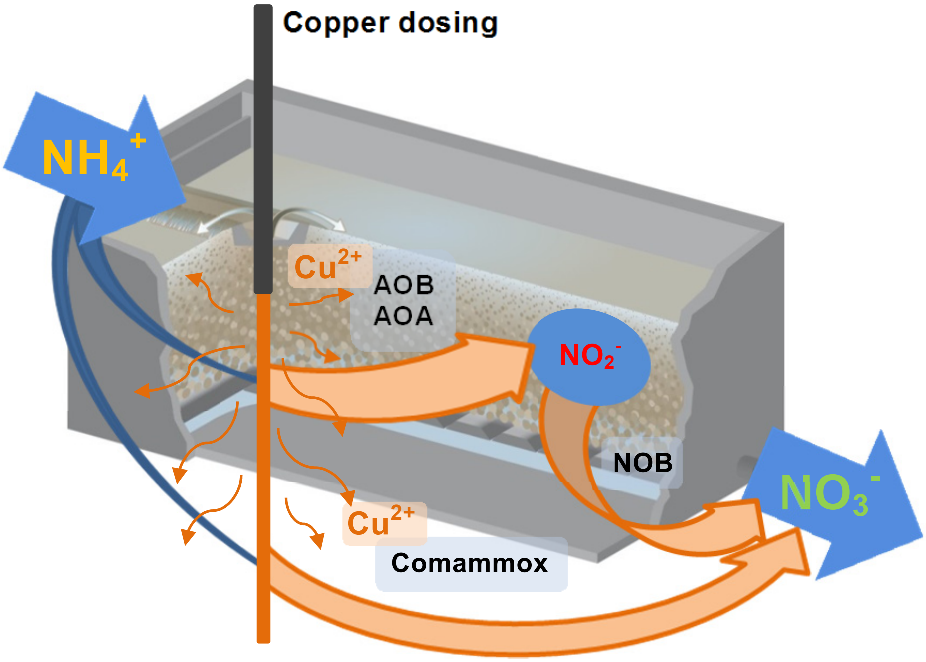


Figure 1 – The copper dependent enzyme AMO is present in AOB, AOA, and comammox bacteria. The arrow widths are indicative for the speed with that the reactions proceed.

1.2 Objectives

This PhD project addressed the importance of copper for efficient nitrification in biological rapid sand filters for drinking water production. More specifically, the research aimed at investigating if copper dosing to filters with incomplete nitrification could stimulate the process and thereby overcome the problems. Investigations were carried out from a problem-solving, engineering point of view, with focus on process optimization and the perspective of improving the treatment efficiency of biofilters. Primary aims which are addressed here were:

- Assess whether or not incomplete nitrification in copper deficient biological rapid sand filters for drinking water production can be stimulated by dosing of the trace metal
- Develop a practically suitable device and method for supplementing copper to biofilters with incomplete nitrification, to stimulate the process
- Determine if remediation of poor nitrification performance through copper dosing is generic and independent of site-specific features at different groundwater treatment plants
- Identify factors affecting the availability of copper and related considerations for copper dosing
- Examine if the stimulation of nitrification is merely a stimulation of nitrification activity or if it involves changes within the nitrifying microbial community

1.3 Experimental approach

Even though laboratory- (Tatari et al., 2013) and pilot-scale experiments (Lee et al., 2014) with full-scale filter sand have been shown to accurately mimic nitrification behavior, full-scale investigations are most accurate, as they are conducted *in situ*. Furthermore, some previous experiments with pilot column filters could not successfully reproduce full-scale filter behavior, possibly due to trace metal release from fittings and piping used for the pilot filters (unpublished data). On the downside, full-scale investigations require meticulous care and hygiene that must be followed, involving on-site sampling equipment sterilization and wearing of protective clothing, when working with these filters which produce drinking water. Furthermore, control of operating conditions (such as well combinations, filter flow, and backwashing) most often requires a plant operator present.

Nevertheless, the vast majority of investigations in this project were carried out at full-scale drinking water treatment plants, as only those can truly reflect filter performance. Given that potential changes within the microbial community in a filter can be comparably slow processes, some investigations had durations of up to one year, in order to achieve steady-states in between changing of operating conditions or parameters.

To characterize the nitrification performance of filters, water samples were collected from filter influent and effluent and analyzed for ammonium and nitrite. However, nitrification is sometimes stratified over filter depth (Lee et al., 2014; Madoni et al., 2001; Tatari et al., 2016), with higher removal rates in the top layers of the filters. Therefore, at some of the investigated treatment plants, water was sampled over depth of the filter. Sampling was carried out with a specially constructed rigid stainless steel sampling probe of 6 mm diameter, housing a Teflon tube (to avoid clogging) with 4 mm diameter. The probe was inserted into the filters at depths of 5, 10, 20, 30, 40, 50, 60, 75, 85, and 95 cm and water was abstracted with a peristaltic pump (Ole Dich, 101 ACR) at 25 mL/min. A 45° angle was used for insertion, so that water could be collected from certain depths without disturbing the flow above the respective sampling points.

Ammonium and nitrite were determined with colorimetric methods, equivalent to APHA 4500-NH3-F and APHA 4500-NO2-B (APHA et al., 2005) and quantification limits were 0.01 and 0.002 mg N/L, respectively. For some long-term experiments, ammonium concentrations were monitored at high frequency (intervals as short as 30 minutes) with an ammonium auto-analyzer (AMTAX™ sc, Hach Lange), with a quantification limit of 0.02 mg N/L. From measured ammonium and nitrite concentrations, volumetric ammonium loading and removal rates were calculated to further assess biofilter performance. The volumetric ammonium loading rate was calculated as

$$ALR = \frac{Q * c_{a,in}}{A * \Delta z}$$

where Q is the filter flow rate [m^3/h], $c_{a,in}$ is the influent concentration of ammonium [g NH_4^+-N/m^3], A is the filter cross section area [m^2], and Δz is the depth of the active layer of the filters [m].

The volumetric ammonium removal rate was defined as

$$ARR = \frac{Q * (c_{a,in} - c_{a,out})}{A * \Delta z}$$

where $c_{a,out}$ is the effluent concentration of ammonium [g NH_4^+-N/m^3]. The unit of ALR and ARR is [g NH_4^+-N/m^3 filter material/h]. Furthermore, under the assumption that all oxidized ammonium was converted to nitrite, which was oxidized further to nitrate, the volumetric nitrite removal rate was calculated as

$$NRR = \frac{Q * ((c_{a,in} - c_{a,out}) + (c_{n,in} - c_{n,out}))}{A * \Delta z}$$

where $c_{n,in}$ and $c_{n,out}$ are the nitrite influent and effluent concentrations [g NO_2^--N/m^3]. The unit of the NRR is [g NO_2^--N/m^3 filter material/h].

From water depth profiles, depth specific volumetric ammonium and nitrite removal rates were calculated. When referring to the depth specific ARR of a specific layer of the filter, $c_{a,in}$ and $c_{a,out}$ are the ammonium influent and effluent concentrations to and from this layer. Δz is the depth of that specific layer (e.g.: for calculating the ARR of the layer from 30-40 cm depth, $c_{a,in}$ and $c_{a,out}$ are the ammonium concentrations at 30 and 40 cm depth, respectively, and Δz is 0.1 m).

2 Background and state-of-the-art

2.1 Drinking water production in Denmark

The drinking water supply in Denmark is entirely based on groundwater (Danish Nature Agency, 2014; IWA, 2014). Before the water is ready for distribution to the consumers, anaerobic groundwater needs to be treated to remove compounds such as hydrogen sulfide (H_2S), methane (CH_4), dissolved iron (Fe^{2+}) and manganese (Mn^{2+}), and ammonium (NH_4^+).

Treatment at the water works typically comprises an aeration step (Fig. 2), where oxygen is transferred into the water and unwanted volatile compounds such as hydrogen sulfide and methane are removed from the water through stripping. Different types of aeration include cascade aeration, diffusor aeration, or oxygen injection (Crittenden et al., 2005). Introduction of oxygen into the water causes oxidation of reduced Fe^{2+} and Mn^{2+} to Fe^{3+} and Mn^{4+}, which precipitate as metal hydroxides. Aeration is followed by granular media filtration (Fig. 2), e.g. rapid sand filtration. Its main purposes are removal of particles and metal hydroxides, and oxidation of dissolved compounds like ammonium, and remaining fractions of dissolved gases and iron and manganese which have not already been removed or oxidized during aeration.

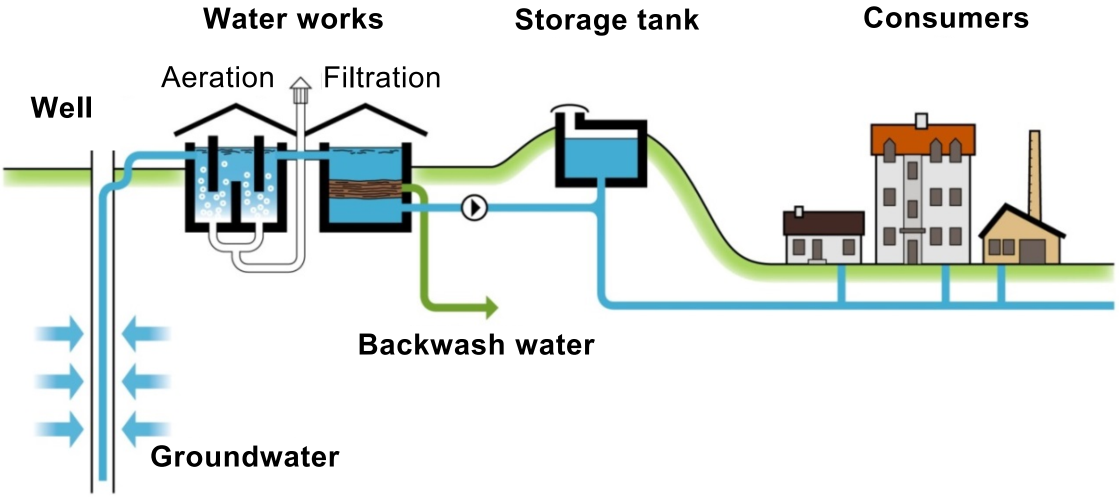


Figure 2 - From groundwater to potable water for consumption (adapted from VandCenter Syd, 2013).

Fe^{2+} and Mn^{2+} are oxidized chemically during filtration, but also biological oxidation by iron- and manganese oxidizing bacteria is possible, largely depending on dissolved oxygen concentrations and pH conditions (Mouchet, 1992). Ammonium is removed biologically in the filters through nitrification. After filtration, the water may be stored in buffer tanks at the water works or elevated storage tanks, before it is distributed to the consumers (Fig. 2). In Denmark, disinfection is not commonly used and utilities do not maintain a disinfectant residual during distribution.

2.2 Rapid sand filtration

Granular media filtration is used worldwide to treat groundwater and surface water (Crittenden et al., 2005), with rapid sand filtration (Fig. 3) being the most commonly used filtration process at Danish water works. “Rapid” refers to the water filtration rate, which is typically between 5-15 m/h (Crittenden et al., 2005). Filtration rates in this range are common for non-biological filters (e.g. when pre-chlorination is used to avoid biofilm formation), whereas rates for biological rapid sand filters are sometimes lower. In Denmark, filtration rates are often 3 m/h, or less. Water usually flows downwards driven by gravity, but closed pressurized systems or up-flow filters are also possible. Filters are periodically backwashed in reverse direction with high flow rates of water or water and air, to remove accumulated particles and precipitates.

Filters are comprised of a filter bed (often also termed ‘active layer’, i.e. active for removal of contaminants) on top of a support layer. Typical filter material for the active layer is quartz with a grain size between 0.8-1.4 mm. Bed depths vary from 0.6 to 1.0 m for open gravity filters, but can be larger for closed pressurized filters (up to 2.5 m).

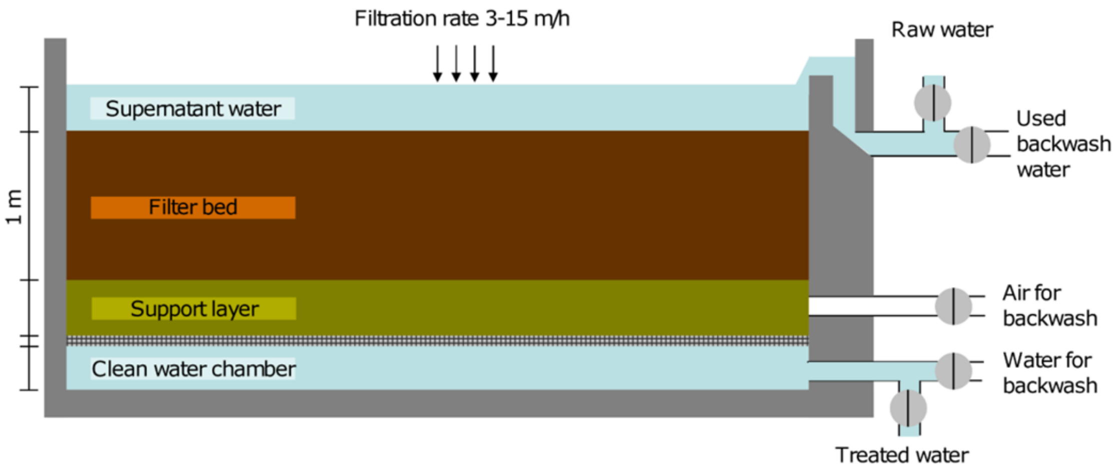


Figure 3 – A typical single media, gravity driven rapid sand filter (Lopato, 2011).

2.3 Nitrification in rapid sand filters

Nitrification is the biological process during which ammonium is oxidized to nitrate. Ammonia oxidizing bacteria (AOB; Prosser, 1989) or archaea (AOA; Martens-Habbena and Stahl, 2011) oxidize ammonium to nitrite (NO_2^-), which is further oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB; Prosser, 1989). AOB and AOA are together often referred to as ammonia oxidizing prokaryotes (AOP; van der Wielen et al., 2009). Well known representatives of AOB are found within the genus *Nitrosomonas*, and of NOB within genera *Nitrobacter* or *Nitrospira.*

The energy yield for the oxidation of ammonia to nitrite is $\Delta G°' = -275$ kJ/mol NH_3 and $\Delta G°' = -74$ kJ/mol NO_2^- for nitrite oxidation to nitrate. For a long time, the ammonia and nitrite oxidizers have been believed to be dividing the labor in nitrification, by strictly oxidizing either ammonium or nitrite. This has puzzled researchers, as complete oxidation of ammonia to nitrate would yield more energy than either separate step ($\Delta G°' = -349$ kJ/mol NH_3). Such complete ammonia oxidizers (comammox; Costa et al., 2006) have recently been discovered within the genus *Nitrospira* (Daims et al., 2015; Palomo et al., 2016; Pinto et al., 2015; van Kessel et al., 2015). Hence, we can distinguish between canonical, betaproteobacterial AOB and complete ammonia oxidizing *Nitrospira* bacteria, within the class Nitrospira. The group AOP can therefore be extended to include comammox *Nitrospira.*

Nitrification consumes alkalinity at 7.1 mg $CaCO_3$/mg NH_4^+-N and a significant amount of oxygen as electron acceptor at 4.5 mg O_2/mg NH_4^+-N (Rittmann and McCarty, 2001). Consequently, even small amounts of ammonium in the distribution system can be a problem due to oxygen consumption. Further nutrients such as phosphorus and micronutrients like trace metals are required for growth and activity.

In rapid sand filters, the nitrifying microorganisms are organized within a biofilm around the filter media (Gülay et al., 2014). Several investigations have reported on the presence of these nitrifiers in rapid sand filters treating groundwater for drinking water production (Albers et al., 2015; de Vet et al., 2011; Gülay et al., 2016; Palomo et al., 2016; Tatari et al., n.d.).

2.4 Problems with ammonium removal

Rapid sand filters are designed and dimensioned to remove a certain content of ammonium from a certain raw water flow. However, nitrification is sometimes inefficient (low nitrification rate) and/or incomplete (ammonium and/or nitrite residues in the finished water). This is problematic for water quality, as it can result in nitrification during drinking water distribution, regardless of whether disinfection as hygienic barrier is used at the water works or not. When chlorination is used, nitrification can consume and alter the disinfection residual (Zhang et al., 2009). Furthermore, and especially in systems without disinfection, nitrification can cause biological regrowth, and associated potential growth of opportunistic pathogens, taste and odor problems, and material corrosion of distribution system materials (Prest et al., 2016; van der Kooij, 2000).

In countries like Denmark, where disinfection is not used, it is of utmost importance to achieve biologically stable water (Rittmann and Snoeyink, 1984) through removal of biodegradable electron donors. The EU guidelines for water works' effluent water are 0.5 mg NH_4^+/L for ammonium and 0.1 mg NO_2^-/L for nitrite (European Comission, 1998). Nevertheless, Denmark enforces stricter values of 0.05 and 0.01 mg/L for ammonium and nitrite (Ministry of Environment and Food of Denmark, 2016). The stringent guideline for ammonium is the most challenging for water utilities to comply with (Table 1).

Table 1 – Water quality parameters from Danish water works treating >1,000 m^3/d between 2002 and 2013 (adapted from Naturstyrelsen, 2014, 2012, 2009, 2007). MAC: Maximum Acceptable Concentration.

Parameter	Danish MAC (Ministry of Environment and Food of Denmark, 2016)	Unit	Analyses between 2002-2013		
			Samples	**Exceedings**	**% exceedings**
Ammonium	0.05	mg NH_4^+/L	5,826	769	***13.2***
Nitrite	0.01	mg NO_2^-/L	6,155	162	2.6
Iron	0.1	mg Fe/L	15,146	548	3.6
Manganese	0.02	mg Mn/L	5,723	189	3.3
Methane	0.01	mg CH_4/L	203	16	7.9
Hydrogen sulfide	0.05	mg H_2S/L	128	1	0.8
NVOC	4	mg C/L	5,109	84	1.6
Coliforms	0 (not detectable)	/100 mL	22,742	684	3.0

Ammonium levels which exceed the water quality standard are problematic as such, however its implications reach further. Increased effluent ammonium concentrations usually indicate that a biological filter has reached its maximum nitrification capacity. If the filter is then subjected to increases in ammonium loading, effluent concentrations increase further (Fig. 4). Such loading rate increases can frequently occur and are caused by variations in ammonium inlet concentrations (Kors et al., 1998) or hydraulic loading (Lee et al., 2014), or a combination of both (Lopato et al., 2013). Poor nitrification performance can therefore constrain water utilities, which may be forced to lower the water throughput, in order to maintain a water quality which complies with the guideline.

Insufficient or incomplete ammonium removal in biolfilters for drinking water production is by no means only a Danish problem. Challenges are reported world-wide (de Vet et al., 2009; de Vet et al., 2011; de Vet et al., 2012; Kors et al., 1998; Lytle et al., 2015; Pedroni et al., 1997; Tekerlekopoulou et al., 2013; van der Aa, 2002; Yoshizaki and Ozaki, 1993), where both inefficient and incomplete nitrification are problematic.

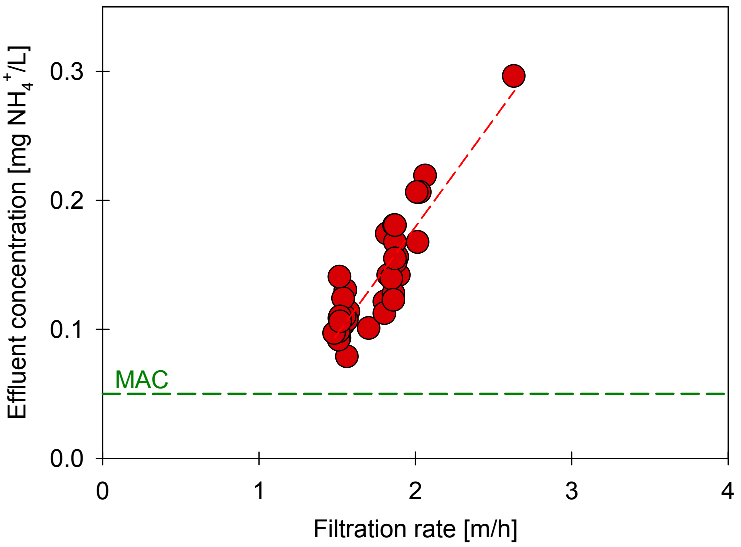


Figure 4 – Relationship between ammonium effluent concentrations and filtration rate (hydraulic loading) of a biofilter operating beyond its nitrification capacity (adapted from Wagner et al., **II**). MAC: Maximum Acceptable Concentration.

2.5 Nutrient limitations and role of copper in nitrification

Like all microorganisms, nitrifiers need essential chemical elements such as hydrogen (H), carbon (C), oxygen (O), nitrogen (N), phosphorus (P), and trace nutrients (Madigan et al., 2002) for growth. Nutrients that are not present in sufficiently high concentrations can limit nitrification. This is especially valid for drinking water systems, where nutrients may be present at relatively low levels (Zhang et al., 2009). For example, limitations of nitrification in biofilters treating groundwater for drinking water production have been reported (de Vet et al., 2012), and dosing of phosphorus was used to enhance ammonium removal in rapid sand filters treating surface water (Kors et al., 1998) or groundwater (Lee, 2014; Lytle et al., 2015, 2013), and in granular activated carbon (GAC) filters for surface water treatment (Yoshizaki and Ozaki, 1993). Phosphorus is required for nucleic acids, cell membranes, coenzymes, and adenosine triphosphate (ATP), the energy carrier of a cell (Westheimer, 1987).

While phosphorus is considered a macronutrient (Madigan et al., 2002), nitrifying microorganisms also need micronutrients, such as trace metals, in comparably low concentrations. Among those, the metal copper is believed essential for the enzyme ammonia monooxygenase (AMO), catalyzing the oxidation of ammonium to hydroxylamine. As the enzyme has not yet been purified, the exact content and composition of copper in AMO is yet to be determined (Sayavedra-Soto and Arp, 2011). However, several lines of evidence point towards the significance of copper in catalysis. In cell extracts of the AOB *Nitrosomonas europaea*, copper addition stimulated AMO activity (Ensign et al., 1993). The authors suggested that initially lost enzyme activity upon cell lysis was due to the loss of copper from the enzyme. In contrast to copper, which successfully recovered activity, a wide range of other metal ions, including Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, and Ag^{+}, could not stimulate AMO activity (Ensign et al., 1993), emphasizing a specific role of the metal. Moreover, the mode of action of nitrification inhibitors such as thiourea and allylthiourea, both Cu-chelating compounds, is thought to be through binding of copper at the active site of AMO (McCarty, 1999). Further indirect evidence is provided by comparison to particulate methane monooxygenase (pMMO), an enzyme similar in structure and catalysis, and evolutionary related to AMO (Bedard and Knowles, 1989; Sayavedra-Soto and Arp, 2011). Studies on pMMO are currently more advanced, and copper content as active enzyme site has been confirmed (Balasubramanian et al., 2010). The ammonia oxidizers AOB, AOA, and comammox *Nitrospira* all have the genetic inventory to express AMO (Arp et al., 2007; Daims et al., 2015; Leininger et al., 2006).

Addition of copper stimulates nitrification only to a certain extent. Depending on the concentration of available copper, different '*metabolic impacts'* (Zhang et al., 2009) on nitrifier growth and activity are possible (Fig. 5). Theoretically, no nitrifier growth and activity are observed in the absence of copper (assuming that no other element can substitute for it). Increasing concentrations will stimulate nitrification, until a concentration range optimal for growth and activity is reached. Increasing concentrations further will have adverse effects, eventually leading to complete inhibition (Fig. 5).

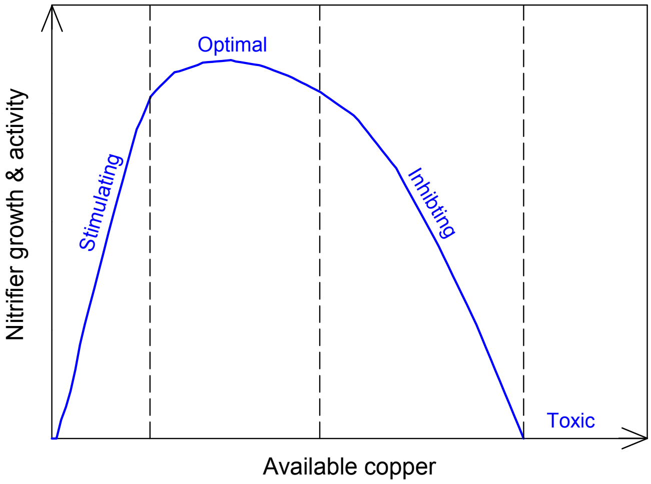


Figure 5 – Conceptual model of the effect of available copper on nitrifier growth and activity (adapted from Zhang et al., 2009).

The inhibition of nitrification by heavy metals such as copper is well documented for waste water treatment (Braam and Klapwijk, 1981; Hu et al., 2004; Li et al., 2016; Loveless and Painter, 1968; Ochoa-Herrera et al., 2011; Song et al., 2016; Tomlinson et al., 1966). Here, copper concentrations are usually higher than in drinking water, and inhibition of nitrification rather than limitation by lack of copper is therefore of concern. The precise mode of action of copper as nitrification inhibitor is currently unknown, however Hu et al. (2003) found no correlation between inhibitory effects and intracellular or sorbed fractions of copper, and suggested the inhibition mechanism may involve disruption of the cytoplasmic membrane (Hu et al., 2004, 2003).

Selected studies, reporting on different copper concentrations causing limiting, stimulating, and inhibiting effects, are presented below (Table 2). Comparing concentrations and effects, we see that there is no exact range which exclusively stimulates or inhibits nitrification. For example, 4 mg Cu/L gave approx. 75 % inhibition of ammonia oxidation in a pure culture of *Nitrosomonas europaea*, but almost 40 times more was necessary to achieve the same effect in nitrifying activated sludge (Tomlinson et al., 1966). The same authors suggested this to be due to complexation of copper with organic matter present in the sludge. Indeed, copper can readily be complexed with inorganic or organic ligands (Sylva, 1976; Xue et al., 1996), thereby decreasing the fraction of free copper (Cu^{2+}).

Table 2 – Selected concentrations reported limiting, stimulating, and inhibiting growth or activity of nitrifiers, for different growth conditions.

	Concentration (total Cu)	**Growth condition**	**Notes**	**Reference**
Limitation	0.32 µg Cu/L	*Nitrosopumilus maritimus* (AOA) in seawater medium	Limited to 55 % of max. specific growth rate	Amin et al., 2013
Stimulation	5-30 µg Cu/L	*Nitrosomonas europaea*	-	Loveless & Painter, 1968
	20 µg Cu/L	Nitrifiers in PVC pipe, enriched from tap water	Ammonia oxidation increased from 45 to 60 %	Zhang & Edwards, 2010
	100 µg Cu/L	*Nitrosomonas europaea*	-	Tomlinson et al., 1966
Inhibition	2 µg Cu/L	Not specified	Reported for waters with low chelating capacity	Waara and Wilander, 1985 in: Zhang et al., 2009
	47.6 µg Cu/L	*Nitrosopumilus maritimus* (AOA) in seawater medium	Inhibited 10 % compared to max. specific growth rate	Amin et al., 2013
	80 µg (soluble) Cu/L	Nitrifiers in brass pipe, enriched from tap water	Significant inhibition	Zhang et al., 2008
	50-560 µg Cu/L	*Nitrosomonas europaea*	-	Loveless & Painter, 1968
	100-500 µg Cu/L	*Nitrosomonas europaea*	0.1 mg/L: slight inhibition; 0.5 mg/L: complete inhibtion	Skinner and Walker, 1961
	4,000 µg Cu/L	*Nitrosomonas europaea*	Approx. 75 % inhibtion	Tomlinson et al., 1966
	Approx. 150,000 µg Cu/L	Activated sludge	Approx. 75 % inhibtion	Tomlinson et al., 1966

In a PVC pipe assay, inorganic complexing with bicarbonate (HCO^{3-}) from alkalinity decreased copper inhibition and stabilized ammonium oxidation (Zhang and Edwards, 2010). Braam and Klapwijk (1981) studied the effect of pH on copper inhibition of nitrification and found a linear correlation between nitrification capacity and concentration of free copper, as influenced by pH. In the same way that copper complexation can ease inhibitory effects, it can aggravate limitations by lack of the metal, as for example reported for marine environments (Amin et al., 2013; Jacquot et al., 2014; Moffett et al., 2012). Speciation is therefore an important factor, as it controls the concentration of free copper, which determines bioavailability, rather than total or dissolved copper concentrations.

A precise requirement of copper for nitrifying microorganisms in biological filters for drinking water production is currently unknown, and also pure culture studies focusing on concentrations <1 µg Cu/L are lacking. For a marine AOA, Amin et al. (2013) reported 45 % reduction in specific growth at a concentration of 0.32 µg total Cu/L. However, AOA may have a higher requirement for copper compared to AOB (Amin et al., 2013). Furthermore, AOA are not the only nitrifying guild present in biological filters (de Vet et al., 2011; Gülay et al., 2016; Lee et al., 2014; Palomo et al., 2016; Tatari et al., 2016). Nevertheless, it is suggested that a low concentration range (≤1 µg Cu/L) is of interest when assessing potential limitations, especially when complexation may be high, as for example in groundwater with high alkalinity or surface water with high concentrations of natural organic matter (NOM).

Information about copper concentrations in the raw water of drinking water treatment plants would therefore be valuable. From the present legislative view point, chemical analysis of the raw water serves the purpose of assessing content of potentially harmful substances, such as pesticides. In practice, the raw water chemistry is also used to evaluate treatability, i.e. to determine necessary treatment steps based on the levels of contaminants in the water (e.g. Fe^{2+}, Mn^{2+}, and NH_4^+). According to current legislation (Ministry of Environment and Food of Denmark, 2016), analysis for copper is not required when determining the raw water chemistry. In the very few cases where copper concentrations are determined, the analytical methods provide detection limits of >1 µg Cu/L and may therefore not be sensitive enough. As available data is only of limited value when assessing potential copper limitations of nitrification, we used a more sensitive method to determine copper concentrations at a potentially limited plant, as described in the following.

3 Effect of copper dosing on nitrification efficiency of biofilters

Based on knowledge from literature concerning the importance of copper for nitrification and a preliminary laboratory-scale investigation with trace metal addition (Wagner et al., **I**), copper concentrations were determined at Nærum drinking water treatment plant (Fig. 6). The plant has had problems with inefficient and incomplete nitrification for several years, dating back to 2012, when filter sand and piping at the plant were replaced. Numerous attempts to increase nitrification rates had failed, including dosing of phosphorus, which had shown promising results in laboratory- and pilot-scale, however no success in full-scale (Wagner et al., 2014).

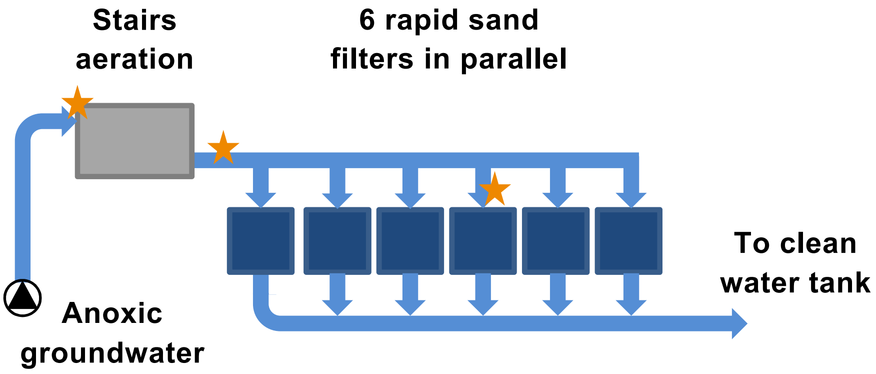


Figure 6 – Treatment of water at Nærum drinking water treatment plant. Orange stars mark locations of water sampling for copper determination.

Given what is known from literature about the magnitude of copper concentrations that may limit nitrification, the commonly used method of analysis with ICP-OES cannot provide a high enough sensitivity (with common detection limits of >1 µg Cu/L). Here, concentrations of total copper in water, sampled at three locations within the treatment train (Fig. 6), were <0.01 µg Cu/L (analyzed with ICP-MS, analogous to US EPA method 6020A (USEPA, 2007a)). The detection limit was thereby approx. 2 orders of magnitude lower than that of ICP-OES analysis and therefore much more relevant for assessing potential copper limitations. The low copper groundwater concentrations may have been caused by adsorption or complexation of copper in soil, and a generally low copper content of, or sorption to the aquifer material (Adriano, 1986) of the limestone aquifer supplying the plant with raw water. The very low copper concentrations were considered a factor likely limiting nitrification. Consequently, it was investigated whether copper dosing could stimulate nitrification activity at the full-scale water works.

Copper was dosed to a filter through active dosing employing electrolysis (see chapter 4 for details), at an average dosing concentration of <1 µg Cu/L, and peak concentrations of up to 5 µg Cu/L (Wagner et al., **I**). This concentration range for dosing was deliberately chosen, in order to avoid inhibition of nitrification. Influent and effluent ammonium concentrations of the filter were monitored at 30 min frequency, in order to quantify a possible effect of the dosing on ammonium removal.

3.1 Stimulation of ammonium removal

Filters at Nærum water works were operated at a volumetric ammonium loading rate (ALR) of 0.5 g NH_4^+-N/m^3/h, corresponding to only 33 % of the plant's design capacity. Nevertheless, prior to copper dosing, nitrification was incomplete with average ammonium and nitrite filter effluent concentrations of 0.18 mg NH_4^+-N/L (Fig. 7) and 0.009 mg NO_2^--N/L.

Ammonium removal responded quickly to the onset of copper dosing (Fig. 7), and ammonium effluent concentrations decreased by 21 % after only 5 days of dosing. Concentrations decreased steadily to <0.02 mg NH_4^+-N/L after 23 days (Fig. 7). At that point, the volumetric ammonium removal rate (ARR) was 0.48 g NH_4^+-N/m^3/h, which was more than double, compared with prior to dosing.

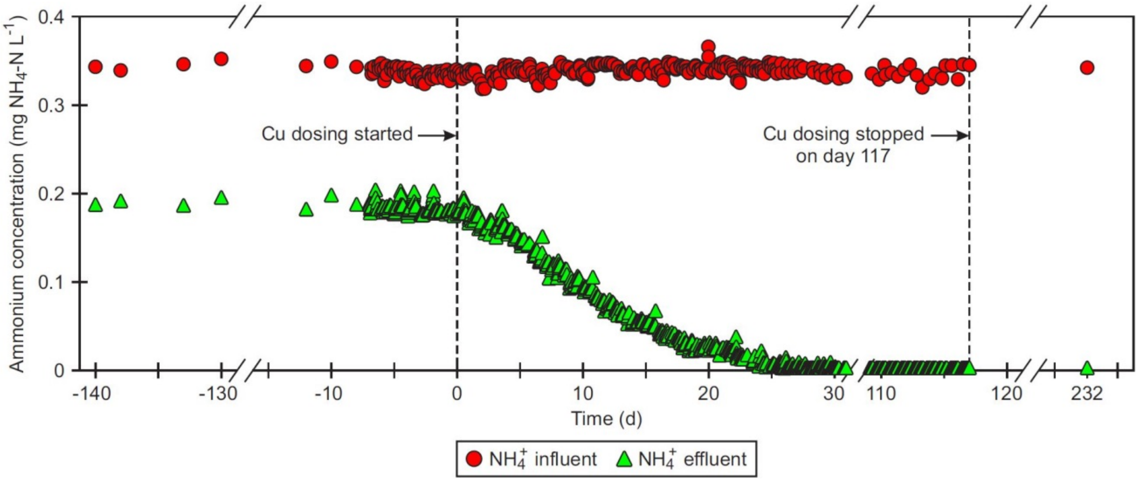


Figure 7 – Effect of copper dosing on ammonium removal in a full-scale filter at Nærum water works. The hydraulic loading rate was constant during the study (Wagner et al., **I**).

Within 22 days of dosing, the filter treated almost 12,000 m^3 of water, and 0.4 µg Cu/L were added. Filter effluent concentrations ranged from <0.05 to 1.3 µg Cu/L (Wagner et al., **I**), which was 2 and 3 orders of magnitude lower than the national standard for water work's effluent water of 100 µg Cu/L (Ministry of Environment and Food of Denmark, 2016) and international health-based guidelines for water quality at the consumer's tap of 2,000 µg Cu/L (European Comission, 1998; WHO, 2011).

Stimulation of nitrification due to copper addition has been reported for lab-scale systems with pure cultures of *Nitrosomonas europaea* (Loveless and Painter, 1968; Tomlinson et al., 1966), and for nitrifying microorganisms enriched from tap water in a PVC pipe lab-scale setup (Zhang and Edwards, 2010). Copper concentrations for which stimulation was reported in above studies ranged from 5-100 µg Cu/L. For full-scale sand filters treating drinking water, Pedroni et al. (1997) reported stimulation of ammonium removal by added concentrations as low as 0.3 µg Cu/L. However, the dosing was a combined trace metal dosing which included molybdenum at 0.01 µg Mo/L, and data quantifying possible effects over time was extremely sparse, with only 3 data points within 2 months (Pedroni et al., 1997). In contrast, our study found a rapid stimulation of nitrification, with a doubling in ammonium removal rate within approx. 20 days by copper dosing to full-scale biofilters at <5 µg Cu/L (Wagner et al., **I**), which has not been reported before.

3.2 Nitrification rate increase in upper filter layers

To investigate the effect of copper dosing on ammonium and nitrite removal over filter depth, water depth sampling was conducted one day before dosing onset, after 23 days with dosing, and after 57 days with dosing.

Within 23 days of dosing, depth specific ARRs were increased from 0 to 80 cm filter depth, compared to rates before dosing onset (Fig. 8C). Ammonium oxidation activity moved further upwards in the filter (Fig. 8A), and within 57 days of dosing, the ARR for 0-10 cm filter depth was increased almost 14-fold (Wagner et al., **I**). Improved ammonium oxidation increased concentrations of nitrite in the upper layer, which was however removed to ≤0.002 mg NO_2^--N/L after 60 cm depth (Fig. 8B). Nitrite concentrations were generally approx. one order of magnitude lower than ammonium; correspondingly, depth specific volumetric NRRs were only slightly lower than ARRs (Fig. 8C&D).

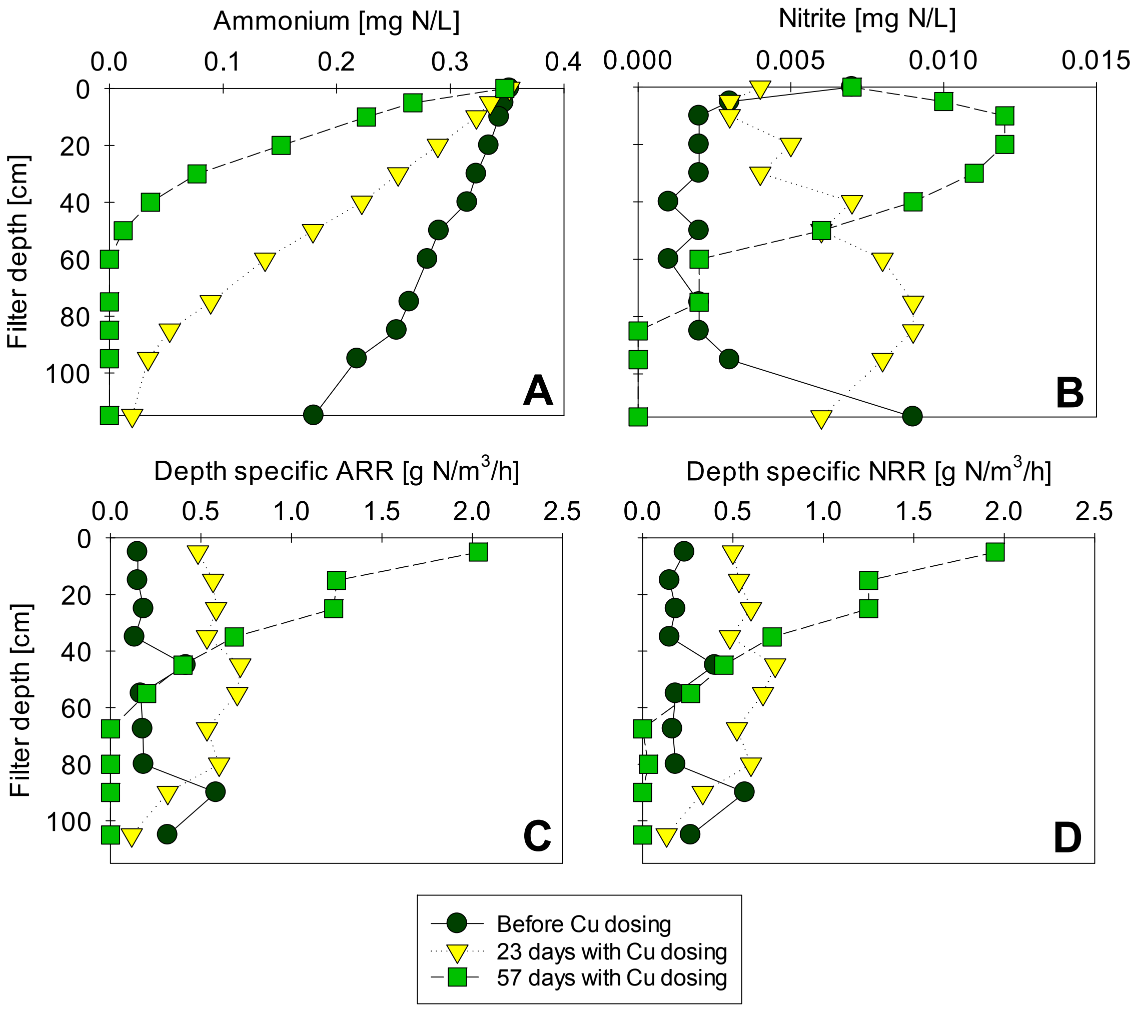


Figure 8 – Effect of copper dosing on ammonium (A) and nitrite (B) concentrations over filter depth at Nærum water works. Volumetric depth specific ammonium and nitrite removal rates (ARR; C and NRR; D) were calculated from the concentrations (Panel A and B: adapted from Wagner et al., **I**).

The depth sampling revealed that lower ammonium concentrations were reached further up in the filter with dosing, due to increased ammonium removal rates in the upper layers. Dosing therefore lead to stratified removal, and the filter achieved ammonium and nitrite effluent concentrations of <0.01 mg NH_4^+-N/L and <0.002 mg NO_2^--N/L. Stratification has been reported for other well-performing sand filters that achieve complete ammonium removal (Lee et al., 2014; Madoni et al., 2001; Tatari et al., 2016). Here, as a result, the filter now complied with stringent national drinking water quality standards (Ministry of Environment and Food of Denmark, 2016). This is relevant for biological stability of the finished drinking water (van der Kooij, 2000).

3.3 Persistence of stimulation after dosing is stopped

Whether or not a continuous dosing of copper is necessary for filters that require dosing can be of high practical relevance. Therefore, the persistence of the effect of copper dosing on nitrification was investigated after dosing stopped (Wagner et al., **I**). A second filter at Nærum water works received dosing in the same manner as the filter described above. Dosing lasted for 42 days, and persistence of the stimulation was monitored through ammonium and nitrite concentration depth profiles (Fig. 9) and effluent measurements (Wagner et al., **I**).

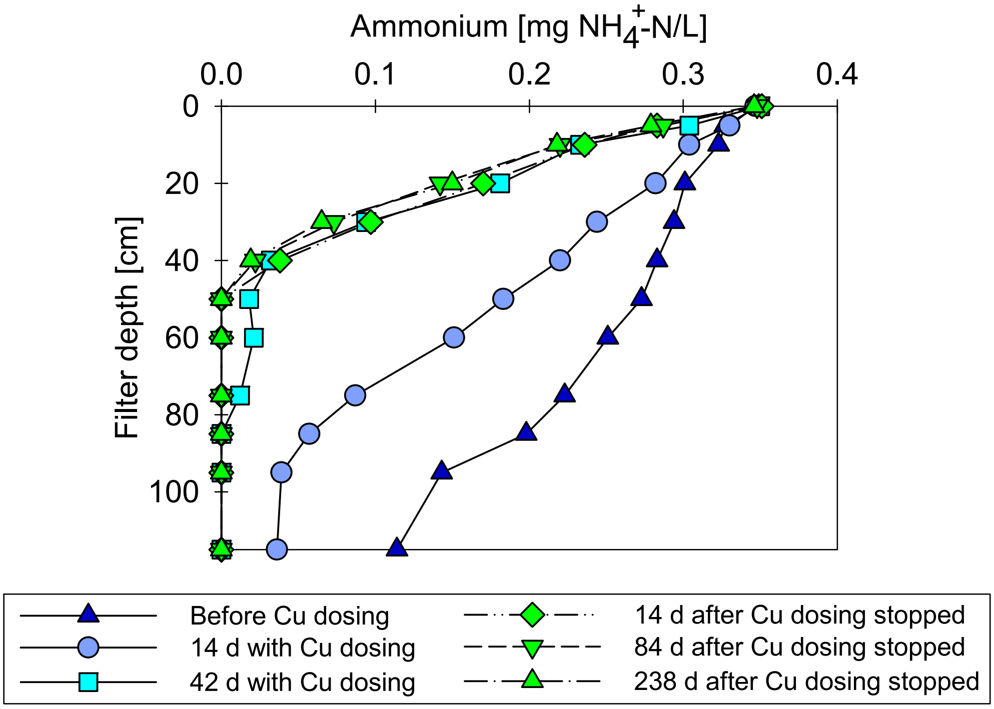


Figure 9 – Persisting effect of copper dosing on ammonium removal over filter depth (Wagner et al., **I**).

After 42 days with dosing, >90 % of ammonium was oxidized within the top 50 cm of the filter (Fig. 9), comparable to the performance of filter 4 with 57 days of dosing (Fig. 8A). After dosing was stopped, ammonium removal activity remained high in the upper layers of the filter (Fig. 9), no matter if probed 14, 84, or 238 days after the dosing was stopped. Influent copper concentrations after dosing stop were <0.01 µg Cu/L; this was therefore excluded as a possible explanation for the continued stimulation. However, measurements of copper content on filter media documented accumulation of copper on the sand during the dosing phase. After dosing was stopped, the amount of copper on filter sand decreased again – however at a slower rate than it had accumulated (Wagner et al., **I**). Effluent measurements of up to almost 1 µg Cu/L confirmed a release of the metal to the bulk phase after dosing stopped, which may be an explanation for the observed persisting stimulation of nitrification. Other factors contributing to this effect likely include a (re)cycling of copper within the biofilm (Costerton et al., 1995; Simpson, 2008), or simply high biomass growth with subsequent low decay (van Loosdrecht and Henze, 1999). The observed persistence of stimulation is of practical relevance, as it shows that continuous dosing is not necessary, but that also periodical dosing can yield a long-term stimulation of nitrification.

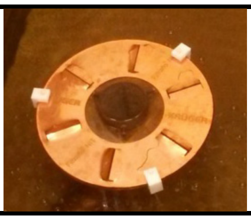
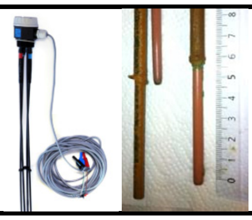
Copper concentrations in surface water can also be very low (Sanchez and Lee, 1973; Xue et al., 1996), and low groundwater copper concentrations are also reported from elsewhere (e.g. the US; (Ayotte et al., 2011)). The above results may therefore potentially impact nitrifying filters at surface water treatment plants and other groundwater treatment plants. Alongside sand filters, nitrification is commonly used in a variety of engineered systems for ammonium removal. Examples include biologically activated carbon (BAC) filtration (Andersson et al., 2001) and trickling filters (de Vet et al., 2011) for drinking water production, or nitrifying membrane bioreactors (Dvořák et al., 2013), moving bed bioreactors (Hem et al., 1994), rotating biological contactors (Boller et al., 1990), and of course activated sludge systems (Tchobanoglous et al., 2004) for waste water treatment. Even though nutrient loading rates and absolute trace metal concentrations are higher in waste water treatment, micronutrient dosing, including copper, has been reported for process enhancement in waste water treatment systems (Burgess et al., 1999a, 1999b; Jefferson et al., 2001; Zandvoort et al., 2006).

4 An innovative method for copper supplementation

4.1 Different forms of dosing

Copper can be dosed from a solution of its salts, which however needs to be acidified in order to keep the copper from precipitating. This in turn requires particular maintenance and storage of the dosing tank. Therefore, we aimed to develop an improved method for copper dosing to biofilters, and investigated copper dosing from solid copper, through passive or active release, as alternative dosing methods. With passive dosing, copper is released to the water simply through contact of the water flow with a solid copper structure (Albrechtsen et al., **II**). In active dosing, the release is triggered through electrolysis of the metal from solid copper electrodes (Albrechtsen et al., **II**). Each of the three methods has certain benefits and deficits (Table 3).

Table 3 – Different methods of copper dosing and their benefits and deficits.

	Passive dosing from solid	**Active dosing employing electrolysis**	**Dosing of chemical solution**
			
Benefits	• Passive dosing • Low maintenance (however dependent on water chemistry) • No chemicals necessary	• Controlled release • Flow proportional dosing • No chemicals necessary • Relatively low maintenance • Requires little installation space	• Controlled release • Flow proportional dosing • Suitable for dosing to pressurized systems
Deficits	• Uncontrolled release • Flow proportional dosing not possible	• Modifications necessary when dosing to pressurized systems	• Chemicals necessary • Solution often needs acidified • Relatively high maintenance • High space and possibly special storage requirements • Overdosing possible • Problematic if leakages • Pumps can fail

4.2 Electrolysis dosing tested in the laboratory

A main requirement on a dosing device is the ability to deliver copper at stable concentrations, which are high enough to stimulate nitrification and yet low enough to prevent inhibition. Passive dosing through a solid copper structure has the benefit of low maintenance. However with this method, the release of copper cannot be controlled, contrary to active dosing through electrolysis. Furthermore with electrolysis, the power supply can be coupled to a water treatment plant's SCADA system (supervisory control and data acquisition system) which enables dosing proportional to the treatment flow. In water treatment, electrolysis with electrodes containing copper has been suggested where copper or the released metal acts as a biocide, to destroy (pathogenic) microorganisms present in the water (Colledge and McKechie, 2003). Possibly because of the lack of awareness of nitrification limitations due to the lack of copper, low concentration copper dosing for stimulation of biological processes has so far not been described in technical literature.

We conducted a batch experiment to test release of copper at low concentrations and to investigate the effect of different applied currents. Copper electrodes (anodes) of 5 mm diameter were tested in batches filled with 1 L of tap water. Surface area exposed to water for submersion depths of 1, 3, and 6 cm was 1.57, 4.71, and 9.42 cm^2. A carbon electrode served as cathode. The surface area in contact with water had only little impact on the release rate (Table 4). However, the rate of release increased with increasing current, following an almost linear correlation (Table 4), confirming that dosing of low concentrations could be controlled through the current. The relationship of applied current and release rate was later transferred to the full-scale system.

Table 4 – Copper released to water in batches with different submersion depths and applied currents (Albrechtsen et al., **II**).

Exposure time [min]	Electrode submersion depth [cm]	Current applied [mA]	Copper conc. in water [µg Cu/L]	Copper release rate [µg Cu/L/min]
0	0	0	<0.04	0
30	1	0.1	46	1.5
30	3	0.1	39	1.3
30	6	0.1	36	1.2
30	3	0.2	95	3.2
30	3	0.4	260	8.7
60	3	0.1	46	0.8
120	3	0.1	130	1.1

4.3 Active electrolysis and passive dosing in full-scale

Copper release and the effect on ammonium removal through passive dosing with a solid copper structure and active electrolysis dosing were investigated at Glostrup DWTP. Treatment at the water works comprises stairs aeration, followed by two single-stage rapid sand filters in parallel. Before dosing onset, ammonium removal was incomplete (Fig. 10), with an average volumetric ammonium removal rate (ARR) of 0.82 g NH_4^+-N/m^3/h at an average volumetric ammonium loading rate (ALR) of 1.30 g NH_4^+-N/m^3/h. Like at Nærum DWTP, copper in the filter influent was <0.01 µg Cu/L. Passive copper dosing by a solid copper plate, shaped like a flat ring, was placed around the upright inlet pipe of the filter (Fig. 11). Right after installation, the copper concentration was 0.12 µg Cu/L in the influent water.

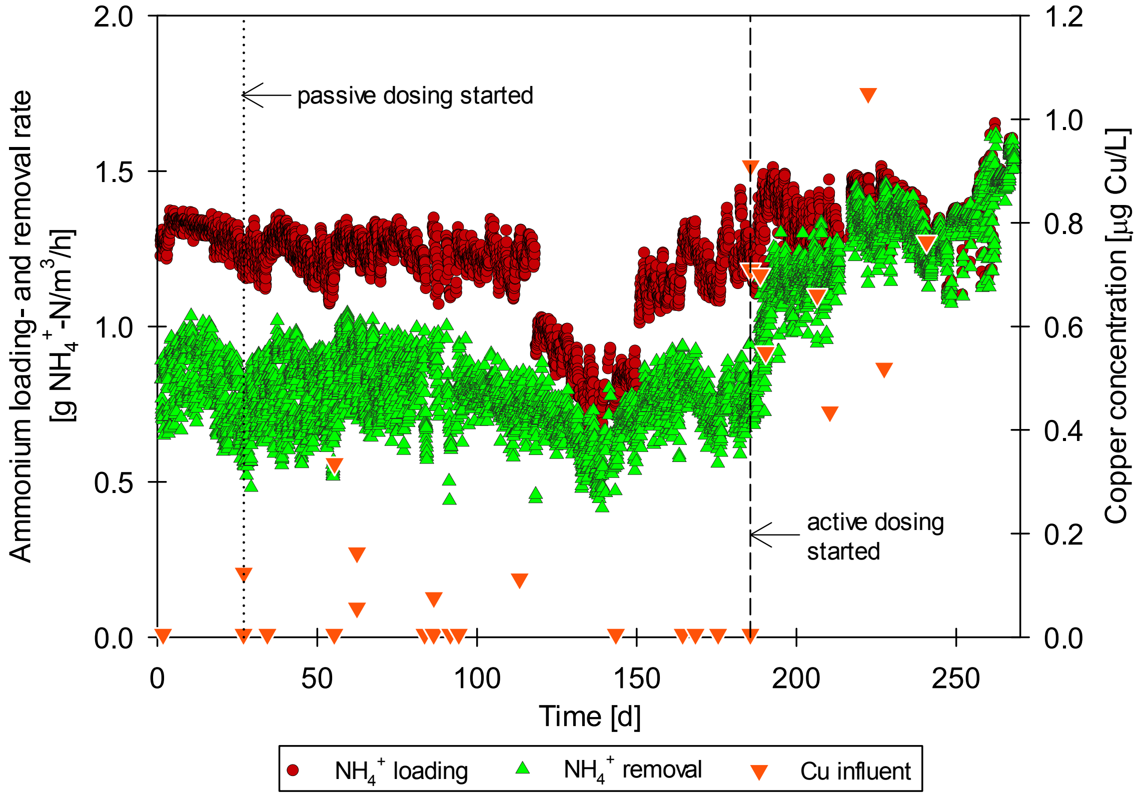


Figure 10 – Passive vs. active dosing of copper. Volumetric ammonium loading rates (ALR) and ammonium removal rates (ARR), and copper concentrations in the filter inlet over time at Glostrup DWTP. The dotted line depicts onset of passive dosing employing a copper plate and the dashed line depicts onset of active electrode dosing. The ALR drop between days 118 and 150 was caused by problems with one abstraction pump.

However, concentrations decreased again to <0.01 μg Cu/L (Fig. 10), possibly because the copper plate became passivated with a layer of iron oxide-hydroxides (Fig. 11). Only after the coating was removed physically with a steel brush on days 55, 63, and 114, the copper concentrations increased temporarily; but this had no persisting effect and copper concentrations decreased again (Fig. 10). Correspondingly, the short-lasting increases in copper concentrations due to brushing of the plate had no positive effect on ammonium removal. Removal remained incomplete, with an average ARR of 0.77 g NH_4^+-N/m^3/h, at an average ALR of 1.18 g NH_4^+-N/m^3/h, between days 150 and 186.

On day 186, the passive copper plate was replaced by active dosing with two electrodes, which increased copper concentrations in the inlet water by up to two orders of magnitude compared with passive dosing (Fig. 10). Ammonium removal increased already shortly after electrode dosing started (Fig. 10), and after only 1 week, ARR was improved from 0.77 to 1.17 g NH_4^+-N/m^3/h, at an average ALR of 1.35 g NH_4^+-N/m^3/h. After 30 days of dosing, ARRs were equal to ALRs and ammonium removal was complete, with effluent ammonium concentrations <0.01 mg NH_4^+-N/L.

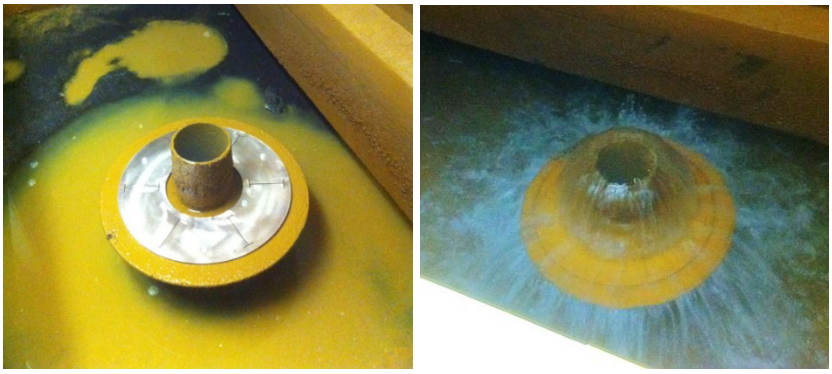


Figure 11 – Left: Passive dosing structure immediately after installation. Right: After approx. 1 week of use, the structure is covered with a layer of iron oxide-hydroxides.

Iron concentration in the influent of the filter receiving dosing was 1.32 mg Fe/L. Passive copper dosing was also investigated at Langerød, Bakkebølle, and Skindermarken DWTPs (Wagner et al., **III**). At those treatment plants, primary filtration is used, which resulted in lower iron influent concentrations to the secondary filters of 0.14, 0.08, and 0.06 mg Fe/L, respectively. Passive dosing installed in secondary filters of the three water works resulted in rapid stimulation of nitrification and in complete ammonium removal after only 12, 17, and 13 days (Wagner et al., **III**).

Overall, dosing from solid copper was successful in stimulating ammonium removal at several water works. When active electrolysis dosing is used, release of copper can be controlled through electrical current. This may be beneficial in cases where increased concentrations of copper are necessary, for example when increased iron loads (Benjamin et al., 1996) or high alkalinity (Zhang and Edwards, 2010) are expected to bind a fraction of the added copper. It is also preferable over passive dosing, in cases where copper release from the passive device is prevented through formation of inorganic deposits. Results furthermore suggest that copper should be dosed to filters receiving prefiltered water, if primary filtration is part of a water work's treatment configuration.

The developed electrode dosing method is a simple yet effective means to stimulate ammonium removal in biological filters. It has relatively low maintenance requirements and can easily be retrofitted into existing DWTPs, at low space requirements. The technology is at present successfully applied at a number of Danish water works, and is commercialized under the name Filtraflo NIT™.

5 Dosing at ten different full-scale treatment plants

Nærum drinking water treatment plant was the first plant where copper dosing was investigated and nitrification was successfully stimulated. However, factors such as water chemistry (Sylva, 1976; Xue et al., 1996; Zhang and Edwards, 2010) or iron load to the filters (Benjamin et al., 1996) affect copper availability, and those factors can vary from plant to plant. Therefore, throughout the project, the effect of copper dosing on nitrification was studied at 10 different treatment plants with incomplete nitrification, in order to evaluate if copper dosing can generically stimulate the process (Wagner et al., **III**).

5.1 Generic remediation of nitrification

The investigated plants are located in Denmark and treat groundwater with aeration and subsequent filtration, where some plants apply two filtration steps (Table 5), with primary (prefiltration) followed by secondary filtration (afterfiltration). Filters are all granular media filters, but vary in construction and hydraulic loading rate. The average raw water temperature at the plants was 9.3 ± 0.4 °C. Ammonium influent concentrations to the filters ranged from 0.15 to 1.17 mg NH_4^+-N/L. As opposed to treatment plants with raw water concentrations of up to 3.3 mg NH_4^+-N/L (Lytle et al., 2015), where the oxygen demand of 4.5 mg O_2/mg NH_4^+-N for complete nitrification can be an issue, the above concentrations should as such not pose a challenge. Alkalinity (as HCO_3^-) ranged from 150 to 448 mg HCO_3^-/L, and pH ranged from 7.1 to 8.2. Iron influent concentrations to the filters receiving prefiltered water were generally lower than concentrations to filters at plants applying only single-stage filtration (Table 5). Thus, the water works represented a wide variety regarding several different parameters.

Copper was supplemented through passive dosing at drinking water treatment plants Langerød, Bakkebølle, Glostrup, and Skindermarken, and through active electrolysis dosing at Nærum, Glostrup, Holmehave, Mørkeskov, Valbygård, and Frederiksgade. At Skive drinking water treatment plant, copper was dosed from solution (Wagner et al., **III**).

Table 5 – Filter design and operation, water quality, and ammonium removal performance indicators before and after copper dosing at all investigated drinking water treatment plants (adapted from Wagner et al., **III**; alkalinity and pH data adapted from National Well Database (Jupiter)).

Treatment plant	Pre-filter? yes/ no	Filter material of filter with dosing	Filter velocity before/ after Cu [m/h]	EBCT [h]*	NH_4^+ influent before/after dosing [mg N/L]	ALR before/ after dosing [g N/m^3/h]	Fe influent [mg Fe/L]	Alkalinity [mg HCO_3^-/L]	pH [-]	Cu influent before/after dosing [µg/L]	ARR before/after dosing [g N/m^3/h]	Response time for complete NH_4^+ removal [days]	Δ ARR/ t
Nærum	n	0.8-1.4 mm quartz	1.56	0.38	0.34/0.35	0.88/ 0.90	2.06	342	7.5	<0.01/ 0.97	0.39/ 0.87	22	0.022
Langerød	y	0.8-1.4 mm quartz	2.40	0.27	0.75/0.76	2.77/ 2.81	0.14	358	7.4	<0.03/ 0.42	1.55/ 2.73	12	0.098
Bakkebølle	y	1-3 mm granular calcium carbonate	1.07	0.65	0.89/0.91	1.37/ 1.41	0.08	406	7.1	<0.03/ 1.50	0.62/1.37	17	0.044
Glostrup	n	0.8-1.4 mm quartz	2.71/2.80	0.30/ 0.29	0.38	1.29/ 1.30	1.32	448	7.5	<0.01/ 1.06	0.71/ 1.26	30	0.018
Holmehave	n	0.8-1.4 mm quartz	1.92	0.39	0.38/0.40	0.97/ 1.03	1.46	308	7.4	<0.01/ 1.34	0.62/ 0.98	21	0.018
Mørkeskov	n	0.8-1.4 mm quartz (200 cm) → 1.2-2.0 mm quartz (50 cm)	6.46	0.39	1.01/1.17	2.61/ 3.02	0.7	408	7.2	NA/NA	1.39/ 3.00	21	0.076
Valbygård	y	1.6-2.2 mm quartz	1.62	0.49	0.70	1.43	NA	398	7.4	<0.02/ 0.15 (effluent)	0.86/ 1.41	20	0.028
Frederiksgade	y	1.0-2.0 mm quartz	2.41	0.30	0.38/0.37	1.27/ 1.24	0.01	349	7.3	<0.01/ 0.66	0.77/ 1.22	14	0.032
Skindermarken	y	Quartz: 0.4-0.8 mm (20 cm) → 0.8-1.4 mm (25 cm) → 1.3-2.0 mm (15 cm)	1.30	0.46	0.35	0.76	0.06	265	8.2	<0.04/ 0.29 (effluent)	0.59/ 0.74	13	0.012
Skive	n	0.8-1.4 mm quartz	1.62/2.43	0.74/ 0.49	0.22/0.15	0.3	0.51	150	8.0	<0.04/ 1.50	0.10/ 0.25	22	0.007

Cu analyses for Langerød, Bakkebølle, Valbygård, Skindermarken, Skive according to ICP/MS ISO 17294:2; * EBCT: Empty Bed Contact Time; NA: not available

Before dosing, copper could not be detected in any of the influents to the drinking water treatment plants, with detection limits ranging from 0.01-0.04 µg Cu/L (Table 5; no data available for Mørkeskov). For several years, the treatment plants had problems with incomplete ammonium removal (Fig. 12).

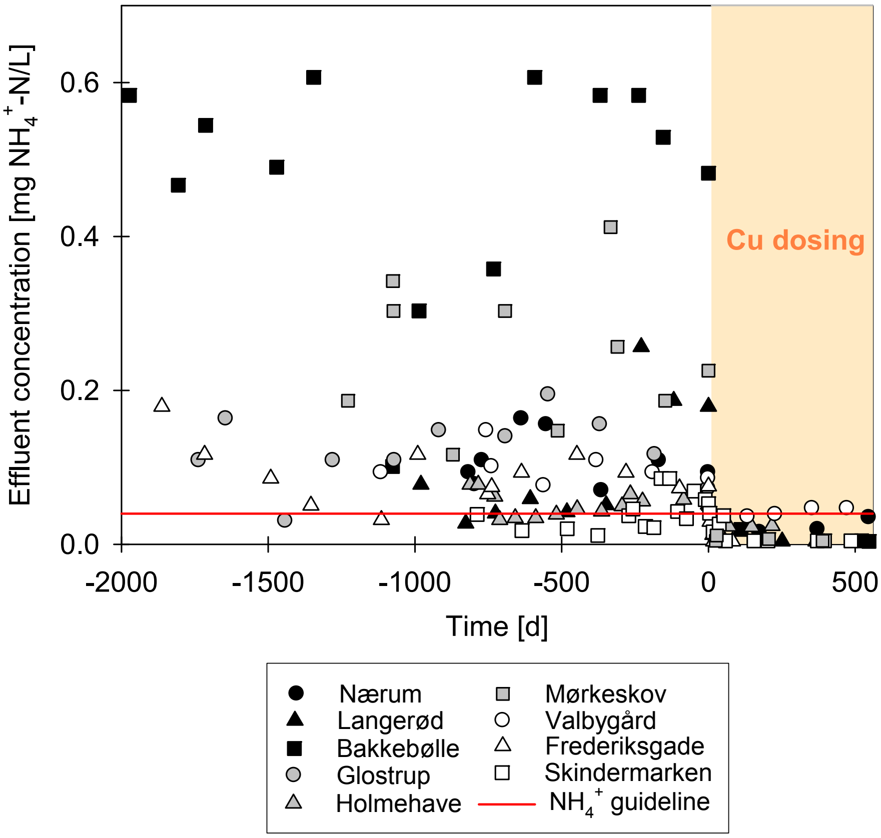


Figure 12 – Effect of copper dosing on ammonium effluent concentrations at the investigated drinking water treatment plants. Part of the data is adapted from the National Well Database (Jupiter). Skive water works was a start-up, hence no long-term data was available (Wagner et al., **III**).

With dosing, ammonium oxidation responded promptly (Fig. 12) and low effluent concentrations of <0.01 mg NH_4^+-N/L were achieved by most filters within only 2-3 weeks (Table 5, Wagner et al., **III**). After dosing onset, 0.42 - 1.50 µg Cu/L copper were measured in filter influent water at the treatment plants.

We tested for possible correlations (with Pearson product-moment correlation) between operational and water quality parameters with the effect of copper dosing on ammonium removal. To quantify the effect of the dosing, the increase in volumetric ammonium removal rate (ARR) [g NH_4^+-N/m^3/h], as induced by the dosing, was normalized by the time it took to reach this increase [d]:

$$\frac{\Delta ARR}{t} = \frac{(ARR_{before\ Cu} - ARR_{after\ Cu})}{Response\ time}$$

Input data and values of ΔARR/t can be found in Table 5, three last columns.

The change in ARR induced by copper dosing over time was not correlated with operational parameters such as filter velocity or empty bed contact time (EBCT) or with water quality parameters like iron, alkalinity, or pH (Table 6). However, ΔARR/t was positively correlated ($r = 0.95$) with the volumetric ammonium loading rate (ALR) and ammonium influent concentrations ($r = 0.78$). This was somewhat expected, given that higher ALRs would result in higher growth, which in turn would lead to higher ARRs (or a faster increase in ARR), under non-limiting conditions.

Table 6 – Correlation matrix for operational and water quality parameters with ΔARR/t. Correlation coefficients in bold indicate a positive correlation.

	ΔARR/t	*Re-sponse time*	*Filter vel.*	*EBCT*	*NH_4^+ infl. conc.*	*ALR*	*Fe*	*Alk*	*pH*
ΔARR/t	1.00								
Response time	-0.40	1.00							
Filter vel.	0.51	0.21	1.00						
EBCT	-0.35	0.00	-0.33	1.00					
NH_4^+ infl. conc.	**0.78**	-0.19	0.53	0.06	1.00				
ALR	**0.95**	-0.23	0.66	-0.45	**0.83**	1.00			
Fe	-0.32	**0.71**	0.03	-0.24	-0.29	-0.22	1.00		
Alk	0.43	0.21	0.29	-0.39	0.62	0.60	0.14	1.00	
pH	-0.54	-0.07	-0.32	0.19	-0.67	-0.59	-0.08	-0.75	1.00

Furthermore, the response time until complete removal was achieved was positively correlated ($r = 0.71$) with the filters' influent iron concentrations. Indeed, at plants applying de-ironing prefiltration, the full effect was reached after an average of 15 ± 3 days, whereas it took 23 ± 4 days on average for plants without primary filtration (Wagner et al., **III**). A two-sample t-test ($\alpha = 0.05$) confirmed that it took significantly longer (p-value = 0.004) at these plants until the full effect of dosing had occurred. This slower response to copper dosing at water works without prefiltration may be explained by reduced availability of copper for nitrifiers, due to sorption of copper to iron oxides (Benjamin et al., 1996) in the filter (see also chapter 6). However, on average for all plants, it took only 19 days to establish full nitrification (Wagner et al., **III**).

Overall, copper dosing could successfully increase nitrification efficiency at all 10 plants, which have had problems with incomplete nitrification for many years. Dosing at ≤1.5 µg Cu/L had a generic effect, independent of site specific parameters such as filter design and operation, ammonium loading rates, water chemistry, or configuration of treatment units. Considering that many more drinking water treatment plants in Denmark have incomplete and/or insufficient nitrification (Danish Nature Agency, 2014), we believe that our findings have great practical implications.

5.2 Nitrification capacity increase at increased ammonium loading rates

Ammonium effluent concentrations were decreased by the dosing (Fig. 12), in connection with the increase in ARR. Removal rates before dosing ranged from 0.1-1.55 g NH_4^+-N/m^3/h, and increased to 0.25-3.00 g NH_4^+-N/m^3/h within the respective response times (Table 5). These ARRs were still rather low, when comparing with removal rates achieved by other nitrifying granular media filters (Table 7). However, at this point, ammonium removal was complete, meaning that ARRs were almost equal to the ALRs applied at the water works (Table 5). Consequently, ammonium removal was limited by the available ammonium loading, and it was therefore not possible to determine the new maximum ARRs (= removal capacity) after copper dosing.

Yet, information about a filter's removal capacity is valuable, as it helps to evaluate if a filter can safely remove ammonium under dynamic operating conditions with varying ammonium loading rates (Lee et al., 2014). Therefore we investigated if copper dosing can increase ARRs further, beyond the ALRs the filters are normally operated under (Wagner et al., **III**).

Table 7 – Volumetric ammonium removal rates achieved at respective loading rates and operating conditions, for different filter systems.

Authors	Filter system	Filtration rate [m/h]	Ammonium infl. concentration [mg NH_4^+-N/L]	ALR* [g NH_4^+-N/m^3 filter material/h]	ARR* [g NH_4^+-N/m^3 filter material/h]	Notes
This study (Wagner et al., III)	Full-scale rapid sand/granular media filters at 10 different drinking water treatment plants (see table 5 for details)	1.1-6.5	0.15-1.17	0.3-3.0	Before copper addition: 0.1-1.55 After copper addition: 0.25-3.0	Ammonium removal was complete when copper was added - ARRs were therefore limited by the ALRs
de Vet et al., 2011	Full-scale trickling filters treating groundwater, one filter treating subsurface aerated groundwater, one filter natural groundwater	2.0-2.6	Approx. 1.5-5	Approx. 2.2-5	1.5-5	Incomplete nitrification in the non-subsurface aerated filter
Kors et al., 1998	Full-scale RSF treating surface water	2.7	1.1	2.5	2.5	Dosing of phosphoric acid necessary (at 1°C temperature)
Laurent et al., 2003	Pilot-scale BAC filters treating river water	5	0.42-0.55	7-9.2	2.5-4.4	-
Lopato et al., 2011	Pilot-scale RSF column treating groundwater	4.2	0.8	3.6	2.7	-
Lee et al., 2014	Pilot-scale RSF column treating groundwater	3.9 & 7.9	0.04-1.68	0.22-10.3	3.4	Investigation of ARR under ALR upshifts
Lytle et al., 2015	Full-scale aeration gravel filled contactor treating groundwater	5.6	3.3	up to 16.2	10.5 (at ALR of 12.4)	0.3 mg PO_4/L is dosed in contactor influent
Štembal et al., 2005	Pilot-scale, pressurized RSF column treating groundwater	10	2.6	NA	Complete removal of 2.6 mg NH_4^+-N/L at 10 m/h hydraulic loading rate	-
Tatari et al., 2016⁺	Lab-scale column setup with sand from top layer of a well-functioning full-scale sand filter treating groundwater	4.4	1	14.6	9.3	Lab-scale system as described in Tatari et al., 2013
van den Akker et al., 2008	Pilot-scale trickling filter with polypropylene media treating surface water	6	0.7-5	2.8-20	12	-
Tekerlekopoulou and Vayenas, 2008	Pilot-scale trickling filter with gravel	9.4	2.3	17	Approx. 16	-

* Volumetric ALRs and ARRs are averages over filter depth, except for ⁺, where sand from 0-10 cm depth was used; RSF: rapid sand filter; NA: not available;

Short-term upshifts of the ALR were conducted at Glostrup and Holmehave treatment plants (Wagner et al., **III**). At Glostrup, the normal ALR was 1.23 g NH_4^+-N/m^3/h, and short-term upshifts as high as 1.85 g NH_4^+-N/m^3/h were performed. Nevertheless, the filter removed the incoming load, with an ARR of 1.84 g NH_4^+-N/m^3/h (Wagner et al., **III**). The filter at Holmehave had been operated mainly at 0.6-1.0 g NH_4^+-N/m^3/h, prior to the upshifts. Before dosing onset, the removal capacity was 0.64 g NH_4^+-N/m^3/h, irrespective of the applied ALRs as high as 1.48 g NH_4^+-N/m^3/h (Fig. 13).

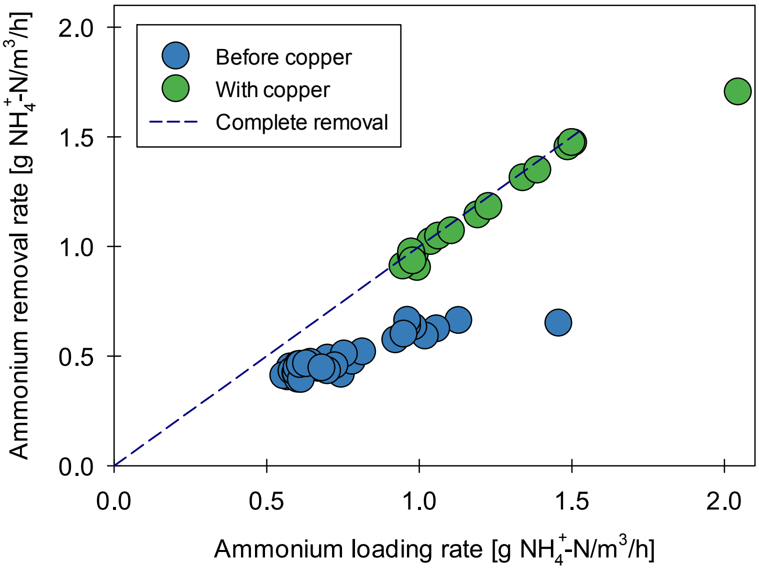


Figure 13 – Effect of copper dosing on ammonium removal at Holmehave treatment plant, when subjected to ammonium loading rate upshifts (adapted from Wagner et al., **III**).

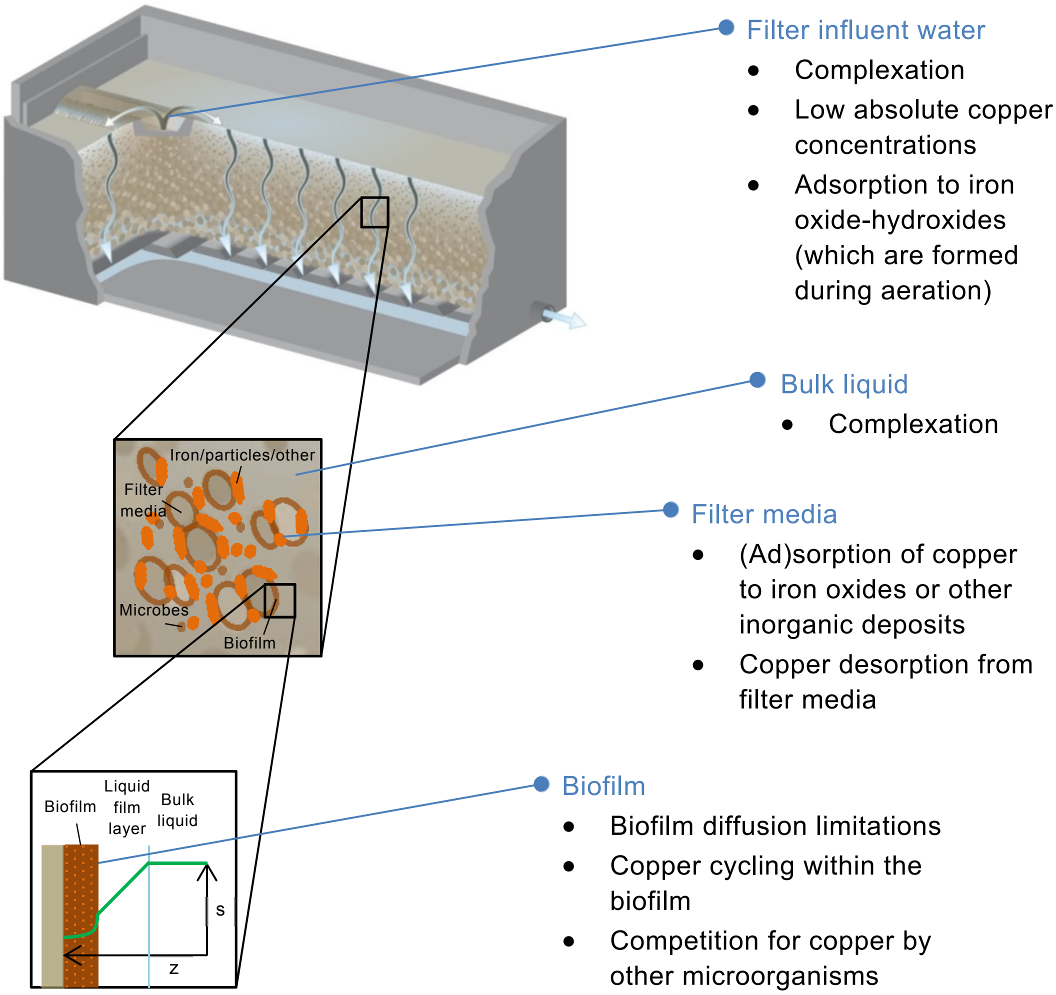
With copper dosing however, ammonium removal was complete, even when upshifted to 1.50 g NH_4^+-N/m^3/h (Fig. 13). The filter could therefore not only remove loadings of around 1.0 g NH_4^+-N/m^3/h which were normally applied, but had acquired extra capacity for removing ammonium, during loading rate up-shifts.

When operated under an ALR of 2.05 g NH_4^+-N/m^3/h, the ARR was 1.70 g NH_4^+-N/m^3/h and ammonium removal was therefore incomplete (Fig. 13). This indicated a new removal capacity around 1.70 g NH_4^+-N/m^3/h, which was more than 2.6 times higher compared to the initial capacity of 0.64 before dosing. This was still lower than ARRs reported for other filter systems (Table 7), although probably because these systems had been operated under generally higher ALRs, and the ALR upshifts at Holmehave were only short-term upshifts, in order to minimize growth (Wagner et al., **III**).

The findings are important from an operational point of view, because dynamic ammonium loading can occur when influent ammonium concentrations are variable (Kors et al., 1998), under changing hydraulic loading (Lee et al., 2014), or a combination of both (Lopato et al., 2013). Perspectives of increasing removal capacity through copper dosing may include the possibility of producing more water with less filter area, thereby decreasing the geographical footprint of drinking water production.

6 Factors affecting copper availability in sand filters

Copper is taken up by microorganisms mainly as the free, cupric ion Cu^{2+} (Cooksey, 1993; Trevors and Cotter, 1990). Copper limitation can occur when Cu^{2+} concentrations are sufficiently low (Jacquot et al., 2014; Moffett et al., 2012). In the aquatic environment, Cu^{2+} often makes up only a small fraction of total copper, due to adsorption, complexation, and precipitation processes (Sylva, 1976). Therefore, we aimed to identify factors which can affect the availability of copper for the nitrifying microorganisms in biological filters. Several factors, at different scales, are at play (Fig. 14):



Filter influent water

- Complexation
- Low absolute copper concentrations
- Adsorption to iron oxide-hydroxides (which are formed during aeration)

Bulk liquid

- Complexation

Filter media

- (Ad)sorption of copper to iron oxides or other inorganic deposits
- Copper desorption from filter media

Biofilm

- Biofilm diffusion limitations
- Copper cycling within the biofilm
- Competition for copper by other microorganisms

Figure 14 – Copper availability is affected by various factors at different scales.

- ***Complexation and precipitation, influencing the distribution (speciation) of copper in the bulk water phase***

Knowing the speciation of copper is crucial to assess the fraction of free Cu^{2+} and thereby the bioavailability. Copper speciation (Fig. 15) was calculated using Visual MINTEQ (Gustafsson, 2013) for filter influent water at Nærum water works (chapter 3), with water quality parameters provided in Wagner et al., **I** and under the assumption of a total water phase copper concentration of 1 µg Cu/L.

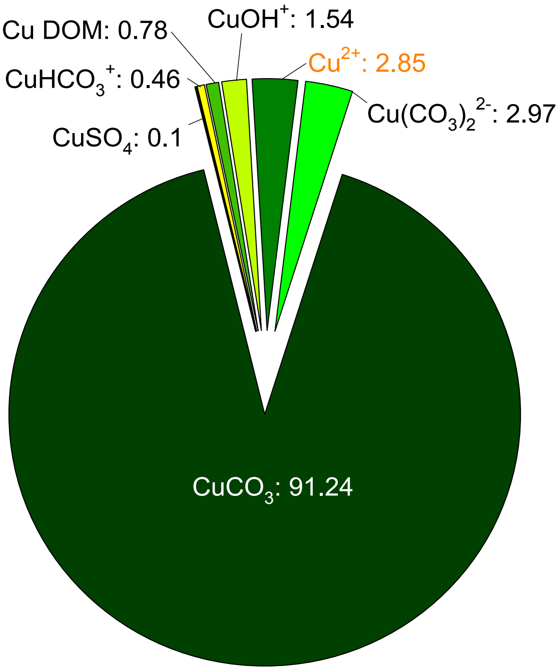


Figure 15 – Speciation of copper in filter influent water at Nærum water works, assuming a total copper concentration of 1 µg Cu/L. Numbers are fractions in [%] of total.

Model predicted fraction of free copper was 2.85 % Cu^{2+}, and most copper (>90 %) was complex-bound as copper(II)carbonate (Fig. 15). Alkalinity (as HCO_3^-) was 348 mg HCO_3^-/L and pH was 7.7. For comparison, Zhang and Edwards (2010) predicted a fraction of free Cu^{2+} of 2 %, for water with a pH of 7, an alkalinity of 500 mg $CaCO_3$/L, and a total copper concentration of 5 µg Cu/L. Complexation at high bicarbonate concentrations may be of special relevance when groundwater from limestone aquifers is used for drinking water production. In river and lake water, copper readily forms complexes with organic ligands, where >99 % of copper can be bound (Xue et al., 1996).

- ***Low absolute copper concentrations***

Adsorption of copper in soil, and generally low content of copper in the aquifer material, are factors contributing to low total concentrations (Adriano, 1986).

In the present study, total copper concentrations in raw water (chapter 3) or filter influent water to investigated filters (chapter 5) were below detection limits of 0.01-0.04 µg Cu/L, stressing that low absolute concentrations are indeed a relevant factor. In surface water, copper can be bound in bottom sediments. Sanchez and Lee (1973) reported the binding capacity to be related to the alkalinity of the sediments, and only a minor contribution of sulfides and organics to the binding.

- ***(Ad)sorption of copper to iron oxides or other inorganic deposits***

Total copper concentrations can be decreased by sorption of copper to inorganic deposits in biofilters or on filter media, e.g. iron oxides (Benjamin et al., 1996) or manganese oxides (Han et al., 2006). Copper sorption can occur with permanent deposits (mineral coating on filter media (Gülay et al., 2014)) and non-permanent, loose deposits of e.g. particles and iron oxide-hydroxide, which are being removed through filter backwashing.

At Nærum water works, where only single-stage filtration is used, the nitrifying filters also receive considerable iron loads (Table 5). The vast majority of iron is removed within the top 10 cm of the filter (Fig. 16, left), and accumulated iron is removed periodically through backwashing of the filter. Copper concentrations over filter depth also show a marked decrease within the top layer (Fig. 16, left). 22 days after dosing onset at Nærum, filter media samples were collected from 0-40 cm filter depth, before and after backwash. Total copper content on filter media was determined by ICP-MS analysis, subsequent to microwave assisted acid digestion, according to US EPA method 3051A (USEPA, 2007b). During copper dosing, a fraction of added copper accumulated together with iron oxide-hydroxide precipitates in the top 10 cm of the filter, and was then removed through backwashing of the filter (Fig. 16, right).

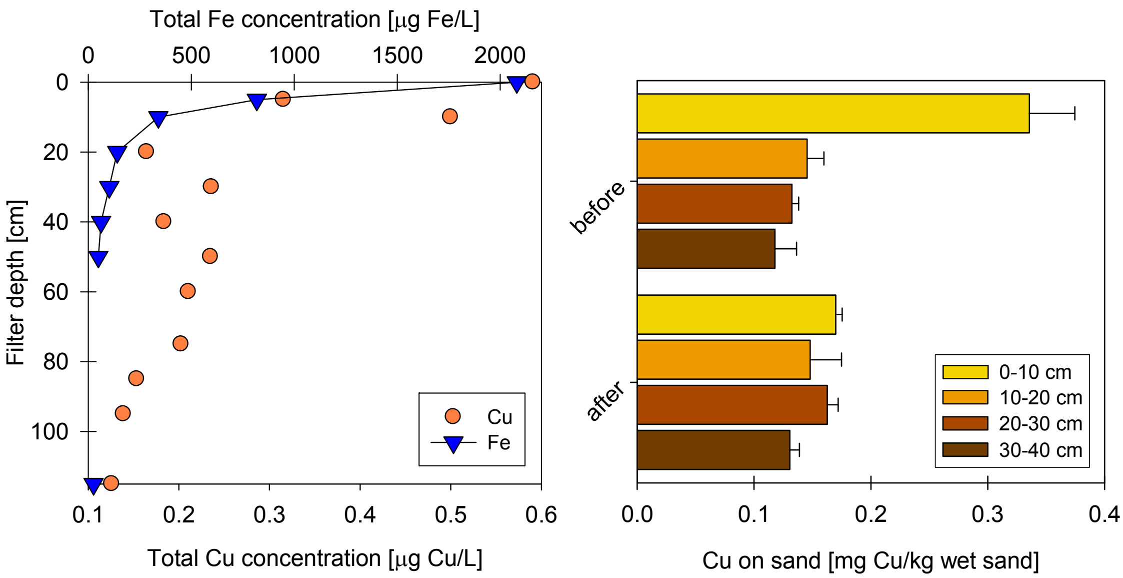


Figure 16 – Left: Total iron and copper concentrations over filter depth at Nærum water works, 23 days after dosing onset. Right: Copper concentrations on sand from 0-40 cm filter depth at Nærum water works, 22 days after onset of dosing, before and after filter backwash.

- ***Copper desorption from filter media***

Besides filter media sampling at Nærum water works 22 days after dosing started, additional samples were taken after 116 days with dosing (last day of dosing) and 115 days after the dosing was stopped. Analysis of copper content on sand showed that copper accumulated on sand during the dosing phase, with approx. twice as much copper on filter media when comparing the last day of dosing with the day before dosing started (Wagner et al., **I**). With the change in equilibrium when dosing was stopped, copper content decreased again, however at a slower rate than during accumulation (Wagner et al., **I**). Hence a copper storage was created, which can make copper available to the bulk phase over longer periods of time, even after dosing is stopped.

- ***Biofilm diffusion limitations***

Microorganisms are organized within a biofilm around the sand or granular media in a biofilter (Gülay et al., 2014; Rittmann and McCarty, 1980). The transport of solutes/nutrients like ammonium or oxygen to the cells is governed predominantly by diffusion through the biofilm (Stewart, 2003), and influenced by factors such as bulk liquid solute concentration, mass-transfer at the biofilm-liquid border, and biofilm porosity and reaction rate (Nicolella et al., 2000). When cells are aggregated as biofilm, the diffusion distance for solutes increases, which can lead to diffusion limitations and gradients in nutrient concentrations (Stewart, 2003). Diffusion limitations can occur in principle for any solute, and have also been reported for copper. For example, accumulation of copper under presence of sulfate reducing bacteria can occur, where copper is bound as copper sulfide (White and Gadd, 2000). Furthermore, retardation of copper in an *E.coli* biofilm was reported (Hu et al., 2007) and linked to the content of extracellular polymeric substances (EPS), which microorganisms in biofilms produce (Costerton et al., 1995; Gülay et al., 2014).

- ***Copper cycling within the biofilm***

Copper can be (re-)cycled within the biofilm (Simpson, 2008). Cell decay and subsequent hydrolysis can make cell components (and therefore also copper) available for growth of new biomass (Boe-Hansen, 2001; van Loosdrecht and Henze, 1999).

- ***Competition for copper by other microorganisms***

Besides ammonium oxidizing microorganisms, which need copper as cofactor for the enzyme ammonia monooxygenase and other proteins, copper is also essential to other microbial groups. Numerous types of copper containing proteins are known to date, among which is cytochrome *c* oxidase (Magnani and Solioz, 2007), essential as terminal electron acceptor of the respiratory chain (Ostermeier et al., 1996) and present in most aerobic microorganisms. Copper is also vital for rusticyanin, a copper-protein regulating electron transfer in the iron oxidizer *Thiobacillus ferrooxidans* (Cobley and Haddock, 1975), and for particulate methane monooxygenase (pMMO) of methanotrophic bacteria (Hakemian and Rosenzweig, 2007). Methane oxidizers, due to their high demand of copper for this essential enzyme, have effective mechanisms for copper acquisition (Magnani and Solioz, 2007): methanobactin, a molecule similar to siderophores for iron acquisition in other bacteria, can transport high amounts of copper and at the same time avoid toxic effects (Kim et al., 2004). Like iron oxidizing bacteria, methane oxidizers are present in rapid sand filters treating groundwater (Gülay et al., 2016; Palomo et al., 2016). Ammonium oxidizing microorganisms are thus not the only microbial group with high copper requirements, present in these environments.

In summary, aforementioned aspects affect copper availability in biofilters at all scales, and it may be difficult to account for all factors accurately, especially when dealing with complex full-scale systems. From investigations at Nærum drinking water treatment plant, it is difficult to determine a precise Cu^{2+}/NH_4^+-N demand. For this, batch systems (Amin et al., 2013) may be more suitable, which however are a simplification of the real world. Nevertheless, from speciation calculations alone, we know that the predicted free Cu^{2+} concentration was 2.85 % of the total concentration; hence, two orders of magnitude lower than total dosing concentrations (which means approx. 0.03 µg Cu^{2+}/L). The true, required concentration of free Cu^{2+} to completely nitrify the incoming ALR was however likely lower, due to the interplay of further factors which decrease availability (especially adsorption of copper to iron oxide-hydroxides (Fig. 16), which contributed substantially).

With factors such as iron and bicarbonate concentrations varying from plant to plant (Table 5), copper dosing concentrations should be adjusted. However, low concentrations of ≤1.5 µg Cu/L were sufficient at the investigated water works. Yet, being able to quantify the impact of said factors on availability can help to determine the dosing concentration necessary for removal of a set ammonium concentration/load. In the same manner that lower limits of dosing can be adjusted, factors affecting availability can also be evaluated to avoid overdosing, and thereby toxic effects of Cu^{2+}.

7 Effect of copper dosing on the nitrifying microbial community

Microbial communities in groundwater-fed biological rapid sand filters are complex, with different guilds utilizing a variety of electron donors (e.g. NH_4^+, Fe^{2+}, Mn^{2+}, etc.) as energy sources (Gülay et al., 2016). The influent water chemistry shapes the microbial community (Albers et al., 2015); as a result, dosing of a (trace) nutrient may affect its composition. Therefore, we aimed at investigating whether the observed stimulation of nitrification was merely a stimulation of cell specific activity, or if it involved a change within the microbial community and the nitrifying guild.

Filter material was sampled at Nærum and Holmehave treatment plants for microbial analyses of nitrifier densities by quantitative PCR (qPCR) and community composition by Illumina sequencing of 16S rRNA gene amplicons. Filter media samples were collected from the top 10 cm of a filter receiving dosing (‘test filter’) and from a reference filter without dosing at Holmehave. At Nærum, one sample was taken from a test filter. Details on microbial and bioinformatics analyses can be found in Wagner et al., **IV**.

7.1 Increase in relative abundance of betaproteobacterial AOB

Copper dosing stimulated nitrification activity at both drinking water treatment plants. The volumetric ammonium removal rate (ARR) for the top 10 cm increased from the initial 0.67 to 4.90 g NH_4^+-N/m^3/h within 67 days of dosing at Holmehave, and from 0.15 to 2.25 g NH_4^+-N/m^3/h within 116 days of dosing at Nærum.

Figure 17 shows the corresponding effect of 67 days of copper dosing on the 13 most abundant genera of the test filter at Holmehave treatment plant. Genus *Nitrospira* was the most abundant, which is in agreement with observations from similar systems, reported elsewhere (Gülay et al., 2016; Tatari et al., n.d.). With dosing, relative abundance of *Nitrospira* increased from 25.9 to 33 %, which was however not significant ($\alpha = 0.05$). Neither of the genera showed significant changes due to copper dosing, except for genus *Nitrosomonas* (betaproteobacterial AOB), which exhibited a highly significant increase (p-value of 5.5E-34) from 1.1 to 8.5 % (Fig. 17). Conversely, for the reference filter without dosing, relative abundance of *Nitrosomonas* was unchanged (1.1 % before, and 1.2 % after 67 days of dosing to testfilter). For the test filter at Nærum, both *Nitrospira* and *Nitrosomonas* increased significantly, from 13.1 to 20.4 % and from 0.5 to 2.7 %, within 116 days of dosing (Wagner et al., **IV**).

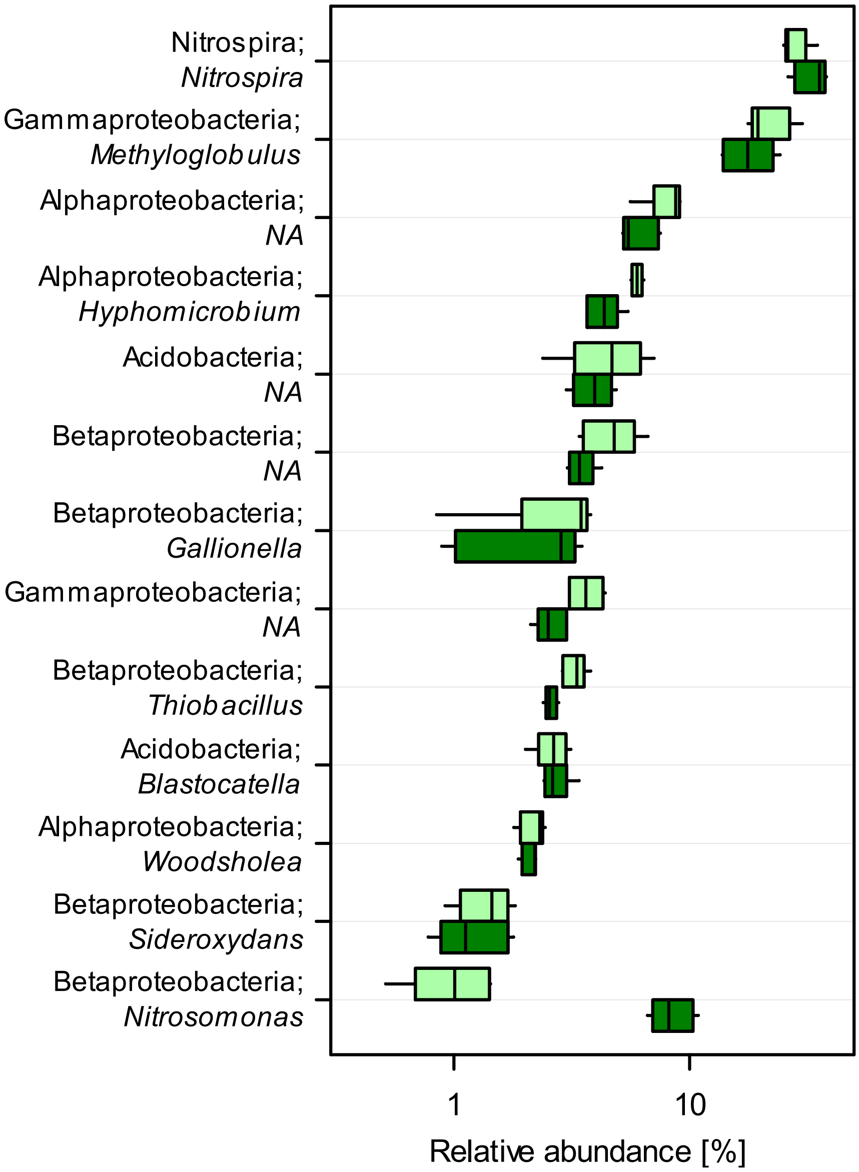


Figure 17 – Effect of copper dosing on relative abundance of the 13 most abundant genera in the top 10 cm of the filter with dosing at Holmehave treatment plant. The taxonomic distribution is based on 16S rRNA gene sequencing, and sequences are grouped according to their assigned genus (labelled as "class; *genus*"; *NA* indicates unidentified genera). Light green 🟩 : before copper dosing; dark green 🟩 : 67 days after copper dosing started. Box plot computed from 5 biological replicates shows 10th and 25th percentiles, median, and 75th and 90th percentiles (adapted from Wagner et al., **IV**).

Even though in absolute numbers *Nitrospira* were higher and initially over 20 times more abundant than *Nitrosomonas*, the relative change of genus *Nitrosomonas* was much higher. Remarkably, its relative abundance in test filters at Holmehave and Nærum increased by factors 7.7 and 5.4, respectively.

7.2 Effect on comammox *Nitrospira* vs. canonical AOP

To assess the relative contribution of comammox *Nitrospira,* betaproteobacterial AOB, and AOA within AOP before and after copper dosing, we added estimates of their abundances and calculated their fractions (Fig. 18).

Quantitative PCR was carried out targeting AOB (16S rRNA gene), AOA (*amoA* gene), and comammox *Nitrospira* (*amoA* gene). Furthermore, phylogenetic analyses of 16S rRNA amplicon sequences suggested that, within genus *Nitrospira* at both plants, some sequence variants were attributed to canonical NOB, and some were closely related to known sequences of comammox *Nitrospira* (Wagner et al., **IV**).

Absolute AOA densities quantified by qPCR ranged from 1.1-7.4 x 10^{10} cells/m^3 filter material (Wagner et al., **IV**), which is approx. 2 to 4 orders of magnitude lower than previously reported for rapid sand filters treating groundwater (Gülay et al., 2014) or GAC filters treating river water (Kasuga et al., 2010). In relative terms, this amounted to only 0.006-0.009 % of total AOP (hence not shown in Fig. 18). Cell specific ammonia oxidation rates of AOA which are generally 1 to 2 orders of magnitude lower than for AOB (Prosser and Nicol, 2012) imply a minor importance of AOA, relative to the total ammonium removal activity in the investigated filters. Their low abundance in our copper limited systems may be explained by a suggested higher requirement for copper, compared to AOB (Amin et al., 2013; Walker et al., 2010). Based on qPCR estimates, the relative abundance of comammox *Nitrospira* at Holmehave decreased from 54 % before dosing to 49 % after dosing start, and from 20 % to only 2 % at Nærum (Fig. 18, left). Based on amplicon sequencing estimates, the fraction of putative comammox *Nitrospira* was significantly larger at both plants (Fig. 18, right). AOA were not detected with amplicon sequencing.

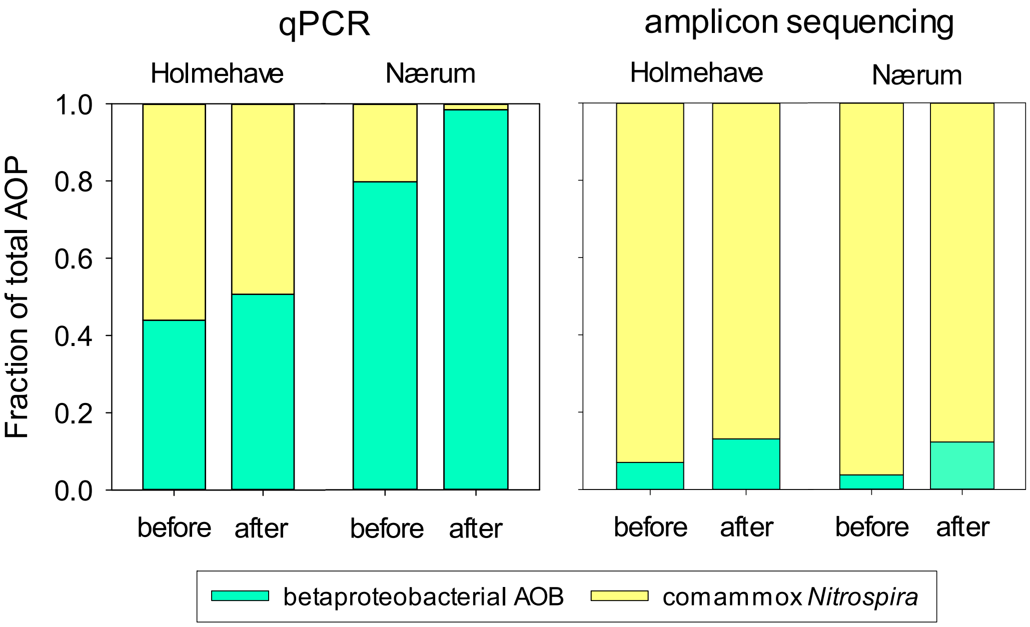


Figure 18 – Effect of copper dosing on contribution of betaproteobacterial AOB and comammox *Nitrospira* to total AOP. Left: based on qPCR. Right: based on 16S rRNA gene amplicon sequencing Wagner et al., **IV**).

The discrepancy between the results of the two different methods may have been caused by an overestimation of AOB abundance by the qPCR targetting the 16S rRNA gene (Dechesne et al., 2016). Furthermore, comammox *Nitrospira* are not a monophyletic group, but are instead interspersed with nitrite-oxidizing *Nitrospira* (Pjevac et al., 2016). Therefore uncertainties are associated with using the 16S rRNA gene as a marker to distinguish comammox and non-comammox (strict NOB) *Nitrospira*. Nevertheless, the trend of a decreasing fraction of comammox *Nitrospira* with copper dosing was consistent (Fig. 18). Our results therefore indicate that, at both DWTPs, copper dosing shifted the AOP ratio away from comammox, towards canonical ammonia oxidizers. Based on genome characteristics, Palomo et al. (2017) proposed that comammox *Nitrospira* could have an advantage in environments with low copper availability. A higher specific growth rate of betaproteobacterial AOB (Costa et al., 2006) may thus explain the proliferation of *Nitrosomonas* when the copper limitation was removed as a result of the dosing.

7.3 Cell specific ammonium oxidation rates of betaproteobacterial AOB

The order of magnitude of cell specific rates of canonical AOB prior to copper dosing was estimated by normalizing the depth specific volumetric ARR before dosing with the absolute (qPCR based) AOB densities (Wagner et al., **IV**). Cell specific ammonium removal rates for betaproteobacterial AOB prior to dosing were 0.03-0.08 x 10^3 fg N/cell/day, which is lower than what is commonly reported in literature for similar systems (Table 8), and which may indicate that a large number of AOB were not actively oxidizing ammonium. However, cell specific rates in the same order of magnitude were reported for a full-scale trickling filter treating groundwater (de Vet et al., 2011). The authors suggested that the low rates point towards most AOB cells to be dead or having severely inhibited activity, the latter being a possible result of nutrient limitations (de Vet et al., 2011). Indeed, it is known that during starvation or nutrient limitation, bacteria can enter a state of dormancy, where they are neither dead nor active (Roszak and Colwell, 1987; Stevenson, 1978; van Loosdrecht and Henze, 1999). This would be in agreement with low cell specific activities in our copper limited filters.

Table 8 – Cell specific ammonium removal rates for AOB in this investigation and in literature.

Authors	**System**	**AOB cell spec. activity [x 10^3 (fg NH_4^+-N/cell/day)]**	**Notes**
This study (Wagner et al., IV)	Full-scale rapid sand filters at two different water works	Before Cu: 0.03-0.08 With Cu: 0.15-0.79	-
Tatari et al. (2016)	Lab-scale column setup with sand from top layer of a well-functioning full-scale sand filter treating groundwater	1.5-14	Different cell specific activities at different ammonium loading rates and filter depths
Lee et al. (2014)	Pilot-scale RSF column treating groundwater	0.96-1.68	-
de Vet et al. (2011), with ref. to Prosser (1989)	Continuous & batch experiments w/ pure or mixed cultures	0.24-7.2	-
de Vet et al. (2011)	Trickling filters treating sub-surface and non-sub-surface aerated groundwater	0.05-12	Specific nitrification rate determined in batch; low cell specific rates for non-sub-surface aerated filter

With copper dosing to the filters at both treatment plants, the relative abundance of betaproteobacterial AOB showed the largest relative change (Fig. 17 and Wagner et al., **IV**). We hypothesized that most of the new removal capacity was due to the increase in betaproteobacterial AOB, and estimated cell specific rates by normalizing the copper-induced change in ARR by the absolute change in betaproteobacterial AOB abundance (Wagner et al., **IV**). AOB cell specific activity was now between 0.15-0.79 x 10^3 fg N/cell/day. These values fit in the range reported in literature (Table 8), which suggests that it is plausible that the canonical AOB were the main active ammonium oxidizers associated with the removal activity increase induced by the dosing. A limitation of comparing cell specific rates to literature values lies however in uncertainties associated with the cell quantification methods. For example, AOB cell numbers quantified with two different qPCR primer sets (targeting *amoA* gene; Rotthauwe et al. (1997), and 16 S rRNA gene Hermansson and Lindgren (2001)) may differ by up to 3 orders of magnitude (Wagner et al., **IV**; Dechesne et al. (2016) reported up to 2 orders of magnitude difference).

Overall, our results showed that the copper-induced stimulation of nitrification involved a change within the nitrifying microbial community. No other filter function than nitrification (such as iron or manganese removal) was affected, and no other microbial guild changed their relative abundance markedly (Wagner et al., **IV**).

Theoretically, growing nitrifier populations can produce considerable amounts of soluble microbial products (Ni et al., 2011; Rittmann et al., 1994), which can serve as organic substrate and support growth of heterotrophic microorganisms (Furumai and Rittmann, 1992). This can result in increased heterotrophic plate counts (HPC) in the filter effluent, and more significantly the potential growth of undesired microorganisms in the filter or the distributed water (Bendinger, 2008). From a practical point of view, the water chemistry and in connection with it growth conditions and possible impacts on other groups in the microbial community should be considered when nutrients are supplemented for stimulation of nitrification.

8 Conclusions

Copper is of vital importance for efficient nitrification in biological rapid sand filters for drinking water production. In conjunction, nitrification in biofilters can be limited by lack of sufficient concentrations of the metal, but insufficient nitrification was shown to be remediated through copper dosing. Specifically, the following can be concluded:

- Absolute copper concentrations in filters with incomplete and inefficient nitrification were low at <0.01-0.04 µg total Cu/L. Nitrification could be stimulated within only a few days of controlled dosing of ≤1.5 µg total Cu/L. At ten investigated drinking water treatment plants with year-long histories of poor ammonium removal performance, copper dosing induced an average 87 % increase in volumetric ammonium removal rates. It took on average only 19 days to achieve complete ammonium removal to effluent concentrations as low as <0.01 mg NH_4^+-N/L.
- Dosing also affected nitrite removal positively. Prior to dosing, nitrite effluent concentrations at three of the ten water works violated the national standard of 0.003 mg NO_2^--N/L, but with dosing concentrations decreased to <0.002 mg NO_2^--N/L.
- Copper dosing can have a long-term positive effect: A persisting stimulation of nitrification in one investigated filter was observed for at least 238 days after the dosing was stopped.
- A novel dosing method and apparatus were developed (patents pending), which employs active release of copper from solid copper electrodes. Compared to passive dosing from solid copper, or active dosing from a copper solution, the new method has the benefits of controlled release of low amounts of copper, dosing proportional to the treatment flow, and that no chemicals and comparably little maintenance are required.
- The copper-induced stimulation of nitrification was independent of different site specific parameters at the treatment plants, such as filter design and operation, ammonium loading rates, water chemistry, or configuration of treatment units. However, it took longer to achieve complete ammonium removal for filters without primary filtration (on average 23 ± 4 days), compared to filters receiving prefiltered water (15 ± 3 days), likely due to higher iron loads to filters without primary filtration.

- Dosing moved ammonium removal upwards in the filters and induced stratified removal. Removal rates for the top 10 cm of a filter increased almost 14-fold, thereby increasing the filters overall ammonium removal capacity.
- Filters acquired extra ammonium removal capacity through copper dosing. Under short-term ammonium loading rate upshifts with loadings twice as high as the normal loading rates, filters successfully removed ammonium, with a 2.6 times increase in removal capacity of a filter. The extra capacity can increase the filters' robustness towards loading rate upshifts during dynamic filter operation.
- A complex interplay of factors can decrease bioavailable free copper Cu^{2+} concentrations in biofilters. These factors include copper complexation and precipitation in the bulk water phase, low absolute copper concentrations, adsorption/desorption of copper to/from iron oxides or deposits in the filter, biofilm diffusion limitations and (re)cycling of copper, and competition for copper by other microorganisms. Filter material analyses and speciation calculations showed that considerable amounts of added copper were bound to iron oxide-hydroxides, and that most copper complexed with carbonate (91 % of total Cu was present as $CuCO_3$), leaving as little as <3 % in the available Cu^{2+} form.
- In practice, dosing should be adjusted when low copper availability is predicted. For example, when iron or bicarbonate concentrations are high, active electrolysis dosing is preferable over passive dosing, as it can provide higher copper concentrations continuously.
- Dosing did not only affect nitrification activity, but also nitrifier densities. The highest comparative increase in relative abundance was that of genus *Nitrosomonas* (betaproteobacterial AOB), which increased from 0.5 to 2.7 % and from 1.1 to 8.5 %, at two water works. Comammox *Nitrospira* were also present in the filters, but their fraction of total AOP decreased, while the fraction of *Nitrosomonas* increased.
- Besides nitrifiers, no other microbial guild was substantially affected by the dosing. While nitrification was the only stimulated filter function, no adverse effects on other functions of the biofilter (such as iron or manganese removal) were observed.

The obtained results add to the scientific understanding of nitrification in biological filters for drinking water production and will influence future filter designs and also operation of biofilters currently running. The developed dosing method is already now successfully applied at a number of Danish drinking water treatment plants, where increased nitrification in the filters enhances biological stability and therefore the quality of the finished drinking water.

9 Perspectives

A main finding of this project was that nitrification in groundwater-fed rapid sand filters for drinking water production can be limited by lack of sufficient copper concentrations, and that dosing of copper can stimulate the process. Compared to numerous, partly experience-based, trial and error remediation attempts prior to dosing (including time-consuming and costly replacement of the filter material), dosing was a very simple measure to increase nitrification efficiency in biofilters, which suffered from insufficient filter performance for several years. The research has therefore highly practical implications and a direct impact on drinking water quality.

Here, groundwater based systems were studied. Consequently, one of the most interesting questions originating from this research is: Are the findings relevant for other systems, such as surface water treatment plants, when nitrification is incomplete? In theory, copper availability can also be low in surface water. However other factors may be at play, like generally higher loads of biodegradable organic matter, which may lead to competition of microorganisms for space in the filters, and may cause insufficient nitrification (Rittmann et al., 2002). Hence, further research is needed, which could start with estimating the amount of available copper, when nitrification problems occur in surface water treatment.

Even though copper dosing concentrations were 1-2 orders of magnitude higher than total copper concentrations in the influent water prior to dosing, the concentrations of approx. 1 µg/L and below were still very low. Yet, the exact ratio of Cu^{2+}/NH_4^+-N needed is still unknown. Further research could be concerned with determining this ratio experimentally. This in turn could be used to extend existing nitrification models (Queinnec et al., 2006; Rittmann et al., 2002) for nutrient limitations by lack of copper, in the way that van der Aa et al. (2002) have included limitations by lack of phosphorus. A developed model would however need to include parameters that can incorporate the complex factors affecting copper bioavailability.

Knowing the precise amount of copper needed for nitrification may also enable dosing concentrations to be decreased even further. In one filter, stimulation of nitrification persisted for at least 238 days after the dosing was stopped, probably linked to a copper storage which accumulated on the sand during dosing. This opens up the question if shorter dosing intervals could be used, or if a pulse dosing of higher concentrations may be suitable. At higher concentration dosing however, possible toxicity effects need to be evaluated prior to dosing.

Ammonium loading rate upshift experiments showed that copper dosing also increased the maximum ammonium removal rates (=capacity). This indicates the possibility of increasing the hydraulic loading (treatment flow) to a filter, or to treat the same amount of water with less filter area, thereby decreasing the geographical footprint. Experiments with long-term loading rate upshifts and copper dosing could investigate whether removal capacities can be increased even further, and in what range a maximum capacity may be situated. Ultimately, it should be possible to increase the capacity further, until another factor becomes limiting (such as mass transfer, nutrients like phosphorus, or space). Such up-shift experiments would however need to be conducted in laboratory or pilot scale, as ammonium loading rates cannot be increased excessively in full scale, without jeopardizing the consumers' water quality.

Dosing induced stratification of nitrification in the filters, which means that both ammonium and nitrite were removed further up in the filters and that less contact time was necessary for complete removal. These results point towards the option of using less filter material, which in turn could lead to (energy) savings when backwashing the filters (due to smaller backwash pumps). However, decreasing the filter bed depth may take away filter length that can serve as a buffer for dynamic loading conditions. Furthermore, this should not be at the expense of other removal processes such as manganese removal, which is often taking place in filter regions after ammonium is completely removed (Tekerlekopoulou and Vayenas, 2008; Vandenabeele et al., 1995), or removal of particles.

Lastly, Costa et al. (2006) postulated that a microorganism capable of complete ammonia oxidation might have a high growth yield, rather than a high specific growth rate. If copper dosing creates conditions that favour faster growing AOB rather than comammox *Nitrospira* (as indicated in this study), then its application may be of interest for start-up of new filters. When starting up new biofilters, it is desirable that biological removal processes are completely functioning as fast as possible. Indications for copper-induced stimulation of nitrification during start-up were already found at Skive water works (Table 5) and at 'New Esbjerg Water works' (unpublished data). Therefore, copper dosing to decrease the start-up time of new nitrifying biofilters should be further investigated.

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11 Papers

I **Wagner, F.B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J., 2016. Copper deficiency can limit nitrification in biological rapid sand filters for drinking water production. *Water Research,* 95:280-288.

II Albrechtsen, H-J., **Wagner F.B.**, Borch Nielsen, P., Boe-Hansen, R., Fischer, E.V., 2015. Apparatus comprising trace element dosage and method for treating raw water in a biofilter. *Patent application published under patent cooperation treaty,* WO2015/132283 A1.

III **Wagner, F.B.**, Borch Nielsen, P., Boe-Hansen, R., Albrechtsen, H-J. Remediation of incomplete nitrification and capacity increase of biofilters at different drinking water treatment plants through copper dosing. *Submitted.*

IV **Wagner, F.B.**, Diwan, V., Dechesne, A., Fowler, S. J., Smets, B.F., Albrechtsen, H-J. Copper-induced stimulation of nitrification in biological rapid sand filters for drinking water production by proliferation of *Nitrosomonas* spp. *Manuscript.*

In this online version of the thesis, **papers I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from:

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