Processes effecting nitrification performance in biological rapid sand filters

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Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Lee, C. O., Binning, P. J., Albrechtsen, H-J., Smets, B. F., & Boe-Hansen, R. (2014). Processes effecting nitrification performance in biological rapid sand filters. Kgs. Lyngby: DTU Environment.

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PhD Thesis
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Printed by: Vester Kopi
October 2014

Cover: Torben Dolin

Preface

This thesis presents the research of a PhD project carried out at DTU Environment, Technical University of Denmark (DTU). The project was funded by the Danish Council for Strategic Research via the DW-Biofilters project and DTU. The Supervisors were Philip J. Binning, Hans-Jørgen Albrechtsen, and Barth F. Smets from DTU Environment, and Rasmus Boe-Hansen from Krüger A/S.

The thesis is based on a synopsis of the work presented in three scientific papers. The papers in the thesis are cited by the author name and roman numerals corresponding to the papers listed below (e.g. Lee et al., I). The included papers are:

I Lee, C. O., Boe-Hansen, R., Musovic, S., Smets, B., Albrechtsen, H.-J., Binning, P., 2014. Effects of dynamic operating conditions on nitrification in biological rapid sand filters for drinking water treatment. Water Research. 64C, 226–236.

II Lee, C. O., Smets, B., Albrechtsen, H.-J., Binning, P. Increasing nitrification in biological rapid sand filters used for drinking water: The positive effects of phosphorous addition. Submitted to Water Research.

III Lee, C. O., Smets, B., Albrechtsen, H.-J., Binning, P. Impact of backwashing on nitrification at different loading conditions in biological rapid sand filters. Submitted to Water Research.

In this online version of the thesis, the papers 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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This PhD work also contributed to the following manuscript in preparation and conference proceedings, including six at international conferences:

Wagner F. B., Lee C. O., Tatari K., Nielsen P. B., Albrechtsen H.-J. Nutrient limitations in drinking water rapid sandfilters with incomplete ammonium removal. In preparation for Water Research.

Lee, C. O., Albrechtsen, H.-J., Smets, B., Binning, P., 2012. Modelling the performance of biological rapid sand filters used to remove ammonium, iron, and manganese from drinking water. American Water Works Association Annual Convention and Exposition (AWWA-ACE), Dallas Texas, USA. Conference proceedings.

Lee, C. O., Albrechtsen, H.-J., Smets, B., Boe-Hansen, R., Lind, S., Binning, P., 2012. Relating dynamic conditions to the performance of biological rapid sand filters used to remove ammonium, iron, and manganese from drinking water. American Water Works Association (AWWA) Water quality and technology conference (WQTC), Toronto Canada. Conference proceedings.

Lee, C. O., Albrechtsen, H.-J., Smets, B., Boe-Hansen, R., Lind, S., Binning, P., 2013. Relating dynamic conditions to the performance of biological rapid sand filters used to remove ammonium, iron, and manganese from drinking water. Danish Water Research and Innovation Platform (DWIRP). Abstract in proceedings.

Lee, C. O., Albrechtsen, H.-J., Smets, B., Boe-Hansen, R., Lind, S., Binning, P., 2013. Phosphate limitation in biological rapid sand filters used to remove ammonium from drinking water. American Water Works Association (AWWA) Water quality and technology conference (WQTC), Long Beach, California, USA. Conference proceedings.

Wagner, F.B.; Lee, C.O.; Tatari, K.; Nielsen, P.B.; Albrechtsen, H.-J. (2014) Phosphorus Limitations in Rapid Sand Filters Studied with Different Packed Column Assays. World Water Congress, International Water Association (IWA), September 21-26, 2014, Lisbon, Portugal (accepted for poster presentation)

Wagner, F.B.; Nielsen, P.B.; 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. Water Quality Technology Conference, American Water Works Association (AWWA), November 16 – 20, 2014, New Orleans, Louisiana, USA (accepted for oral presentation)

Wagner, F.B.; Nielsen, P.B.; 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. Water Quality Technology Conference, American Water Works Association (AWWA), November 16 – 20, 2014, New Orleans, Louisiana, USA (accepted for poster presentation)

Acknowledgements

Firstly, I would like to thank my main supervisor, Philip Binning, and my other co-supervisors at DTU for their help and support during my PhD. Their professional guidance and years of experience greatly contributed to all aspects of my PhD. I would also like to thank my co-supervisor, Rasmus Boe-Hansen, for his expertise and creativeness in the experiments. Thank you to Søren Lind and George Amoako at HOFOR, for allowing us access to Islevbro water works, and for their dedication to giving us whatever was needed to make this study successful. Thank you to Krüger for the use of their pilot plant and other equipment and to Peter Nielsen for his always rapid and valuable responses to any crisis I had with the pilot plant. A special thanks to my master's students Robbe Deleu and Florian Wagner for their hard work and dedication, and to the lab expertise of Bent Skov, Mona Refstrup, Sabrina Nedell, and Sihn Nguyen for their help on the project.

A big thank you to my DW-Biofilters family including Charlotte Corfitzen, Katie Lin, and Sanin Musovic. A special thanks also to Karolina Tatari for all the great science and good times! This is also extended to all of the wonderful office mates I have had over the years, including Mathilde, Sune, Olga, Yuhoon, Laure, and especially Sarah, who has also taken it upon herself to teach me such wonderful Danish! DTU has been a wonderful place to work over the last few years because of the fantastic people here. A big thank you to Elham, Yoshi, Fabio, Luca, Claire, both Julies, Bentje, Ana, Hugo, Anas, Borja, Alex, Carlos, Kos, Mike, Jon, Katerina, Valintina, Pau, Pauly, Helena, Marta, Pedram, Pernille, Ramona, Rene, Clause, Manfred, and all of the other people who made my PhD bearable!

A special thanks to all my wonderful Turks, Gizem, Arda, Suzi, and Gamze, for my Turkish training, and to my class coach, Arnaud, for trying his best to class me up! Also, thank you to Flo and Uli for being so German!

I am also grateful to my family and friends elsewhere, especially my parents for their life long support. I would also like to thank Kerry Howe for all of his patience and support, and for the excellent training in all matters of science and research, without which I would not be here today.

Most importantly, I would like to thank my wife, Danielle, for putting up with me and my PhD, and all of her wonderful support through good times and bad.

Summary

Biological rapid sand filters are commonly used in some parts of the world to remove ammonium from drinking water via nitrification. Currently the design and operation of these filters is based on rules of thumb and operator experience, and solutions to nitrification problems are often a trial and error process. This PhD thesis aimed to obtain more insight into the effects of operating conditions on nitrification in these filters. A scientifically based understanding of nitrification and the responsible nitrifying organisms is needed to design, operate, and troubleshoot these filters to ensure safe and reliable drinking water supplies.

One complicating factor in these filters is that they are very dynamic in nature. Operating conditions such as hydraulic flow rate and water quality can change rapidly in these filters, leading to excessive effluent concentrations of ammonium and nitrite. Two pilot scale filters were used to investigate the effects of various operating conditions, substrate loadings, and nutrient limitations on nitrification. Depth profiles showed that the filters could mimic the full scale filters in terms of biological and particle removal, which gives confidence that the results obtained would be relevant to the full scale filters.

Under the normal operating conditions, ammonium removal was determined to be a function of total ammonium load and not hydraulic loading or inlet ammonium concentration individually, and the density of AOB was determined to set the ammonium removal rate. The filters were also found to have additional ammonium removal capacity that was five times larger than the average ammonium loading rates.

Other operating parameters such as filter run length and backwashing can influence both the amount of nitrifying biomass and nitrification performance in these filters. Backwashing had little effect on both ammonium removal and AOB under normal operating conditions and the ammonium removal capacity did not change with time after backwash, despite long filter runs (23 days).

Ammonium loads to the pilot filters were increased by approximately an order of magnitude to examine the effects of backwashing and nutrient limitations at increased ammonium loads. Although ammonium removal and nitrifying biomass increased over a 23 day filter run, most of this increase was transient, as backwashing removed much of the newly developed ammonium removal capacity and biomass. However, subsequent backwashes were found

to only have minimal impacts on nitrification in the filters, suggesting that little time is needed for the nitrifying biomass to become firmly established on the filter media.

Because nitrification is a biological process, sufficient substrate and nutrients, including phosphorous and other micronutrients, are needed to ensure proper activity and growth. During increased ammonium loads, nitrification and nitrifying biomass increased despite being operated under low phosphorous conditions. Phosphorous addition rapidly increased ammonium removal in the filters, especially at the top of the filters, where the highest densities of AOB were.

Flow through lab columns, capable of reproducing loading conditions seen in the full scale filters, were used to determine if phosphorous or other micronutrients could be causing poor nitrification in two other Danish water works. Using the columns, phosphorous was found to be the limiting in one of the water works, while micronutrients were found to be the cause of poor nitrification at the other water works. These findings were later verified with a pilot and a full scale study.

Depth profiles quantifying AOB, AOA, NOB (*Nitrospira* and *Nitrobacter*), and Eubacteria were used to examine the changes of these microorganisms under various operating, and loading conditions. The NOB, Nitrospira, was found to be 3 to 4 orders of magnitude more abundant than *Nitrobacter*. AOB and AOA were approximately equally abundant at the normally low inlet ammonium concentrations. Under increased ammonium loads, there was a shift in the relative abundance of AOB to AOA. After 45 days of increased ammonium loads, AOB were approximately an order of magnitude more abundant than AOA. The relative abundance of AOB and *Nitrospira* to Eubacteria also increased with increased ammonium loads, showing their importance in these filters.

The results in this thesis show that rapid sand filters are a robust method for ammonium treatment via nitrification, with an inherent capacity for handling short term increases in ammonium load. The work has shown that there are several opportunities to enhance and tune the performance of the filters, by managing nutrient addition and by examining how the depth distribution of microorganisms relates to nitrification performance under varying loading and operating conditions.

Dansk sammenfatning

Over hele verden anvendes biologisk aktive sandfiltre i drikkevandsbehandlingen til at fjerne ammonium ved hjælp af nitrifikation. Designet og driften af disse filtre er baseret på tommelfingerregler og operatørernes erfaringer, og problemer med nitrifikations-processen løses oftest ved at prøve sig frem.

Målet for denne PhD-afhandling var at undersøge, hvordan driftsforholdene påvirker nitrifikationen i hurtige sandfiltre. Det er nødvendigt at skabe en grundlæggende forståelse for nitrifikationen og de ansvarlige organismer for at kunne designe, drive og løse problemer med filtrene og dermed skabe grundlaget for en sikker og pålidelig drikkevandsforsyning.

Sandfiltre er meget dynamiske af natur, hvilket komplicerer forståelsen af dem. Driftsforholdene såsom hydraulisk flow rate og råvandskvaliteten kan ændre sig hurtigt, hvilket kan medfører for høje udløbskoncentrationer af ammonium og nitrit. To forsøgsfiltre (height: 70 cm; diameter: 30 cm) blev anvendt til at undersøge, hvordan nitrifikationen blev påvirket af forskellige driftsforhold, substratbelastninger og begrænsning af næringsstof. Dybdeprofiler af forsøgsfiltrene viste, at de imiterede den biologiske fjernelse og partikelfjernelsen, der blev observeret i fuld skala. Dette understregede, at de resultater der blev opnået i forsøgsfiltrene også ville være relevante for filtre i fuld skala.

Under normale driftsforhold var ammoniumfjernelsen afhængig af den totale ammoniumbelastning og ikke af den hydrauliske belastning eller indløbskoncentration af ammonium som individuelle parametre. Derudover blev det bestemt, at densiteten af ammonium oxiderende bakterier (AOB) fastlagde fjernelsesraten af ammonium. Filtrene havde kapacitet til at fjerne fem gange så meget ammonium, som de gennemsnitligt blev belastet med.

Andre driftsparametre så som hvor længe sandfiltrene er aktive mellem returskyllene og længden af selve returskyllet kan påvirke størrelsen af den nitrificerende biomasse og nitrifikationen. Returskyl havde kun en lille effekt på både ammoniumfjernelsen og AOB under normale driftsforhold og på trods af lange aktivitetsperioder mellem returskyllene (23 dage) var fjernelseskapacitet for ammonium uændret i tiden efter et returskyl.

Ammoniumbelastningen på forsøgsfiltrene blev øget med cirka en faktor ti for at undersøge effekten af returskyl og begrænsning af næringsstof under disse forhold. Selvom ammoniumfjernelsen og den nitrificerende biomasse steg over den 23 dage lange aktive filterperiode, var størstedelen af denne

stigning kortvarig, eftersom det meste af den ny skabte fjernelseskapacitet og biomasse blev fjernet i løbet af det efterfølgende returskyl. Målinger ved efterfølgende returskyl antydede dog, at den nitrificerende biomasse efter kort tid blev solidt etableret i filtersandet, og at returskyllene kun havde minimal påvirkning på nitrifikationen i filtrene.

Da nitrifikation er en biologisk proces er en tilstrækkelig tilførsel af substrat og næringsstoffer, inklusiv fosfor og andre mikronæringsstoffer, en nødvendighed for at sikre en passende aktivitet og vækst. Da ammoniumbelastningen blev forøget steg nitrifikationen og den nitrificerende biomasse på trods af, at der kun var lidt fosfor tilstede. Tilførsel af fosfor øgede hurtigt fjernelsen af ammonium i filtrene, især i den øverste del af filtrene hvor densiteten af AOB var størst.

Kolonner, der kunne simulere belastningsforholdene i fuld skala filtre, blev anvendt til at undersøge om fosfor og mikronæringsstoffer kunne forårsage en utilstrækkelig nitrifikation i sandfiltrene på to andre danske vandværker. Ved hjælp af kolonnerne blev det påvist at fosfor var den begrænsende faktor på det ene vandværk, imens mikronæringsstoffer begrænsede nitrifikationen i det andet vandværk. Disse konklusioner blev verificeret af undersøgelser i forsøgsfiltrene og i filtrene i fuld skala.

Dybdeprofiler af mængden af AOB, ammonium oxiderende arkæer (AOA), nitrit oxiderende bakterier (NOB) (*Nitrospira* og *Nitrobacter*) og Eubacteria blev anvendt til at undersøge ændringer af disse mikroorganismer under forskellige drifts- og belastningsforhold. Der var 10^3 til 10^4 gange så mange NOB *Nitrospira* som der var *Nitrobacter* i filtrene. Under normale, lave indløbskoncentrationer af ammonium var der cirka lige mange AOB og AOA filtrene, men efter 45 dage med forhøjet ammoniumbelastning var der cirka ti gange så mange AOB som AOA tilstede. Den relative mængde af AOB og *Nitrospira* i forhold til den totale mængde af Eubacteria steg også med forøget ammoniumbelastning, hvilket tydeliggjorde deres betydning i sandfiltrene.

Resultaterne i denne afhandling viser, at sandfiltre er en robust metode til at fjerne ammonium ved hjælp af nitrifikation, og at de har iboende kapacitet til at kunne fjerne en kortvarig forøget ammoniumbelastning. Der er adskillige muligheder for at forbedre og tilpasse ydelsen af filtrene ved at styre tilsætningen af næringsstoffer og ved at undersøge, hvordan fordelingen af mikroorganismer over dybden af filtret relaterer til nitrifikationen under forskellige belastnings- og driftsforhold.

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1 Introduction

1.1 Biological filters for drinking water

Biological filters are a commonly used drinking water treatment technology that combines biological processes, used to remove organic and some inorganics, with particle removal. The main difference between biological and non-biological filtration is that biological filtration has an active microbial community on the filter material. This is illustrated in Figure 1A, which shows a sand grain from a biological rapid sand filter. By using Syto 9 staining to show the microbial community (stained green), the biological aspect of the same sand grain becomes apparent, as illustrated in Figure 1B (images courtesy of Arda Gulay).

Both ground and surface source waters generally contain the necessary microbial community needed for biological filtration. In non-biological filters, microbial growth and activity is suppressed, usually through continuous chlorination. Without biological suppression, the filters would naturally revert to biological filters.

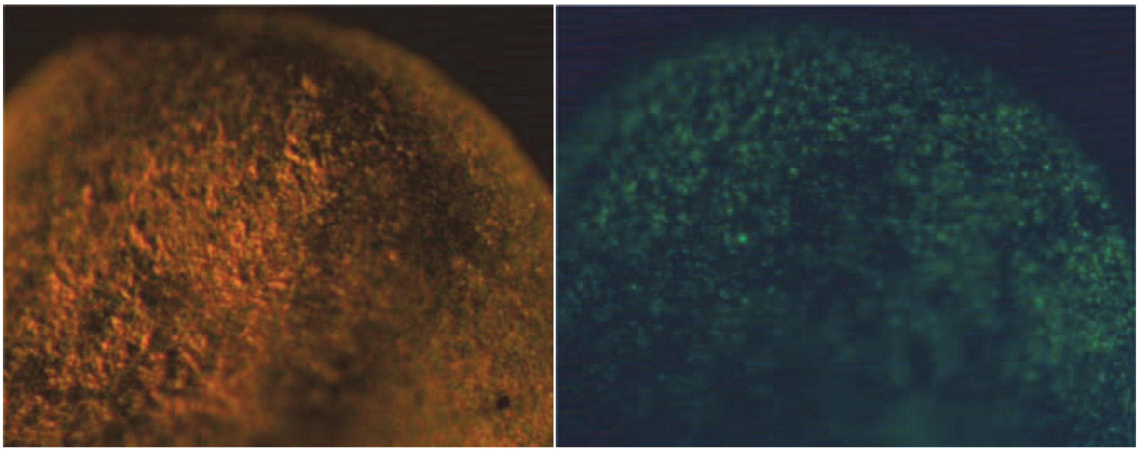

Figure 1. A) Stereo microscope image of a sand grain from a biological rapid sand filter. B) Syto 9 staining with Stereo microscope showing microorganisms on the same sand grain (green staining, images courtesy of Arda Gulay).

Biological filters used in drinking water are most often used to remove biodegradable organic matter (BOM) following ozonation (Crittenden et al., 2005). For groundwater, organics are generally not a concern. In anoxic ground water sources, reduced inorganics such as Fe^{2+}, Mn^{+2}, and NH_4^+, are often present above regulatory guidelines levels, and biological filters are commonly used to remove ammonium via nitrification. Nitrification is a two-step biological process involving aerobic autotrophs. Ammonium (NH_4^+) is first oxidized to nitrite (NO_2^-) by Ammonia-oxidizing Prokaryotes (AOP), which consist of both Ammonia-Oxidizing Bacteria (AOB) and Archaea (AOA), and then further oxidized to nitrate (NO_3^-) by Nitrite-Oxidizing Bacteria (NOB).

Several problems are associated with ammonium in drinking water. Nitrification in drinking water distribution systems can lead to aesthetic problems (taste and odour) (Rittmann et al., 1989), corrosion (Murphy et al., 1997), and alkalinity consumption and decreased pH (Rittmann et al., 2012). Incomplete nitrification can also lead to nitrite production, a toxic intermediate of nitrification. Ammonium can also increase chlorine demand, which can increase disinfection by-products (DBPs), and can cause biological instability in the distribution system leading to unwanted microbial growth (Rittmann et al., 2012). Ammonium removal is therefore especially important in regions that do not use disinfectants in the distribution system. Proper management of the drinking water treatment processes is needed at the water works, to ensure quality of delivered water, and to avoid problems in the distribution system.

1.2 Nitrification problems

Although biological filters are effective for removing ammonium from drinking water, problems can still occur causing increased levels of ammonium or nitrite. Several factors can influence the performance of nitrification including:

- Factors controlling the biochemical processes such as temperature (Aa et al., 2002; a Andersson et al., 2001; L. J. Kors et al., 1998) and pH (Antoniou et al., 1990).
- Factors controlling the supply of substrate and nutrients. These include inlet ammonium concentrations (van den Akker et al., 2008), availability of phosphorous (de Vet et al., 2012), alkalinity (Biesterfeld et al., 2003), dissolved oxygen (DO) (Lytle et al., 2013), and other essential micronutrients (Wagner 2013).
- Competition for space and nutrients from heterotrophs (Chen et al., 2006).
- Accumulation of flocks and particles on the filter material (Niquette et al., 1998) and the interactions of iron with phosphorous and other nutrients (de Vet et al., 2011).

- Design and operating conditions including flow rate, filter run length, and backwash procedures (Lopato et al., 2013).

When filters are not performing properly, factors such as pH, DO, and alkalinity can be checked first, as these problems can be easy to diagnose. Other problems with nitrification can be more complicated and are not well understood.

Operational parameters, such as flow rate and filter run length, are generally well understood in non-biological filters, but are more complicated and less understood in biological drinking water filters used for nitrification. Fluctuations in both hydraulic and ammonium loads are common in the operation of the filters, and can lead to levels of ammonium and nitrite in the effluent that are above safety guideline values (Lee et al., I). It is important to know the safe operating windows for these filters, in terms of both flow rate and influent ammonium concentration, in which these filters can operate and still meet safety guideline values. By developing an understanding of the processes effecting ammonium removal, the design and operation of the filters can be optimized, creating filters that are robust and reliable.

One of the major operating parameters for biological filters is the backwash. Backwashing is needed to remove particles and biomass that accumulate on the filter material over a filter run. Backwashing is performed based on acceptable head loss, decreasing flow rate, or turbidity breakthrough, although for ease of operation, is usually a predetermined filter run time (Bhargava and Ojha, 1989). Although the effects of backwashing have been widely studied in biological filters used for NOM removal, there is still only limited knowledge about the effects of backwashing on nitrification. This is exasperated by the many factors that can contribute to the effects of backwashing, including filter type, temperature, bed fluidization, backwash procedures (including duration, flow rate, and if air is used), and type of filter material. Despite its importance, procedures for biological filters are generally based on rules of thumb (Bhargava and Ojha, 1989).

The effects of nutrient limitations, generally phosphorous, on nitrification has recently been studied, with most research focusing on increasing nitrification at low water temperatures with phosphorous addition (Aa et al., 2002; L. Kors et al., 1998; Yoshizaki and Ozaki, 1993). Phosphorus, generally in the form of phosphate (PO_4^{3-}), is required by all organisms for growth and activity and is needed by microorganisms in the synthesis of DNA and RNA, and in the production of adenosine triphosphate (ATP) (Westheimer, 1987). Other micronutrients are needed in smaller amounts, but are also essential to microorganisms. For example, copper is part of the ammonium monooxygenase enzyme used to carry out the first two steps of in the biological oxidation of ammonium to nitrite (Gilch et al., 2009).

There is still little known about the effects of phosphorus and other micronutrient limitations on nitrification and nitrifying biomass, which is further complicated in source waters containing iron. Phosphorous can be absorbed to iron during the oxidation of iron(II) to iron(III) (Lijklema, 1980) and the co-precipitation of iron with phosphorous and other nutrients could lead to limitations that decrease nitrification (de Vet et al., 2011). Iron flocks that accumulate on the filter media could also form a physical barrier that could limit diffusion into the biofilm.

1.3 Motivation and thesis overview

Although nitrification in rapid sand filters is a common and important process for removal of ammonium from drinking water, problems can occur in these filters and they are difficult to diagnose. Solutions are generally obtained by trial and error, and such a process can be expensive, time consuming and sometimes does not even solve the problem. This is because the operation of these filters is often based on operator experience and general guidelines, instead of being on a scientific basis (Lawler et al., 2006). Several aspects effecting nitrification, including filter operation and nutrient limitations, were investigated in this thesis. An overview is presented in Figure 2.

Operation of these filters is further complicated by dynamic operating conditions. Changes in hydraulic, ammonium, or iron loading rates can significantly impact both the short and long term performance of these filters. The safe operating windows for rapid sand filters was determined in this thesis which aimed to provide a scientific understanding of the operating conditions controlling nitrification in terms of filter run lengths, hydraulic loading rates, and backwashing procedures for better management of these filters. Proper management of these filters is essential in ensuring drinking water that meets or exceeds safety guideline values.

Influent water
- Ammonium (Lee et al., I)
- Phosphorus and other nutrients (Lee et al., II & Wagner et al., IV)

Rapid sand filter

Factors affecting nitrification
- Ammonium loading rate (Lee et al., I)
- Backwashing (Lee et al., III)
- Filter run time (Lee et al., III)
- Availibility of nutrients (Lee et al., II & Wagoner et al., IV)
- Nitrifying biomass (Lee et al., I, II, & III)

Filter assessment tools
- Full scale filter (Lee et al., I)
- Pilot scale filter (Lee et al., I, II, III)
- Lab scale columns (Wagner et al., IV)

Figure 2. Overview of the experiments and aims of this thesis.

The key aspect to biological rapid sand filters is the microorganisms on the filter material. This thesis examines which nitrifying organisms contribute to nitrification in these systems. It has generally been thought that AOB where responsible for ammonium oxidation, and the nitrite oxidizing bacteria, *Nitrobacter*, was responsible for nitrite oxidation. Recent research has challenged these assumptions though with the discovery of ammonium oxidizing archaea (Könneke et al., 2005) and findings that show the importance of the nitrite oxidizer *Nitrospira* (Kim and Kim, 2006; Schramm et al., 1999) in engineered systems. Direct quantification of nitrifiers at different times in the operational cycle, and under different loading conditions, was used in combination with in-situ nitrification rate measurements to further elucidate our understanding of the nitrifiers in these systems.

Besides substrates, nutrients such as phosphorous and other trace metals are needed for growth and activity. Although the importance of nutrients is common knowledge, nutrient limitation is often overlooked as a reason for poor nitrification in these filters, and therefore the effects of nutrient limitations on growth and activity was investigated. Poor nitrification caused by nutrient limitations was assessed for two different Danish water works using flow through lab columns.

Increased scientific based understanding of both the effects of operating procedures and nutrient limitations on nitrification is needed. Further insight into how these factors effect both the nitrifying activity and biomass in these filters would lead to better design, operating, and troubleshooting procedures, that can ensure these filters are robust and able to consistently produce reliable and safe drinking water.

1.4 Objectives

The overall objective of this PhD was to investigate factors impacting nitrification performance in biological rapid sand filters. These include operating conditions, such as hydraulic loading rates, filter run lengths, and backwashing, as well as the effects of ammonium loads and nutrient limitations.

More specifically, the thesis aims to:

- Determine if a normally well operating filter can safely remove ammonium and nitrite under sudden short term increases in ammonium loading.
- Determine the effects of backwashing on both nitrifying activity and biomass under steady state and increased ammonium loading conditions.
- Examine the development of both the nitrifying activity and biomass, with depth in a filter, under increased ammonium loading conditions, both under phosphorous limiting and non-limiting conditions.
- Determine, using flow through lab scale columns, if problems associated with nitrification can be attributed to nutrient limitations.
- Determine the importance and relative abundance of AOB and AOA for ammonium oxidation, and the genera *Nitrospira* and *Nitrobacter* for nitrite oxidation, under various ammonium loading conditions.

2 Investigating nitrification in biological drinking water filters

2.1 Removing ammonium from drinking water

There are many treatment processes that can remove ammonium from drinking water including reverse osmosis, ion exchange, and air stripping, although these applications are limited and generally not used for the sole purpose of ammonium removal (Lytle et al., 2013). The most common employed ammonium removal processes are biological removal (nitrification) or chlorination until breakpoint. In breakpoint chlorination, chlorine is added until a free chlorine residual is met. At elevated ammonium levels, increased chlorine is needed, which could increase levels of DBPs to above regulatory limits. Breakpoint chlorination can also be problematic if ammonium concentrations are not stable. Varying ammonium concentrations, due to operational or source water fluctuation, make it difficult in maintaining safe levels of chlorine in water, both in terms of maintaining a proper chlorine residual and because of the risk of adding too much chlorine.

Biological filters are also used to remove ammonium from drinking water, and although they are widely used in western Europe, they have only recently started to be used for this purpose in North America (Rittmann et al., 2012). Biological filters offer several advantages over conventional physicochemical processes including decreased use of chemicals, and therefore reduced by-products, and lower operational and maintenance costs (Tekerlekopoulou et al., 2013).

2.2 Granular biological filters used for nitrification

There are many different types of biological filters used to remove ammonium from drinking water, with the most common being slow sand filtration, trickling filters, and rapid sand filters. Slow sand filters are characterized by very low hydraulic loading rates (< 0.5 m h^{-1}) and long filtration cycles (weeks or months). Slow sand filters are not backwashed, but instead require regular removal of the top few centimetres of filter material. One of the major advantages to slow sand filers is that they are simple to operate, although large space requirements has limited their use (Crittenden et al., 2005).

Biological rapid sand filters and trickling filters differ from slow sand filters in that they are operated under higher hydraulic loading rates, are backwashed, are cleaned more frequently, and require much less space. Trickling filters are generally associated with wastewater treatment, although they are also used in drinking water applications (de Vet et al., 2011; Tekerlekopoulou and Vayenas, 2003; van den Akker et al., 2008). The major difference between biological rapid sand filters and trickling filters is that rapid sand filters are fluidized, whereas trickling filters are not. A schematic showing the cross section of typical rapid sand filter is shown in Figure 3A. Although biological rapid sand filters are more commonly used for ammonium removal in drinking water, trickling filters do offer the advantage of not being limited by oxygen (De Vet et al., 2009). Nitrification requires significant amounts of oxygen, with a theoretical oxygen consumption of 4.6 g O_2/g NH_4-N (Rittmann et al., 2002). Therefore, nitrification in biological rapid sand filters is limited by oxygen in sources waters with ammonium concentrations greater than approximately 1.5-2.0 mg NH_4-N L^{-1} (de Vet et al., 2009; Lytle et al., 2013).

Different types of media are used in biological rapid sand filters. Generally, the filter material consists of sand, anthracite, GAC, or in the case of dual media filters, a combination of two. GAC is thought to have advantages over other filter materials due to its irregular shape and macroporous structure which allows more space for microbial growth and provides better protection from shearing (Urfer et al., 1997). Although GAC might offer some advantages in biofilm development and retention (Billen et al., 1992), sand has also been shown to be an effective filter material in biological filters. The research presented here will only focus on biological rapid sand filters, as they are the primary treatment process for drinking water in Denmark, where this study was conducted.

Both biological rapid sand filters and trickling filters use microorganisms that are attached to the filter media rather than being suspended in solution. These attached microbes are generally thought to form a biofilm on the media. A conceptual model of a biofilm is shown in Figure 3C. Biofilm kinetics is controlled by both internal and external mass transport resistance. External mass transport resistance is controlled by diffusion across the water film layer, from the bulk water to the biofilm, and internal mass transport resistance is controlled by molecular diffusion of substrate into the biofilm (Rittmann and MacCarty, 2001).

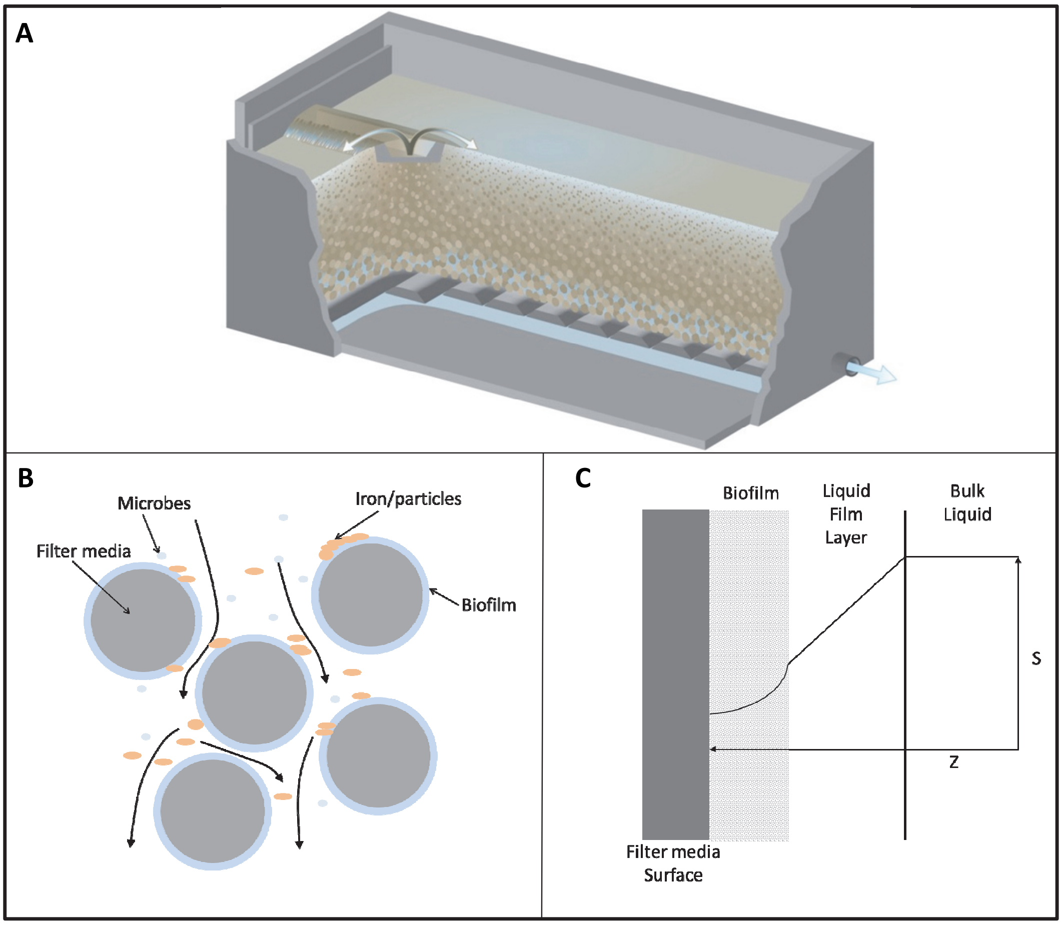

Figure 3. (A) Cross section of a typical rapid sand filter (B) Conceptual model of flow through filter media of a biological rapid sand filter (C) Conceptual model of a biofilm.

2.3 The black box approach

Several studies have examined nitrification in biological rapid sand filters, with many having a black box approach. The black box approach focuses on the influent and effluent of a filter. Although this approach can shed some light on the overall performance of a filter, deeper insight is needed to understand the processes occurring inside the filter.

For example, several authors have examined the effects of phosphorous limitation on nitrification in full scale biological rapid sand filters, by measuring ammonium in the influent and effluent. In all of these studies, problems with ammonium removal occurred after the onset of low temperatures (Aa et al., 2002; L. Kors et al., 1998; Yoshizaki and Ozaki, 1993). Phosphoric acid was added and after some time ammonium removal increased. Although these results are useful in showing that at low temperatures the addition of phosphoric acid may improve nitrification, they do not offer any insight into what is occurring in the filter, which leaves many unanswered questions. A more recent study by Lytle et al. (2013), used pilot scale biological aerated filters (BAF) to examine the effects of phosphorous limitations on filters with high ammonium loads (> 3 mg NH_4-N L^{-1}). The drawback with this study is that they also only measured inlet and outlet water samples, and like the previous studies, offer no insight into processes inside the filter. De Vet et al. (2012), used batch experiments, measuring both ammonium removal rates, and growth of AOB at different phosphate concentrations to show phosphorous could be limiting in some nitrifying trickling filters. Although these experiments further our knowledge of phosphorous limitations on nitrification, they do not describe or account for the processes occurring inside a real filter, such as filter hydraulics and the influence of iron.

2.4 The importance of scale

When examining these filters, there are generally three scales to choose from: full, pilot, and lab scale. The choice of which scale to use is dependent on the objectives of the study, as each scale offers their own benefits and drawbacks. Ideally, all studies would be done at full scale, as this offers accurate results at an industrial scale. Filter hydraulics, loading rates, filter design and operation, and influent water characteristics can be difficult or impossible to capture or represent in lab scale experiments. Despite the obvious advantages to full scale studies, there are several drawbacks that often preclude their use. Sampling and monitoring processes inside a full scale filter can be difficult and time consuming, and extreme caution should be taken to not contaminate the filter or deteriorate filter performance. Also, changes to operating conditions or spiking of compounds into the filter may not be an option unless the filter is taken offline and the treated water is wasted, which is generally not feasible for operational filters.

Pilot column studies can be used as an alternative to full scale filter studies, and offer many of the same benefits without the large drawbacks of doing a full scale study. Pilot columns can accurately represent the hydrodynamics, influent water matrix, and operating conditions (filter run length, hydraulic loading rate, backwash flows and time) of a full scale filter, although confirmation to full scale filter performance should be confirmed. Although heterogeneity and horizontal flow may not be captured in a pilot column, some of this concern can be alleviated by increasing the diameter of the pilot column. Pilot columns also offer the advantages of being easier to sample, are less expensive to operate, and produce much less water than full scale filters, making water disposal less of an issue. The overall advantage that well designed and operated pilot columns offer is that they can accurately mimic the behaviour of full scale filters, while performing experiments that could not otherwise be done at full scale. The disadvantages of these systems are that it can be difficult or impossible to isolate specific processes and that these systems require time and expense to operate.

Lab scale experiments give the most control over experiments and offer the advantage of isolating certain parameters. These experiments are generally quicker and less expensive to set up and maintain, and results can often be obtained in a shorter period of time. One of the large drawbacks with these systems is that they may not accurately capture important parameters observed in larger systems, such as the hydraulics or dynamic changes in substrates and nutrients with depth. Therefore, great care should be used in designing lab scale experiments and in interpreting their results.

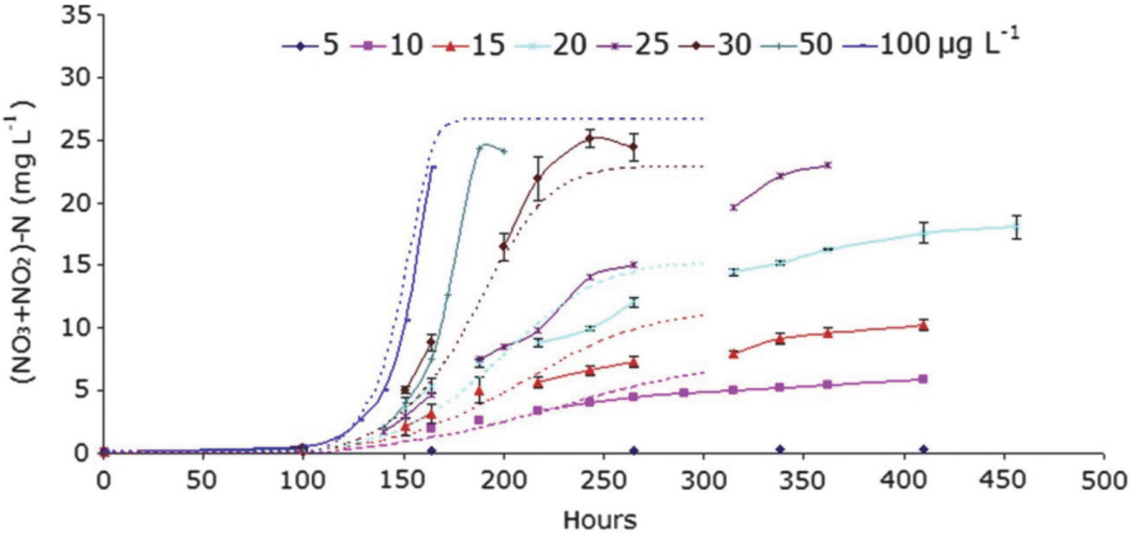

Figure 4. Nitrate plus nitrite formation as a function of time at different MAP concentrations (de Vet et al., 2012)

An example of this is illustrated in Figure 4. Using lab scale batch experiments with cultured medium, de Vet et al. (2012) found increased nitrification rates with increasing microbial available phosphorous (MAP) concentrations of up to at least 100 μg P L^{-1}) with little to no nitrification occurring at MAP concentrations of < 10 μg P L^{-1}. In pilot scale experiments by Lee et al. (II), increased nitrification was observed at total inlet phosphorous concentrations of 6 ± 2 μg P L^{-1} with little of this phosphorous actually being consumed. Increasing phosphorous to 430 μg P L^{-1} in the pilot filter was found to increase nitrification, although less than 70 µg P L^{-1} was removed from the filter, and it was highly likely that at least some of the phosphorous was not used by microbes, but instead absorbed to iron hydroxides on the filter material.

2.5 Examined water works

The results of this thesis came from full, pilot, and lab scale experiments, which were used to examine nitrification at several different water works in Denmark. The water works examined had varying influent water characteristics and operating conditions at two of the examined water works had existing problems with nitrification.

2.5.1 Islevbro water works

Both full and pilot scale studies were performed at Islevbro water works, a plant operated by HOFOR, the largest water supplier in Copenhagen. Islevbro water works treats anoxic groundwater with the main compounds of concern being iron, manganese, and ammonium. The treatment schematic for Islevbro is shown in Figure 5. Typical Danish drinking water treatment consists of aeration, followed by either one filter step, or a primary filter and secondary filter. In Denmark, water is treated and distributed without chemical disinfection. The primary filters are designed to remove most of the iron in the water, while the secondary filters are primarily used for nitrification.

The pilot and full scale studies in this research focused on nitrification in the secondary filters. The water works was chosen because it was a well operating system that was operated continuously at relatively stable flow rates and inlet water quality. This made it possible to establish the pilot columns so that could accurately mimic the full scale filters. The filters at Islevbro are characterized by very low organics removal and low inlet iron, phosphorous, and ammonium concentrations as shown in Table 2. The term ‘reference operating conditions’ is used frequently throughout this thesis and refers to water quality and operating conditions found in this Table. The low loading conditions allowed for easy manipulation of the influent water and also allowed us to examine nitrification at various loading conditions. Specific design and operational parameters of the filters are shown in Table 1.

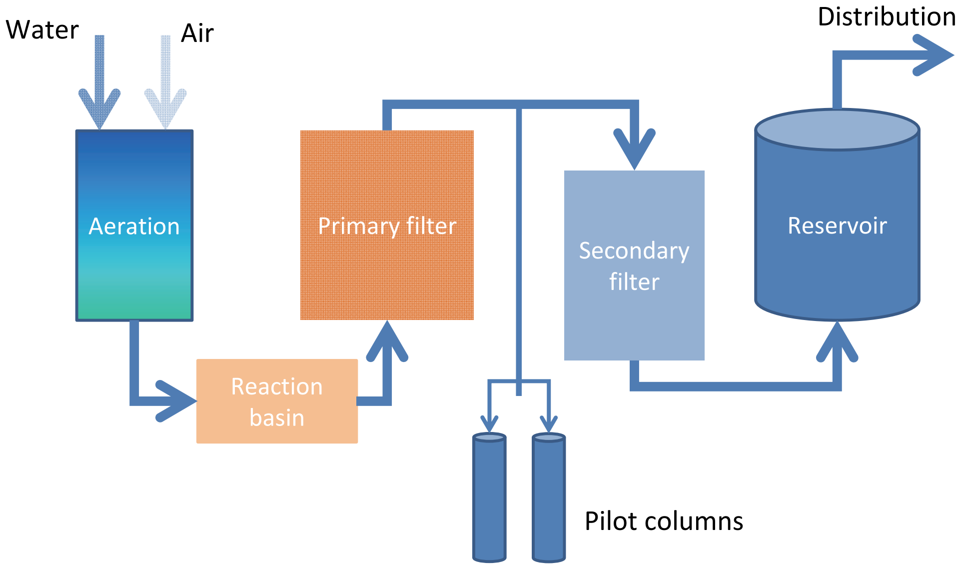

Figure 5. Schematic of the treatment processes at Islevbro water works

Besides Islevbro water works, two other Danish works, Langerød and Nærum, were also examined using flow though, lab scale columns. Both of these water works had ongoing problems with nitrification, and were unable to meet the Danish drinking water guideline value of 0.05 mg NH_4 L^{-1}. These water works had different operating and water quality characteristics, compared to Islevbro, and both had low influent phosphorous concentrations, which could mean the poor nitrification in these filters could be caused by phosphorous or other micronutrient limitations.

2.5.2 Langerød water works

Langerod has two identical treatment trains, which consists of step aerators followed by a primary filter, which then feeds two secondary filters (Figure 6). The treatment scheme is similar to Islevbro waterworks, except that Langerød does not have a contact chamber following aeration. Operating and design parameters for Langerød are shown in Table 1. Inlet ammonium iron, and phosphorus concentrations to the secondary filters at Langerød were 0.66 mg NH_4-N L^{-1}, 0.14 mg Fe L^{-1}, and 0.028 mg P L^{-1}. Similar to Islevbro, there was no observed phosphorous removal in the filter and analysis for micronutrients such as copper, where all below quantifiable limits, making this filter a good candidate to determine possible nutrient limitations.

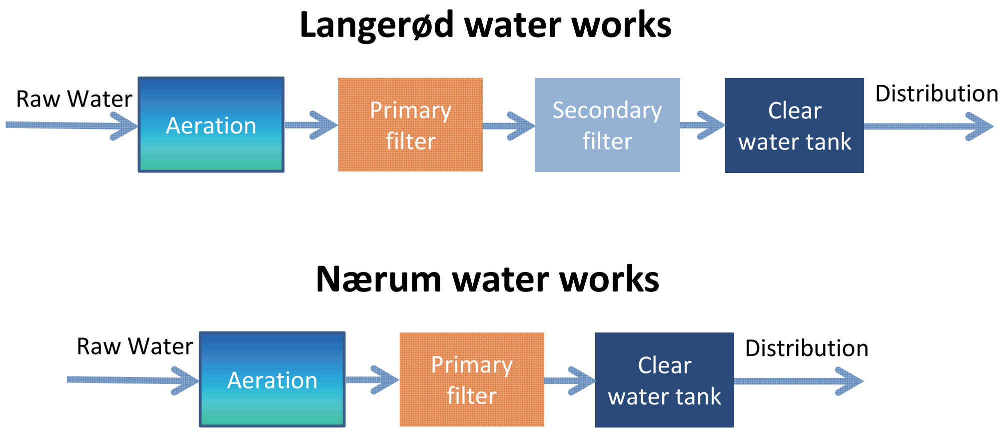

Figure 6. Schematic of the treatment processes at Langerød and Nærum water works.

2.5.3 Nærum water works

Nærum water works is different from the other water works examined in that the treatment train consists of step aeration followed only by primary filtration (no secondary filter used), as shown in Figure 6. There are six primary filters operated in parallel. Water quality parameters for Nærum are shown in Table 2 and general operating and design parameters are shown in Table 1. Although inlet ammonium concentrations to the filters are relatively low at 0.34 ± 0.04 mg NH_4-N L^{-1}, very little ammonium is removed and effluent concentrations average 0.24 ± 0.02 mg NH_4-N L^{-1}. Because there is no secondary filter at Nærum, iron loads to the filters are high at 2.5 mg L^{-1}, and although inlet phosphorus concentrations are higher than those at the other examined water works, effluent phosphorus concentrations were the lowest of the examined plants at < 0.005 mg L^{-1} (Table 1). These filters show that nitrification could be limited by a lack of phosphorous or other micronutrients, which was examined using the flow through lab columns.

Table 1. Design and operating parameters, as well as inlet ammonium, iron, and manganese concentrations for the secondary filters at Islevbro and Langerød water works, the pilot columns at Islevbro, and the primary filter at Nærum water works.

	Islevbro		**Langerød**	**Nærum**
Parameter	Secondary filter	Pilot columns	Secondary filter	Primary filter
Filter area	18 m^2	.071 m^2	20 m^2	19.2 m^2
Filter depth	0.7 m (filter material), 0.3 m (support material)	0.7 m (filter material), 0.3 m (support material)	0.65 m	0.6 m
Filter Volume	12.6 m^3	~50 Liters per column	13 m^3	11.5 m^3
Empty bed contact time	10.5 minutes	10.5 minutes	15.6 minutes	23.0 minutes
Volumetric flow rate (average)	72 m^3/h	0.275 m^3/h (275 L/h)	50 m^3/h	30 m^3/h
Filtration velocity (average)	4 m/h	3.9 m/h	2.5 m/h	1.6 m/h
Backwash (air)	3 min at 90 m/h	3 min at 90 m/h	5 min at 60 m/h	6 min
Backwash (water)	10 min at 25 m/h	8 min at 40 m/h	3.5 min at 35 m/h	4.5 min
Filter run length	10-14 days (22 days, maximum observed)	10-14 days (23 days maximum)	6-14 days	6 days
Inlet iron (mg/L)	0.38 ± 0.16	0.28 ± 0.18	0.14	2.5
Inlet NH4 (mg N/L)	0.13 ±0.05	0.1 ± 0.024	0.66 ± 0.01	0.34 ± 0.04
Inlet Phosphorous (mg P/L)	0.008 ±0.005	0.01 ± 0.004	0.028	0.02

2.6 Full scale sampling

Aqueous water samples were collected with depth in one of the full scale filters at Islevbro water works using sampling tubes (Figure 7). Stainless steel sampling pipes collected aqueous samples in the middle of the filter at depths of 10, 20, 30, and 40 cm. An additional sampling pipe was fitted two cm above the filter bed to sample the influent water. A five channel peristaltic pump was used to simultaneously collect samples at all depths. The pipes were positioned five cm apart to avoid any hydraulic interference between sampling pipes.

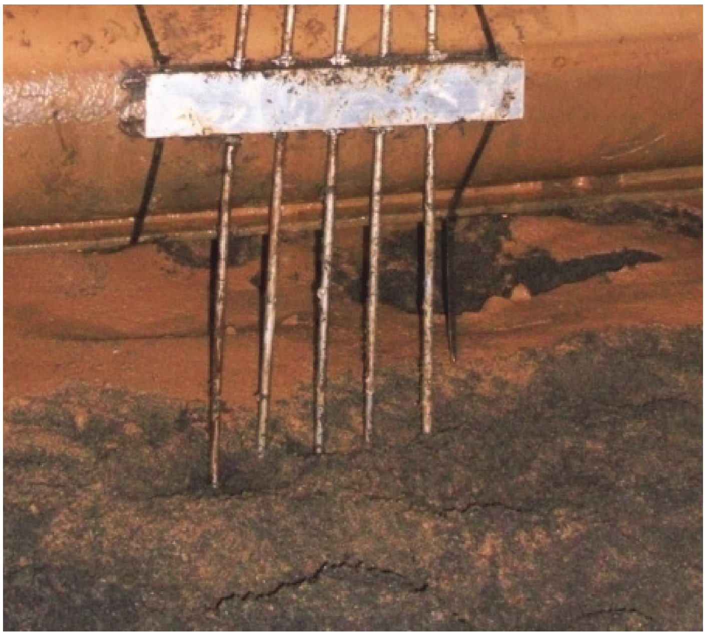

Figure 7. Picture of the sampling tubes used to take aqueous samples with depth in a full scale filter at Islevbro water works.

2.7 Pilot plant

2.7.1 System design and operation

A pilot container was provided by the engineering consulting company, Krüger, which contained two pilot columns that were operated in parallel. Several steps were taken to ensure that the columns accurately represented the full scale filters. First, sand was taken from one of the full scale filters and used in the pilot columns. Caution was taken to ensure that the entire 70 cm sand depth profile was preserved in the columns. Water was pumped from the channel supplying the secondary filters to ensure the pilot columns were supplied with the same water. Also, the pilot filters were operated in a similar manner as the full scale filters in terms of hydraulic loading rate, filter run times, and backwashing procedures (Table 1).

Figure 8. Picture of the two pilot columns used in the study at Islevbro water works (photo courtesy of Philip Binning)

2.7.2 Validation of the pilot columns to the full scale filters

For our pilot study, it was important to validate that the pilot columns mimicked the full scale filters in both biological removal and particle retention. To validate their performance, ammonium, iron, and manganese depth profiles were taken on the same day in both a full scale filter and pilot column 1 (Figure 9 taken from Lee et al., I).

A nitrogen balance showed that ammonium removal occurred biologically, and is removed in the top 20 cm of both the full scale filter and pilot column. The inlet concentrations vary slightly, due to the samples being taken several hours apart. The DO, pH, (Table 1), and contact time strongly suggest that iron is most likely Fe(III), in the form of precipitates or flocks (Stumm and Lee, 1961), and therefore, removal was attributed to filtration. Under these conditions, the manganese removal was most likely due to biological and autocatalytic processes on the filter media (Sahabi et al., 2009). Data on the removal of all three compounds gives confidence that the pilot columns performed similarly to the full scale filters (Figure 9). This validation of the columns is important because it gives confidence that results obtained from the experiments in the pilot columns, are representative of the full scale filter.

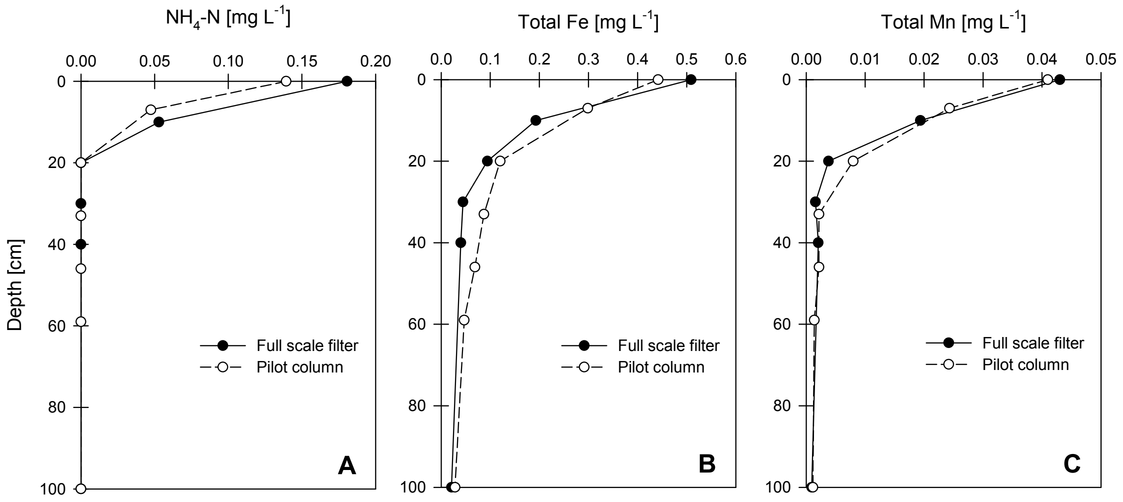

Figure 9. Depth profiles in the full scale filter and pilot column 1 for ammonium (A), total Fe (B), and total Mn (C). All samples were taken on the same day, 4 months after start-up of the pilot column. (Taken from Lee et al., **I**)

Table 2. Water quality parameters for the raw water, secondary filter influent (at Islevbro and Langerød), pilot filter effluent, and full scale filter effluent at the examined water works.

			Islevbro				**Nærum**		**Langerød**		
Parameter	Units	Guideline	Raw water	Influent full scale secondary filter	Effluent full scale secondary filter	Effluent pilot column [a]	Raw water	Effluent	Raw water	Influent secondary filter	Effluent
NH4-N	mg NH_4-N L^{-1}	0.04	0.32 ± 0.16	0.13 ±0.05	0.01 ± 0.005	< 0.02	0.34 ± 0.04	0.24 ± 0.02[a]	0.84 ± 0.13	0.66 ± 0.01[a]	0.29 ± 0.03
Fe	mg L^{-1}	0.1	1.8 ± 1.1	0.38 ± 0.16	0.011 ±0.002	0.009 ±0.007	2.5	0.05[a]	3.0 ± 0.2	0.14[a]	0.011 ± 0.001
Mn	mg L^{-1}	0.02	0.06 ± 0.02	0.035 ±0.072	< 0.005	0.001 ±0.0008	0.12	0.007 ± 0.004	0.118 ± 0.011	NA	0.008 ± 0.004
P	mg L^{-1}	0.15	0.025 ± 0.01	0.008 ±0.005	0.009 ± 0.004	0.01 ±0.004	0.02	<0.005[a]	0.274 ± 0.046	0.028[a]	0.022 ± 0.005
Alkalinity	meq L^{-1} HCO_3	-	5.67 ± 0.79	5.5	5.74	NA	5.6	5.57 ± .02	5.95 ± 0.13	NA	5.85 ± 0.13
NVOC	mg L^{-1}	4	2.3 ± 0.3	2.4	2.2 ± 0.1	2.65 ±0.14	2.14	1.92 ± 1.4	2.6 ± 0.2	NA	2.5 ± 0.3
pH		7.0-8.5	7.4 ± 0.3	7.4	7.4 ± 0.1	7.32 ±0.04	7.46	7.77 ± 0.09	7.4 ± 0.1	NA	7.7 ± 0.1
Temp.	°C	-	9.3 ± 0.4	9.1	9.6 ± 0.9	9.8-10.8		9.7 ± 0.4	9.1 ± 0.3	NA	9.3 ± 0.9
DO	mg L^{-1}	> 5 (at consumer tap)	1.09 ± 0.59	9.3	8.7 ± 0.4	9.4-9.7	0.55	10.0 ± 0.4	0.31 ± 0.13	NA	9.7 ± 0.5

a: Analysis taken from sampling. All other reported values from Jupiter data base and is part of a DWBiofilters

2.8 Lab scale columns

Continuous flow lab scale columns were used to investigate if nutrient limitations could be the cause of poor nitrification performance at Langerød and Nærum water works. The lab scale columns were operated at both hydraulic and ammonium loading conditions that are seen in full scale filters. The continuous flow through real filter sand, coupled with hydraulic and ammonium loads observed in the full scale filter, give the advantage of being able to access nitrification at different depths in the filters, without the drawbacks of performing full scale investigations (Tatari et al., 2013).

To investigate possible nutrient limitations as the cause of poor nitrification, sand from the investigated filters was collected and rinsed. The columns were filled to a bed height of 5 cm and had a diameter of 2.6 cm ($V_{col} = 26.5$ cm^3). The columns were fed with effluent water from the examined water works, and hydraulic and ammonium loading rates were set to match those of the examined waterworks (Tatari et al., 2013). All columns were supplemented with additional ammonium. One of the columns was used as a control and supplemented with ammonium only. The other three columns were additionally supplemented with phosphorus, micronutrients, and phosphorus and micronutrients. Information on the experimental set up and operation of the columns during the experiments is found in Wagner 2013.

2.9 Application of the different scale experiments

The full and pilot scale experiments performed at Islevbro water works were used to investigate how operating conditions such as hydraulic loading rates (Lee et al. I), backwashing, and filter run times influence nitrification (Lee et al. III). Further experiments showed how increased ammonium loads and supplementing phosphorus effected both nitrification and nitrifying biomass (Lee et al. II). These experiments would have been difficult or impossible to examine at full scale, but validation of the pilot columns with the full scale filters, gives confidence that the findings accurately portray that at full scale. The flow through, lab scale columns were a quicker and less expensive alternative to pilot filters or full scale investigations, in determining if poor nitrification in two Danish works could be caused by nutrient limitations (Wagner 2013). The knowledge gained from the experiments at the different scales is valuable in and understanding the processes controlling nitrification performance in these filters, and in determining safe operating windows and troubleshooting filters with existing nitrification problems.

3 Effects of operating conditions on ammonium removal

Breakthrough of ammonium can occur when the ammonium loading rate exceeds the ammonium removal rate. The volumetric ammonium loading and removal rate is a function of both the hydraulic loading rate (Q/A) and ammonium concentration in a filter, and is determined using Equations 1 and 2.

$$r_L = Q\frac{C_{in}}{A\Delta z} \tag{1}$$

$$r_R = Q\frac{C_{in} - C_{out}}{A\Delta z} \tag{2}$$

Where r_L and r_R is the volumetric ammonium loading and removal rates (g NH_4-N m^{-3} h^{-1}), Q is the volumetric flow rate (m^3 h^{-1}), $c_{,in}$ and c_{out} is the influent and effluent ammonium concentration (mg NH_4-N L^{-1}), *A* is the cross sectional area of the filter (m^2), and Δz is the active depth of the filter bed (0.7 m), and does not include the support material. When considering the removal rates with depth in the column, c_{in} and c_{out} are the corresponding influent and effluent concentrations at the beginning and end of each considered depth.

The hydraulic loading rate is one of the major operating parameters that can be designed for and controlled in these filters. At Islevbro water works, the inlet ammonium concentration was observed to vary from 0.04 to 0.20 mg NH_4-N L^{-1}, and hydraulic loading rates have been observed to double in a few hours (Figure 10). These large changes in ammonium loading rate could contribute to excessive amounts of ammonium and nitrite observed in treated water, exceeding the Danish drinking water guideline values of 0.04 mg NH_4-N L^{-1} and 0.003 mg NO_2-N L^{-1}. It is therefore important to determine the safe operating windows, in terms of both inlet ammonium concentration and hydraulic loading rate, in which these filters can operate and still meet the safety guidelines.

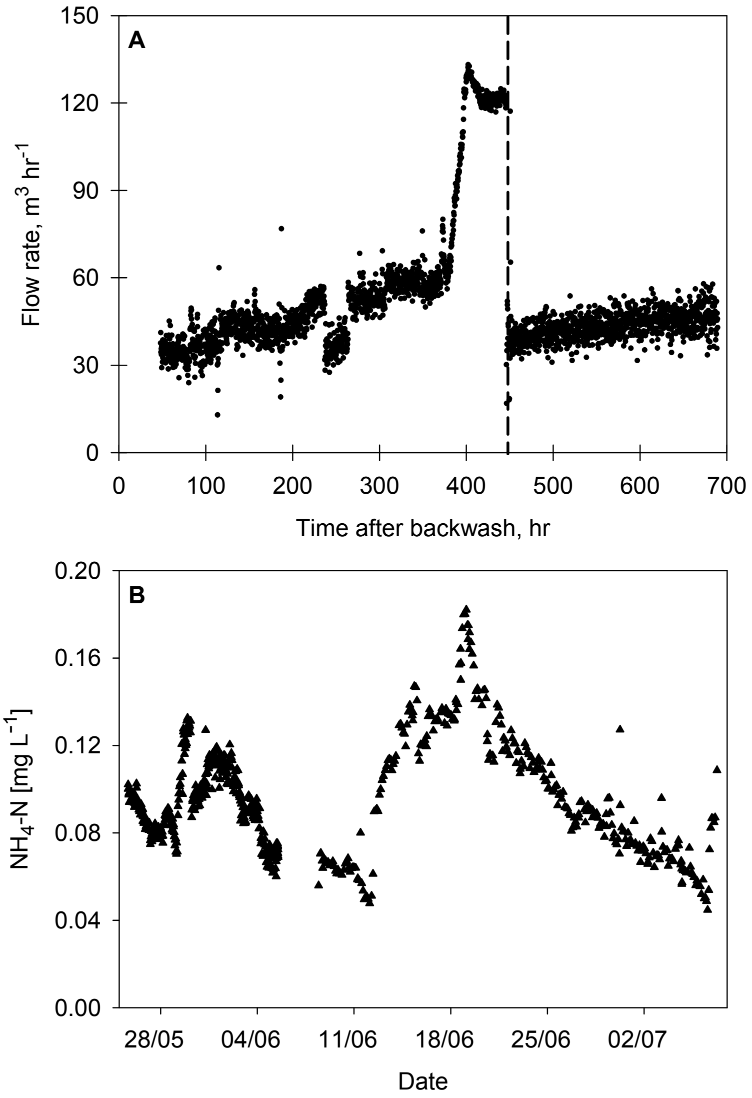

Figure 10. (A) Flow rate in one of the full scale filters at Islevbro water works, as a function of time after backwash. Dashed line shows time of backwash. (B) Inlet ammonium concentration to the pilot and full scale filters at Islevbro water works (Lee et al, I).

3.1 Short term increased ammonium loading experiments

Short term increased ammonium loading experiments were performed in pilot filter 1 to determine the maximum ammonium removal rates under increased influent ammonium concentrations, hydraulic loading rates, and time after backwash. The premise of the experiments was that the ammonium load shifts should be long enough so that steady state is established in terms of DO consumption and effluent ammonium concentration, and short enough to not change the biological make-up of the filter or alter the overall filter performance. Initial experiments showed that 6 hours of increased ammonium loading was enough time to allow steady state conditions to be established (Figure 11).In addition, the columns were operated under reference loading conditions for at least 3 days between experiments. The experiments were conducted over two filtration cycles (23 days and 22 days), which was the longest time between backwashes observed in the full scale filters (Lee et al., I).

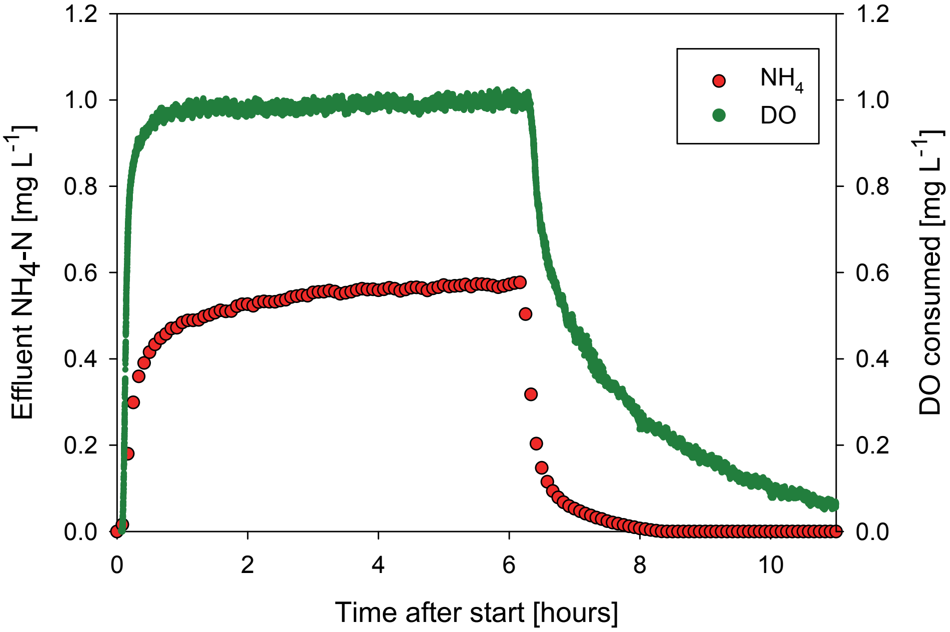

Figure 11. Effluent NH_4-N and DO during initial short term ammonium loading experiments at a hydraulic loading rate of 3.9 m h^{-1}.

3.2 Inlet ammonium concentration

Substrate supply into the biofilm is a diffusion transport limited process, while substrate demand is governed by properties of the nitrifiers, such as the growth rate, yield, and the amount of nitrifying biomass (Chen et al., 2006). Biofilm kinetics are controlled by both the diffusion of substrate between the bulk water and biofilm (external mass transport), and across the biofilm (internal mass transport) as illustrated in Figure 3C. This diffusion is controlled by the concentration gradient of substrate across both of these layers, and therefore increased inlet ammonium concentration, will increase ammonium removal rates, up to the maximum substrate oxidation rate, R_{max}.

Ammonium loading rates were increased by increasing influent ammonium concentrations to a pilot filter at the reference hydraulic loading rate of 3.9 m h^{-1}, to determine the effects of the inlet ammonium concentration on ammonium removal. Short term loading shifts, were used to ensure the increased loading rates did not change the biological make-up or performance of the filter. Inlet ammonium concentrations were shifted from the reference operating concentrations, which varied from 0.04 to 0.20 mg NH_4-N L^{-1} (0.22 to 1.13 g NH_4-N m^{-3} h^{-1}), to applied concentrations between 0.40 to 1.68 mg NH_4-N L^{-1} (2.07 to 9.35 g NH_4-N m^{-3} h^{-1}).

Ammonium was removed to below detectable limits at loading rates of up to 3.1 g NH_4-N m^{-3} h^{-1}, and there is a rapid shift from one-to-one first order removal, to zero order removal (Figure 12). The maximum volumetric ammonium removal rate achieved was 3.4 g NH_4-N m^{-3} h^{-1}, and did not increase despite loading rates of up to 9.35 g NH_4-N m^{-3} h^{-1} (Lee et al., I). These results show that these filters can effectively remove ammonium up to the maximum removal rate, and that increased ammonium concentrations beyond this, do not increase ammonium removal.

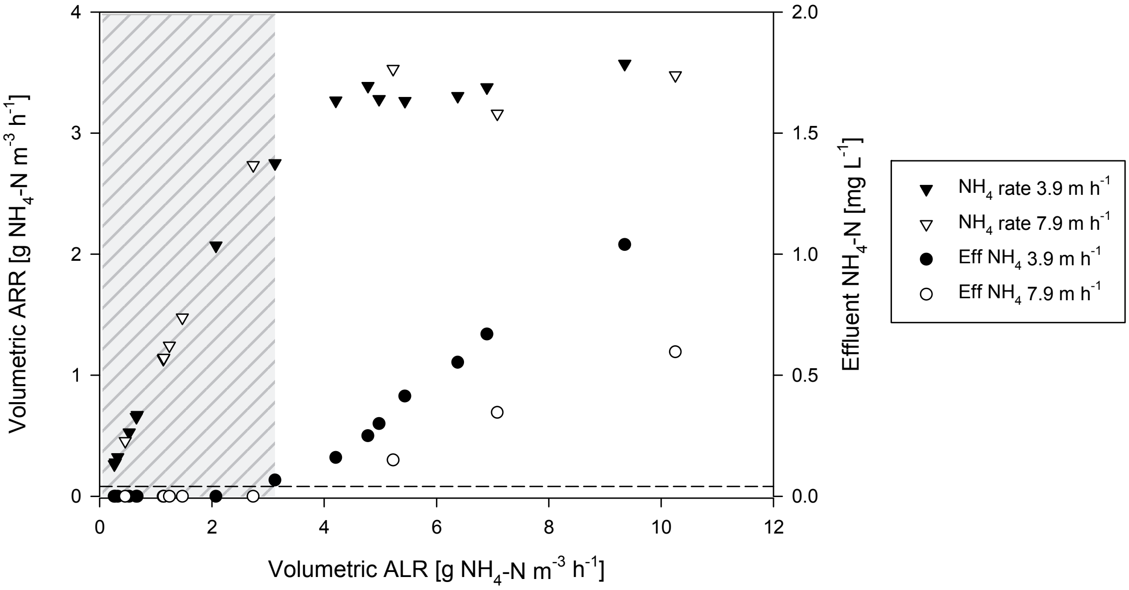

Figure 12. Volumetric ammonium removal rate (ARR) and effluent ammonium concentrations at hydraulic loading rates of 3.9 and 7.9 m h^{-1} as a function of volumetric ammonium loading rate (ALR) (Lee et al., I).

3.3 Hydraulic loading rate

Hydraulic loading rates can also affect the removal rates of ammonium, because the thickness of the water film layer (Figure 3) is a function of the hydraulic loading rate. Increased ammonium loads were applied by increasing both the inlet ammonium concentration and hydraulic loading rate. It was expected that the ammonium removal rate would increase with increased hydraulic loading rate due to the decrease in the external mass transfer resistance (Rittmann and MacCarty, 2001). However, even though the hydraulic loading rate increased from 3.9 to 7.9 m h^{-1} and the external mass transfer layer decreased by 21%, there was no observed increase in ammonium removal. The same maximum volumetric ammonium removal rate of 3.4 g NH_4-N m^{-3} h^{-1} was achieved, even at loading rates as high as 10.3 g NH_4-N m^{-3} h^{-1} (Lee et al., I). This was further illustrated when examining the maximum volumetric ammonium removal rates with depth (Figure 13). The ammonium removal rates are approximately the same at all examined depths in the filter, showing that the examined hydraulic loading rates has little influence on ammonium removal throughout the depth of the filter.

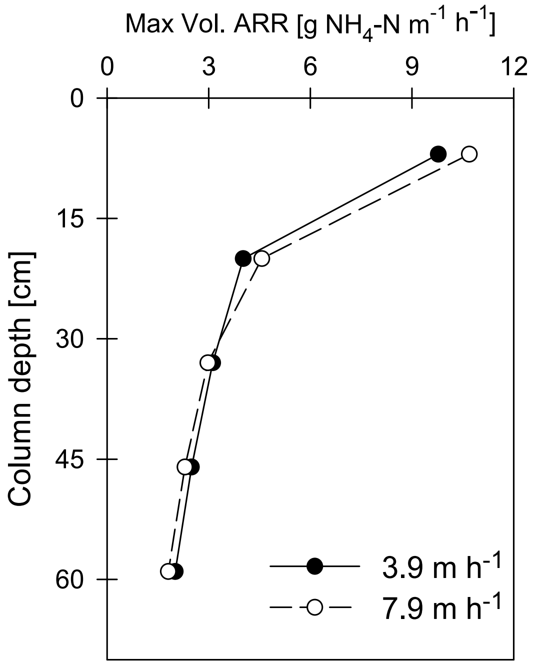

Figure 13. Maximum volumetric ammonium removal rates with depth in column 1 at hydraulic loading rates of 3.9 and 7.9 m h^{-1} (taken from Lee et al., I).

This finding is contrary to other findings (Lopato et al., 2013; Stembal et al., 2005; van den Akker et al., 2008), which showed increased removal rates with increased flow. This is explained by comparing the maximum utilization rate of the biofilm to the external mass transfer resistance (K*). The calculated K* values during these experiments was between 2 and 4 (Lee et al., I). Values greater that one indicate that ammonium removal was not limited by external mass transport.

One of the important findings of these experiments is that ammonium removal was not a function of inlet ammonium concentration or flow rate individually, but instead a function of the total ammonium loading rate. This is likely due to the thin biofilms associated with the low ammonium loads applied during reference operating conditions in these filters. With filters operated at increased ammonium loading rates, a thicker biofilm would be expected and external mass transfer might be limiting. Increasing the hydraulic loading rate would therefore increase ammonium removal. This can be seen in the nitrifying trickling filters used by van den Akker et al. (2008), which showed increased removal with increased hydraulic loading rates (Figure 14).

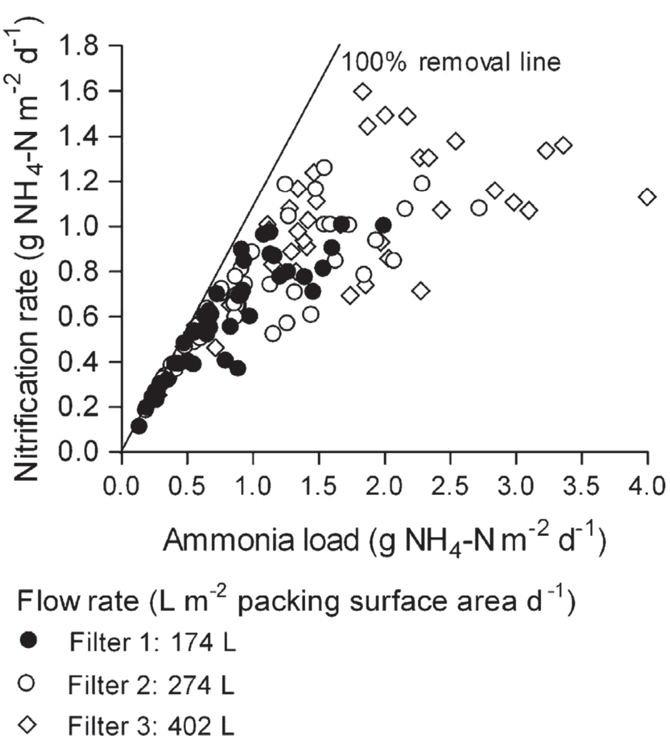

Figure 14. Nitrification rate as a function of ammonium load at three different hydraulic loading rates in a nitrifying trickling filter. Inlet ammonium concentrations were 4.1 ± 2.2 mg NH_4-N L^{-1} (van den Akker et al., 2008).

Another important conclusion is that these filters have additional ammonium removal capacity well beyond their normal loading rates. The pilot column was able to remove ammonium to below Danish drinking water guidelines at 5 times the average ammonium loading rate observed under reference operating conditions (Lee et al., I). The extra capacity of these filters gives confidence that despite sudden and large shifts in ammonium loading rates, they can still produce water that meets or exceeds water quality guideline values.

3.4 Backwash

Backwashing is a major operational parameter that is necessary in these filters. Backwashing removes particles and biomass that accumulate over a filter run and is necessary to reduce head loss and prevent water quality from deteriorating. Although the effects of backwashing on biological filters used to remove BOM has been studied extensively (Ahmad and Amirtharajah, 1998; Ahmad et al., 1998; Emelko et al., 2006; Goldgrabe et al., 1993; Hozalski and Bouwer, 1998; Miltner et al., 1995), the effects of backwashing on nitrification is less well known. What is known, is that the effects of backwashing on nitrification is complicated and dependent on many parameters including filter media (Kihn et al., 2002), temperature (A. Andersson et al., 2001), filter type (De Vet et al., 2009), bed expansion (Laurent et al., 2003) and if air is used in the backwash (Li et al., 2011). This is illustrated in Figure 15 (Laurent et al., 2003), which shows the varying effects backwashing has on ammonium removal in columns operated at different temperatures and using different filter material. Backwashing has been reported to both improve nitrification, by removing flocks and particles that cause a diffusion barrier (Niquette et al., 1998), and decrease nitrification by removing nitrifying biomass (Laurent et al., 2003).

What is clear is that current research on backwashing biological filters has shown mixed results both in terms of its effects on BOM removal and nitrification. Table 3 is used to get an overview of the current research, including types of systems, scales of investigations, and major findings. There are several items missing from our current understanding on the effects of backwashing on nitrification. Currently most backwashing research on nitrification indirectly quantifies nitrifying biomass (ammonium plus nitrite oxidizers) and/or nitrification using lab essays that do not take filter hydraulics or biofilm density into account. Most research also focuses on surface water, which can have high variations in temperature and substrate loading rates. This thesis shows the effects of backwashing on groundwater filters under both steady state and increased ammonium loading conditions by quantifying both AOB and AOA while relating the nitrification to insitu filter measurements. This high level of analysis is necessary to better understand and operate these filters to ensure safe drinking water.

3.4.1 Backwashing under steady state conditions

The effects of backwashing on both ammonium removal and AOB were examined under both steady state (reference operating conditions), and increased ammonium loading conditions. Under steady state conditions, backwashing had little effect on ammonium removal (Figure 16B). Backwashing also had little effect on AOB (Figure 16A) with only 9% of the AOB being removed by backwashing, after a 23 day filter run (Lee et al., III). The low removal of AOB is likely due to the very low ammonium loads applied to the filters under reference operating conditions, which limits biomass growth. These results also show that the biomass that is there is well established on the filter material, and not easily removed by backwashing.

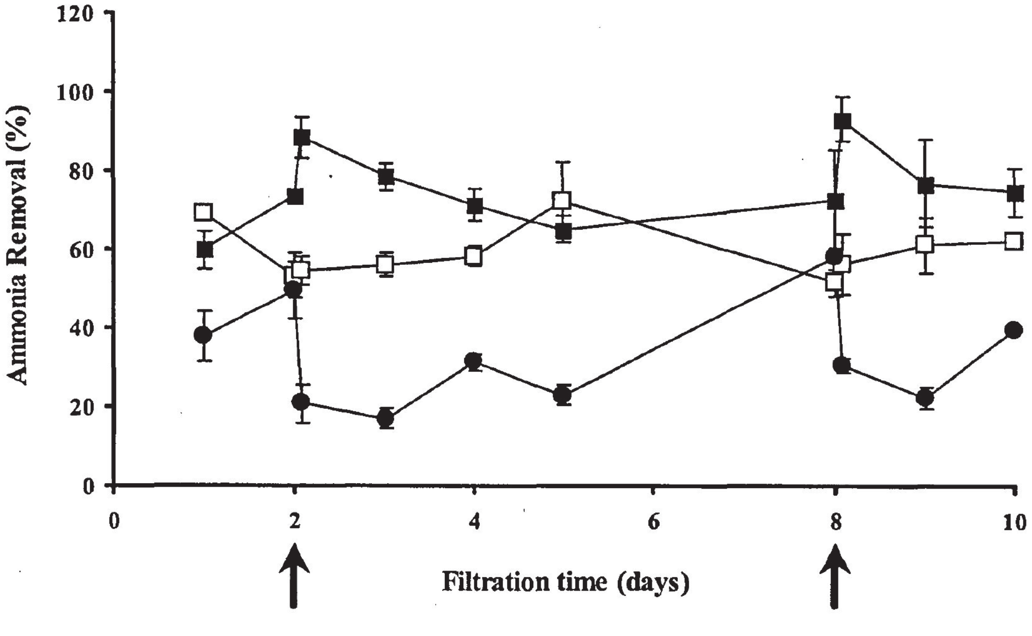

Figure 15. The effects of backwashing on 3 different pilot filters. Pilot filter 1 (filled squares) is operated at temperatures of 18.1 °C and has an open structure GAC as filter material. Pilot filter 2 (open squares) was operated at 18.1 °C and used closed structure GAC as filter material. Pilot filter 3 (filled circles), was operated at 4 °C and used open structure GAC. Black arrows indicates time of backwash (Laurent et al., 2003).

Table 3. Information on the effects of backwashing in biological drinking water filters used to remove organics and/or ammonium.

Author	System	Hydraulic loading rate/filter run time	Backwash procedure	Method to quantify biomass	Water quality parameters	Major findings
Laurent et al. (2003)[1]	Primary pilot and full scale secondary GAC rapid sand filters fed with surface water: Pilot: 30 cm depth open and closed GAC. Full: Open and closed GAC	Pilot: 5 m/h. Full: 3.9-5 m/h 1-3 weeks	Pilot: 70 m/h air for 2-3 min; low expansion of 25-30% with 20-25 min water at ~25 m/h. One high BW at 40 m/h with 60% bed expansion. Full: air at 30 m/h for 3 min, air and water (20 m/h), water for 15 min at 40% bed expansion (ClO2 water)	Used Kihn et al. (2000) method to estimate Potential nitrifying activity (PNA) to estimate total nitrifying biomass (Nitrite and ammonium oxidizers)	High organics: 4-10 mg C/L Low alkalinity: 20-50 mg CaCO3/L Large temperature fluctuations: 1-28 °C. Pilot: 0.55-0.42 mg NH4-N/L Full: 0.02-0.15 mg NH4-N/L	BW had greater effect on primary filters. In primary pilot columns, BW improved NH4 removal in warm water for open GAC and decreased removal in closed GAC. At colder temperatures (< 4 °C), BW decreased nitrification capacity in open GAC. In full scale secondary filters, BW had slight negative effect at 8 and 12 °C. The overall biomass remained unchanged. Biomass redistributed at high expansion BW, but not low expansion. Better to use non-chlorinate BW water.
De vet et al. (2011)[1]	Full scale trickling filters fed with ground water. One is sub-surface aerated. 2 m depth of filter sand.	2.2 m/h 48h	Low flow of water with air followed by higher flow of water	qPCR to quantify AOB and AOA using amoA at two depths; 0-50 cm and 100-150 cm	See de Vet et al. (2009) for more info. pH 7.67 ± 0.13, temperature 11.6 ± 0.3 °C. NH4-N, 1.7- ~5 mg NH4-N/L	Proper BW can prevent a thick biofilm and inorganic deposits, which can limit substrate and nutrient diffusion. Poor nitrification not caused by excessive detachment or washout of AOP.
De vet et al. (2009)[1]	Two full scale trickling filters fed with ground water. Dual media filter: 1 m anthracite, and 1 m of sand. Subsurface aerated filter: 2 m of sand	2.2 m/h NA	In subsurface aeration, filter is BW for long periods at 4 m/h and air scour.	NA	Dual media: Iron, 5.4 mg/L. HCO3, 229 mg/L, TOC 2.7 mg C/L, Temperature, 12 °C. pH, 7.24. 4.5 mg NH4-N/L. Subsurface aerated: Iron, 3.6 mg/L. HCO3, 229 mg/L. TOC, 2.2 mg C/L. pH, 7.33. 1.7 mg NH4-N/L	For efficient deposit removal, a minimum 10% bed expansion is required. Dual media BW with expansion or subsurface aeration can prevent loss of nitrification
Madoni et al. (2001)[1]	Full scale biological rapid sand filter treating ground water. Primary filter: 3 m sand. Secondary filter: 3 m GAC	8.5 m/h 24-42 h	BW for 21 minutes	Volatile solids as an indicator of biomass	Iron, 1.48 mg/L. Temp, 13 °C. pH, 7.2. P, 0.12 mg/L. 1.56 mg NH4/L	GAC might provide better protection for microorganisms during BW.

Author	System	Hydraulic loading rate/filter run time	Backwash procedure	Method to quantify biomass	Water quality parameters	Major findings
Niquette et al. (1998)[1] [2]	4 pilot filters. Pretreated river water (coag/floc) followed by ozone. Filter 1 and 2: 2m GAC over 20 cm sand. Filter 3: 2m anthracite over 20 cm sand. Filter 4: 2.1 m GAC	10 m/h 36-48 h	Sand had bed expansion of 20% while GAC & anthracite had 40% bed expansion. All had air scour (2.5 min total) with 30 sec high rate water BW at 30 m/h (GAC/sand) and 80 m/h (anthracite/sand). All had 15 min. normal water flow of 25 m/h except anthracite/sand had 55 m/h.	^{14}C glucose respiration potential	Temp, 5-14 C. DOC 2.9-3.3 mg C/L.	In general, organics and ammonium removal were not affected by BW. Particles and flocks may hinder BOM in GAC layer by 1) precipitated metals on activity, 2) shortening contact time due to channelling, and 3) creation of a diffusion barrier. In warm water, BW improved BOM removal in top layer and in cold water improved deeper in the filter.
Goldgrabe et al. (1993)[2]	8 pilot columns fed with transported river water.	4.9 m/h NA	NA	Biomass measure with phospholipid technique developed by Findley et al. (1989)	Temp, 12-29 °C. TOC, 7.0-11.4 mg TOC/L. pH, 7.23-8.49.	BW with chlorinated water inhibits biomass growth. Biomass in Non-chlorinated water did not change after BW. Head loss in Biological filter greater and reached acceptable head earlier
Emelko et al. (2006)[2]	4 filters. 2 filters with 137 cm GAC over 30 cm sand: 2 filters of 122 cm anthracite over 30 cm of sand. Fed with treated surface water (coag/floc/ ozone/filter)	4.2-5.8 m/h ~15-24 h	3 BW procedures. 1) air scour then low rate water at 10.7 m/h, high rate at 42.7 m/h 2). No air scour, low flow water followed by high rate (same as 1). 3.) "collapse pulsing", then sub fluidization wash with air (7.3 m/h).	Phospholipid method by Findlay and colleagues (1989)	Temp, 11 (1-23 °C). TOC 6 (5-7 mg/L). pH, 8.2 (7.9-8.4). Alkalinity, 4.2 meq/L HCO3	BOM removal was not affected by the different BW conditions, although conventional filtration was. Collapse pulsing significantly decreased filter run times. More biomass without air scour, but not as active. At low temps, BW without air scour had lower oxalate removal than with air scour.
Bhargava and Ojha (1989)[2]	Pre-treated river water (Coag/floc) followed by ozonation.	NA	NA	NA	NA	Rules of thumb are often used when deciding when to BW. BW is done based on acceptable head loss, decrease in flow rate, or turbidity breakthrough, although it is usually a predetermined time for operator convenience.

Author	System	Hydraulic loading rate/filter run time	Backwash procedure	Method to quantify biomass	Water quality parameters	Major findings
Ahmad et al. (1998)^2	5 pilot filters with 0.6 m anthracite on top of 0.3 m sand. Pre-treated river water (coag/floc) followed by ozone for filters 2-5	10 m/h 24 h	Filter 2 (non-chlorinated BW water) and 5 (chlorinated BW water): Air scour (sub fluidized water flow with collapse pulsing), followed by 25% bed expansion. Filter 3: Water only at bed expansion of 15, 25, and 50%.	NA	Mean AOC, 0.11 mg C/L.	A major concern of air scour BW is that it may decrease biological performance by removing to much biomass. AOC removal did not change after BW with air scour and no significant difference in AOC removal between air scour and non air scour BW. Chlorinated BW water decreases biological performance.
Ahmad and Amirtharajah (1998)^2	Bench scale filters with 3.8 cm diameter with 0.6 m GAC on top of 0.3 m sand. Fed with raw and settled river water	5 m/h 48 h	BW at 10 and 60% bed expansion. Collapsed pulsing air scour for 3 min (0.9 m/min air and 12.5 m/h water) followed by 10 min of water only BW.	HPC on filter media	Temp, 21 °C. NPOC, 2.1-2.5 mg C/L.	Biomass has higher adhesion strength than non-biological particles. BW should not be so efficient that it removes the necessary biomass. No results were given on the effects of BW on biological performance.
Hozalski and Bouwer (1998)^2	Bench scale filters 31 cm bed height of either glass beads or sand. Fed with enriched DI water.	2.4 m/h 48 h	BW with water only for 10 min. at flows of 23-53 m/h (15-50% bed expansion)	NA	Temp, 22.5 °C. pH, 7.2. TOC, ~2.25 mg TOC/L	BW removed between 20-40% of biomass, and filter material and bed expansion did not significantly affect the bacteria or TOC removal.
Miltner et al. (1995)^2	2 columns with anthracite over sand, fed with transported river water	4.9 m/h ~4 days	BW for 10 minutes at bed expansion of 50%	Biomass measure with phospholipid technique developed by Findley et al. (1989)	Temp, 12-29 °C. TOC, 7.0-11.4 mg TOC/L. pH, 7.23-8.49.	BW with non-chlorinated water had no effect on organics removal, while BW with chlorinated water decreased biomass an average of 22% and decreased organics removal. BW with chlorinated water also decreased overall biomass.

BW: Backwash

NA: Not analyzed or reported

1: Filters used for ammonium removal

2: Filters used for organics removal

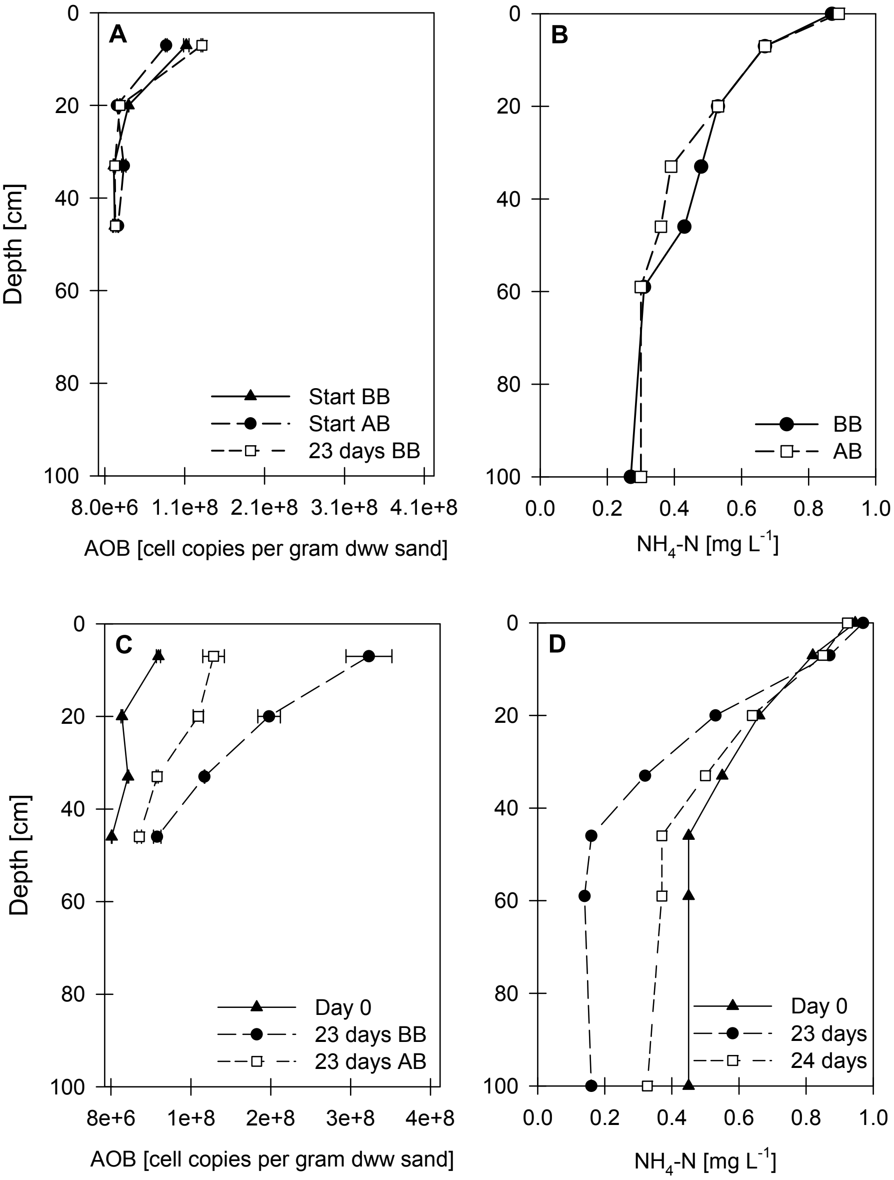

Figure 16. (A) AOB depth profiles in pilot filter 1, during the start of the short term loading experiments before backwashing (BB), after backwashing (AB), and at the end of a 23 day filter run. (B) NH_4-N depth profiles in pilot filter 1 under reference operating (steady state) conditions. (C) Depth profiles of AOB at the start, after 23 days BB, and 23 days after backwashing in pilot filter 2 during the continuously increased ammonium loading experiments. (D) NH_4-N depth profiles during the same continuously increased loading experiments at the start, after 23 days (BB), and at 24 days (1 day AB) (Parts of figures taken from Lee et al., II and Lee et al., III)..

3.4.2 Backwashing under increased loading conditions

Under continuously increased ammonium loads in pilot filter 2, both the ammonium removal and AOB increased after 23 days (Figure 16C and D). Unlike the effects of backwashing at steady state though, much of the increase in ammonium removal and AOB was removed with backwashing (Lee et al., III). The newly formed biomass is likely not well established on the filter media and therefore was easily removed by backwashing. Ammonium removal increased over the subsequent 22 day filter run, and although backwashing did decrease the removal slightly, the pilot filter was still able to remove ammonium to below detectable levels (Lee et al., (III). To assess the stability of nitrification in the filter, an intense backwash was applied 4 days later where the time for air and water in the filter was doubled. Again, the ammonium removal rates declined slightly, but ammonium was still removed to below detectable limits by a depth of 46 cm, implying that the AOB can quickly become established on the filter material.

3.4.3 Filter run length

The length of a filter run is generally based on acceptable head loss, effluent turbidity, or for simplicity, time (Tekerlekopoulou et al., 2013). During a 23 day filter run under reference operating conditions, the maximum ammonium removal capacity of pilot filter 1 did not change.

During both the steady state and increased ammonium loading experiments the effluent turbidity remained below 0.03 NTU, and head loss less was than 0.7 m which is the acceptable head loss in the full scale filters (Lee et al., III). The head loss in these filters was likely contributed to iron flocks, while operating at steady state, and increased biomass, while operating with increased loads. This is an important finding because it shows that filters with low ammonium (or other substrate) and iron (or particle) loads can be operated for extended periods without backwashing, and without deteriorating water quality.

3.5 Overall effect of operating conditions on nitrification

Under reference operating conditions, these filters were found to be robust, with increased ammonium removal capacity well beyond the normal ammonium loading rates (Lee et al. I). The filters saw very little variation in removal capacity over long filter runs and backwashing had little effect on the both nitrification and ammonium oxidizing biomass (Lee et al., III). With increased ammonium loads, the initial backwash greatly reduced nitrification and nitrifying biomass, but subsequent backwashes, including an ‘intense backwash’ had little effect on nitrification, showing that these filters can quickly adapt and operate under increased ammonium loads. These results are important in showing what could be expected under different loading and operating conditions. This increased understanding of the effects of various operating conditions on nitrification can be used to better design and operate these systems.

4 Nutrient limitations on nitrification

In addition to ammonium, nitrifying biomass need sufficient carbon, oxygen, and phosphorous, as well as certain trace elements (micronutrients), for activity and growth. Phosphorous is essential to all microorganisms and is needed in the synthesis of DNA and RNA, and in the production of adenosine triphosphate (ATP) (Westheimer, 1987). Microorganisms usually take up phosphorous in the form of phosphate (PO_4). Micronutrients are generally needed in much smaller quantities, but are still essential to cellular function (Madigan and Martinko, 2008).

Most research examining the effects of phosphorous limitation on nitrification in drinking water filters, has primarily considered nitrification problems caused by low temperatures (Aa et al., 2002; L. Kors et al., 1998; Yoshizaki and Ozaki, 1993). Other recent studies have examined phosphorous limitations in BAF pilot columns (Lytle et al., 2013) and using batch experiments (de Vet et al., 2012). All these studies, except the batch experiments, focus on aqueous inlet and outlet measurements (the black box approach). What is missing is a comprehensive understanding of the processes occurring inside the filter. This includes, not just ammonium removal, but also the effects of phosphorous limitations on the growth and activity of nitrifying organisms.

4.1 Phosphorus limitations under increased ammonium loads

Under reference operating conditions, inlet phosphorous concentrations to the secondary filters (and pilot columns) at Islevbro was low, at 11 ± 4 µg L^{-1}, and no phosphorous was observed to be removed. Although, these phosphorous levels were enough to support proper nitrification at the low ammonium loading rates observed under reference operating conditions, they could be limiting growth and activity with increased ammonium loads. Continuously increased ammonium loading experiments were done both with and without phosphorous addition to determine if phosphorous could be limiting the growth or activity of nitrifiers in these filters.

4.1.1 Phosphorus limiting ammonium oxidation

Inlet ammonium concentrations in column 2 were increased from 0.01 to 0.90 mg NH_4-N L^{-1} for a total of 50 days. The column was initially operated for 33 days under low phosphorous conditions (6 ± 2 µg L^{-1}) to determine the effects of increased loads in a phosphorous limiting environment. Even under low phosphorous conditions, the increased ammonium loads increased the ammonium removal capacity of the pilot filter, although there was a large initial delay of 9 days. The total ammonium removal capacity of the filter increased from 2.8 to 4.5 g NH_4-N m^{-3} h^{-1} over 23 days, (Lee et al., II).

What was not expected was where the ammonium removal increased in the filter. Ammonium removal rates slightly decreased in the top 7 cm of the filter going from 7.2 to 5.6 g NH_4-N m^{-3} h^{-1} (Figure 17A). The ammonium removal rates at depths of 7-20 cm and 20-33 cm approximately doubled in the same 23 days, with little change happening at the lower depths. This was especially surprising because there was 60% more AOB at a depth of 7 cm than 20 cm (Figure 18A).

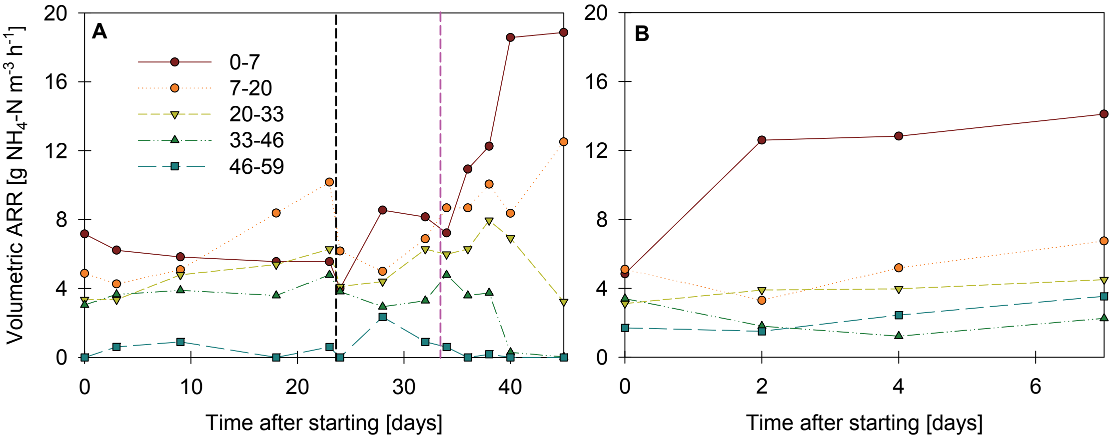

Figure 17. Volumetric ammonium removal rates (ARR) at various depths in (A) Column 2 during continuously increased ammonium experiments. The black dashed line shows when the column was backwashed on day 23, 45, and 49, and the pink line shows when phosphorous addition was started on day 33. (B) Column 1 during continuously increased ammonium loading with phosphorous addition. Black dashed lines show when the column was backwashed on day 7 and day 11 (Taken from Lee et al., II).

After starting phosphorous addition on day 33, the ammonium removal capacity in the filter quickly increased, and within 3 days ammonium was removed to below detectable limits (Lee et al., (II)). The ammonium removal rate increased rapidly in the top 7 cm of the filter, more than doubling in 7 days, going from 8.2 to 18.6 g NH_4-N m^{-3} h^{-1} (Figure 17). De Vet et al., (2012), reported slow but steady growth of AOB at low phosphorus concentrations in batch experiments, and that most of the cells were inactive at large populations. The rapid and large increase at the top of the filter, where the highest densities of AOB were, suggests that the activity of the ammonium oxidizers was limited by phosphorus.

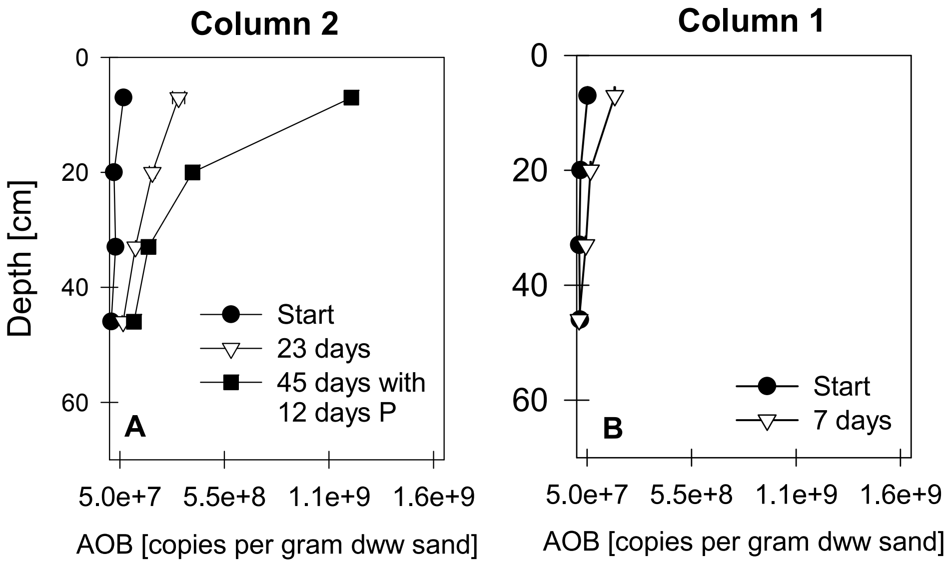

Figure 18. (A) Depth profiles of AOB in column 2 at the start, after 23 days, and after 45 days (with 12 days of phosphorous addition) on continuous increased ammonium loads. (B) Depth profiles of AOB in column one at the start of a filter run and after 7 days of continuously increased ammonium and phosphorous loads. (Taken from Lee et al., II).

In pilot filter 1, continuously increased ammonium loads were started while simultaneously starting phosphorus addition. An almost immediate increase in ammonium removal was observed with removal increasing almost exclusively in the top 7 cm of the filter (Figure 17B), where AOB concentrations were highest (Figure 18B). Within 7 days of increased ammonium loads with phosphorus addition, pilot filter 1 had approximately the same ammonium removal capacity (4.3 g NH_4-N m^{-3} h^{-1}) as pilot column 2 achieved after 23 days without phosphorus addition (4.5 g NH_4-N m^{-3} h^{-1}). Pilot filter 1, also had much less AOB than filter 2 (Figure 17). The rapid increase at the top of the filter, suggests phosphorous was limiting the activity of ammonium oxidizers. These results go beyond the black box approach used by others and show how phosphorous addition can be beneficial in increasing nitrification and nitrifying biomass in these filters.

4.1.2 Phosphorus limitations on nitrite oxidation

It is also important to consider effluent nitrite concentrations in these filters, since nitrite is a toxic oxidation product of ammonium oxidizers. Effluent nitrite concentrations, in pilot filter 2, were consistently higher than the Danish drinking water guideline value of 0.003 mg NO_2-N L^{-1}, until shortly after phosphorous was added on day 33 (Figure 19A). Effluent nitrite concentrations rapidly decreased from 0.023 to 0.005 mg NO_2-N L^{-1} within 3 days of starting phosphorous addition, and decreased to 0.003 mg NO_2-N L^{-1} 4 days later. Although *Nitrospira* increased at all depths in the filter, both before and after phosphorous addition, the sudden increase in nitrite removal suggests phosphorus additions increased activity (Lee et al., II).

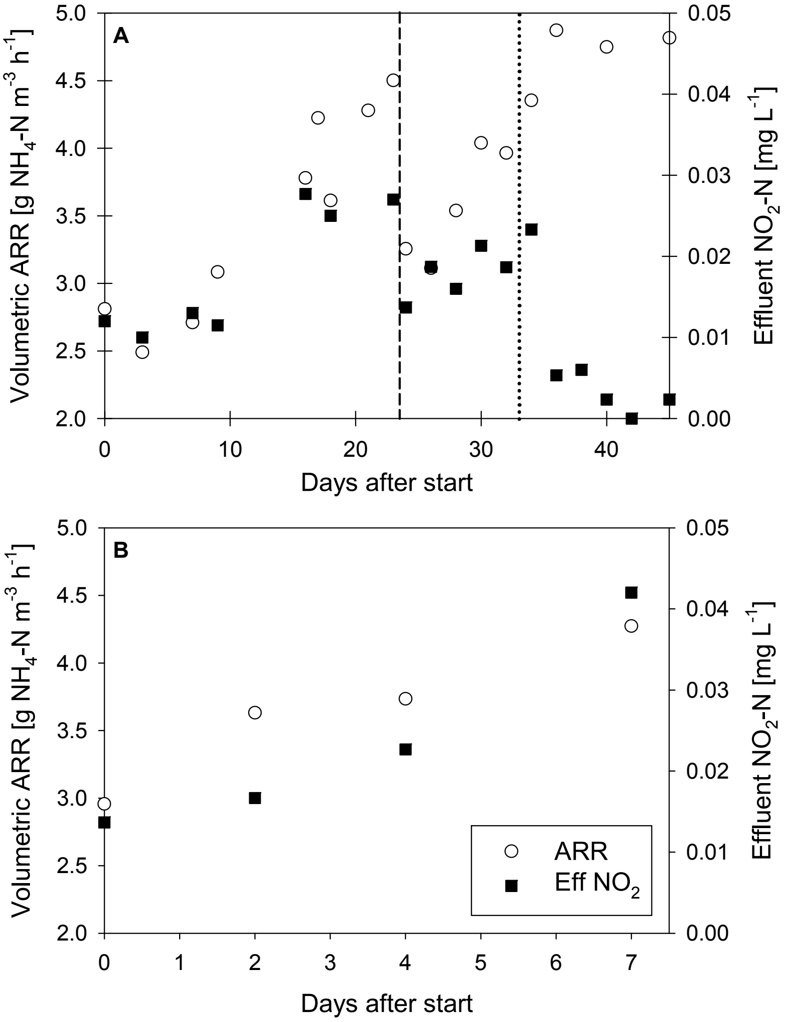

Figure 19. Volumetric ammonium removal rates (ARR) and effluent nitrite concentrations during continuously increased ammonium loads for (A) pilot filter 2, where the dashed lines show when the column was backwashed on day 23 and the dotted line shows when phosphorous was added on day 33, and for (B) pilot filter 1, where ammonium and phosphorous were continuously added from day 0 (taken from Lee et al., II).

In pilot filter 1, where phosphorous addition was started at the same time as the continuously increased ammonium loads, effluent nitrite concentrations increased to 0.042 mg NO_2-N L^{-1} (Figure 19B). There were no large changes in *Nitrospira* at any of the measured depths (Figure 20D). Because nitrification is a sequential process, the increase in effluent nitrite is likely due to the increase in ammonium oxidation, with not enough time for the nitrite oxidizers to be established at the proper depths. This is observed in the nitrite depth profiles in column 1 (Figure 20), which show that nitrite is not sufficiently removed in the bottom of the filter. In pilot filter 2, there was already a large growth of *Nitrospira* throughout the depth of the column during the increased ammonium loads without phosphorous addition (Figure 20B). The nitrite depth profiles for column 2 show that after phosphorous addition was started, there is increased nitrite removal in the lower depths of the column (Figure 20A). The sudden increase in nitrite removal, after phosphorous addition, implies that the activity of the nitrite oxidizers was also limited by a lack of phosphorous.

Phosphorous addition could be an easy and inexpensive solution to nitrification problems that occur in these filters, and could be used as a tool to rapidly increase nitrification during periods of increased ammonium loads. One of the challenges that remain though is in determining if a poorly performing biological drinking water filter is limited by phosphorous or possibly other nutrients.

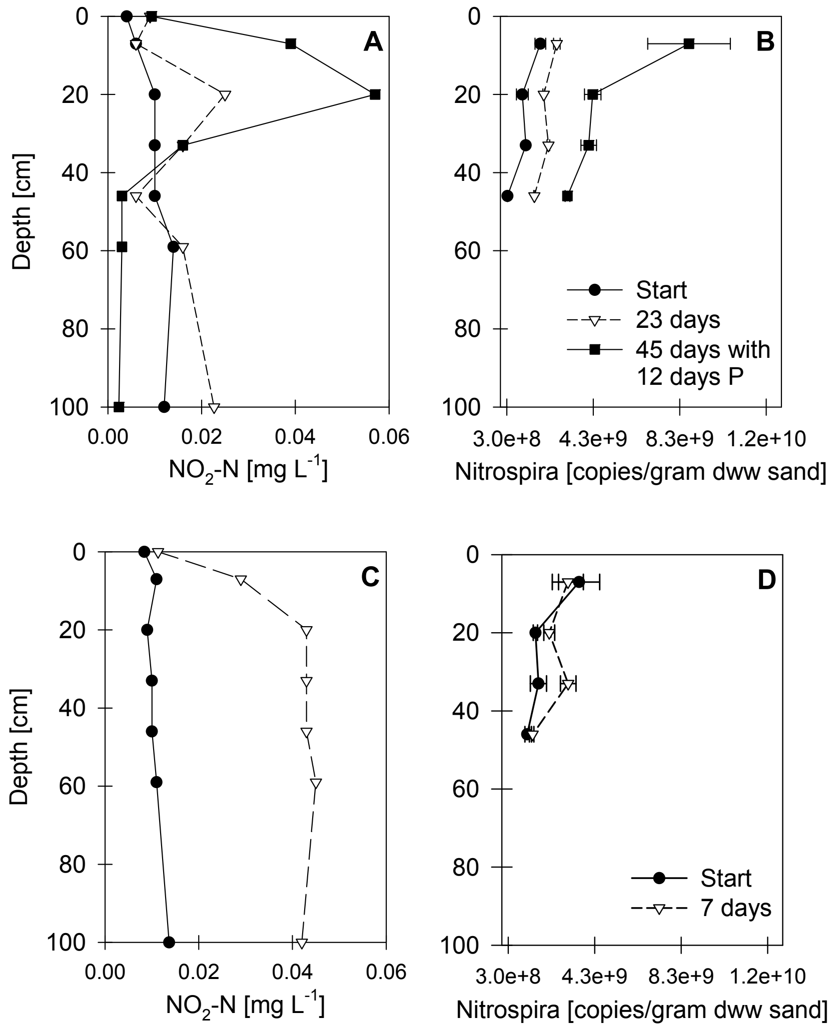

Figure 20. (A) Nitrite and (B) *Nitrospira* depth profiles in column 2 at the start, after 23 days, and after 45 days (with 12 days of phosphorous addition) of continuously increased ammonium loads. (C) Nitrite and (B) *Nitrospira* depth profiles in column 1 at the start and after 7 days of continuously increased ammonium loads with phosphorus addition (Taken from Lee et al., II).

4.2 Determining nutrient limitations in filters with poor nitrification

In Denmark, 20% of water works have trouble complying with ammonium regulatory guidelines (Jupiter, 2014). One problem is that it is often expensive and time consuming to diagnose and fix these problems. Continuous flow lab columns, developed by Tatari et al. (2013), were used to determine if poor nitrification in these filters could be caused by phosphorus or other nutrient limitations. This method is less costly than full or pilot scale experiments, and results can be obtained rapidly. In all experiments, 4 columns were operated in parallel with all columns using effluent from the respective water works with increased ammonium concentrations. Column 1, the control, was operated with ammonium only, column 2 was supplemented with micronutrients, column 3 was supplemented with phosphorous, and column 4 was supplemented with phosphorous and micronutrients.

Two Danish water works with existing ammonium removal problems, Langerød and Nærum water works, were examined using the lab columns. Information about the water works, including water characteristics, operating conditions, and treatment trains, is discussed in Section 2.8. The most notable differences in treatment processes at the examined water works is that Langerød has a secondary filter and Nærum only has a primary filter Figure 6. Because of this, the iron loadings on the filters are considerably different. At Langerød, inlet iron concentrations to secondary filter are 0.14 mg L^{-1}, while at Nærum inlet iron concentrations are 2.5 mg L^{-1}. Inlet phosphorous (measured as phosphate) to both filters is low at 0.03 (Langerød) 0.02 mg PO_4-P L^{-1} (Nærum). Iron could have a significant impact on the availability of phosphorous and other micronutrients (de Vet et al., 2011) and therefore is an important factor when troubleshooting nitrification problems in these filters.

Sand from two different depths, 0-10 cm and 30 cm, was examined at Langerød. There was no substantial difference in ammonium removal in the top 10 cm of the column. At 30 cm, the columns supplemented with micronutrients had removal rates 28% and 24% higher than the columns not supplemented with micronutrients (Wagner 2013). This suggests that at the lower depths of the filter, the lack of available micronutrients could be limiting nitrification. The necessary nutrients might be consumed at the top of the filter, leaving little available for nitrifiers at lower depths.

At Nærum water works, the lab columns were used to investigate the top 10 cm of the filter. The columns supplemented with phosphorous had ammonium removal rates 79% and 73% higher than those not supplemented with phosphorus (Wagner 2013). Nærum water works has high iron loadings onto the filter, which could be limiting phosphorous availability to the nitrifiers due to absorption or co-precipitation of phosphorous to iron (de Vet et al., 2012; Lijklema, 1980).

Both the results from Nærum and Langerød, imply that phosphorous or other micronutrients are responsible for the poor nitrification observed in these filters. Further pilot column experiments also support that phosphorus was limiting nitrification at Nærum water works (Wagner 2013), and full scale supplementation of a micronutrient verified that this was indeed the case at Langerød waterworks.

4.3 Increasing nitrification performance with nutrient supplementation

The results reported in Lee et al. (II), have important implications in biological drinking water treatment. Although phosphorous addition may not fix nitrification problems in all filters, it is a solution that could be easy to implement, and can increase drinking water quality and safety. The flow through lab scale columns designed by Tatari et al. (2012), have been adapted to diagnose phosphorus and other possible micronutrient limitations in these filters (Wagner 2013), and can be a valuable diagnostic tool that is rapid, inexpensive, and effective.

5 Nitrifying community

Nitrification is a biological process that is controlled by both the quantity and activity of nitrifying organisms (Tränckner et al., 2008). Although much is known about the nitrifying community in wastewater systems, there is less known about nitrifiers in drinking water filters, and how operating and loading conditions affect these organisms.

Several studies have estimated total nitrifying biomass (ammonium and nitrite oxidizers together) in drinking water filters, based on activity measurements (a Andersson et al., 2001; Kihn et al., 2000; A. Kihn et al., 2002; Laurent et al., 2003; Tränckner et al., 2008). Although this can be a useful, they do not give any information on the abundance and activity of the nitrifying organisms responsible for nitrification, and do not account for biofilm structure or mass transfer, which is important when determining activity.

Recent research has used qPCR to directly quantify nitrifying organisms on sand in these filters (de Vet et al., 2011; Kasuga et al., 2010; Niu et al., 2013). Direct quantification of the different genera of nitrifiers, provides better insight into nitrification in these filters, and improves our understanding of the major microbial contributors to nitrification. What is missing from the current body of knowledge is how the nitrifiers change with depth, time, and varying operating conditions, and how this relates to nitrification performance. This study utilizes direct quantification methods to characterize the density of different genera of nitrifying organisms at scales that are comparable to the full scale filters, and at a high resolution with depth. These results provide a more detailed description of the relationship between some of the major operating conditions controlling nitrification and how it affects the microorganisms responsible for nitrification.

5.1 Relative abundance of AOA and AOB

Until recently, it was thought that AOB were mainly responsible for ammonium oxidation, although recent discoveries have also shown certain archaea can also oxidize ammonium to nitrite (Könneke et al., 2005). Since then AOA have been reported in biological drinking water filters and in distribution systems, although their total contribution to ammonium oxidation is still undetermined (de Vet et al., 2011; Kasuga et al., 2010; Niu et al., 2013; van der Wielen et al., 2009).

In drinking water filters with high ammonium loads (1 to 6 mg NH_4-N L^{-1}), AOA have been found to only provide a minimal contribution to ammonium oxidation (de Vet et al., 2011). White et al. (2012) found no AOA in biological drinking water filters with average inlet ammonium concentrations of 1.1 mg NH_4-N L^{-1}, and concluded that AOB are the dominant ammonium oxidizers in their system. AOA are thought to have a very high affinity for ammonium compared with AOB (Martens-Habbena et al., 2009) and could be inhibited at higher ammonium concentrations (Prosser and Nicol, 2012). In systems with very low ammonium loads (< 0.1 mg NH_4-N L^{-1}), AOA, rather than AOB, are thought to be the dominant ammonium oxidizers (Kasuga et al., 2010; Niu et al., 2013).

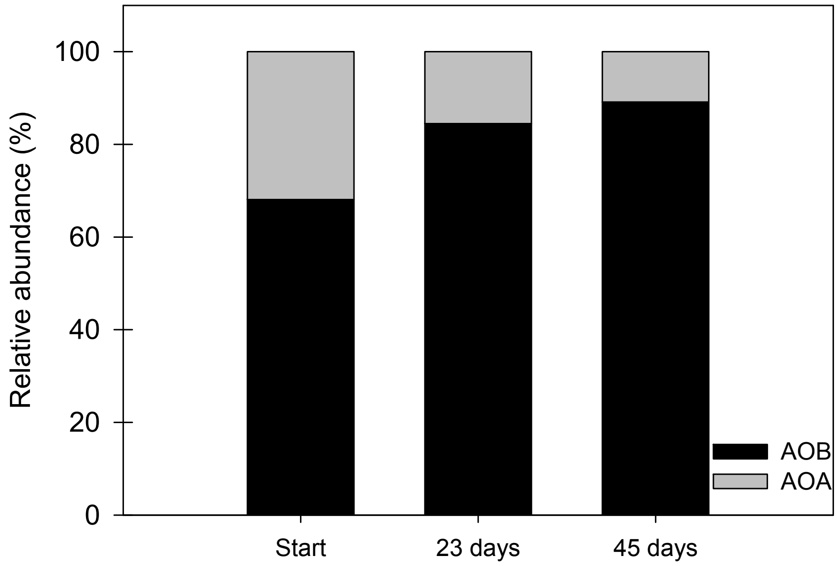

Figure 21. Relative abundance of AOB and AOA in pilot column 2 during the continuously increased ammonium loading experiments.

At Islevbro water works, the average ammonium concentration to the filters was low at 0.1 mg NH_4-N L^{-1}, and AOA and AOB were found to be approximately equally abundant. Under reference operating conditions, AOB made up 60% and 69% of the ammonium oxidizing community of pilot filters 1 and 2. Under the increased loading experiment, the fraction of AOB increased to 84% in 23 days, and 89% after 45 days of increased ammonium loads (Figure 21). Although AOA made up a considerable fraction of the ammonium oxidizing population in these filters, the cell specific rates for AOB have been reported to be 1 to 2 orders of magnitude greater than AOA (Prosser and

Nicol, 2012; Tatari, 2014). Based on the assumption that the cell specific rates for AOA are 10 times less than AOB, we assume that the contribution to ammonium oxidation by AOA is minor, and they are therefore not considered in the presented results.

5.2 Relationship between AOB and ammonium removal

Under reference operating conditions in pilot filter 1, a strong relationship was observed between the maximum ammonium removal rates, and the number of AOB (Figure 22A). This suggests that under low ammonium loading conditions, ammonium removal is controlled by the quantity of AOB. The cell specific ammonium oxidation rate was $0.6 \pm 0.2 \times 10^2$ fg NH_4-N h^{-1} $cell^{-1}$ and varied little with depth as seen in the low standard deviation (Lee et al., I). Cell specific rates were determined by division of the volumetric ammonium removal rate by the number of AOB cell copies per volume. During the continuously increased ammonium loads in pilot filter 2, the cell specific ammonium oxidation rate decreases, despite an overall increase in ammonium removal. The rates decrease from $0.9 \pm 0.1 \times 10^2$, at start of the experiments, to $0.2 \pm 0.06 \times 10^2$ fg NH_4-N h^{-1} $cell^{-1}$, after 23 days. They continue to decrease to $0.1 \pm .04 \times 10^2$ fg NH_4-N h^{-1} $cell^{-1}$ after 45 days of increased loads with 12 days of phosphorous addition (Figure 22B). Under reference operating conditions, the biofilms were most likely thin. With increased ammonium loads, the biofilms got denser, which would increase mass transfer resistance, and therefore decrease the cell specific rates.

The phosphorous results presented earlier also suggest that the rates were also limited by a lack of available phosphorous during the first 23 days of increased ammonium loads. The rates are within the range reported by others for nitrifying trickling filters (0.02 to 5×10^2 fg NH_4-N h^{-1} $cell^{-1}$, (de Vet et al., 2011)), and in continuous and batch reactors (0.1 to 3.2×10^2 fg NH_4-N h^{-1} (Prosser I., 1989)). The results are important in showing that increased biomass does not necessarily provide the most efficient ammonium removal, and that the cell specific rates in an operating filter may vary greatly depending on how the filters are loaded. The rates reported here was determined insitu, and account for hydraulic and loading conditions that are generally not accounted for in batch experiments.

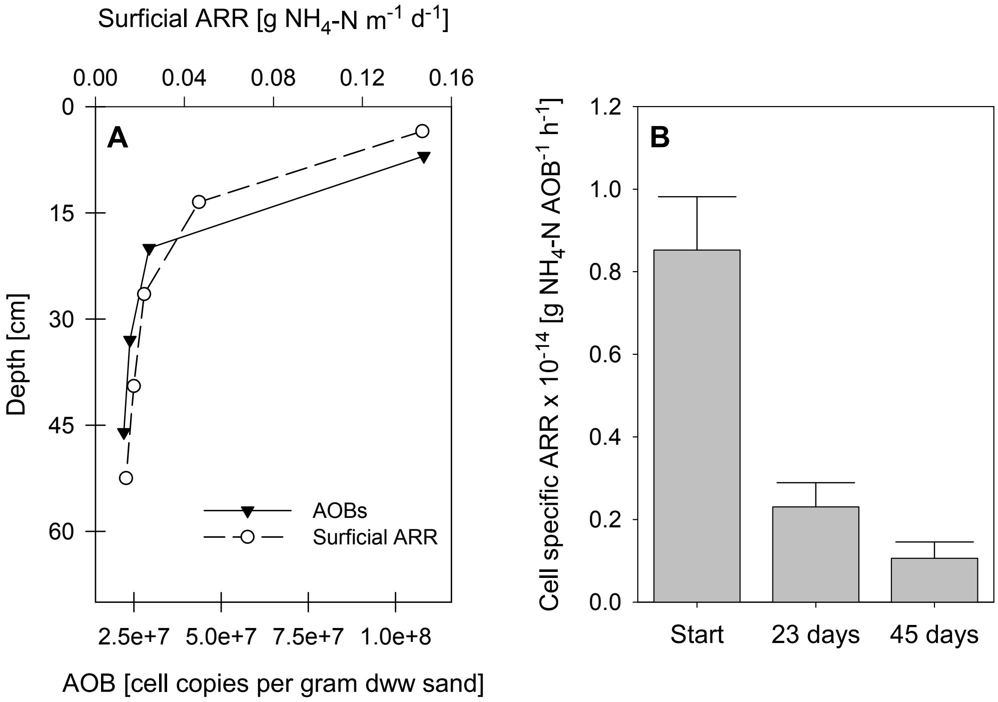

Figure 22. (A) Average surficial removal rates and AOB with depth in pilot filter 1, during the short term loading experiments (Lee et al., I). (B) Cell specific ammonium removal rates (ARR) in pilot filter 2 during the continuous increased loading experiments, at the start, after 23 days, and after 45 days (with 12 days phosphorous addition).

5.3 Nitrite oxidizers

Two different NOB are thought to be responsible for nitrite oxidation in these systems, *Nitrospira* and *Nitrobacter*. It was previously thought that *Nitrobacter* were mainly responsible for nitrite oxidation (Bock and Koops, 1992), although recent research has shown the importance of *Nitrospira* in many engineered systems. *Nitrospira* were found to be the dominant nitrite oxidizers in our filters, being between 3, to more than 4 orders of magnitude more abundant than *Nitrobacter* (Lee et al., II). *Nitrospira* have been found to be the dominant nitrite oxidizer in several biological drinking water filters (de Vet et al., 2011, 2009; Feng et al., 2012; Qin et al., 2007; White et al., 2012) and in drinking water distribution systems (Martiny et al., 2005). At low nitrite concentrations, such as those typical of drinking water systems, *Nitrospira* are expected to dominate due to their higher affinity for oxygen and nitrite (Manser et al., 2005; Schramm et al., 1999).

5.4 Relative abundance

Although AOB made up only 0.2% to 6.5% of the Eubacteria in the pilot filters, *Nitrospira* composed from 11% to 49% of the Eubacteria (Lee et al., II). Other systems have also observed *Nitrospira* to be from one to two orders of magnitude more abundant than AOB (Cébron and Garnier, 2005; Schramm et al., 1999). Feng et al. (2012) also observed a much larger community of *Nitrospira* compared with the AOB, *Nitrosomonas*, in drinking water filters, with *Nitrosomonas* accounting for less than 1% of the total bacteria, and *Nitrospira* accounting for 17%.

The relative abundance of both AOB and *Nitrospira* to Eubacteria, increased with increased ammonium loads and was generally highest at the top of the filters. At the start of the increased loads in column 2, the percentage of N*itrospira* to Eubacteria was 20% ± 6%, which increased to 27% ± 1% after 23 days of increased ammonium loads and continued to increase to 41% ± 6% after 45 days with 12 days of phosphorous addition (Lee et al., II). The mean increase in Eubacteria on day 45 was 139% ± 40%, but when factoring out the contribution of N*itrospira* the increase drops to 61% ± 24% and when also factoring out AOB the increase drops to 53% ± 27%. Before increasing ammonium loads approximately 20% of the total bacteria were composed of nitrifying bacteria, which slightly increased to approximately 29% after 23 days of increased ammonium loads and increased to 45% after 45 days with 12 days of PO4 addition. The percentage of AOB to Eubacteria also increased with increased ammonium loads going from an average 0.6% ± 0.3% at the start, to 3.4% ± 2.4% after 45 days of increased loads. The increase was especially noticeable at the top of the filter where the percentage of AOB to Eubacteria increased from 0.9% to 6.5% after 45 days.

The increase in ammonium was the only change to substrate loading during these experiments. The large increase in both the measured AOB and *Nitrospira* after increasing the ammonium loads show both their importance to nitrification in these systems, and how these filters can shift in biological makeup under different loading conditions. These results further our understanding of the importance of AOB, and the nitrite oxidizing bacteria, *Nitrospira*, under both low and increased ammonium loading conditions, and show how different loading regimes can change the composition of the microbiology in these filters.

6 Conclusions

Nitrification in biological drinking water filters is an important process used to remove ammonium from drinking water. Despite its widespread use, there is still a lack of scientific understanding concerning nitrification in drinking water filters, which can lead to improper design and operation of these filters, causing elevated ammonium and nitrite levels above safety guideline values. The results presented in this thesis show the relationship between operating conditions and ammonium removal as well as how different substrate and nutrient loadings can affect nitrification performance and biomass. More specifically, the main conclusions of this work are:

- Pilot scale filters can accurately mimic full scale filter performance in terms of biological and particle removal. Caution should be taken in the set up and operation of the pilot filters and confirmation should be done to verify filter performance.
- Despite the normally low ammonium loading rates observed under reference operating conditions, the pilot filters were able to remove ammonium at 5 times the average ammonium loading rate loads observed under reference operating conditions, showing that these filters have extra capacity that can handle sudden and large changes in ammonium loads.
- Under reference operating conditions (low ammonium loads), ammonium removal in this filter was found to be dependent on total ammonium load, and not influent concentration or hydraulic loading rate individually. The density of AOB was closely linked to ammonium removal suggesting that AOB density set the observed removal rates.
- Backwashing the filters had little effect on both nitrification and nitrifying biomass under steady state conditions. At increased ammonium loads, backwashing initially removed much of the newly developed nitrification capacity and biomass. Subsequent backwashes slightly decreased nitrification, but to much less extent. Backwashing also had little effect on the relative abundance of AOB to AOA.
- Phosphorous addition produced an almost immediate increase in nitrification, specifically at the top of the filter, indicating that low phosphorous availability was limiting nitrifying activity.

- Lab scale flow through columns were successfully used to determine if phosphorous and other micronutrients caused poor nitrification in two different water works.

- The relative abundance of AOB to AOA were similar under low ammonium loading conditions (0.1 mg NH_4-N L^{-1}), although the low cell specific rates of AOA, based on the current knowledge, might suggest there contribution to ammonium oxidation in our filters is minimal. After 45 days of increased ammonium loads, the relative abundance of AOB to AOA increased to 90%, suggesting that AOB are the dominant ammonium oxidizers at higher ammonium loads.

- *Nitrospira* was found to be the dominant nitrite oxidizer in these filters, and the relative abundance of AOB and *Nitrospira* to Eubacteria, increased with increased ammonium loads, suggesting their importance for nitrification in these systems.

7 Perspectives

The knowledge gained from this study can be directly applied to improve operational procedures for rapid sand filters, and can also be used to assist in troubleshooting poorly performing filters. One of the important conclusions of this work, was that these filters can safely remove ammonium to very low levels, despite sudden and drastic changes in inlet ammonium concentrations and hydraulic loading rates (Lee et al., I). Another conclusion of this work is that under steady state conditions, these filters could be operated with long filter runs, and that backwashing was found to have minimal effect on nitrification and nitrifying biomass (Lee et al., III). Caution should be taken when transferring these results to other systems though, as other factors such as iron and ammonium loading rates as well as other influent water parameters, can contribute to nitrification performance.

The secondary filters at Islevbro water works is characterized by low organics removal and low ammonium, particle, and iron loads. High organics loading can cause increased competition for space and other nutrients, which could decrease nitrification (Rittmann et al., 2002). Filters operating under increased iron or particle loadings could experience channelling or diffusion limitations (Niquette et al., 1998), and high iron loads can also lead to nutrient limitations (de Vet et al., 2011). High iron loads increased ammonium in the effluent at Islevbro water works, but we do not know why. The effect of high iron loads on nitrification in these filters is still unknown and is an area that should be researched further.

One of the obvious benefits to the depth profiles used in this thesis is that these can give a more detailed description of the processes occurring inside the filter. One of the findings in our research was that ammonium removal in the Islevbro filters was stratified with the highest amount of ammonium removal occurring at the top of the filter (Lee et al., I). This coincided with the highest density of AOB. This stratification in both ammonium removal and AOB was not removed during backwashing, both under low and increased ammonium loading rates. The stratification was likely due to the build-up of precipitates on the filter material, which leaves the filter material stratified with larger, less dense material at the top of the filter. It is unknown as to whether this stratification is actually beneficial to nitrification performance in these filters, and future studies could focus on this area as it could be an important aspect when designing and operating these filters.

Phosphorus addition was found to be useful for increasing nitrification under increased ammonium loads and the addition of nutrients could be a useful tool for increasing the biological performance in these filters (Lee et al., II). Flow through lab scale columns showed that nitrification problems in two poorly performing filters were caused by nutrient limitations, possibly due to the interference of iron from the source water (Wagner et al., 2013). This further illustrates the need for further research on the interactions between iron, nutrients, and nitrification.

Although dosing nutrients to poorly performing filters could increase nitrification performance, the interactions with iron could make it difficult to supply these nutrients. Iron coagulants are often used as a tool to remove phosphorous from water, and phosphorous supplementation could increase flocks in the filter, causing increased head loss and actually decreasing performance. Future research should focus on determining if poor nitrification is caused by nutrient limitation, and whether dosing techniques can be implemented to supply the required nutrients in an efficient manner that does not compromise filter performance. Other research could consider optimizing filter design, for example by using pre-filters for source waters that are affected by nutrient limitations caused by high iron concentrations.

This work showed that AOA and AOB were approximately equally abundant under low ammonium loading conditions, and that AOB increased in abundance with increased ammonium loads (Lee et al. II). However, current knowledge about the microbial communities in different biological rapid sand filters is still lacking, and more research is still needed. Although not covered in this thesis, mathematical modelling is a useful tool in the design and operation of these filters. Currently, there are mathematical models that describe nitrification in these systems (Queinnec et al., 2006; Rittmann et al., 2002), although there is still room for improvement. What is lacking in current models is the ability to incorporate phosphorous and other nutrient limitations into both the growth and activity of the nitrifying biomass. Nitrification models should also be able to accurately describe nitrification under various loading conditions. The cell specific nitrification rate was found to decrease with increasing loads in these filters. Models describing nitrification in these filters should be able to incorporate this into them to accurately predict the changes in both nitrification and nitrifying biomass, under various loading conditions.

The start-up time of these filters is a serious concern, as nitrification has been shown to take up to several months to become established in these filters (Lytle et al., 2007). This can be problematic as the water treated during this period may have to be wasted if it does not meet regulatory requirements. There could be several opportunities to increase the efficiency of nitrification in these filters during the startup period. This would include nutrient supplementation and optimization of the backwash during the startup period. The results presented here showed that under non-steady state conditions (increased ammonium loading), biomass and nitrification decreased after backwashing (Lee et al. III). Optimizing both the filter run length and backwash procedure during a filter's start up, could be beneficial in establishing and maintaining the required biomass needed for proper nitrification. Nutrient supplementation could also be a way of ensuring the microbial community is optimally growing and active, which could also greatly decrease start up time.

There are many treatment processes that can be used to remove ammonium from drinking water, although most are either energy intensive, or rely on chemical addition which can lead to unwanted byproducts. Nitrification in biological rapid sand filters offers a simple, safe, and effective way to remove ammonium from drinking water, without the drawbacks associated with other technologies. Current water works, using rapid sand filters, can be easily converted to biological rapid sand filters by simply stopping pre-chlorination before filtration. Increased understanding into the factors effecting nitrification in these filters is needed in the design, operation, and troubleshooting of these filters to ensure that they are robust and able to continuously meet drinking water guidelines. The results presented in this thesis have shown how both nitrification and nitrifying biomass are effected under various loading and operating conditions and offer much needed insight into the processes controlling nitrification in these filters.

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I Lee, C. O., Boe-Hansen, R., Musovic, S., Smets, B., Albrechtsen, H.-J., Binning, P., 2014. Effects of dynamic operating conditions on nitrification in biological rapid sand filters for drinking water treatment. Water Research. 64C, 226–236.

II Lee, C. O., Smets, B., Albrechtsen, H.-J., Binning, P. Increasing nitrification in biological rapid sand filters used for drinking water: The positive effects of phosphorous addition. Submitted to Water Research.

III Lee, C. O., Smets, B., Albrechtsen, H.-J., Binning, P. Impact of backwashing on nitrification at different loading conditions in biological rapid sand filters. Submitted to Water Research.

In this online version of the thesis, the papers 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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The Department of Environmental Engineering (DTU Environment) conducts science-based engineering research within four sections:
Water Resources Engineering, Urban Water Engineering,
Residual Resource Engineering and Environmental Chemistry & Microbiology.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

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