Inhibition of Nitrification in Industrial Wastewater
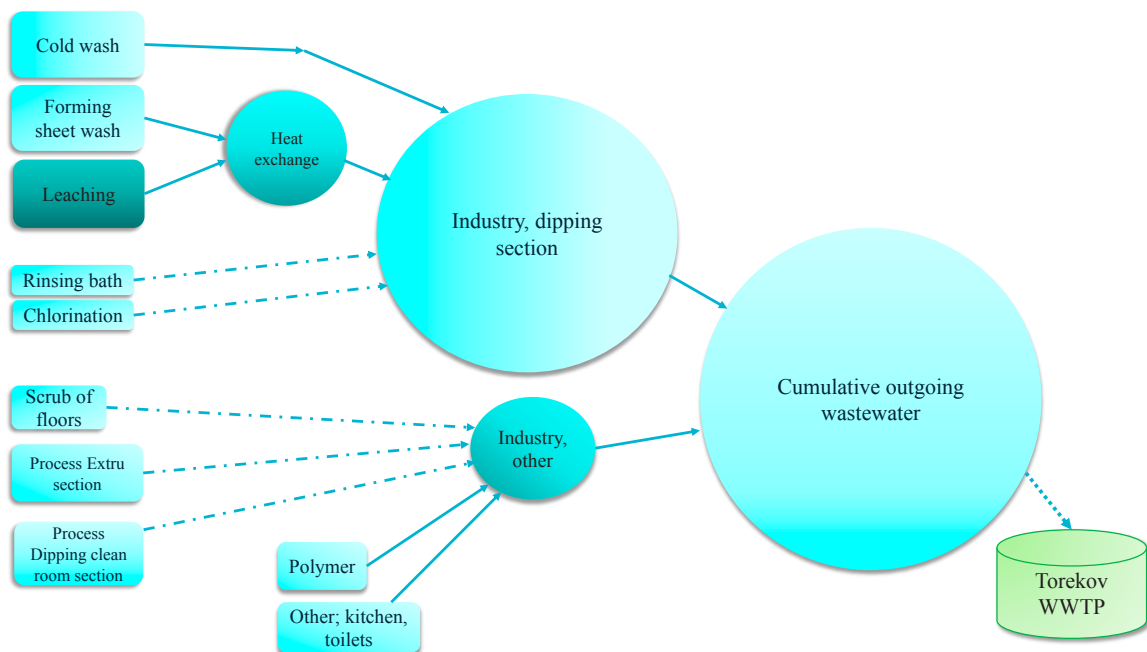## – Identification of Sources

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Inhibition of Nitrification in Industrial Wastewater
– Identification of Sources

by

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Figure on front page: Flows of process waters at Nolato MediTor.

Preface

This master thesis is carried out on request from the company Nolato MediTor, who needed to investigate their wastewater. It has been a learning journey with a lot of communication, questions and concern. We think we speak for all parts involved in this study when we say it was very exciting! The thesis can be of interest to people working with problems related to inhibition of nitrification, and those that are quite interested in the environment and industrial wastewater. To work with a real case and real WW have let us understand that there is (often) no easy answers. Wastewater and sludge can both be tricky to study and to understand, but most of all interesting. We would like to thank all personnel at the chemical engineering department, whom have been very kind and have helped us with all different kinds of questions. The time at the department during this project have felt like a smooth start of going into the "real" working life of engineering. It feels good! Special thanks to Karin Jönsson, our supervisor at LTH, for being an exceptional sounding board and a great source of knowledge in the science of inhibition of nitrification. Our supervisors at Nolato MediTor Jimmy Nilsson and Kristian Sandberg, thank you for answering our many questions about the industry, chemicals and wastewater streams; without your answers, we would not have been able to conclude much at all.

We are especially thankful for all help from Gertrud Person, the safety representative. Also, a lot of thanks to Klagshamn WWTP, and especially Ivelina Dimitrova, for welcoming us at the WWTP and for helping us to collect activated sludge, the very necessary material to perform our investigations.

Thanks to Herje Schagerlöf for providing us with insight in which methods that can be used to measure concentrations of the different compounds studied and to Frank Lipnizki for sharing his knowledge in membrane filtration. Thanks to Jes de la Cour Jansen for giving us feedback regarding our work on additivity and statistical analysis, and for sharing his knowledge in the field of nitrification inhibition. Thanks to Maja Ekblad for your help regarding writing of the report and the spectrophotometer used in the lab. Thank you Camilla Roslander, process manager at NSVA and Jan-Erik Petersson, specialist at wastewater treatment at NSVA, whom have been a nice contact from the municipal side of the problem and thank you for welcoming us at a study visit. Finally, thanks to Åsa Davidsson for being the examiner of our master thesis, and not to forget, the very first person who introduced us to this exciting detective work!

Pleasant reading.

II

Abstract

The discharge of industrial wastewaters (WWs) to wastewater treatment plants (WWTPs) can be problematic due to their sometimes elevated concentrations of toxic compounds compared to municipal wastewater. Also, industrial WWs often contain compounds that are unique to the specific production, and the receiving WWTP is not always designed to remove these compounds. Hence, there is a risk that these compounds pass through the WWTP untreated. In addition, they tend to accumulate in the sludge, decreasing its quality and suitability as fertilizer. These compounds can also disturb the biological processes utilized at the treatment plant, making the removal of nutrients as for instance nitrogen perform worse. A consequence of this can be that elevated concentrations of these nutrients are discharged from the WWTP, ultimately causing eutrophication and oxygen depletion in the receiving water body.

In this study, the inhibitory effect on the nitrification process by industrial WW from the company Nolato MediTor was investigated. Nitrification is a process commonly used at WWTPs to remove nitrogen, and it is used at the WWTP receiving WW from Nolato MediTor. Previous measurements have shown that the WW is inhibitory to the nitrification process; particularly the leaching WW have given high inhibition of nitrification. In this study, the investigation was taken one step further in an attempt to distinguish what is causing the inhibition and the fluctuations of inhibition in different internal streams at the industry.

Laboratory tests with activated sludge were set up in order to mimic the nitrification process as carried out at WWTPs, and the inhibition among the different streams of WW were studied. In addition, solutions of single compounds were prepared and the inhibition in those were compared to the inhibition in the streams of industrial WW to estimate the individual contribution from several chemical components used at the industry. Analyses were performed regarding composition of nitrate, nitrite, ammonium and chemical oxygen demand (COD) in the test substances. Finally, the combined and separate effects from nitrate and nitrite, which are both present in the industrial WW in comparatively high concentrations, were studied in an additivity test in order to find out if the inhibitions from these compounds can be summed in order to estimate the total inhibition, or, alternatively, if they counteract or amplify the inhibition of one another, called antagonism and synergism, respectively. The standard test was further modified so that it applies to the non-linear dependence of inhibition on concentration of nitrate and nitrite, and the compounds were found to act additive at the low concentrations studied, while they tended towards antagonism at higher concentrations.

IV

Sammanfattning

Utsläpp av industriellt avloppsvatten till avloppsreningsverk kan medföra problem då det ofta innehåller förhöjda koncentrationer av toxiska ämnen jämfört med avloppsvatten från hushåll. Industriellt avloppsvatten innehåller även ofta kemiska ämnen som är specifika för produktionen i fråga. Det mottagande reningsverket är inte alltid utformat för att rena dessa ämnen, vilket gör att de riskerar att passera verket utan att renas. Utöver detta tenderar dessa ämnen att ackumuleras i slammet, vilket försämrar dess kvalitet och minskar dess lämplighet att användas som gödningsmedel. Dessa ämnen kan även störa de biologiska processerna som utförs på reningsverket och hämma reningen av näringsämnen som till exempel kväve. En konsekvens kan bli att förhöjda koncentrationer av näringsämnen släpps ut från reningsverket som i sin tur leder till övergödning och syrebrist i mottagande vatten.

I detta examensarbete har nitrifikationshämningen i industriellt avloppsvatten från företaget Nolato MediTor utretts. Nitrifikation är en vanlig reningsmetod för att ta bort kväve och den används på det reningsverk som tar emot avloppsvatten från Nolato MediTor. I tidigare studier har industrivattnet visat sig vara hämmande för nitrifikationsprocessen, och speciellt lakvattnet som innehåller mycket kemikalier var nitrifikationshämmande. I den här studien har undersökningen utökats till att utreda orsaken till hämningen, hur den är kopplad till användningen av olika kemikalier samt variationen av hämning i de olika delströmmarna i fabriken.

Försök i laboratorium med aktivt slam sattes upp för att simulera nitrifikationsprocessen som den utförs på avloppsreningsverk, och hämningen i prover från de olika strömmarna med avloppsvattnen studerades. Även rena kemikalier som används i produktionen testades i hämningsförsök och hämningen av dem jämfördes sedan med den i avloppsvattnet för att uppskatta bidraget till den totala hämningen från dessa kemikalier. Analyser av nitrat, nitrit, ammonium och kemisk syreförbrukning (COD) utfördes också på testsubstanserna. Slutligen studerades den kombinerade och de separata hämningseffekterna från nitrat och nitrit, vilka båda finns i jämförbart höga koncentrationer i det industriella avloppsvattnet, i ett additivitetstest för att ta reda på om de individuella hämningarna kan summeras för att uppskatta den totala hämningen från dessa ämnen, eller alternativt, om de motverkar eller förstärker varandras hämning, känt som antagonism och synergism. Standardtestet var vidare modifierat för att passa det icke-linjära sambandet mellan hämning och koncentration av nitrat och nitrit, och ämnena visade sig vara additiva vid de låga koncentrationerna som studerades, medan de tenderade mot antagonism vid högre koncentrationer.

Abbreviations

AOB	Ammonia oxidizing bacteria
AUR	Ammonia uptake rate
COD	Chemical oxygen demand
EC50	Half effective concentration; concentration that causes half of the maximum response effect under a given period of time
IC50	The concentration of an inhibitor where the response is reduced by half
NO$_x$	Oxidized nitrogen ($NO_2 + NO_3$)
NOB	Nitrite oxidizing bacteria
LC50	Half lethal concentration; the concentration that is required to kill half of the test organisms under a given period of time
L(E)C50	Half lethal (Effective) concentration
LOD	Limit of detection
OUR	Oxygen uptake rate
sOUR	Specific oxygen uptake rate
PNEC	Predicted no effect concentration
SS	Suspended solids
TN	Total nitrogen
TP	Total phosphorus
VSS	Volatile suspended solids
WW	Wastewater
WWTP	Wastewater treatment plant

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1. Introduction

One of today's environmental challenges is the eutrophication of surface waters. Eutrophication is caused by an oversupply of nutrients, leading to increase of primary production of biomass, algal blooms and decreased oxygen supply at the bottom among other issues. Nitrogen is one of the key nutrients for biological growth, but when the concentration have gotten too high for the ecosystems to handle, then it becomes a pollutant. Therefore nitrogen is commonly treated in wastewater treatment plants (WWTPs). Discharges containing nitrogen from agriculture, industries and domestic areas, together with nitrogen from the atmosphere, are all leading to eutrophication of the seas (Swedish EPA, 2016a). To reach the state of no eutrophication is one of the national environmental quality objectives in Sweden, adopted by the Swedish Parliament (Swedish EPA, 2016b).

1.1 Nitrogen removal in wastewater treatment

Nitrogen removal is obligated at Swedish WWTPs taking care of wastewater (WW) from more than 10 000 persons and whose emissions reach the coast between Norrtälje and Strömstad (Svenskt Vatten, 2016). A common method to remove nitrogen is nitrification and denitrification; where ammonium is oxidized to nitrate (nitrification) followed by conversion into nitrogen gas (denitrification) (Hammer and Hammer, 2011). Nitrification and denitrification are performed by bacteria in activated sludge, and as all living organisms they have different requirements on environmental, physical and chemical conditions. Many chemical substances and mixtures are toxic to bacteria if they are present in too high concentrations and can cause a decrease of the biological process. As the nitrifying bacteria are most sensitive to toxic chemicals, they are often used as test organisms to detect chemicals in toxic concentrations. The toxicity to nitrifying bacteria can be measured by inhibition of nitrification, which has been observed at many WWTPs in Sweden (Jönsson, 2001). To reduce the impact on the nitrification process at municipal WWTPs that receive industrial WW, industrial WWs that are too toxic are not accepted.

1.2 Problem description

In this master thesis the inhibition of nitrification in the WW from Nolato MediTor was investigated, as the inhibition in some previous measurements have exceeded the maximum accepted level stated by the municipality (NSVA, 2011). Nolato MediTor AB is an industrial company producing medical plastic products, such as anaesthesia breathing bags and catheter latex balloons. The process waters from Nolato MediTor are discharged for treatment at the municipal WWTP in Torekov, and contain toxic compounds causing inhibition of nitrification (Malmqvist, 2013). The WWTP has earlier observed problems with inhibition of nitrification, and suspected WW from Nolato MediTor as a probable cause. The requirement on inhibition of nitrification is less than 10% inhibition at 20% dilution of the WW, which has to be met before the end of 2017. To map out the nitrification inhibition at the industry, a programme was carried out to identify sources in the different process waters (Sandberg, 2016). In earlier measurements high values of inhibition of nitrification were confirmed and the study pointed out the latex dipping process as the most likely cause. Treatment with UV was tested for the leaching WW, without substantial results. Further analysis of the WW, identification of the source(s) of the inhibition, and if necessary, a treatment to reduce the toxicity has to be done to meet the requirement of less than 10% inhibition of nitrification.

1.3 Aim

The main aim with this project has been to identify causes to the nitrification inhibition observed at the outlet of the industry. Results and methods have been going hand in hand, as one result led to the next test set up. To come to a conclusion, the following research questions were addressed:

- What inhibition of nitrification can be expected from the outgoing WW from Nolato MediTor and in partial process WWs?
- What are the sources of the inhibition?
- Are the concentrations of nitrate and nitrite in the outgoing WW high enough to cause inhibition?
- Is the inhibition by nitrate and nitrite additive?
- What are the concentrations of the production chemicals Tamol, WAQ, SMO and Emulan that can be leached out in the worst-case scenario?
- How inhibitory are these production chemicals in that concentration range?
- With respect to inhibition of nitrification, should the WW be treated?

1.4 Limitations

To determine the scope of this study, some limitations had to be outlined. The perspective was to get a good understanding of the whole system and an overview of the causes of the inhibition of nitrification. Solutions of how to detoxify the industrial process water before it is discharged to the WWTP could have been suggested, but it was not possible within the frame of this study. The chemical composition of the WW is not known, and to find methods to identify the components and determine their individual concentrations would be a master thesis in itself. However, to know the chemical composition could have been very helpful when investigating the inhibition of nitrification. Due to the limit of time, the report does not include a deeper literature review of the toxicity of each production chemical. Likewise, characterization of the toxic chemicals in terms of how they interact with organisms is not included. This could have been done to create a more complete picture of the toxicity of the WW.

2. Literature review

In this chapter, background information is presented. A description of the nitrification process and the different ways it can be inhibited is given. The company Nolato MediTor will be described, and the previous findings on inhibition of nitrification at the industry, as well as a data analysis on their WW and toxic chemicals used in the production.

2.1 The nitrification process

In biological WW treatment, bacteria can perform nitrification, if the right conditions are applied. The nitrification reaction can be divided into two steps and is described hereunder; reaction 1 describes the overall reaction while reactions 2 and 3 describes the two partial steps. In reaction 1 ammonium is oxidized to nitrate, and in reaction 2 and 3 the intermediate product nitrite is formed and further oxidized to nitrate. The chemical reaction formula of nitrifying biomass growth can be seen in reaction 4. AOB (ammonia oxidizing bacteria) and NOB (nitrite oxidizing bacteria) are in principle the two groups of nitrifying bacteria, also called nitrifiers. They are autotrophic, as they oxidize inorganic nitrogen and use it as an energy source (Halling-Sørensen and Jørgensen, 1993).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{1}$$

$$NH_4^+ + 1,5O_2 \rightarrow NO_2^- \tag{2}$$

$$NO_2^- + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{3}$$

$$NH_4^+ + 1.38\,O_2 + 1.98\,HCO_3^- \rightarrow$$

$$0.021\,C_5H_7NO_2 + 0.98\,NO_3^- + 1.041\,H_2O + 1.88\,H_2CO_3 \tag{4}$$
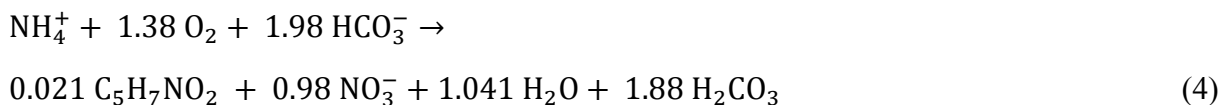
(Halling-Sørensen and Jørgensen, 1993)

In biological WW treatment, it can in a simplified way be said that the available substrate (ammonium) and the contact time with the substrate determine the growth of the nitrifiers. The nitrifiers are growing more slowly and are more sensitive to toxic compounds compared to heterotrophic bacteria, making them more vulnerable to disturbances at WWTPs (Blum and Speece, 1991). Heterotrophs and nitrifiers are competing in the same sludge reactor for oxygen, nutrients and space. To avoid outcompeting the nitrifiers, the chemical oxygen demand (COD) has to be balanced by sufficient nitrogen, and the sludge age should be high enough to maintain the nitrifying population (Hammer and Hammer, 2011).

2.1.1 Factors affecting nitrification
The nitrification rate is determined by a number of factors that are affecting the environment for the microorganisms (Halling-Sørensen and Jørgensen, 1993). The kinetics of the nitrification process can be expressed in concentration of ammonia (which is in equilibrium with ammonium), temperature, pH, organic content and dissolved oxygen concentration. As the ammonium is the substrate, it must be available in sufficient amounts. In a study by Shammas (1986) it was found that the nitrification rate was almost eliminated at low concentrations of ammonia. The concentration of dissolved oxygen is also important for the growth of the nitrifiers, as it is used in the synthesis. The effect of oxygen concentration on nitrification rate has been studied by Stenström and Song (1991) using mathematical models, and the general relation is that the more oxygen there is, the higher the nitrification rate. In the same study it

was found that the effects of oxygen concentration on the nitrification are also depending on the oxygen transportation. Under steady state conditions the nitrification limiting dissolved oxygen concentration lies between 0.5 to 2.5 mg/l, and for shock loads of organic matter 4 mg/l dissolved oxygen or less can be limiting for nitrification. In practice it is desirable to achieve a high nitrification rate to the lowest possible cost, and it has to be controlled for each case. The dissolved oxygen concentration is usually kept higher than 2 mg/l in conventional WW treatment, to prevent oxygen depletion (Wilén, 2010).

The temperature is of great influence on the growth of the nitrifiers, with its optimum at around 30°C although the optimum is likely a plateau between 15°C and 35°C (Halling-Sørensen and Jørgensen, 1993). Furthermore the pH optimum for nitrification has been investigated in several studies, and one author found it to be 8.4 (Wild *et al.*, 1971). In more general terms the range of optimum pH is between 8.0 and 8.4 according to Gray (2004). The pH is changing due to the nitrification reaction itself, and the chemical reaction formula of nitrifying biomass growth can be seen in reaction 2. As the ammonium is oxidized, dissolved bicarbonate is consumed, also called alkalinity, which makes the pH drop. Halling-Sørensen and Jørgensen (1993) concluded that the more ammonium that is consumed, the lower the pH will be at the end of the reaction. It was found that this could be counteracted by aeration to strip off carbon dioxide which is part of the equilibrium with water and carbonic acid (H_2CO_3).

Naturally, the biomass of the nitrifying bacteria is affecting the nitrification rate. According to a study made by Wild *et al.* (1971), the amount of nitrifiers is directly proportional to the time required for nitrification. In a test performed to investigate interactions of temperature, pH and biomass on the nitrification rate, activated sludge with nitrifying biota was developed, and the concentration of MLVSS (mixed liquor volatile suspended solids) was considered as the biomass. The study could conclude that the concentration of MLVSS was significantly interacting with pH and temperature so that it was influencing how much the pH and temperature affect the nitrification. A higher MLVSS gave a stronger influence of both pH and temperature. On the other hand, no interaction between pH and temperature was found, the studied concentrations of MLVSS were 430, 1200 and 3200 mg/l (Shammas, 1986).

2.2 Inhibition of nitrification

In this chapter some compounds present in the WW from Nolato MediTor that are suspected to have inhibitory effects are described. Not all of these compounds have been measured before, making it difficult to predict the extent of inhibitory effects among those. First, a short insight of different modes of action of inhibition are presented, followed by an explanation of inhibition caused by metals. Among metals, zinc is presented to a greater extent as zinc is used in significant amounts in the process at the industry. Inhibition of nitrification caused by nitrate, nitrite and ammonia are also described below, as well as the influence on inhibition of nitrification due to the type of sludge.

Inhibitory compounds can alter the nitrification process through different modes of action. The type of inhibition can be divided into two categories, reversible and irreversible inhibition. Reversible inhibitors binds to an enzyme only when the equilibrium between bound and dissolved forms of the inhibitor allows. This means that when the inhibitor is removed from the solution, the inhibitory effect will disappear as the inhibitor dissociates from the enzyme. In contrast, irreversible inhibitors bind covalently to an enzyme, altering its function permanently. As a result, shock loads of irreversible inhibitors cause a more persistent effect than those of reversible inhibitors (Jönsson, 2001).

Reversible inhibitors can in turn be divided into two categories, competitive and non-competitive inhibitors. Competitive inhibitors bind to the same active site of the enzyme as the substrate, depressing the catalytic activity of the organism. As a result, the inhibitory effect is depending on the relative concentrations of substrate and inhibitory compound rather than the concentration of inhibitory compound itself. A non-competitive inhibitor instead binds to a site on the enzyme other than the active site. Hence, it does not compete with the substrate for access to the active site, but affects the activity of the enzyme in other ways (Jönsson, 2001).

The inhibition of nitrification can be related to the toxicity of the substance, or the substances in the mixture. Acute toxicity was tested by the European Centre for Ecotoxicity and Toxicology of Chemicals (ECETOC) and analyzed in the Technical Report No 80 (ECETOC, 2001). In this report, it is stated that the toxicity of a mixture of substances can be either additive, synergistic or antagonistic. A conclusion that could be drawn was that, generally, mixtures of substances had an additive acute toxicity, for substances that were chemically related or had the same mode of action. This was not true for mixtures including metals, whom were less predictable in their behavior. ECETOC also concluded that generalizations are not possible to make about chronic toxicity of metal containing mixtures. When it comes to toxicity of industrial discharge, the compositional and temporal variability makes the effects difficult to predict.

2.2.1 Industrial wastewater and inhibition of nitrification
Inhibition of nitrification at WWTPs is often suspected to be caused by industrial WW. According to a survey made by Jönsson (2001) on Swedish WWTPs, higher inhibition of nitrification can be expected at WWTPs receiving WW from a large number of different industries. When investigating an industry and inhibition of nitrification, some general questions should first be addressed to identify or exclude sources. If the industry have any weekly routines with concentrated discharges, those might be important events. It is also good to identify the streams and their individual importance in terms of flow and toxicity. If the industry is connected to the municipal sewer system, the inhibition of nitrification is probably measured at the connection point, and a dilution might occur at the industry which should be considered. For many industrial WWs, it is more reasonable to talk about toxic concentration, rather than toxic substances (Jönsson, 2001).

2.2.2 Metals
Some metals become toxic in higher doses while others that are not needed by organisms often are toxic in considerably smaller doses (Svenskt Vatten, 2009). Today, a wide range of metals are used in industrial processes. A fraction of these metals end up in the process WW and are transported to the WWTP. Due to the physical-chemical processes associated with activated sludge WW treatment, metals entering the WWTP tend to adsorb and accumulate in the sludge (Álvarez *et al.,* 2002).

Zinc (Zn^{2+}) is toxic for organisms since it inhibits the uptake of other essential metals (Svenskt Vatten, 2009). Zinc carbonate ($ZnCO_3$) is used in large amounts at Nolato MediTor. The solubility of this salt in water is 1.3 mg/l. The PNEC (predict no effect concentration) of zinc on microorganisms in WWTPs is 52 µg/l (Brenntag Nordic AB, 2015). The EC50 (3h) for zinc carbonate on microorganisms is 5.2 mg/l (ECHA, 2016). Zinc has been shown to be inhibitory to the nitrification process. However, the degree of inhibition is not clear cut since the result differs among different studies. This could be due to different methods being employed and different ways of measuring zinc, for example only the dissolved fraction of zinc can be measured as opposed to the total zinc concentration including insoluble particles. The sludge

used could also be of importance since sludge that has been exposed to a certain substance for a longer time can become adapted and more resistant to that substance (Jönsson, 2001). Another aspect of inhibition by metals is metal interactions with sludge, and the uptake of metals increases with increasing pH, up to a certain level (Cheng *et al.*, 1975).

Juliastuti *et al.* have carried out two studies examining the inhibitory effect of zinc on nitrification. In one of these studies the OUR method was employed, resulting in an IC50 value of 0.35 mg/l, while 0.5 mg/l caused an inhibition of 70% (Juliastuti *et al.*, 2003a). In the other study another method was employed, ISO 9509, giving the same IC50 value of 0.35 mg/l, but 0.5 mg/l caused an inhibition of about 90% instead (Juliastuti *et al.*, 2003b). The concentration of zinc was in these studies measured as free zinc ions (Zn^{2+}) and the sludge used was derived from a municipal WWTP. In another study carried out by Madoni *et al.* (1999), two different methods were employed to estimate the inhibitory effect of a series of metals including zinc - an AUR test and a sOUR test. The AUR test measured the inhibition to be about 60% at 0.93 mg/l, while about 90 % inhibition was caused by 2.10 mg/l. The corresponding inhibitory effects according to the sOUR test was about 70% and 80%, respectively. In the studies zinc was measured as free ions and the test sludge originated from an industrial WWTP. Dalzell *et al.* (2002) performed a study where the inhibition of nitrification was estimated using the so-called screening method (ISO 9509, 1989). The study revealed an IC50 of 10 mg/l. However, it is not quite clear whether zinc was measured as free ions or as total zinc in that study.

X. Li *et al.* (2015) discuss the current state of science regarding measurements of nitrification inhibition by metals in WW systems. The authors found that the metal bioavailability and metal speciation are two important factors for nitrification inhibition, as well as the growth mode of the nitrifying bacteria. A difference in inhibition was found in tests made in continuous and batch mode, which have different contact time between the metal and the bacteria.

Metal speciation
The speciation of metals is important for the inhibition, and to only consider the total concentration of a metal may give questionable results of inhibitory behavior (Çeçen *et al.*, 2010). How the metal species appear depends on equilibrium between the species. The metal may also form complexes with other substances in the solution, which can change the bioavailability of the metal. A study investigated the effect of speciation on nitrification inhibition for a number of metals including zinc. The actual speciation in comparison with the theoretical could conclude that the species of zinc causing inhibition was most probably the free and labile ones (Çeçen *et al.*, 2010).

2.2.3 Chemical compounds
Blum and Speece (1991) studied the toxicity of organic chemicals for different groups of bacteria used in treatment processes. AOB was ten times more sensitive than the NOB. NOB showed about the same sensitivity as the heterotrophs. The different toxicity to the two partial steps of nitrification can be important to consider when measuring inhibition of nitrification, as it is usually measured in terms of either decrease of ammonium or increase in nitrate. Sulfur-containing compounds are considered typical potential inhibitors of nitrification (Halling-Sørensen and Jørgensen, 1993). Some relevant chemical compounds used at Nolato MediTor and their toxicity will be discussed in chapter 2.3 Nolato MediTor AB.

2.2.4 Ammonia, nitrate and nitrite
The two groups of nitrifying bacteria, AOB and NOB, are sensitive to both the substrate of their own and each other. Anthonisen *et al.* (1976) found that ammonia starts to show inhibitory

effects on the first step of the nitrification carried out by AOB at much higher concentrations (10-150 mg/l) than on the second step, carried out by NOB (0.1-1.0 mg/l). In the same study. It was shown that the concentration of free nitrous acid, which is in equilibrium with nitrite, the substrate of NOB, started to be inhibitory to both species of bacteria at concentrations between 0.22 and 2.8 mg/L . The concentrations at which inhibition was initiated were given as bands rather than specific values since the outcome was depending on factors such as the ambient temperature, which ranged from 10°C to 23°C. The inhibitory effects of ammonia and nitrite has been confirmed by other authors. In domestic WWs in WWTPs the ammonia and nitrite concentrations are normally not within an inhibiting range (Halling-Sørensen and Jørgensen, 1993) and the inhibitory concentration may depend on the test conditions, such as pH and the type of test organisms (Jönsson, 2001). In an investigation of factors affecting nitrification rates, the effect of the ammonia concentration was tested in a pilot trickling filter and a batch reactor. It was found that at ammonia nitrogen concentrations less than 60 mg/l, there was no inhibition (Wild *et al.*, 1971).

Jönsson (2001) also investigated the inhibition of nitrification caused by nitrate and nitrite. The inhibition of nitrate was tested in concentrations ranging from 10-10 000 mg NO_3-N/l. At for example concentrations of 100 and 1000 mg NO_3-N/l, the inhibition was 13 and 53%. Nitrite caused about ten times higher inhibition than nitrate. At a concentration of 10 mg NO_2-N/l the inhibition of nitrification was close to 18%, while that corresponding to a concentration of 100 mg NO_2-N/l was slightly over 66%. The high inhibition is likely to be a cause of the toxic nature of nitrite. It is toxic to many bacterial processes, including those of nitrification (Philips *et al.*, 2002). A clear relation between nitrite concentration and inhibition have not been found in the literature, as reported concentrations and inhibition percentages differ greatly. Listed in the review of Philips *et al.* for example 100 mg NO_2-N/l gives 20 and 40% inhibition of nitrification respectively (Philips and Verstraete (2000); Dahl *et al.* (1997)) and Jönsson (2001) found yet another inhibition value by nitrite at 100 mg NO_2-N/l. The different results can depend on the test conditions, as well as the type of sludge.

2.2.5 Sludge
Inhibition of nitrification varies depending on the sludge type at WWTPs, because sludge bacteria that are exposed to certain toxicants under a period of time might adapt to these and work up a steady growth under the chemical exposure (Jönsson *et al.*, 2001). The results of measurements of inhibition of nitrification differ greatly depending on the type of bacterial culture, when comparing pure cultures and activated sludge derived from WWTPs, from negligible to substantial inhibition for the same industrial WW (Li *et al.*, 2015). An analysis on inhibition of nitrification in activated sludge could conclude that unexposed biomass in short-term tests may not give accurate results on inhibition of nitrification in a long-term and full-scale treatment plant (Kelly *et al.*, 2004). The authors also found a difference in inhibition values when using different sludge types, as well as a dependency on pH, which confirms the study made by Jönsson *et al.* (2001). The study also suggests that there are two mechanisms of inhibition, as the two groups of nitrifying bacteria (AOB and NOB) were differently impacted by the chemical inhibitors. This confirms the different toxicity to AOB and NOB in the study made by Blum and Speece (1991). It is also important to be aware of the sensitivity of the sludge to chloride. Since many industrial WWs contain high amounts of chloride, a sludge that is not adapted to these high concentrations of chloride could show high degrees of inhibition only due to the chloride present in the WW. It could then be important to separate this effect from the inhibitory effect from other substances present in the WW (Swedish EPA, 1995).

2.3 Nolato MediTor AB

Nolato MediTor AB is an industrial company located in Torekov in Båstad municipality, producing medical plastic products. Extruded medical tubing, latex dipping and injection-moulded rubber are offered through clean room manufacturing. In the product areas of anaesthesia breathing bags and catheter balloons, Nolato MediTor is one of the leading companies worldwide. The product components are produced in TPE (thermoplastic elastomer), silicone and latex rubber, by usage of a number of chemicals. The chemical components of the products have been approved by the costumers and are not wanted to be replaced. The WWTP in Torekov suspected Nolato MediTor to be one source of the problems observed in the nitrification process, whereby an identification program of inhibition of nitrification was initiated at the industry in 2014. The requirements on industrial WW are set by NSVA who runs the WWTP in Torekov.

Of the different parts of the industry, the dipping process was identified as a likely source of inhibition of nitrification, as a bigger partial stream including leaching WW is produced. The dipping process starts with cleaning of the forming sheets, in a tank with continuous flow. The forming sheets are then dipped in a coagulation agent tank, whereafter they are dipped in the latex tank. In a separate room the latex recipe is prepared, where chemicals are mixed depending on product of choice, followed by a step where the concentration of the total solids is increased, called creaming. After the latex dipping, the forms are dipped in another coagulation agent tank. Finally they are dipped in leaching tanks to leach out unwanted chemicals. The water from the leaching tanks is exchanged continuously, replaced by clean water which is heated up to about 50 degrees. WW from the leaching and the washing of forming sheets are led to a heat exchanger; these waters are sufficiently warm so that they can heat up the incoming clean water that will be used in the same processes. The leaching WW contains chemicals from the whole process described above, and especially the water-soluble chemicals are expected to be leached out.

The finished latex products are washed to get rid of residual chemicals, and talc is added. The WW from the last washing step is called "cold wash". WW from cleaning the forming sheets, called "rinsing bath", and WW from a chlorination process are also led to the sewerage, but these streams are relatively small and released batchwise, about 400 l/week. Figure 2.1 is presenting the streams of WW at the industry. The size of a circle/box is an indication of the size of the flow, and the circles are continuous flows whereas rectangular boxes are released as batchwise loadings. The industry also has a production part called Nolato Polymer, which also produces WW. The WW from Polymer is consisting of the partial streams of process waters, water from scrubbing of the floors and the domestic WW. Moreover an extension of the production has been made since the initiation to investigate inhibition of nitrification, and some changes in cleaning routines have also been made. The production is more or less constant, and the last year the normal production cycle at Nolato MediTor was Sunday to Friday, with 126 hours per week (Nilsson, 2017).
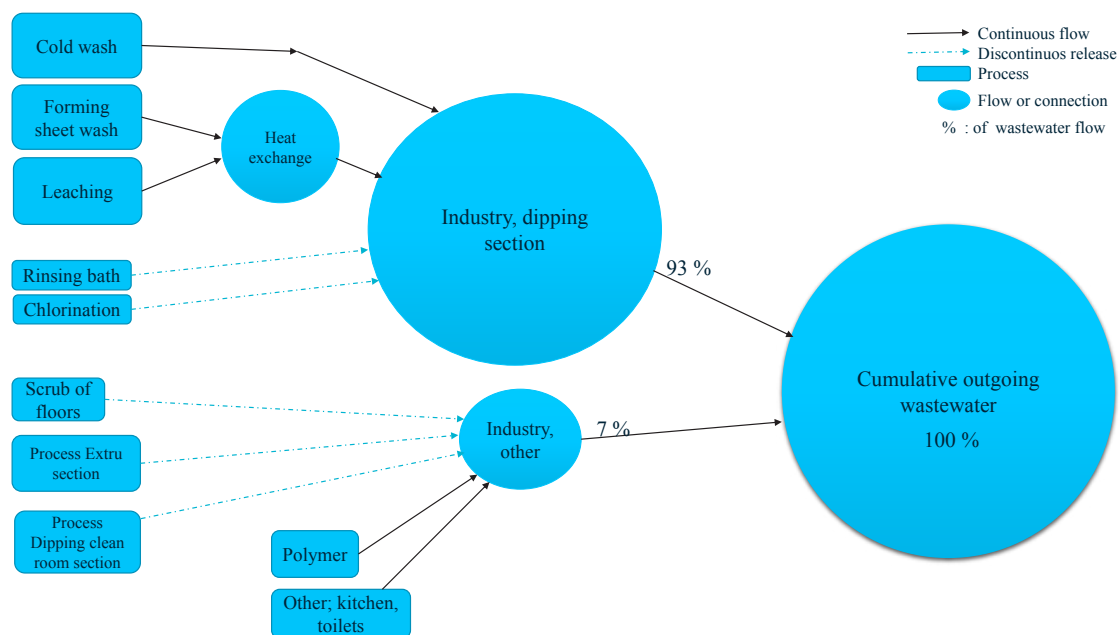
Figure 2.1. Flow of WW at Nolato MediTor (Nolato MediTor, 2017a).

In Table 2.1, a description of the streams of WW tested for inhibition in this study, along with the associated process or connection point is presented.

Table 2.1. Processes or connection points and corresponding streams of WW at Nolato MediTor.

Process/connection point	Description of WW
Cold wash	WW produced from washing the finished latex breathing bags
Leaching	WW produced from leaching out unwanted chemicals in the latex
Heat exchange	WW consisting of leaching WW and WW from the forming sheet wash, used for heating incoming water
Rinsing bath	WW produced from extra cleaning of the forming sheets
Industry, other	WW produced from the rest of the industry, including the production at the Polymer side of the industry
Industry, dipping section	WW produced in the dipping section of the industry (named "Dipping WW" in this study)
Cumulative outgoing wastewater	Cumulative wastewater consisting of all the partial streams, going out from the industry to the WWTP (named "outgoing WW" in this study)

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2.3.1 Inhibition of nitrification at Nolato MediTor

Data from earlier executed inhibition of nitrification tests at Nolato MediTor are presented in Table 2.2. The tests have been performed by the company AnoxKaldnes according to a modified ISO method called SS-EN ISO 9509:2006. The measurements focused on the outgoing WW from either the cumulative flow or on separate streams from the leaching section of the production. The inhibition of nitrification from daily cumulative outgoing flow under normal production was highly fluctuating. Measurements from 2012 until 2016 have shown a variety on inhibition of nitrification from 8 to 55% (at a volume fraction of 20%), see Table 2.2. AnoxKaldnes used sludge from Öresundsverket WWTP in Helsingborg, which should be considered as the inhibition of nitrification may vary with different sludge types (Jönsson *et al.,* 2001). In November 2014 the inhibition was low, only 8%, which is thought to be a result of no production in the latex dipping section. The most recent test performed in December 2016 showed remarkable results, only 8% inhibition, despite full production (Nolato Meditor, 2017b).

Table 2.2. Previously measured values of inhibition of nitrification in the outgoing WW from Nolato MediTor diluted to 20% volume fraction (Nolato MediTor, 2017b). The asterisk appearing besides one of the dates indicates that there was no production in the dipping section at that point in time.

Date	Inhibition (%)
2012-06-15	23
2012-09-11	55
2012-10-22	26
2013-03-22	47
2013-06-13	28
2013-11-28	34
2014-06-16	31
2014-11-17*	8
2015-06-03	31
2015-12-09	34
2016-06-07	18
2016-12-08	8

Figure 2.2 below is illustrating how the inhibition of nitrification at the industry has varied since the start of measurements. The measurements are plotted in chronological order, and were all made by AnoxKaldnes.
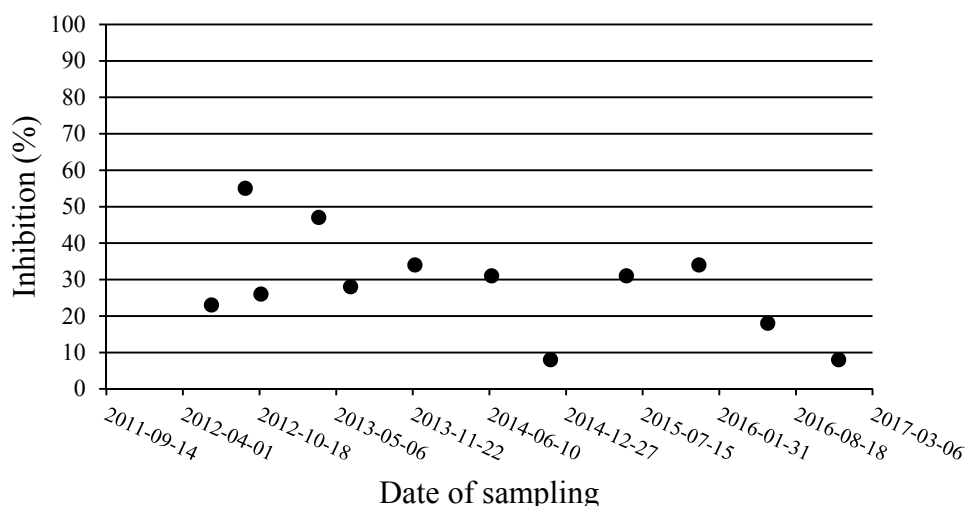
Figure 2.2. Previous measurements of inhibition of nitrification in 20% diluted WW from Nolato MediTor (Nolato MediTor, 2017b).

An investigation in 2013 conducted by Embreco AB analysed recorded data on flow, pH, BOD, COD, nitrogen, phosphorus, TSS, chloride, zinc and inhibition of nitrification (Malmquist, 2013). An investigation of the production chemicals suspected to cause inhibition was made, by literature research. The chemicals are discussed below. Embreco also investigated inhibition of nitrification in different partial streams. High inhibition of nitrification was found in the streams called "rinsing water" ("sköljvatten"), "leaching WW 1 and 2" ("lakningsvatten 1 och 2") and "scrub water" ("skurvatten"), where the names inside the parenthesis are the names of the streams used in the report by Embreco. Also the load of zinc in the outgoing WW was relatively high, although Embreco remarks that the zinc is likely to be in the particulate form which is not inhibiting but at very high concentrations the equilibrium could be shifted towards the dissolved form, which is inhibitory (Malmqvist, 2013). It is not stated why the zinc is suspected to be in particulate form. WW is also produced from scrubbing of the floors at the mixing process area. The inhibition of nitrification of this water was found very high; 80% (Nolato MediTor, 2017b). Though the mixing process has changed in 2013, from adding vulcanizer chemicals in powder form to dissolved chemicals in water, probably leading to less chemicals on the floor. No measurements on the scrub/swab water have been made after the change of mixing procedure (Nilsson, 2017).

Leaching WW and treatment experiments

As the leaching process was suspected to be the major source of the inhibition of nitrification, further inhibition tests were done on leaching WWs. The leaching consists of four tanks with leaching WW, identical in size and discharge. In the leaching section all water-soluble addition chemicals that are not part of the actual latex are wanted to be leached out. Some of them are suspected to be the cause of the inhibition of nitrification. At the leaching step WW is continuously produced, called leaching WW. The most recent previous measurement confirm that the inhibition of nitrification is higher in the leaching WW than in the outgoing WW; 71-72%, compared to 31% inhibition measured in the same year (Sandström, 2014).

In 2014, the consultancy company WSP evaluated methods to reduce the inhibition of nitrification (Sandström, 2014). Membrane filtration, UV, advanced oxidation process, ozone, activated carbon and aeration were analyzed regarding advantages, drawbacks and investment- and operational costs. Aeration treatment was tested in laboratory, and showed a decrease in

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inhibition of nitrification, but also caused a heavy foam formation which potentially could give operational problems. Though the inhibition of nitrification of the leaching WW decreased from 72% to 43%, indicating that inhibitory compounds were stripped off. Treatment with UV was after recommendation tested at Nolato MediTor, but unfortunately without substantial results. The inhibition of nitrification was tested before and after treatment on only one part of the water from the leaching section, in a small pilot scale setup. The inhibition of nitrification was decreased from 71% to 49%, which was not enough, even though some inhibitory compounds should have been destroyed by the UV. The measurements done on leaching WWs and treatment method trials are presented in Table 2.3 (AnoxKaldnes, 2014a,b,c).

Table 2.3. Inhibition measured on the leaching WW, and measurements before and after aeration and UV treatment. Leaching WW 1 and 2 denote tank 1 and tank 2, respectively. (Malmqvist, 201(*)3; Sandström, 2014(**); AnoxKaldnes, 2014a,b,c (***)).

Date	Water	Inhibition (%)
2012-10-22	Leaching WW 1 *	24
2013-03-22	Leaching WW 1 *	60
2013-03-22	Leaching WW 2 *	57
2013-03-22	Scrub water *	80
2013-06-13	Leaching WW 1 *	38
2013-06-13	Leaching WW 2 *	27
2014-03-18	Leaching WW before aeration **	72
2014-03-18	Leaching WW aerated **	43
2014-07-16	Leaching WW before UV ***	71
2014-07-16	Leaching WW after UV ***	49
2014-08-29	Leaching WW after UV ***	23

Other sources of WW at Nolato MediTor are wash-water from final cold-water wash in washing machines of the breathing bags and water from a rinsing bath where nitric acid (HNO_3) is used. Furthermore, the forming sheets are washed every Friday. These streams are not of importance for the inhibition of nitrification, as the amounts of water is small. The water from the cold water wash have been tested for inhibition of nitrification and was not over the limit value (5%). The stream from the forming-sheet cleaning showed only 8% inhibition of nitrification (Malmqvist, 2013).

2.3.2 Wastewater characteristics

Measurements have been done regularly on the cumulative outgoing WW on flow, pH, chloride, BOD_7, COD, TN, TP, SS, NO_x, nitrate, nitrite and zinc. The outgoing flow from the industry from 2014 to 2016 was on average 70 m^3/day and the pH was on average 7.9. The data show that the outgoing WW contains high levels of nitrogen. Among the forms of nitrogen, nitrate is shown to be dominant (140 mg/l on average) while nitrite and ammonium are found in substantially lower concentrations (on average 3 mg/l and 30 mg/l, respectively). Since the concentration of ammonium has not been measured explicitly, it was instead estimated by taking the difference between TN and the concentrations of nitrate and nitrite added together. This probably gave an overestimation of the concentration of ammonium, since other forms of nitrogen are expected to be present in the WW. The BOD_7 indicated that there is some readily biodegradable organic material. When comparing COD and BOD_7, a conclusion can be drawn that the major part of the organic material is not readily biodegradable as the COD/BOD_7 ratio lies between 4 and 7 (Malmqvist, 2013).

The relation between measured inhibition of nitrification and different parameters was studied by Embreco, but without any clear trends. As Malmqvist (2013) states, this is not remarkable, as the measurements of parameters such as COD/BOD, nitrate and zinc cannot alone verify the cause of the inhibition of nitrification. The concentrations of nitrate was measured twice a year from 2013 to 2016 and was ranging from 1.4 to 220 mg/l. The concentration of nitrite was 0.26 to 6.8 mg/l, and the inhibition of nitrification was measured at the same day. The inhibition of nitrification with respect to the concentrations of nitrate and nitrite are shown in Figure 2.3 and 2.4. No clear trends could be seen between the inhibition and the concentrations of nitrate and nitrite.

Figure 2.3. Inhibition of nitrification measured in the cumulative outgoing WW at a volume fraction of 20% as a function of the concentration of nitrate nitrogen (Nolato MediTor, 2017b).

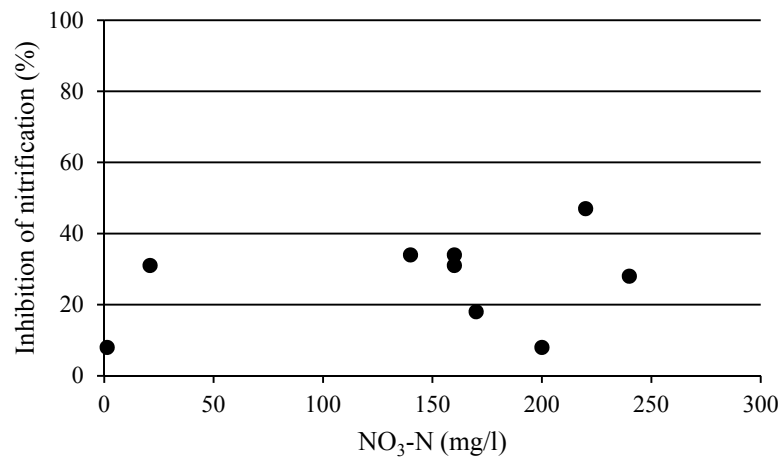*Figure 2.4. Inhibition of nitrification measured in the cumulative outgoing WW at a volume fraction of 20% as a function of the concentration of nitrite nitrogen (Nolato MediTor, 2017b).*
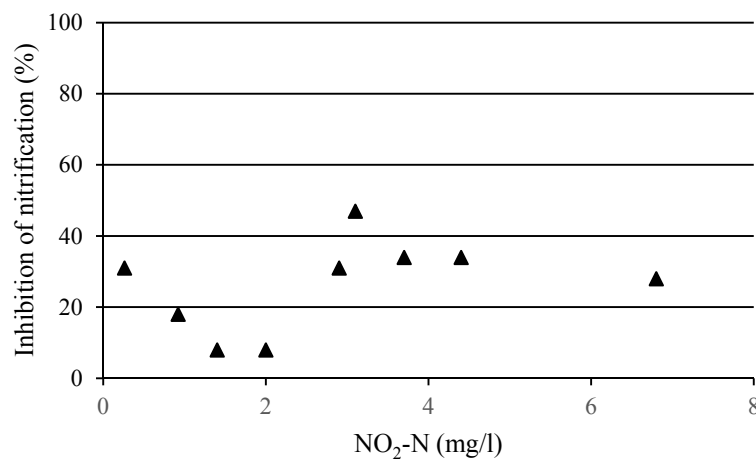
2.3.3 Chemicals used in the production

Nolato MediTor uses a wide range of chemicals in the production process. They can be categorized into function in the production; raw latex ingredients, stabilizers, vulcanizers,

coagulation chemicals, creaming chemicals, pigment, process supporting chemicals and infilling chemicals. The components that are soluble in water and used as stabilization- and vulcanizer chemicals are expected to be leached out in the leaching step. The chemical components are listed in Table 2.4, in falling amounts and divided in water soluble and less/not water soluble components.

Table 2.4. Chemical compounds used in polychloroprene latex at Nolato MediTor and the annual consumption of each of them (Nolato MediTor, 2017c).

Chemical components	Consumption (kg/y)
Water soluble components	
Calcium nitrate tetrahydrate	28000
Tamol	5880
WAQ	5500
Emulan	3500
SMO	2900
Less/not water soluble components	
Industrial spirits (ethanol)	16398
Zinc carbonate	12447

Toxicity of the water soluble compounds
The chemical components suspected to be leached out and therefore present in the WW have been investigated further, some regarding their aquatic toxicity, and found to be potentially toxic in the estimated concentration in the WW flow. Hereunder follows a short description of toxicity of WAQ, Emulan and SMO. These compounds does not appear to have been studied very much; to investigate them further, and to understand how they interact with each other in terms of toxicity are outside the scope of this study. Their individual toxicity may give an indication of the toxicity in the mixed WW.

WAQ is a mixture surfactant containing sulfuric acid and is toxic to aquatic life at concentrations above 10-100 mg/l, and the PNEC (Predicted No Effect Concentration) on microorganisms in sewage treatment is 1084 mg/l (Safic Alcan, 2015). The two values will be used as guidelines for toxic concentrations in the WW.

Emulan is a mixture of mostly water and ethoxylated alcohol. Toxicity to sewage treatment plant organisms is low, EC10>10 g/l (HERA, 2009).

SMO is a mixture dispersing agent, mostly constituted of water and sulfated methyl oleate sodium salt. According to the provided safety sheet, SMO has a hazardous ingredient which has a toxicity to aquatic organisms at an L(E)C50 over 100 mg/l (Ertecto Rubber and Plastics AB, 2014). Further information about the compound is very limited. What can be said is that SMO is containing sulfur, which is a component that makes it suspected of inhibition of nitrification.

Tamol is a polymer sodium salt that is toxic to fish at concentrations over 100 mg/l and to microorganisms over 5000 mg/l respectively. Tamol is soluble in water up to ca 400 g/l at 20°C (BASF SE, 2014). Tamol is a formaldehyde compound (BASF SE, 2014). Formaldehyde is a known toxicant, why Tamol is suspected to cause inhibition of nitrification. A study showed that at an initial formaldehyde concentration above 350 mg/l, a decrease of nitrification could be seen (Eiora *et al.*, 2004). In the same study, nitrification in the presence of both methanol and formaldehyde was investigated, showing a decrease in nitrification at formaldehyde

concentrations above 175 mg/l. The lower affecting concentration of formaldehyde was suggested to be related to the higher COD/N ratio, as it is beneficial for the heterotrophs and therefore inhibiting the nitrification.

Components related to inhibition of nitrification
In the report investigating nitrification inhibition at Nolato MediTor (Malmqvist, 2013), chemical components in one latex product are listed. From these, five were marked as suspect to cause inhibition of nitrification. The chemicals are: a thiourea compound, a formaldehyde sulfate compound (Tamol), zinc, a p-cresol compound and a copper compound. The quantities in the WW are not known, except for zinc which is measured by a consultancy company along with routine parameters. Diphenylthiourea is used as a component in the latex itself. Allylthiourea is a known toxicant to nitrification and was found to be fully inhibiting of nitrification at concentrations from 0.6 to 1 mg/l, depending on sludge type (Jönsson *et al.*, 2001), why also diphenylthiourea is suspected to cause inhibition. As diphenylthiourea is used as a part of the latex, it should not likely be dissolved in the WW. Another study investigated inhibition of nitrification by p-cresol. The study found out that in a nitrifying culture, 17 mg/l p-cresol inhibited by 70% nitrate formation, but at 10 mg/l, no effect was found (Texier and Gomez, 2002). The chemical Lowinox is used at Nolato MediTor, which is a p-cresol compound. Lowinox is not suspected to cause inhibition as it is neither soluble in water nor wanted to be leached out. Ethanol is consumed by the nitrifying sludge, but it can also be inhibitory in high concentrations (Gomez *et al.*, 2000). The effect on nitrification rate by volatile organic compounds was studied in batch reactors where the inhibition of nitrification was found to be 80% at an ethanol concentration of 2000 mg/l. The ethanol used in the production is likely to evaporate, and not be found in any high concentrations, why ethanol inhibition will not be investigated (Nilsson, 2017).

One step in the latex processing at Nolato MediTor is chlorination to make the surface of the rubber smooth, using hydrochloric acid (Nilsson, 2017). According to literature, chloride is inhibiting nitrification, but at a much higher concentration than those measured in the outgoing WW. The study found that at a chloride concentration of 5 000 mg/l, the nitrification was negatively affected (Lu and Shim, 2015). At Nolato MediTor, the average concentration of total active chloride is 0.2 mg/l, so inhibition caused by chloride will not be investigated.

Salts
The WW from Nolato MediTor contains salts, in form of both inorganic (*e.g.* calcium nitrate tetrahydrate) and organic salts (large organic compounds in the form of sodium salts). An investigation of osmotic stress on nitrification was made in a lab-scale bioreactor, finding the performance of the reactor decreasing at very high salt concentrations (Jin *et al.*, 2007). The experiment was made with increasing sodium sulfate dosage over time. Although sodium sulfate is not found in the WW investigated in this study, the salts present may give rise to a higher than normal osmotic pressure.

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) is another chemical used extensively at Nolato MediTor, serving as a coagulant making the latex mixture attach better to the aluminum forms (Nilsson, 2017). Since this salt is used in high amounts; 28 000 kg/year (Nolato Meditor, 2017c) and dissolves well in water; 2 600 mg/l (Univar, 2016), the previously measured, high concentrations of nitrate in the outgoing water is expected to be a result of this salt dissolving in the leaching tanks. Nitrate is not a compound known to be very toxic to nitrifying bacteria. However, in high concentrations it can become inhibitory, even though it is the end product of

the nitrification process itself. This was found in a study by Jönsson (2001), from which the results are showed in Table 2.5.

Table 2.5. Concentrations of nitrate-nitrogen and corresponding inhibition of nitrification (Jönsson, 2001).

Concentration NO$_3$-N (mg/l)	Inhibition of nitrification (%)
100	14
500	32
1000	53
5000	100

Since the outgoing WW from Nolato MediTor contains on average 140 mg/l NO$_3$-N (Nolato MediTor, 2017b) and the leaching WW is expected to contain even more since the calcium nitrate tetrahydrate is leached out in this step (Nilsson, 2017), a non-negligible fraction of the total inhibition observed in the streams is expected to be caused by nitrate.

2.4 Torekov WWTP

Torekov is a small town, meaning that the WW discharged from Nolato MediTor is an important contributor to the composition and magnitude of the flow entering the WWTP. The contribution from Nolato MediTor to the incoming daily flow at the WWTP is approximately 2%, calculated from yearly averages in 2015. Nolato MediTor's contribution to the total nitrogen load is roughly 12% and of the incoming nitrate-nitrogen Nolato MediTor contributes with approximately 56%, see Appendix VII for calculation data. The plant has a biological treatment process and performs nitrification in an aerated activated sludge process (Roslander, 2017). The question about nitrification inhibition was first addressed when the plant faced problems with the biological treatment process. The upstream industries were suspected to release toxic WW, and Nolato MediTor was one of them. Therefore the WW discharged from Nolato MediTor was tested for nitrification inhibition at the point where it connects to the main sewer, and it was found inhibitory. The WWTP in Torekov is managed and operated by NSVA, why "General regulations for usage of Scania Northwest municipalities' general water- and WW plants" applies (NSVA, 2011). The limit value is as mentioned before 10% inhibition of nitrification in 20% dilution of daily sample of WW. The limit values on copper and zinc are for both 0.5 mg/l (NSVA, 2011). The inhibition of nitrification at the inlet of Torekov WWTP has been measured to be 0% in July 2014 and June 2015, and 5% in July 2015, at 80% dilution. The inhibition was tested with the screening method (Petersson, 2017), described in chapter 3.

During the time of this study (spring 2017), Torekov WWTP was experiencing serious problems with its sludge and nitrification process (Roslander, 2017). To better understand the current state at the WWTP, the authors of this report went for a study visit. The ammonium removal was almost zero, SS (suspended solids) was very high and sludge foam was welling out of the basins. The problem was hoped to be solved by replacing a large part of the sludge with new sludge from Öresundverket in Helsingborg, to restart the process by inserting new nitrifying bacteria. These recent problems are not obviously caused by the industry Nolato MediTor (Roslander, 2017).

3. Materials and methods

In the following section the screening method will be described, along with analytical methods used for pre-test data collection and afterwards analysis, as well as the method of calculation used to obtain the inhibition of nitrification percentage. The original screening method description made by Jönsson (2001) can be found in Appendix VI. The screening method is further used in a so-called additivity test, to test the type of the inhibition of nitrification. A description of uncertainties associated with the methods is later provided; the result of the screening method does not only rely on the method itself, but on all methods used before, during and after the screening test.

3.1 The screening method

The screening method used in this study is based on ISO 9509, a toxicity test assessing the inhibitory effect of test substances on activated sludge (ISO 9509, 1989). An overview of the method can be seen in Figure 3.1, where the steps of preparation, incubation and filtration are visibly presented. During the incubation time, the test tubes are shaken on a shaker, enabling the reaction of nitrification (see Figure 3.2). The method is convenient to use for analysis of large series of test samples, since the incubation time is relatively short (120 minutes) and several test samples can be run simultaneously (Jönsson, 2001). The method is adapted for use on sludge with nitrification rates commonly found in activated sludge from municipal WWTPs, but in principle any type of sludge can be used provided that the nitrification rate lies within the range for which the test is applicable. However, it is possible to prolong or shorten the incubation time to match the nitrification rate. The method is suitable for assessing inhibitory effects of WWs, but pure toxicants can also be tested provided that they are soluble or at least can be homogeneously distributed within the solution during the exposure time of the test (Swedish EPA, 1995).
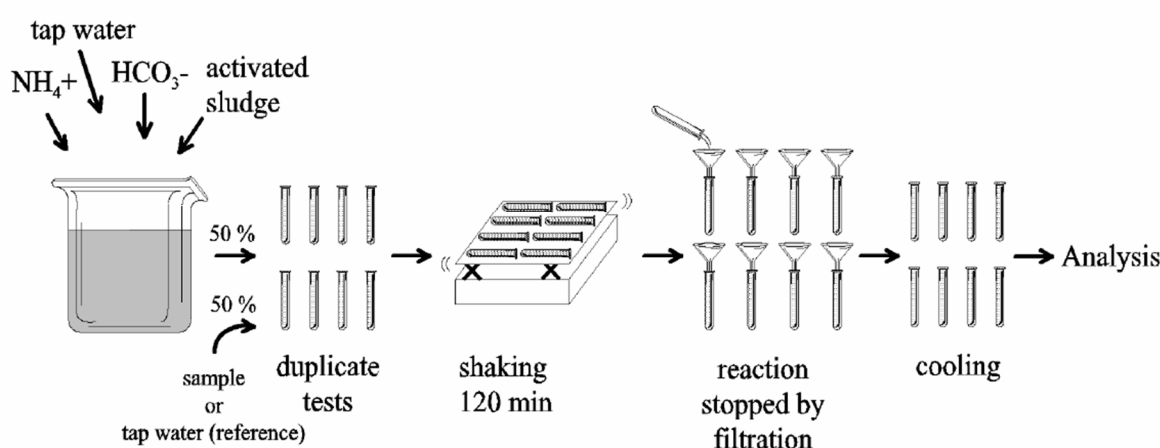
Figure 3.1. Schematic view of the screening method (Jönsson, 2001). Published with permission from Karin Jönsson.

The test is performed at constant temperature, generally between 20°C and 25°C, and at a pH within the range 7.5±0.5. The nitrification process takes place in capped test tubes with a volume of 30 ml where 1/3 of the volume is liquid and the other 2/3 is air, to provide the sludge with oxygen during the test. The liquid consists of a sludge suspension mixed with either the test substance or with tap water, corresponding to the test samples and the control samples,

respectively. The control samples are used as references, to determine the inhibition of nitrification later on. The reaction starts when the sludge suspension is added to the test substance, followed by shaking, whereafter filtration stops the nitrification reaction, see Figure 3.1. The test is done with duplicate samples. After filtration the filtrates are cooled and can be analyzed regarding their content of nitrate and nitrite or ammonium, depending on the analysis method (Swedish EPA, 1995).

A stock solution with nutrients is prepared by dissolving ammonium sulfate (($NH_4)_2SO_4$), sodium bicarbonate ($NaHCO_3$) and monopotassium phosphate (KH_2PO_4) in tap water. To prepare the sludge suspension, the stock solution is added to activated sludge. In most cases, tap water is also added in order to dilute the sludge. To ensure a well-functioning nitrification process, the oxygen concentration should not drop below 4 mg/l during the course of the test. This is prevented by providing good mixing between the liquid and the air in the headspace of the test tubes, why the test tubes are placed on a shaker. The oxygen supply is kept on a sufficiently high level also by ensuring that the COD concentration and the heterotrophic consumption rate in the test tubes do not exceed 400 mg COD/l and 90 mg O_2/l, respectively. However, test samples with a COD concentration above 400 mg COD/l might be suitable for the method provided that the COD contains a relatively small proportion of easily biodegradable matter. If this is not the case, the test can be modified by replacing the air in the headspace with pure oxygen, preventing oxygen depletion (Swedish EPA, 1995).
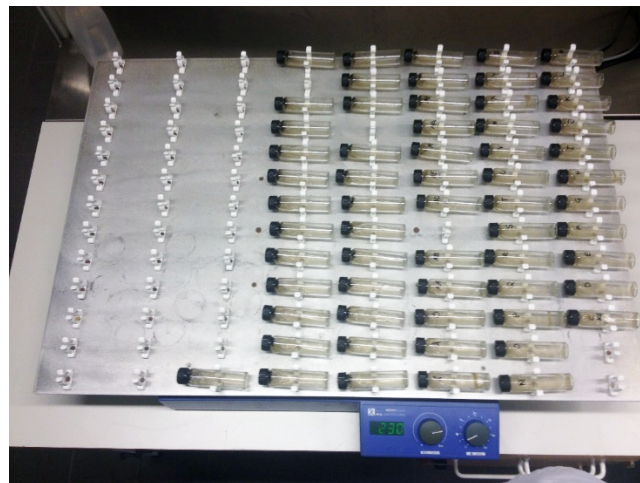
Figure 3.2. The shaker in action during the fifth screening test.

Furthermore, the ammonium concentration should be at least 3 mg NH_4-N/l at the end of the test to ensure that the nitrification rate will not be limited by lack of ammonium. This is achieved by adjusting the sludge content in the test solution based on an estimated nitrification rate. A too high ammonium concentration can also inhibit the nitrification. Hence, the initial concentration of ammonium should not exceed 100 mg/l. In addition, the final VSS concentration of the sludge suspension should lie in the range of 1.5-6 g VSS/l. If needed, the sludge can be concentrated using sedimentation or centrifugation (Swedish EPA, 1995).

A picture of the sludge suspension used during one of the tests can be seen in Figure 3.3. To ensure an as good as possible homogeneous suspension, an E-flask containing the sludge suspension was put on a magnetic stirrer. The stirring was set on slow phase, to avoid oxygen to be mixed with the sludge suspension before it was added to the test tubes, which would have started the nitrification reaction too early.

Figure 3.3. From left to right; filtered test samples from a screening test and sludge suspension. During the test, the sludge suspension was kept on slow agitation.
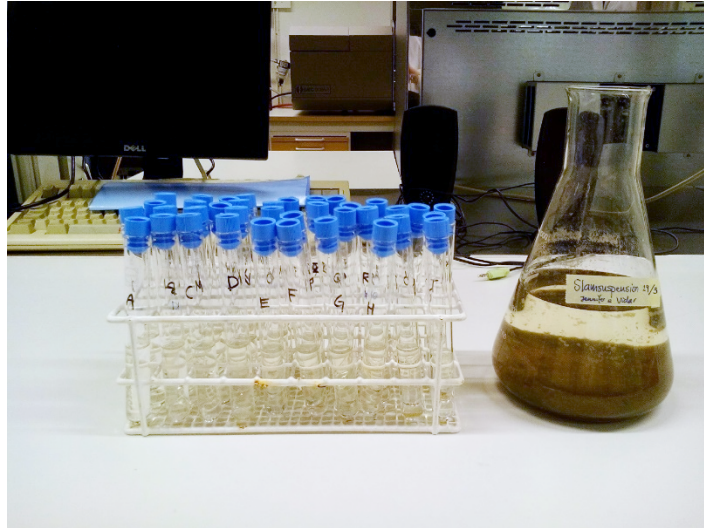
The nitrification rate is determined either based on the decrease of ammonium or the increase of oxidized nitrogen. It is related to the sludge content in the test solution since it supplies the mixture with VSS, and is expressed in mg NH_4^+-N/(g VSS·h) or mg NO_x-N/(g VSS·h) depending on which form of nitrogen that is measured (Jönsson, 2001). In this study, the consumption of ammonium (NH_4^+) was measured since the test samples WW from Nolato MediTor had been shown to contain high concentrations of nitrate according to previous measurements (Nolato MediTor, 2017b). This means that the concentration of oxidized nitrogen (NO_x) is also high, and to measure a consumption that is small compared to what is initially in the test tube would result in unnecessary uncertainties. Hence, the consumption of ammonium was chosen to be measured since the content of ammonium is much smaller than the content of oxidized nitrogen.

To calculate the inhibition of nitrification, the ammonium consumption rate in the test sample is compared with the one in the references containing tap water. The difference in ammonium consumption rate is understood to be the inhibitory effect of the test substance. The inhibition of nitrification is given as a percentage.

The test runs in this study were carried out at room temperature, with normal air as oxygen supply and with an incubation time of 2 hours. The initial concentration and the consumption of ammonium in the test tubes were aimed to be 25 mg/l and 12.5-17 mg/l, respectively; the latter depending on the measured nitrification rate of the test sludge and the desired VSS concentration in the sludge suspension.

3.1.1 Sludge

In previous measurements of inhibition of nitrification caused by the WW from Nolato MediTor, sludge from Öresundsverket WWTP has been used (AnoxKaldnes 2014a). The initial plan was to use aerated sludge from Öresundsverket, line 3, and the idea was that comparison between our results and previously obtained ones would be more straight-forward if the sludge type was the same. However, due to problems with the nitrification process observed at Öresundsverket it was decided to choose aerated sludge from another WWTP. The WWTP in Klagshamn, Sweden, was chosen since it mainly receives domestic sewage with a negligible load from industries (VA Syd, 2014). Hence, the sludge from this WWTP is assumed to be relatively unadapted to compounds that are specific for industrial discharges. More specifically,

the aerated sludge was taken from zone 8, line 1. The WWTP in Klagshamn is using pre-precipitation which decreases the organic load into the biological step, giving lower SS and VSS concentrations in the activated sludge (Jönsson *et al.*, 2008). Furthermore, measurements of the incoming and outgoing WW at the WWTP in Klagshamn show relatively high concentrations of chloride (varying around 500 mg/l), meaning that the sludge used in the screening tests has been continuously exposed to chloride and is most likely not affected by chloride in the WW from Nolato MediTor, which is expected to be present in relatively low concentrations.

In addition to the assumingly unadapted sludge, adapted sludge from the receiving WWTP in Torekov was planned to be used as well. However, due to the problems experienced at the WWTP in Torekov yielding unacceptably low nitrification rates, it was not recommended to use this sludge in the tests (Roslander, 2017).

3.1.2 Test sample liquids

The inhibition of nitrification was tested on different partial streams of WW, pure chemicals solutions and mixtures of chemical solutions. The WWs are: cumulative outgoing WW (called outgoing WW in the remainder of the text), leaching WW, heat exchanger WW, rinsing bath WW, cold wash WW, WW from the other (Polymer) part of the industry and WW from the dipping sections' connection point.

Preliminary studies have shown that the chemical solutions at Nolato MediTor contain several chemicals that are suspected to cause inhibition of nitrification. It is suspected that the toxicants in the mixture will interact with each other; a synergistic or antagonistic effect.

Wastewaters

Samples from the leaching process were tested in four separate screening tests. The leaching WW collected on February 14, 2017, was tested twice in two different tests, at a volume fraction of 20% in the test tube. This was done to check the method reliability and the comparability between tests. Since the same sludge type was used in all five tests, it seems fair to assume that results from different tests can be compared.

The outgoing WW was tested on one hand to investigate the variance over time and dependency of the weekday, to do so; the results were compared with previous studies. The purpose of testing different days was to find any fluctuations over the week and if the production chemicals are accumulating over time. On the other hand the outgoing WW was used as a reference to the other WW streams. All the other WW streams were tested to track the inhibition of nitrification. The WW stream from the rest of the industry apart from the dipping section (the suspected inhibiting process) is called Polymer by Nolato MediTor, but actually contains water from other parts of the industry in addition to the Polymer section. Hence, this stream is referred to as "Industry, other" in the results.

Calcium nitrate and sodium nitrite

Concentrations corresponding to the outgoing WWs and the leaching WWs were done with calcium nitrate tetrahydrate to compare inhibition caused by solely nitrate. Similarly, solutions of sodium nitrite were tested to study the inhibition from nitrite. In addition, mixtures of sodium nitrite and calcium nitrate tetrahydrate were tested, corresponding to the concentration of the respective substance measured in the outgoing WW.

A test of additivity was chosen to be set up according to a Danish method (Birkved *et al.*, 2004). The nitrate-nitrogen concentrations were selected to represent a curve ranging from lower concentrations causing very low inhibition up to concentrations assumed to cause 80-90% inhibition of nitrification. Calcium nitrate tetrahydrate was used as nitrate chemical. The concentrations were chosen so that the plotted results should be easily fitted into a curve. The concentration range was 100-5000 mg/l. The same was done for nitrite-nitrogen, using sodium nitrite. The concentration range was 10-500 mg/l. Two mixtures, A and B were prepared; one with high concentration of nitrite and low concentration of nitrate (A), and vice versa (B).

Chemicals
The inhibitory effect caused by solutions of four different production chemicals was tested; Emulan, SMO, WAQ and Tamol. A stock solution of each chemical was prepared containing 1000 mg/l. These stock solutions were then diluted so that the final concentration of production chemical in the test tube was 200 mg/l and 500 mg/l for Emulan, SMO and WAQ and 200 mg/l and 300 mg/l for Tamol. Tamol was only tested up to 300 mg/l since a higher concentration would have led to a COD exceeding the upper limit of 400 mg COD/l. Two concentrations of each chemical were tested in order to get an idea of the increase in the inhibition of nitrification with respect to the concentration of each chemical compound.

3.2 Analytical methods

In order to perform the screening test, several analyses had to be done before the test to control limiting values. Temperature and pH in the test substances were also measured in order to ensure that the test would be carried out under the desired conditions. In addition, temperature and pH in the test tubes were measured at the end of the test, just before filtration, in order to keep track of the change in these condition variables during the test. After filtration of the test samples the remaining concentration of ammonium was measured.

3.2.1 Hach Lange analyses
Before running the screening test, the test substances were analyzed with regards to their content of COD, nitrate, nitrite and ammonium. COD was measured to determine the maximum acceptable volume fraction of the test substance that could be used in the test, considering the upper limit of 400 mg COD/l in the test tube, above which heterotrophs are thought to start disturbing the nitrification process. Nitrate and nitrite were measured to find out if the concentrations were at inhibiting levels. The concentration of ammonium was measured to be able to calculate the initial concentration of ammonium in each of the test tubes. Hach Lange cuvettes were used, where the test substance reacts with a reaction reagent during a specified period of time, before they are read in a spectrophotometer that measures the absorbance in the cuvette. The type of cuvettes used can be found in Table 3.1. During the first two screening tests, nitrate was analyzed with LCK339, while for the following tests LCK340, with a higher range, was used.

After filtration of the test samples, each sample was analyzed with regards to ammonium concentration using the same type of Hach Lange cuvettes as before the test (LCK303). A small amount of test solution is put by pipette to the cuvette containing reaction reagent. The method is very easy to follow and the ammonium concentration is measured with spectrophotometry. Ammonium cuvettes after measurement can be seen in Figure 3.4.
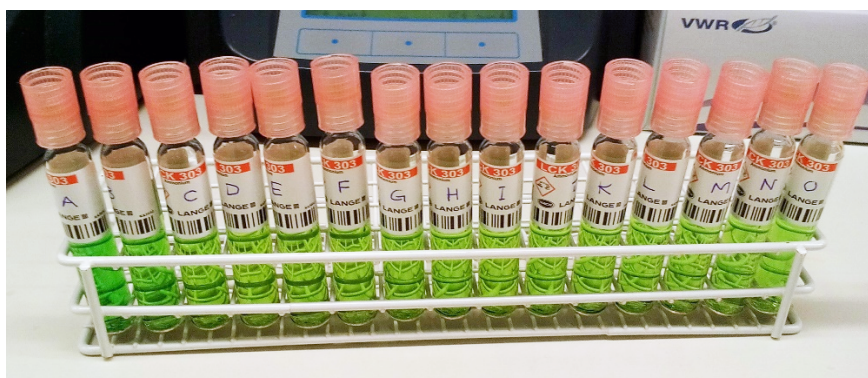
Figure 3.4. Hach Lange ammonium cuvettes in different shades of green. The analysis measures the color to obtain the ammonium concentration.

Table 3.1. Chemical analyses.

Type of cuvette	Type of analysis	Range (mg/l)
LCK114	COD	150-1000
LCK339	NO_3-N	0.23-13.5
LCK340	NO_3-N	5-35
LCK341	NO_2-N	0.015-0.6
LCK303	NH_4-N	2-47

3.2.2 pH

A pH meter of type *Christian Berner AB, WTW pH 320* was used and checked with buffer solutions. The pH meter also measures the temperature.

3.2.3 Conductivity

A conductivity meter of type *Christian Berner AB, WTW Cond 340i* was used to check the conductivity of the pure test solutions. It was done to check that the concentration of ions was within the expected range. In addition, it was used as an extra check to confirm that the dilutions of nitrate and nitrite were prepared as aimed for and to point out potential solutions that was deviating from the expected concentrations. This was done by plotting the conductivity as a function of the NO_3-N, NO_2-N and NO_x-N concentrations in the pure nitrate solution, the pure nitrite solution and the mixture of the two, respectively. The resulting Figures are found in Appendix II.

3.2.4 SS and VSS

The content of SS and VSS were measured according to the method "Water quality - Determination of suspended solids - Method by filtration through glass fibre filters" (STD-40282), provided by Swedish Standards Institute (ISO 11923, 1997). This analysis was done to ensure a bacteria load that would be rich enough to perform the nitrification during the given period of time. The analysis was done in triplicates and given as an average. In the case of this study, the SS and VSS was measured the day before the test on the raw sludge, to calculate the required volume of sludge to be mixed with tap water and stock solution. The aim was to have a VSS content in the sludge suspension of at least 1.5 g/l.

3.2.4 O_2-meter

A *Hach HQ 40d multi* O_2-meter was used for measurements of dissolved oxygen in the sludge and sludge suspension.

3.2.5 Zinc analysis

To obtain the concentration of zinc in the outgoing WW, the samples were sent for analysis at the Biology Department at Lund University, where inductively coupled plasma mass spectrometry was performed (ICP-MS).

3.3 Calculations in the screening method

Calculations of ammonium concentration average, nitrification rate and inhibition of nitrification were done in Microsoft Excel 2013.

The nitrification rate (R) was calculated with Equation 5, where C_0 is the concentration of ammonium at time zero, and C_{end} is the concentration at the end of the test. C_{end} was analyzed directly and calculated as the average of the duplicates. C_0 was calculated from the ammonium content of each test substance added to the average ammonium concentration in the sludge suspension. The ammonium concentration in the sludge suspension was calculated from the three control-zeros; the test tubes with 5 ml tap water and 5 ml sludge suspension which were directly filtered. The factor VSS in Equation 5 is the concentration (g/l) of VSS in the test tube, and t is the reaction time in hours.

$$R = \frac{C_0 - C_{end}}{VSS \cdot t} \tag{5}$$

The inhibition of nitrification was calculated as the difference between the nitrification rate in the controls with reaction time of two hours ($R_{control}$) and the nitrification rate in the test samples (R_{sample}), divided with the nitrification rate in the controls:

$$I_{nitr} = \frac{R_{control} - R_{sample}}{R_{control}} \cdot 100 \tag{6}$$

where:

I_{nitr} − inhibition of nitrification (%)
$R_{control}$ − nitrification rate in the controls (mgNH₄-N/(gVSS·h))
R_{sample} − nitrification rate in the sample (mgNH₄-N/(gVSS·h))

Chemicals
Also, worst-case concentrations of chemical i in the outgoing WW and in the leaching WW, $C_{WC,i}$, were calculated based on the annual consumption (kg) at Nolato MediTor of that chemical, ΔM_i, and the daily outgoing flow or the flow from the leaching tanks, Q, according to Equation 7 (derived by the authors). The yearly production is estimated to correspond to 274 full days per year, the outgoing flow to 70 m³ per day and the flow from the leaching tanks to 22 m³ per day.

$$C_{WC,i} = \frac{\Delta M_i}{274Q} \tag{7}$$

The worst-case concentrations calculated are based on a worst-case scenario where all of the chemicals are leached out in the leaching tanks. This is probably still an overestimation since some of the chemicals are expected to be contained in the product or end up elsewhere in the process.

Overall, the uncertainty is high, and the resulting estimation should only be considered as rough. The worst-case concentrations of the different chemicals in the leaching WW can be found in chapter 4.4 and in Table A3 in Appendix IV.

3.4 Inhibition of nitrification additivity test

As described in the literature study, inhibition of nitrification can be either additive or synergistic/antagonistic. In order to attain a deeper understanding of the combined inhibitory effect from nitrate and nitrite, an additivity test was performed. Inhibition curves were generated by measuring inhibition of nitrification as a function of the concentration of NO_3-N and NO_2-N. The nitrate and nitrite solutions were prepared by dissolving calcium nitrate tetrahydrate, $Ca(NO_3)_2 \cdot 4H_2O$, and sodium nitrite, $NaNO_2$, respectively, in distilled water. In addition, the inhibition from mixtures of the two species was measured. As a quantitative measure of the type of interaction between nitrate and nitrite, an interaction index, II, was calculated for each mixture. As an example, the II for mixture i could be calculated according to Equation 8 (Birkved *et al.*, 2004). The II is dimensionless.

$$II_i = \frac{d_{NO_3-N,i}}{D_{NO_3-N,i}} + \frac{d_{NO_2-N,i}}{D_{NO_2-N,i}}$$

(8)

Here,

$d_{NO_3-N,i}$ − concentration of nitrate in mixture i (mg/l)
$D_{NO_3-N,i}$ − concentration of nitrate that alone is causing the total inhibition measured (mg/l)
$d_{NO_2-N,i}$ − concentration of nitrite in mixture i (mg/l)
$D_{NO_2-N,i}$ − concentration of nitrite that alone is causing the total inhibition measured (mg/l)

The species behave antagonistic (less than additive) if $II > 1$, additive if $II = 1$ and synergistic (more than additive) if $II < 1$. However, for this Equation to be meaningful, the inhibition is required to increase linearly with concentration. This turned out not to be the case in this study, and hence the Equation was modified to include the non-linear relationships between the two components and the respective inhibition according to Equation 9.

$$II_i = \frac{f(d_{NO_3-N,i})}{f(D_{NO_3-N,i})} + \frac{g(d_{NO_2-N,i})}{g(D_{NO_2-N,i})} = \frac{f(d_{NO_3-N,i})}{I_{nitr,i}} + \frac{g(d_{NO_2-N,i})}{I_{nitr,i}}$$

(9)

Here,

$f(d_{NO_3-N,i})$ − function describing the relationship between inhibition from nitrate alone and concentration of nitrate, giving the assumed inhibition from only nitrate in mixture i (%)
$g(d_{NO_2-N,i})$ − function describing the relationship between inhibition from nitrite alone and concentration of nitrite, giving the assumed inhibition from only nitrite in mixture i (%)
I_{nitr} − inhibition of nitrification measured in mixture i (%)

The functions *f* and *g* were derived by the authors and based on the experimental data points obtained when measuring the inhibition from solutions with different concentrations of either nitrate or nitrite. Since *D*'s as before denotes the concentration of each species that alone results in the measured inhibition, the respective function of these values should equal the measured inhibition by the mixture, $I_{nitr,i}$. Hence, each term in Equation 9 is a fraction of the total inhibition. An example calculation can be seen in Appendix III.

3.5 Method uncertainty

Due to variations in the performance of the screening method along with uncertainty linked to the measurement of ammonium, results produced are not exact. Results of inhibition in samples without inhibiting substances are varying around zero, and how much they vary depends on the performance of the method and the following analysis. In order to be able to interpret obtained results in a meaningful way, it is crucial to be aware of the degree of uncertainty of the method. To investigate the uncertainty linked to the screening method with subsequent analysis of nitrogen, two studies were performed by Jönsson (2001). In these studies, the limit of detection (LOD) was determined. The LOD is a measure of the lowest concentration that, with 95% certainty, can be concluded to be a result of inhibitory substances present in the sample. If the inhibition percentage lies under this value, the inhibition is taken as zero. In the two studies, the LOD was found to be 7.4% and 9.2%, respectively. For duplicate samples, this corresponds to 5.2% and 6.5% inhibition for the mean value of the duplicates (Jönsson, 2001).

A similar analysis was carried out in this study to determine the LOD among the samples analyzed. The control samples were used to calculate the LOD, based on the standard deviation, according to Equation 10 (VKI, 1997).

$$LOD = \frac{2 \cdot t_{1-\alpha}(df) \cdot \sigma_{controls} \cdot \sqrt{1 + {}^1\!/_n}}{\sqrt{N_s}} \qquad (10)$$

Here,

$t_{1-\alpha}(df)$ − given fractile of the student's t distribution based on α and *df*
α − level of confidence (in this case α was set to 5%)
df − number of degrees of freedom
$\sigma_{controls}$ − standard deviation among the controls included
n − number of control samples used for correction in routine analysis (in this case *n* = 3 since three sets of controls are used in every screening test)
N_s − number of samples analyzed for each test substance (in this case N_s = 2 since duplicates are analyzed

The LOD was here based on the sets of controls run in the test. In each test, three sets of controls were used, where each set of controls in turn included three control samples. One set was placed in the beginning of the test, the second in the middle and the third in the end. To calculate the standard deviation among the sets of controls, Equation 5 was used, where $R_{control}$ was, as before, the average nitrification rate among the sets of controls and R_{sample} in this case was the nitrification rate in each individual set of controls (start, middle or end). Since the difference between the average nitrification rate among the sets of controls and the nitrification rate in each set of controls is *divided* with the average nitrification rate among the sets of controls, the

inhibition is a normalized expression and the standard deviation does no longer depend on the magnitude of the nitrification rate. Hence, sets of controls from all 5 tests within this study could be used, and the standard deviation could be based on 15 samples (3 sets of controls in each test) instead of 3 samples only. It turned out that the performance of the first test was quite poor, while the performance improved after this test. Hence, it was decided to base the LOD on the standard deviation among sets of controls from the remaining 4 tests (12 samples), and interpret results from the first test extra carefully. The number of degrees of freedom was obtained according to Equation 11 (VKI, 1997).

$$df = N_{samples} - 1 \hspace{4cm} (11)$$

Here, $N_{samples}$ is the number of samples used for calculating the standard deviation (in this case 12), which resulted in a number of degrees of freedom of 11. The fractile of the student's t distribution could then be picked from tabulated values (VKI, 1997). The resulting LOD for our tests can be found in the results part.

In addition to the uncertainty in general, a more specific source of error was detected during screening test 2 when it was found that the cuvette must be shaken generously so that all the reagent powder in the cap of the cuvette is mixed with the liquid. Thus, if the cuvette is not shaken enough, the analysis will show the wrong concentration.

Determination of SS and VSS should not affect the outcome of the screening test. The result from the measurement of VSS is used in the calculation of the nitrification rate. What is an uncertainty is the behavior and performance of the sludge, which varies depending on batch. This uncertainty is however taken care of as the inhibition is compared with control samples with the same sludge.

4. Results

Hereunder follows a presentation of the results obtained in the different screening tests. The results are given in a percentage of inhibition, or a statement of no or clear inhibition of nitrification. First, the results of the inhibition in the WWs are presented, followed by a map of the industry with inhibition of nitrification in the different streams. The results regarding inhibition by nitrate and nitrite, and their combined effect, are presented as well as the results of the additivity test. Thereafter the results of the inhibition among the latex production chemicals are presented, and the result from an analysis of dependency of day and time of sampling on the inhibition. In all figures showing values of inhibition of nitrification in outgoing WW at 20% by volume, the maximum accepted inhibition of nitrification stated by NSVA is plotted, represented by a line called "Limit (NSVA)". This limit is valid only for outgoing WW at 20% by volume; hence it is only relevant to compare the limit with those samples. In addition, a line indicating the limit of detection for this study, called "LOD", is included in all figures. Values falling below this line should be interpreted carefully since the outcome may be due to method uncertainty as well as actual inhibition. The raw data are given in Appendix V, Table A3.

4.1 Wastewaters

In this section, the results of the inhibition in the outgoing WW, the leaching WW and minor streams are presented. The concentration of zinc in the outgoing WW are also presented.

4.1.1 Outgoing wastewater

The samples of outgoing WW collected at different dates showed significantly varying values of inhibition. In all samples the inhibition was increasing with increasing volume fraction of outgoing WW in the test solution. The sample from April 7 showed the highest inhibition of nitrification; 42% inhibition at volume fractions of 20%. The sample that showed least inhibition of nitrification was the one taken on February 27, with 9% and 21% inhibition at volume fractions of 20% and 50%, respectively. Values of inhibition less than 12% are under the limit of detection in this study, why the sample from February 27, at 20% fraction by volume, should be considered as non-inhibitory. However, when considering the values of inhibition at 50% by volume, even the sample from February 27 is over the limit of detection, indicating that this sample contains inhibitory compounds.
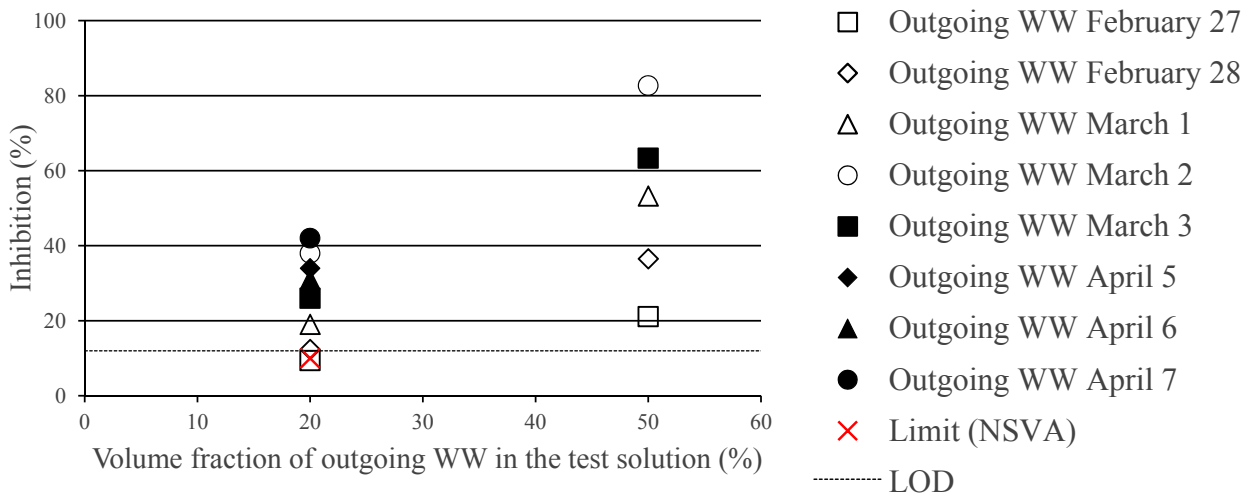
Figure 4.1. Inhibition of nitrification in the outgoing WW in samples taken on five days, in two dilutions (20% and 50%) and the Limit of detection line.

Zinc

The concentration of all zinc elements was analyzed in the outgoing WW samples taken on the dates February 27 to March 3 and April 4 to April 7, all in 2017. The zinc concentration was found to be higher on April 5, which counted 2.44 mg/l. In the remainder of the samples, the concentration of zinc was lower, ranging from 0.19-0.63 mg/l. In Figure 4.2 these samples are plotted together with previous measurements on zinc.

Figure 4.2. The concentration of total zinc plotted over time. Triangles represent previous measurements while circles represent the measurements done in this study.

The inhibition of nitrification in the outgoing WW diluted to 20% as a function of zinc concentration is depicted in Figure 4.3.

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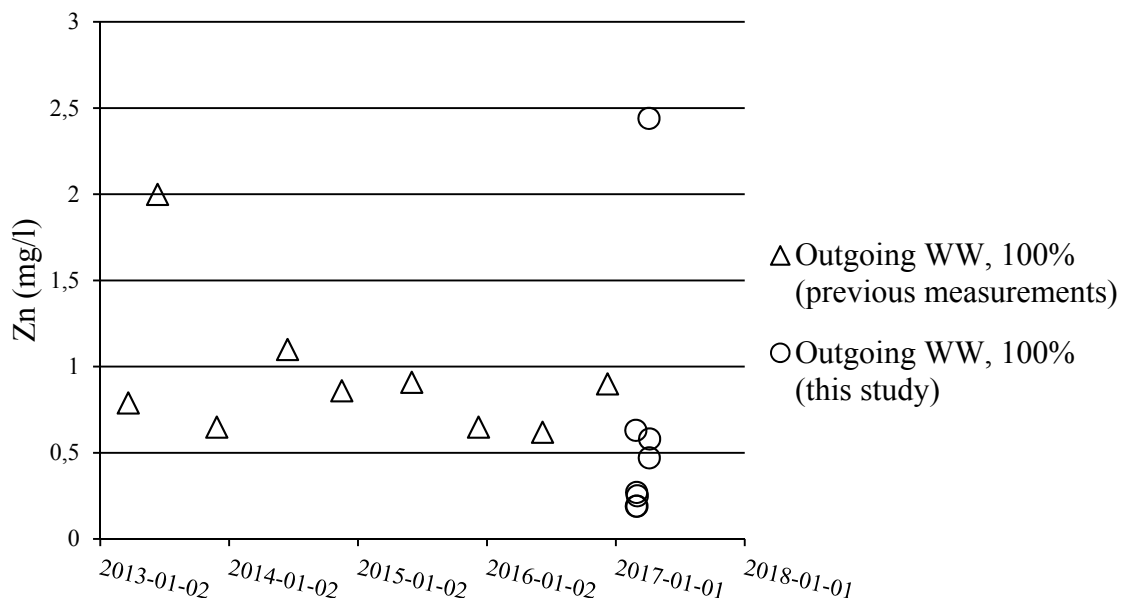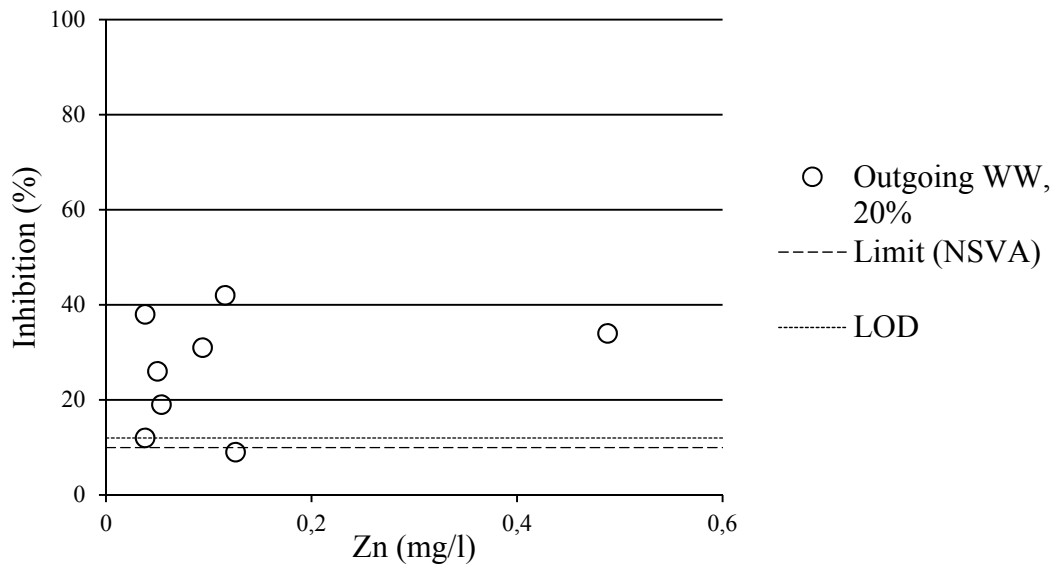*Figure 4.3. Inhibition of nitrification as a function of the concentration of total zinc in the outgoing WW at 20% by volume.*

4.1.2 The leaching wastewaters

The results from testing inhibition of nitrification in the leaching WW are presented in Figure 4.4. The inhibition of nitrification is similar in the different leaching WWs, ranging from 39% to 48% and from 49% to 52% at volume fractions of 20% and 30%, respectively. For the leaching WW collected on February 14 that was tested twice in two different tests at a volume fraction of 20%, the inhibition of nitrification was similar in the two tests (38% and 39%) and the average value is plotted in Figure 4.4 (39%). The leaching water tested in 50% by volume gave an inhibition of 66%. Overall, the inhibition of nitrification is observed to increase with increasing volume fraction of leaching WW in the test solution.

Figure 4.4. Inhibition of nitrification in leaching WWs collected at different dates as a function of the volume fraction of leaching WW in the test tube.

4.1.3 Other streams

The inhibition in the samples of WW from the heat exchanger, the rinsing bath and the cold wash are presented in Figure 4.5. The sample of WW from the heat exchange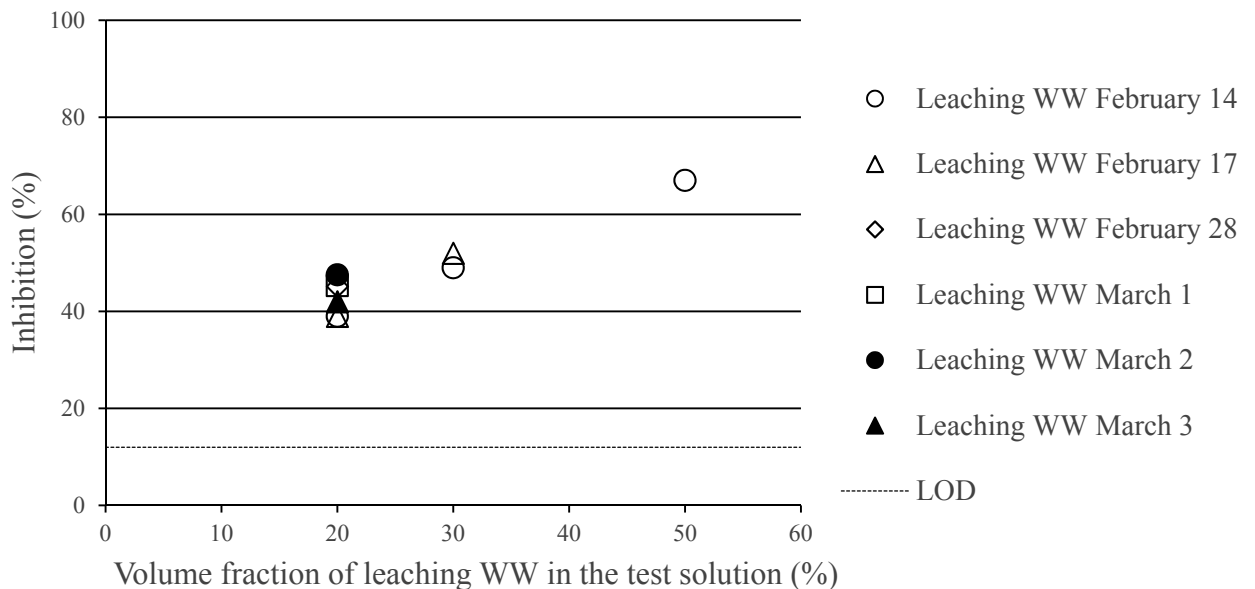r showed less but still significant inhibition. The one from the rinsing bath did not show a clear inhibition of nitrification. The inhibition in the sample with storm water was negative, in other words there was no inhibition (see Appendix V). The sample of WW from the cold wash gave an insignificant inhibition of nitrification, 11%, which is just below the limit of detection. The WW from the cold wash was tested in only one volume fraction.
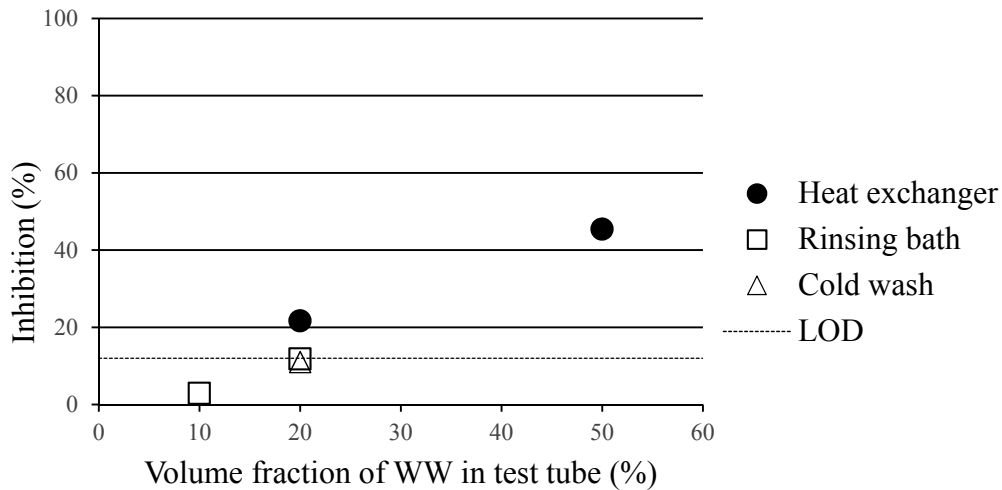
Figure 4.5. Inhibition of nitrification as a function of the volume fraction of WW in the test tube.

A comparison of the WW from the dipping section, the outgoing WW and the WW from the other part of the industry is shown in Figure 4.6, where all samples were tested in volume fractions of 20%. The WW from the dipping section showed inhibition of nitrification ranging from 26 to 36% while the inhibition in samples of WW from the other part of the industry ranged from 7 to 35%. One data point is under the limit of detection, (7% in one sample of WW from "Industry, other").
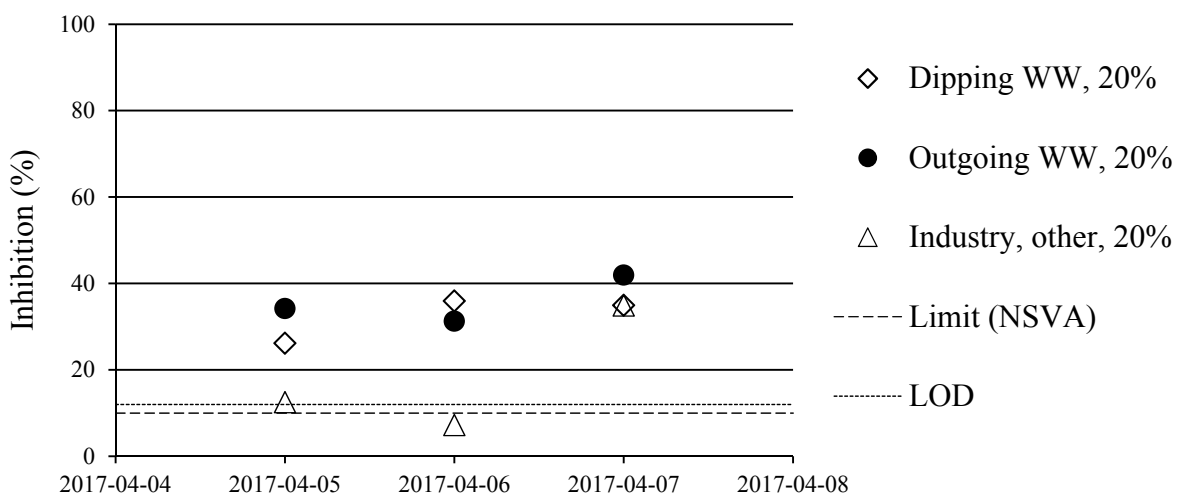
Figure 4.6. Inhibition of nitrification in WW from the dipping section, the other part of the industry and the total outgoing WW. The volume fraction of WW in the test was 20%, and was the same for all samples.

4.2 Mapping of the streams and their inhibition of nitrification

The highest inhibition can be seen in the WW from the leaching process. The WW from the dipping section is more inhibiting than all the partial streams coming to the dipping section. The flows of the different streams and their fractions of the total flow are presented in Table 4.1.

Table 4.1. The flows of the WW streams. (Personal reference: Nilsson, 2017)

| Stream	Flow (l/min)	% of total outgoing flow
Outgoing WW	49	100
Dipping section	46	93
Industry, other	3	7
Streams in the dipping section		
Leaching	15-21	31-43
Forming sheet wash	6-10	12-21
Cold wash	15-25	30-51
Rinsing bath	600 l, one discharge weekly	0.14
Chlorination	400 l, one discharge weekly	0.09

Figure 4.7 shows the partial streams and their inhibition of nitrification (%), in 20% volume fraction in the tests. Some of the measured values of inhibition were under the limit of detection, which are marked. The inhibition of nitrification by the WW from the forming sheet wash was done in previous measurements.
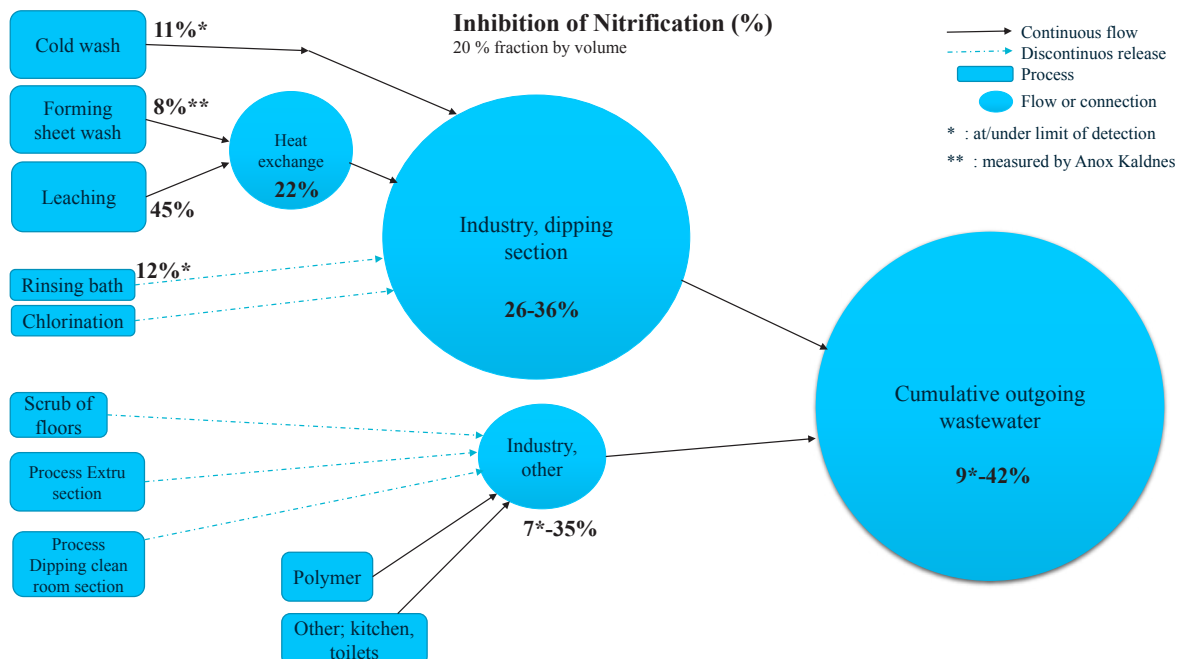

Figure 4.7. Inhibition of nitrification in the partial streams and outgoing WW. All measurements are performed with 20% dilution, and generated by the authors, except for the WW from forming sheet wash which was measured by AnoxKaldnes. When showing an interval, it is the variation during the sampling days.

4.3 Nitrate and nitrite

Both nitrate and nitrite caused inhibition of nitrification, in sufficiently high concentrations. The inhibition curves are presented below. Hereunder the effect from only nitrate is also plotted together with inhibition from leaching WWs containing similar concentrations of nitrate. Similarly, the effect from nitrate solutions, nitrite solutions and mixtures of the two are plotted together with inhibition in samples of outgoing WW with the same composition regarding nitrate and nitrite. Since the concentration of nitrite in the samples of leaching WW analyzed was low, the effect from only nitrite in the leaching WWs was not studied since it was expected to show insignificant inhibition. The concentrations of nitrate and nitrite in the analyzed samples of leaching WW and outgoing WW can be seen in Table 4.2 below.

Table 4.2. Concentrations of nitrite and nitrate in the samples of leaching WW and outgoing WW analyzed.

Sample	NO_2-N (mg/l)	NO_3-N (mg/l)
Leaching WW February 14	4.12	604
Leaching WW February 17	0.05	776
Leaching WW February 28	5.08	599
Leaching WW March 1	4.76	774
Leaching WW March 2	7.80	618
Leaching WW March 3	6.95	543
Outgoing WW February 27	2.69	90.6
Outgoing WW February 28	22.8	92.8
Outgoing WW March 1	10.9	220
Outgoing WW March 2	44.9	139
Outgoing WW March 3	13.9	219
Outgoing WW April 5	11.2	147
Outgoing WW April 6	13.5	141
Outgoing WW April 7	6.5	169

4.3.1 Nitrate

In Figure 4.8 the inhibition in leaching WWs and in pure nitrate solutions, as a function of concentration of NO_3-N, can be seen. The purpose of the figure is to compare the total inhibition in samples of leaching WWs to the inhibition from only nitrate in these samples. The results show that high concentrations of nitrate-nitrogen is causing inhibition of nitrification. When further comparing the inhibition in the leaching WWs with pure nitrate solutions in the same nitrate-nitrogen concentrations, the inhibition in the leaching WW is approximately double as high as in the pure nitrate solutions. Although, in this comparison the interactions with other chemical components in the WW is not considered, why it is difficult to estimate a precise value of the contribution to the inhibition from nitrate. Nitrate could possibly behave antagonistic or synergistic with other components, which would have an impact on the effective inhibition from nitrate.
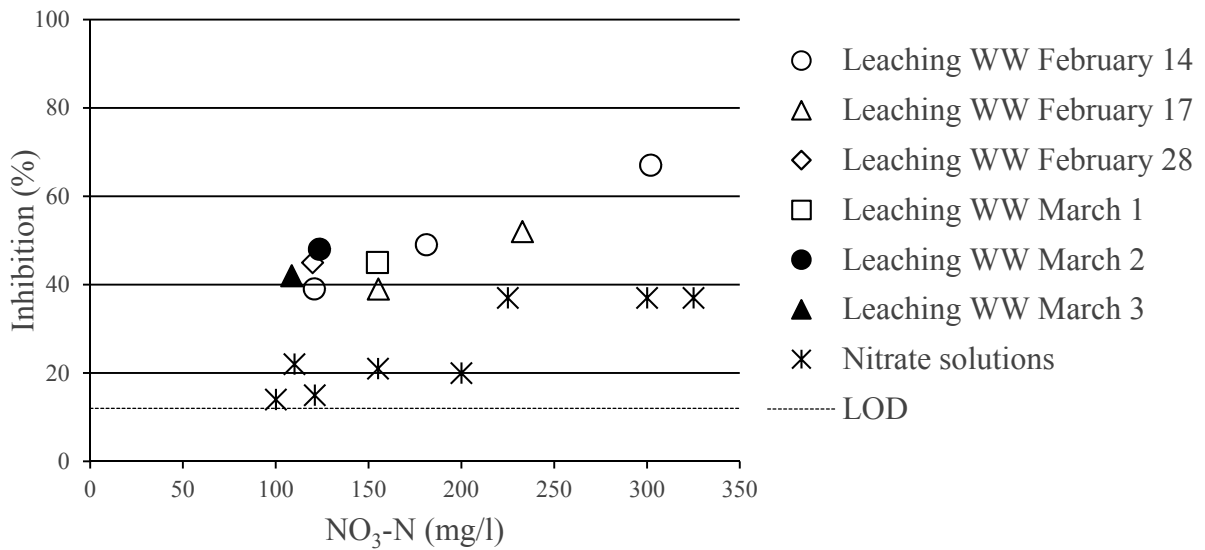
Figure 4.8. Inhibition of nitrification in leaching WWs and in nitrate solutions as a function of NO₃-N concentration. Leaching WWs from February 28, March 1, March 2 and March 3 were only tested in one dilution (20%), while the leaching WW from February 14 was tested in three dilutions (20%, 30% and 50%) and the leaching WW from February 17 was tested in two dilutions (20% and 30%).

The inhibition of nitrification as a function of the concentration of NO_3-N on a logarithmic scale ranging from 100 to 5000 mg/l is depicted in Figure 4.9. All concentrations showed significant inhibition, ranging from 14% to 100% in concentrations of 100 mg/l and 5000 mg/l, respectively.
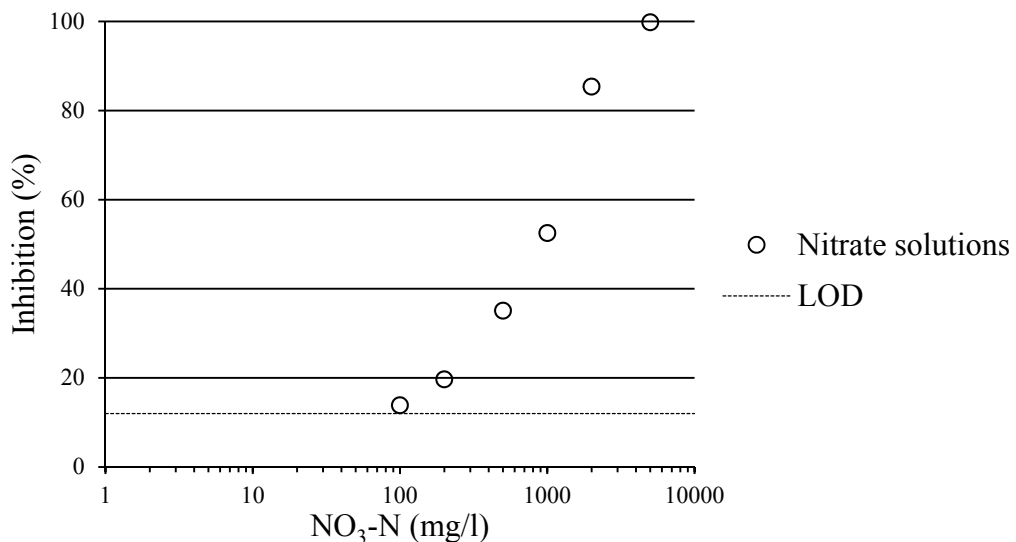
Figure 4.9. Inhibition of nitrification as a function of concentration NO₃-N (mg/l).

4.3.2 Nitrite

Figure 4.10 shows that the inhibition in most of the nitrite solutions are insignificant (<10%), albeit the three dilutions with the highest concentration show significant inhibition (10%, 28% and 35%). The samples of outgoing WW with the same concentration of NO_2-N as the dilutions giving inhibition of 10% and 35% showed inhibition of 37% and 83%. The purpose of Figure

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4.10 is to present a comparison of the total inhibition of nitrification in the outgoing WW, with the one caused by only nitrite, neglecting interactions with other chemical components in the WW.
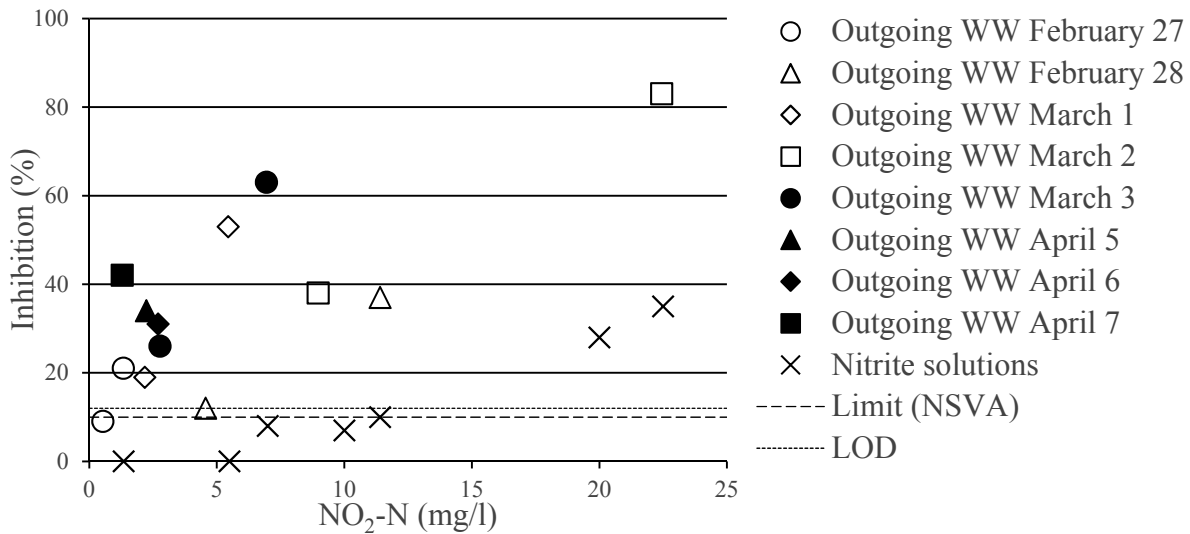
Figure 4.10. Inhibition of nitrification in outgoing WW and in nitrite solutions as a function of NO2-N concentration. Samples of outgoing WW from April 5, April 6, and April 7 were only tested in one dilution (20%), while samples from February 27, February 28, March 1, March 2 and March 3 were tested in two dilutions (20% and 50%).

Pure nitrite solutions did not show any significant inhibition in the lowest concentration (10 mg/l). However, all other concentrations tested showed significant inhibition where the highest concentration (500 mg/l) resulted in an inhibition as high as 91%. The inhibition of nitrification as a function of the concentration of NO_2-N on a logarithmic scale is depicted in Figure 4.11.

Figure 4.11. Inhibition of nitrification as a function of concentration NO2-N (mg/l).

4.3.3 Nitrate and nitrite

In figure 4.12, the inhibition in samples of outgoing WW at a volume fraction of 50% and in solutions of nitrate, nitrite or both combined is depicted. Symbols placed on the same date are equal in terms of their composition of either nitrate, nitrite or both. The mixtures containing

both nitrate and nitrite were all inhibitory except for the mixture representing the sample of outgoing WW from February 27, which had the lowest concentration of both nitrate and nitrite. In most of the studied cases the inhibition from solutions with both nitrate and nitrite were higher than the ones from the corresponding solutions containing only nitrate or nitrite. But in two cases, March 1 and March 3, the inhibition from the solution containing only nitrate was higher or equal to the one from the solution containing both nitrate and nitrite. The inhibition in the outgoing WW was consistently higher than in the pure solutions of nitrate, nitrite and both combined.

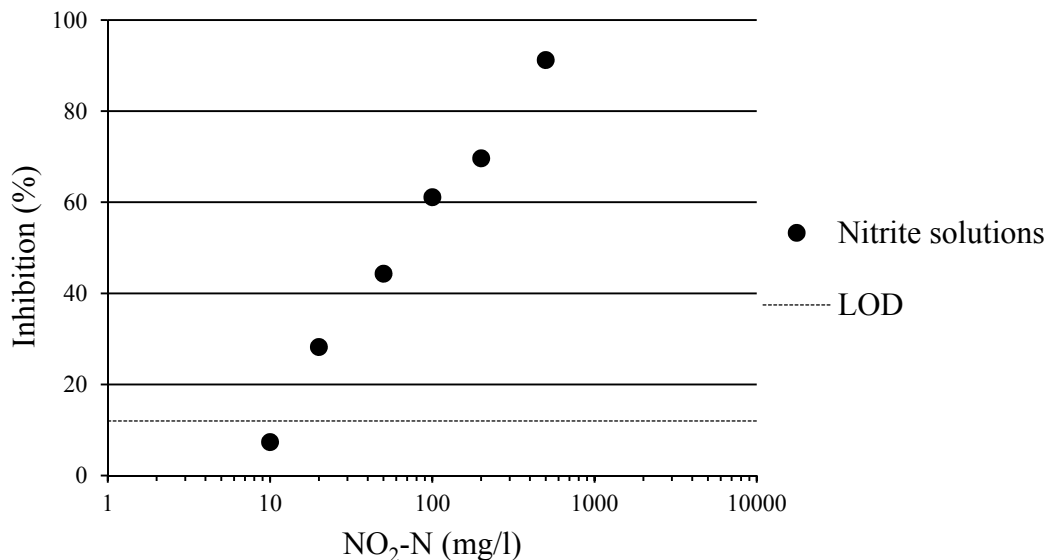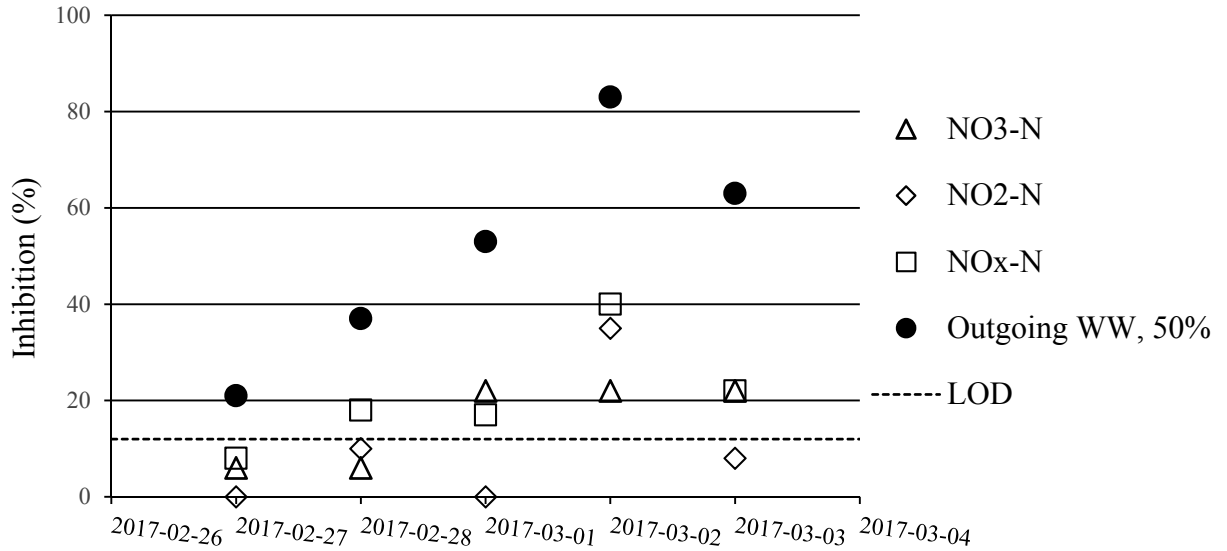*Figure 4.12. Inhibition of nitrification in the outgoing WW at a volume fraction of 50% and in solutions containing only nitrate, only nitrite, and nitrate and nitrite combined. The data points with no fill represent solutions with the same concentration of either only nitrate or only nitrite or both nitrate and nitrite as the outgoing WW plotted on the same date.*

4.3.4 Additivity test

The mixtures of $Ca(NO_3)_2 \cdot 4H_2O$ and $NaNO_2$ all showed significant inhibition of nitrification. The concentrations of nitrate and nitrite in the mixtures, and the measured values of inhibition, can be found in Table 4.3. The interactive index for each mixture were calculated using inhibition curves of NO_3 and NO_2. The method of calculation is described in Chapter 3.

Inhibition functions

From the known concentrations and obtained inhibition percentages which are presented in Figures 4.9 and 4.11, a curve for each compound could be fitted to the data using Excel. The Equation describing the inhibition of nitrification (*f*) as a function of the concentration of NO_3-N (d_{NO_3-N}) is presented here below:

$$f(d_{NO_3-N}) = -7 \cdot 10^{-6}(d_{NO_3-N}) + 0.0508(d_{NO_3-N}) + 9.6279 \tag{12}$$

Respectively for nitrite, the Equation describing the inhibition of nitrification (*g*) as a function of the concentration of NO_2-N (d_{NO_2-N}) is as follows:

$$g(d_{NO_2-N}) = 20.625 \cdot \ln(d_{NO_2-N}) - 36.748 \tag{13}$$

For the given concentrations in each mixture tested in the additivity test, the corresponding inhibitions from nitrate and nitrite were found by calculating *f* and *g*. The calculated inhibition

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by each single substance was divided by the total, measured inhibition of each mixture according to Equation 9. The interactive indices for all mixtures were either equal to or more than one, indicating additivity or antagonism, depending on mixture (see Table 4.3).

Table 4.3. The given effects (inhibition of nitrification) in dilutions of the two mixtures of calcium nitrate and sodium nitrite and calculated interaction indices (II) for each dilution.

Mixture	NO_2-N* (mg/l)	NO_3-N* (mg/l)	Measured inhibition in mixture (%)	II
A	60	300	70	1.1
A/1.5	40	200	49	1.2
A/3	20	100	40	1.0
B	36	800	65	1.2
B/2	18	400	48	1.0
B/2.8	13	290	38	1.0

* concentration in the test tube

4.4 Production chemicals

The worst-case concentrations in the outgoing WW and in the leaching WW in 20% fraction by volume are presented in Table 4.4, together with the measured inhibition of nitrification by each chemical in a concentration of 200 mg/l in the test tube. The inhibition of nitrification in the leaching WW ranged from 39% to 48%, as a comparison, in 20% fraction by volume.

Table 4.4. Worst-case concentrations and inhibition of nitrification are presented for each production chemical.

Chemical	Worst-case concentration of chemical in outgoing WW (mg/l)	Worst-case concentration of chemical in leaching WW diluted to 20% by volume (mg/l)	Inhibition of nitrification in samples with 200 mg/l of chemical (%)
Tamol	307	140	23
WAQ	287	116	40
Emulan	182	220	17
SMO	151	236	4

Figure 4.13 shows the inhibition among the production chemicals tested. Among those, WAQ was the most inhibiting; at 500 mg/l WAQ caused 74% inhibition of nitrification. Emulan, SMO and Tamol also showed clear inhibition of nitrification at 50% by volume (500 mg/l). Among the solutions with 200 mg/l of chemical, all showed significant inhibition of nitrification except SMO, which showed only 4% inhibition (<10%). Tamol showed a strange behavior with less inhibition in the solution with 300 mg/l than in the one with 200 mg/l of the pure production chemical.
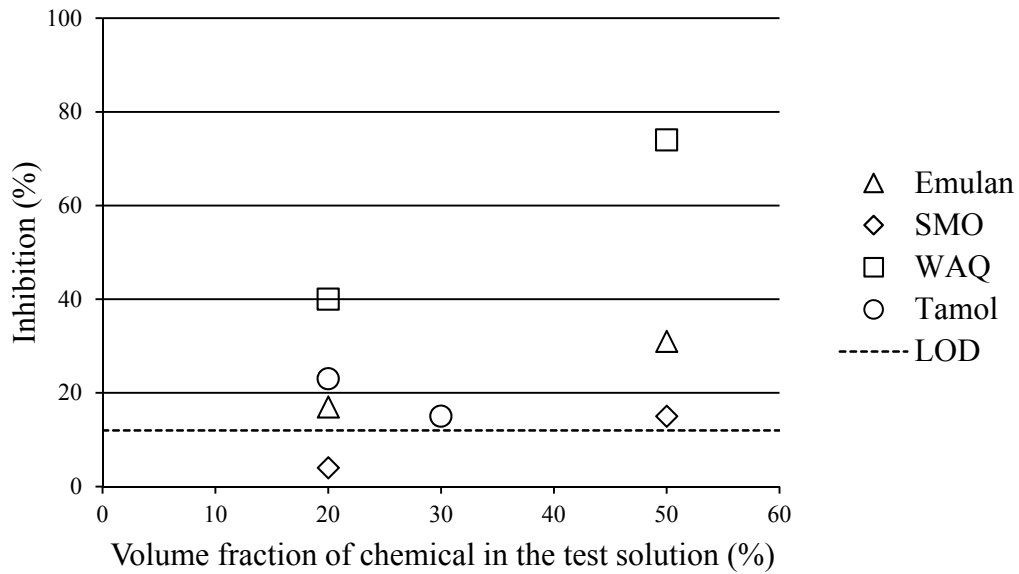
Figure 4.13. Inhibition of nitrification among tested chemicals as a function of the volume fraction of each chemical in the test solution. The chemicals are here defined as solutions with 1000 mg/l of the pure production chemical tested. Volume fractions of 20%, 30% and 50% hence mean solutions with 200 mg/l, 300 mg/l and 500 mg/l of the chemicals.

4.5 Dependency of day and time

Inhibition of nitrification was plotted with respect to time. No general trend was found, see Figure 4.14. Further was the inhibition's dependency on weekday analyzed, see Figure 4.15. The two highest measurements of inhibition of nitrification in this study were found on Thursdays, but compared with previous studies Thursdays did not show higher inhibition than other days.
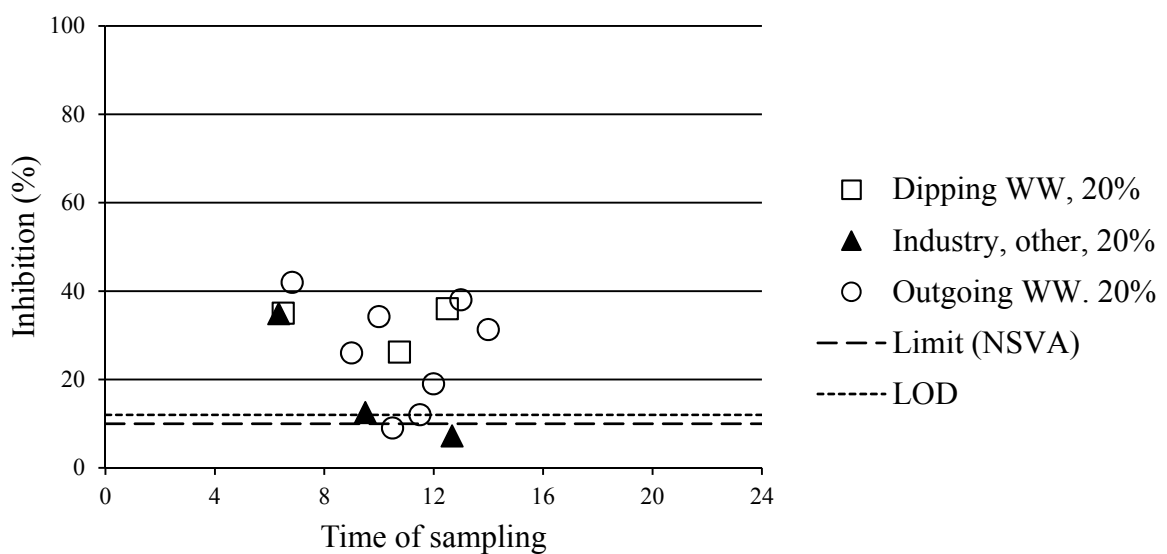
Figure 4.14. Inhibition of nitrification as a function of time of sampling, from 6 a.m. to 2 p.m. in water from the dipping section, the industry, other and from the outgoing WW.

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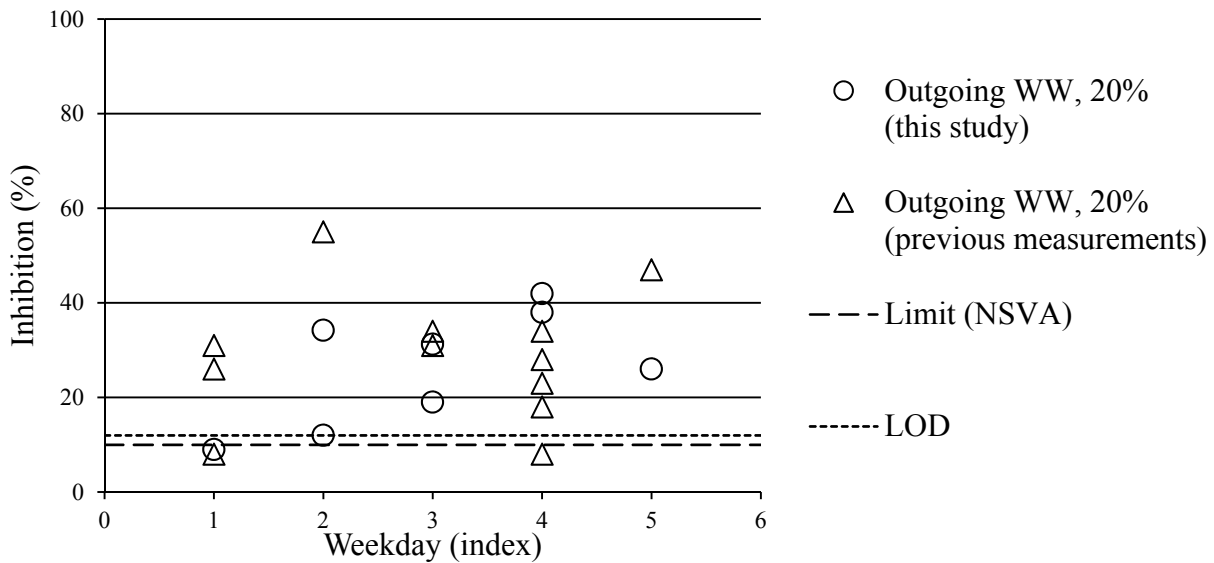
Figure 4.15. Inhibition of nitrification as a function of weekday, where 1-5 represent Monday to Friday.

The measurements of inhibition of nitrification in the outgoing WW and the leaching WW are plotted in chronological order of sampling in Figure 4.16. It can be seen that the leaching WWs caused higher inhibition than the outgoing WW from the same dates, and that there is no general trend over time (February 14 to April 7, 2017).
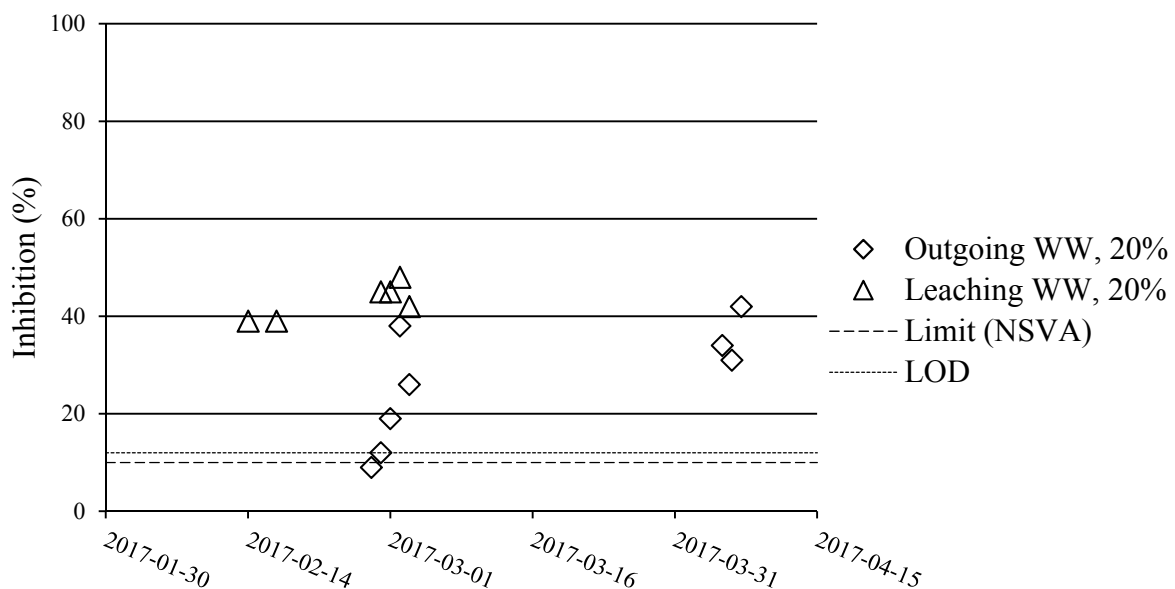
Figure 4.16. Inhibition of nitrification in samples of outgoing WW and leaching WW (at 20% by volume) taken at different dates.

4.6 Limit of detection

In Table 4.5, the values used to calculate LOD and the resulting values of LOD are presented for all tests separately, for all tests together and for test 2-5 together. The first test generated an unsatisfactorily high value of LOD, which was expected since the performance of this test was known to be poor, judging from the difference in the ammonium consumption rate among the sets of controls observed (see Appendix I, Figure A1). Hence it was decided to exclude this test when calculating the overall LOD used in this study. This LOD is thus based on test 2-5, and the value is seen in the row "Test 2-5" in Table 4.5, which turned out to be 11.8%. As a result, the LOD of this study was, after rounding, determined to 12%, meaning that a sample should show at least 12% inhibition to be regarded as inhibitory, with 95% certainty. All values below 12% are according to the same reasoning insignificant, since those values cannot be concluded to be a result of true inhibition.

Table 4.5. The values used to calculate the LOD and the LOD obtained for all test separately, all tests together and test 2-5 together.

Tests included	$N_{samples}$	df	α	$t_{1-\alpha}$	$\sigma_{controls}$ (%)	LOD (%)
Test 1	3	2	0.05	2.920	8.13	41.1
Test 2	3	2	0.05	2.920	1.85	9.29
Test 3	3	2	0.05	2.920	2.37	12.0
Test 4	3	2	0.05	2.920	6.04	30.6
Test 5	3	2	0.05	2.920	4.44	22.4
All tests	15	14	0.05	1.761	5.13	14.7
Test 2-5	12	11	0.05	1.796	4.04	11.8

5. Discussion

To find the cause of the inhibition of nitrification detected in the WW at Nolato MediTor, the inhibition was tested with the screening method on different streams of WW and on chemical compounds used at the industry. The possible sources of the inhibition of nitrification are discussed; relatively high concentrations of nitrate and nitrite, leaching of production chemicals and possibly an undiscovered source of toxic WW. The streams and compounds that can be excluded from being sources of inhibition are also discussed. The results are put in context with the requirements from the municipality, followed by a discussion about uncertainties in the method.

5.1 Sources of inhibition

The inhibition of nitrification in the leaching WW was as supposed high, and was higher than in the outgoing WW all days tested, which means that the leaching WW becomes less toxic on the way to the outgoing WW. The different samples of leaching WW were quite similar in inhibition, indicating an even discharge of inhibitory compounds. The outgoing WW itself varied a lot between the dates of sampling. One explanation could be that the samples were taken as random samples and not daily samples, resulting in that fluctuations in the chemical composition caused by batch-wise release of different process streams plays a major role. The other process streams also contain inhibitory chemicals but none of the other streams tested had higher inhibition of nitrification than the leaching WW.

The map of inhibition of nitrification (Figure 4.7) was made to picture where at the industry the source of the inhibition comes from. The dipping section had high inhibition of nitrification, but in two of three days it was higher in the outgoing WW. In Figure 4.6 the inhibition of nitrification in samples of WW from the dipping section and the outgoing WW, taken on the same date can be seen. Comparing the samples taken on April 5 and April 7, the inhibition of nitrification is 34% and 42% in the outgoing WW compared to 26% and 35% in the WW from the dipping section. An explanation for this could be uncertainty of the method, which taken into account could mean that the inhibition was the same in the WW from the dipping section as in the outgoing WW. That seems reasonable as the flow from the dipping section is 93% of the total flow. The stream "Industry, other" was shown to sometimes be inhibitory, but the flow should be so small that the addition would not contribute to substantially higher inhibition in the outgoing WW. The streams flowing into the connection point of the dipping section all had lower inhibition than the WW from the whole dipping section, called "Industry, dipping section", which is not convincing. One reason for the higher inhibition in the WW from the dipping section could possibly be that one of the discontinuous flows was adding inhibitory chemicals at the time of sampling.

The results from the tests performed in this study can confirm previous studies on substrate and product inhibition; solutions of nitrate and nitrite in sufficiently high concentrations did cause inhibition of nitrification. The outgoing WW showed consistently higher inhibition of nitrification than the pure solutions of nitrate, nitrite and both combined in concentrations corresponding to those in the outgoing WW. This seems reasonable, since the outgoing WW is known to also contain other inhibitory compounds. How much of the total inhibition that is caused by nitrate and nitrite is discussed in the additivity section. From the view of Nolato

MediTor, it was interesting to find out if a larger part of the inhibition of nitrification could be explained by these nitrogen species since they are typical for municipal WW and the receiving WWTP is designed to remove these.

5.1.1 Nitrate

The concentration of nitrate in the leaching WW seems fairly constant over time, indicating that there is no major variation in the leaching process. This is consistent with the known fact that the flow from the leaching tanks is continuous, preventing compounds to accumulate over time. However, some variation is still observed which could arise from small variations in the process or be a result of the leaching tanks not being completely mixed, or measurement uncertainties. Further comparing the nitrate concentrations in the leaching WW with those in the outgoing WW tells us that nitrate is consistently lower in the outgoing WW. This seems reasonable since the only step known to supply the WW with significant loads of nitrate is the leaching step. Hence, the nitrate should be highest in this step, while the outgoing WW should be more diluted due to other, connecting WW streams. When comparing previous measurements of nitrate concentration with those measured in this study, they are similar, see Figure 2.3 and Table 4.2. The similarity in nitrate concentration indicates that the discharge of nitrate has not changed over time.

With the results of this study, the inhibition of nitrification by nitrate is successfully visualized as an inhibition-curve in Figure 4.9. The data points are consistently showing higher inhibition with increasing concentration. A similar consistent graph could be pictured also for nitrite (see Figure 4.11). These results are a validation of the screening method and the certainty of the obtained inhibitions. The results are in line with the study of inhibition by nitrate and nitrite made by Jönsson (2001).

Figure 4.8 which shows different samples of leaching WW together with pure nitrate solutions in the same nitrate-nitrogen concentrations gives a rough estimation that nitrate plays a significant role in the puzzle of the inhibition. As seen in the figure, inhibition of nitrification in the leaching WWs are almost double as high as in the pure nitrate solutions with the same nitrate-nitrogen concentrations.

5.1.2 Nitrite

Looking at the inhibition from solutions of nitrate and nitrite with equal concentrations (i.e. NO_2-N in the nitrite solution equals NO_3-N in the nitrate solution), the inhibition from nitrite was found to be more severe than the one by nitrate, as according to Anthonisen *et al.* (1976). However, nitrite is present in much lower concentrations than nitrate in both the leaching WW and the outgoing WW, meaning that the inhibition from nitrite in these WWs is not necessarily more pronounced than the one from nitrate.

The fact that the outgoing WW generally contained more nitrite than the leaching WW was unexpected since most of the chemicals in the WW are understood to come from the leaching WW. Furthermore, none of the other streams studied contained high nitrite and could have contributed to the unexpectedly high concentration in the outgoing WW. Our results tell us that the nitrite does not origin from the dipping section, nor the other part of the industry. This indicates that there could be an additional process stream, not included in this study, serving as the source of nitrite. Alternatively, since samples were taken as random samples, a possible explanation could be that the different time of sampling among samples taken on the same day makes these samples less comparable. Discharges of high nitrite could have been captured when

sampling outgoing WW, while they were missed when sampling the other streams. The pattern of uneven discharge is confirmed by noting that samples of outgoing WW taken on different days showed large variation in nitrite concentration, where some samples also showed low nitrite. The time allocated for this study was not enough to map out what is causing the high concentration of nitrite in the outgoing WW, and this could be a problem suited for future, continued studies. Another consideration is whether there was interference in the analysis of nitrite. If chemicals with the same "color" are present in the WW, the spectrometer registers those as nitrite. However, no interference data could be found for this analysis, and hence it is assumed that the measured value is merely a result of nitrite.

The nitrite concentrations measured in this study have shown a greater variety than in previous measurements, see Figure 2.4 and Table 4.2. As Figure 4.10 shows, the nitrite concentration varied quite a lot in the different samples of outgoing WW.

When comparing prepared nitrite solutions that showed significant inhibition of nitrification with samples of outgoing WW, the contribution from nitrite could be 30-40% of the total inhibition. However, this is based on the assumption that the substances present in the outgoing WW act additive. The additivity of nitrate and nitrite are discussed below, but the additivity of inhibition among all the chemicals in the WW is unknown. Furthermore, it is difficult to draw any conclusions regarding the contribution from nitrite in the outgoing WWs with low nitrite concentrations, since the corresponding nitrite solutions gave insignificant inhibition of nitrification ($< 12\%$) and method uncertainty becomes substantial, see Figure 4.10.

5.1.3 Additivity
The solutions of nitrate and nitrite corresponding to the outgoing WW from March 1 and March 3 at 50% by volume showed unexpected results, see Figure 4.12. Only nitrate gave higher or equal inhibition of nitrification than the solution containing both nitrate and nitrite. The combination of the two was expected to give more inhibition. However, the samples of WW taken on March 1 and March 3 contained quite low concentrations of nitrite, under 8 mg NO_2-N/l, which means that the contribution to the inhibition from nitrite can be expected to be insignificant in those samples. The low nitrite concentrations could hence explain the observation that nitrate gave the same inhibition as NO_x. The results showing inhibition lower than the limit of detection are not reliable to interpret in terms of additive inhibition. Overall, method uncertainty could explain these unexpected results.

The solutions corresponding to the samples of outgoing WW from February 27 and February 28 showed a more additive behavior, since the inhibition from the solutions containing nitrate and nitrite was approximately equal to the sum of the values of inhibition from the corresponding solutions containing only nitrate and only nitrite. However, as for the nitrite solutions corresponding to the samples of outgoing WW from March 1 and March 3, most of these solutions were falling under the LOD, meaning that method uncertainty plays a major role, and the outcome cannot be concluded to be a result of true inhibition.

The nitrite solution with the highest concentration of nitrite (22.45 mg/l), corresponding to the sample of outgoing WW in 50% fraction by volume from March 2, showed inhibition in the same range as the inhibition by the corresponding mixture which also contained nitrate, which was unexpected since the mixture was expected to give higher inhibition than the solution containing only nitrite. The concentration of nitrate in the mixture was 70 mg/l, which when tested as single substance gave an inhibition of 22%, which is not negligible. This indicates that nitrate and nitrite in these concentrations are not additive, but rather antagonistic since the

inhibition from the mixture was lower than expected. It should be mentioned that this date (March 2) was the only one where all the corresponding solutions (nitrate, nitrite and NO_x) showed inhibition higher than the limit of detection, meaning that this is the most reliable date to interpret in terms of additivity.

The results of the modified additivity test pointed on additive or antagonistic inhibition depending on the magnitude of the inhibition. The three solutions that gave additive inhibition of nitrification (II=1), had a total inhibition of 38%, 40% and 48% and the three mixtures that gave antagonistic inhibition (II>1) had an inhibition of 49%, 65% and 70%. It appears that nitrate and nitrite are additive when the total inhibition is in the intermediate range (40-50%), while they seem to turn antagonistic when the inhibition increases (50-70%). This result is valid for only nitrate and nitrite, but the WW contains many more compounds. The results from the modified additivity test are considered more reliable than the results when comparing individual values of inhibition from nitrate, nitrite and NO_x (Figure 4.12), as the test was set up after a scientific method and the values of inhibition lies higher above the LOD.

The mechanism of inhibition of nitrification in the WW can be understood to be complex, and any general subtractions of inhibition by one compound from the total inhibition of nitrification should not be made without caution. If this statement is foreseen for a minute, a rough estimation of the inhibition by nitrate and nitrite can be done. The average outgoing WW concentration of nitrate is 140 mg/l and of nitrite 3 mg/l, which corresponds to 17 and 0% inhibition according to Equation 12 and 13, making only nitrate contribute to the overall inhibition of nitrification. If instead the peak concentrations measured in the outgoing WW are considered, 220 NO_3-N mg/l and 45 NO_2-N mg/l, the inhibition would be 20% and 42%: in total 62%. These concentrations were not found at the same date, but it could represent a worst-case situation. However, as seen in the additivity test the inhibition seems to be antagonistic at high inhibitions, hence counteracting the inhibition of each another. Nevertheless, the contribution to the overall inhibition from nitrate and nitrite could be large.

5.1.4 Production chemicals
Since the concentrations of the production chemicals in the WW are not known, the inhibition in the outgoing WW by these chemicals cannot be determined. Testing of these chemicals rather gave an indication of the toxicity among these chemicals in the range of concentrations tested. Although the results of the inhibition of nitrification by the production chemicals did not give clear answers, toxic compounds that inhibit the living mechanism of nitrifiers were found.

When looking at the COD in the pure chemicals and comparing the values with the COD in the leaching water, the sum of the worst-case concentrations of COD from the chemicals is much higher than the COD in any of the samples of leaching WW (see Appendix IV). A conclusion can be drawn that the calculated worst-case concentrations of the production chemicals are too high.

WAQ and Tamol seem to be inhibitory at 200 mg/l, which for both compounds is lower than the estimated worst-case concentration in the outgoing WW, making them suspected to cause inhibition of nitrification in the WW. For SMO, the worst-case concentration is estimated to be 116 mg/l, being lower than 200 mg/l which gave no significant inhibition. Thus the inhibition of nitrification of SMO is probably zero in the outgoing WW. To be certain about this, SMO should be further investigated regarding its effect together with the other chemicals; synergistic behavior could for example magnify the effect from SMO. The inhibition of nitrification by

Emulan was assumed to be small, but appeared to be rather significant; 17 % inhibition at 200 mg/l. The estimated worst-case concentration in the outgoing WW was 182 mg/l which is close to 200 mg/l, making Emulan a potential source of inhibition of nitrification.

All chemicals showed an increased inhibition of nitrification with increased concentration, except Tamol which according to our results was less toxic in higher concentration. However, this seems unreasonable since Tamol should have been the only inhibiting substance in the test tube. A plausible explanation could be that the test tubes with different concentrations of Tamol could have been mixed up and labelled wrong; in that case the tube containing the less concentrated solution of Tamol was taken as the one containing the more concentrated solution, and vice versa. The production chemicals were tested in test 2, in which a source of error was found in the analysis of ammonium. The strange result of Tamol could have been caused by a poorly shaken cuvette; and thus the extent of the reaction making the green color for ammonium analysis was questionable. The results of inhibition by the other chemicals were increasing with concentration, which makes them reliable, even though the analysis of ammonium concentration was suspected to might have been uncertain.

5.1.5 Minor streams
According to our results, the rinsing bath WW and the cold wash WW can be excluded from sources of inhibition of nitrification. The rinsing bath WW is only released once per week, and did not show significant inhibition. Nor did the cold wash WW show significant inhibition, and since it is a continuous flow that is mixed with leaching WW, it could instead be a source of WW that is decreasing the inhibition of nitrification in the outgoing WW.

5.1.6 Zinc
In the previous investigation at Nolato MediTor, Malmqvist (2013) remarks that the zinc is likely to be in the particulate form which is not inhibiting, but the report does not state why the zinc is suspected to be in particulate form. In the safety sheet for zinc carbonate by Brenntag Nordic AB it is both stated that the product is soluble and insoluble in water, but according to ECHA zinc carbonate is soluble in water to 1.3 mg/l (ECHA, 2016). This would mean that in theory, zinc could be present as dissolved ions in concentrations up to 1.3 mg/l, which is higher than the zinc concentration in all the test tubes of samples of outgoing WW. However, we cannot say for certain that all zinc is present as ions even though the solubility of zinc carbonate is high enough, since the method employed for measuring zinc measures all elements of zinc.

According to literature, zinc present as free ions at 0.5 mg/l is inhibiting the nitrification to an extent of 70-90%, depending on study. However, in these studies the zinc was measured as free ions, which is more bioavailable than particulate zinc, and hence more prone to cause inhibition. In the outgoing WW from Nolato MediTor, at 20% by volume, one sample showed a zinc concentration as high as 0.49 mg/l, while the rest of the samples had significantly lower concentrations of zinc ranging from 0.04-0.13 mg/l. However, according to Figure 4.4 which shows the inhibition of nitrification in the outgoing WW at 20% by volume there is no trend with zinc concentration, and the sample with significantly higher zinc content than all other samples did not show the highest inhibition of nitrification. The highest inhibition was instead found in a sample with a much lower zinc content of 0.12 mg/l. This is a quite strong indicator that the zinc present in the outgoing WW does not play a major role in determining the inhibition of nitrification. To confirm this, an additivity test of zinc could be set up to evaluate the behavior of zinc in combination with other chemicals.

As can be seen in Figure 4.3, all samples except the one from March 2 show a zinc concentration lower compared to previous measurements. This indicates that the zinc content is somewhat lower in the outgoing WW today than what it used to be. Nevertheless, only 8 samples of outgoing WW have been analyzed, and more data would be needed to conclude this for sure.

5.1.7 Dependence on time of sampling

Inhibition of nitrification can be related to certain routines at industries, why the results were plotted with respect to time and weekday. No general trends were found, as can be seen in Figure 4.14 and 4.15. The different streams dipping section, industry other and outgoing WW all had high inhibition of nitrification early in the morning (6:20-6:50), see Figure 4.14. This might as well be a coincidence, since we only have three data points of samples taken this early. Similarly, no clear trend with weekday is observed. The highest inhibition measured in this study was found on Thursdays, but in comparison with previous measurements the values of inhibition in samples taken on Thursdays are not significantly higher than the ones in samples from other days.

5.2 Reliability and uncertainty analysis

The screening method uncertainty is comparably high in this study, and we can only give the results as indicators of the actual inhibition of nitrification. There are many steps in the method that adds up to the total uncertainty. Several chemical solutions were prepared by calculation of needed mass, weighting of mass and dilution with distilled water. The concentrations of those chemical solutions were verified with Hach Lange cuvettes with certain measuring intervals. They therefore had to be diluted to be within this range, giving a source of error. Furthermore the stock solutions were diluted when preparing the different concentrations that were to be used in the tests. Therefore we cannot say with certainty that the concentrations of the chemical solutions are exactly what we consider them to be. A value on the method uncertainty for Hach Lange analysis have not been found, but as the analysis is based on spectrometry, thus interference by other compounds than the one analyzed is possible.

5.2.1 Comparison of concentrations of nitrate

Concentrations of nitrate were prepared only in small volumes of 15 ml in test 1, which increases the uncertainty that comes with pipetting small volumes of liquid. One part of a drop can easily remain in the pipette tip, or not be sucked up. In this test, 325, 300 and 275 mg/l NH_4-N gave the same inhibition of nitrification. Those concentrations aimed at are not differing substantially, and the similarity in concentrations together with uncertainty linked with preparing those solutions could explain the similarity in inhibition observed.

Moreover, when comparing results from the different tests, it is seen that the inhibition of nitrification for similar concentrations of nitrate differs. For example; 150 mg/l gave 14% inhibition in test 1, and 155 mg/l gave 21% inhibition in test 2. From the reasoning above regarding uncertainties of test 1, the result from test 2 might be more reliable in terms of inhibition of nitrification. The difference between 150 and 155 mg/l nitrate is small and the two concentrations would probably have given similar inhibition in a flawless test. In test 3, 110 mg/l nitrate was tested and gave 22% inhibition, which is high. In the last test (test 5), 100 mg/l nitrate gave 14% inhibition. The varying results is a clear indication that the performance of the tests has been varying, and that it might be wise to keep comparisons of results within the same test. In some cases data from different tests were plotted together, for example outgoing WW and concentrations of nitrate and nitrite, as this comparison was needed to interpret the results. The samples that were tested in the same tests are presented in Appendix V.

5.2.2 Detected errors in the tests

The leaching WW from February 14 tested in test 1 in 50% volume fraction had 432 mg/l COD, but the upper limit of COD is 400 mg/l. A high COD content can lead to higher inhibition than the inhibition by the test liquid since lack of oxygen can also be an inhibitory factor. Though the limit of 400 mg/l COD is set to ensure that no inhibition occurs due to competition with heterotrophs, and the extra 32 mg/l might not be significant. The result from this measurement is included in the results of nitrate.

The rinsing bath WW and heat exchanger WW were tested in test 2, which had a source of error in the method of the ammonium analysis. But since the result showed increasing inhibition with concentration, the test seems reliable. The same was shown for leaching WW and pure nitrate solutions, also tested in test 2.

The VSS in the sludge suspension was often close to or under the lower concentration limit. The lower concentration limit is specified to ensure that a sufficient amount of bacteria is present in the test tube to consume enough ammonium. The smaller the change in ammonium compared to what is initially in the test tube, the more uncertain the calculated inhibition becomes. However, since the nitrification rate in our case was so high, a VSS slightly below the lower concentration limit was accepted and is not expected to have caused biased results.

To summarize, measuring inhibition of nitrification is not a precise science. The obtained results can be used and interpreted, but not give exact numbers or answers. What is important is that an inhibition of nitrification measured in a test tube *may well* also be found at a WWTP and in nature, since the nitrification as a process is in principle the same wherever it is occurring.

5.3 Actions at Nolato MediTor

Although the samples of outgoing WW in this study were taken as random samples, the range of inhibition of nitrification from our measurements in 20% dilution was 9-42%, and six out of seven samples were for certain exceeding the limit stated by NSVA. The results have also revealed inhibition of nitrification by several components present in the WW. Hence the authors would suggest that actions to meet the requirement of 10% inhibition of nitrification needs to be done. As the existing information about the chemical composition of the WW is incomplete, it is problematic to suggest a treatment method. When choosing a treatment method, the input chemicals and their concentrations should be known, to be able to design for the desired reduction. The outgoing WW from Nolato MediTor is indeed inhibiting nitrification, and a large part of the total inhibition of nitrification can possibly be explained by the relatively harmless compounds nitrate and nitrite. The biological step at WWTPs is designed to remove these compounds, but in lower concentrations, usually found in municipal WW, why the results of this study opens up for a discussion about the requirement.

6. Conclusions

The inhibition of nitrification in the outgoing WW is unpredictable, due to varying concentrations of chemicals. The inhibition in the outgoing WW in a volume fraction of 20% was measured to be 42% at its highest, which is well above 10% being the upper limit decided by NSVA. Although the limit value from NSVA should be measured from a daily sample, the random sample showed a clear indication of toxic compounds being discharged. The company should take actions to reduce the inhibition of nitrification in their outgoing WW to under 10% at 20% by volume.

All the sources of the inhibition of nitrification have not been found, but the results say that nitrate and nitrite are giving rise to parts of the inhibition when the concentrations are high. The concentration of nitrate is high – and inhibitory, in the leaching WW. The concentration of nitrite is fluctuating and sometimes inhibitory in the outgoing WW, but low in the other partial WW streams.

From the result of the additivity test, it can be concluded that the inhibition of nitrification by nitrate and nitrite is additive in the intermediate range of inhibition (40-50%), but antagonistic in the higher range (50-70%). In this study, the outgoing WW from Nolato MediTor ranged from 9 to 42% inhibition and had an average inhibition of 26% in a volume fraction of 20%, meaning that the inhibition by nitrate and nitrite in the outgoing WW is expected to be additive, since the closest inhibition measured at (the intermediate range) showed additivity.

WAQ, Emulan and Tamol were revealed to be inhibitory at 200 mg/l, but no conclusion about the inhibition of nitrification can be drawn, as the true concentrations of those chemicals in the outgoing WW are not known.

7. Future Work

An overall view of the sources of inhibition of nitrification in the WW discharged from Nolato MediTor has been provided in this study. However, there are many things that could be studied in more detail, to better understand the problem. Due to the limitation of time, little is known about the remainder of the compounds present in the outgoing WW. The WW composition of the water soluble production chemicals WAQ, SMO, Emulan, Tamol is not known, and to determine the concentrations of these would be a good starting point to further investigate which compounds that are contributing to the inhibition observed. Further analysis of the composition of the WW would also be necessary to make qualified guesses about which treatment methods that are suitable for reducing its toxicity. Further measurements of the concentration of zinc, and an approximation of the speciation of zinc could be done to exclude zinc as a source of the inhibition of nitrification in the WW. Furthermore, the effect of these chemicals combined could be studied in an extended additivity test, by producing inhibition curves and fitting analytical, unique expressions for each chemical in the same manner as for nitrate and nitrite in this study. Another way to mitigate the problem of inhibition of nitrification in the WW can be to investigate the usage of the chemicals in order to decrease the consumption of some chemicals if that that possible. It can also be investigated if it is possible to exchange chemical products, such as WAQ, to chemicals that is less inhibitory.

Another well-suited continuation on this study would be to take daily samples instead of random samples of all streams. Random sampling brings with it uncertainty since discharges with unusually high or low concentrations of inhibitory compounds may be captured, and to perform daily sampling would make the samples more reliable since the composition becomes an average over a whole day rather than over a few seconds as in random sampling. Furthermore, samples taken on the same day would be more comparable since the effect from variation over the day is eliminated, which would make it easier to map out how the streams are affecting each other. If all process streams are included, this could also point out the source of nitrite observed in the outgoing WW. In addition, the requirement from NSVA with less than 10% inhibition at 20% sample by volume is based on daily samples, which further speaks for daily sampling being the most appropriate.

Finally, it would be a good complement to study the performance of treatment methods to reduce the toxicity of the WW. Lab-scale trials could be performed, testing for example biological treatment which is suitable to reduce both nitrate and nitrite and organic compounds. Hopefully, the activated sludge used in biological treatment could become adapted, which could make it able to reduce the high concentrations of nitrate and nitrite, and other compounds present in the WW as well.

8. Reference list

AnoxKaldnes AB. (2014a) investigation of inhibition of nitrification on sample from Nolato MediTor AB "Before UV, 140718", *investigation report* [internal communication].

AnoxKaldnes AB. (2014b) investigation of inhibition of nitrification on sample from Nolato MediTor AB "After UV 200 l/h, 140718", *report* [internal communication].

AnoxKaldnes AB. (2014c) investigation of inhibition of nitrification on sample from Nolato MediTor AB "with UV-light, 140829", *report* [internal communication].

AnoxKaldnes AB. (2016a) investigation of inhibition of nitrification on sample from Nolato MediTor AB "Daily sample 20160607/08", *report* [internal communication].

AnoxKaldnes AB. (2016b) investigation of inhibition of nitrification on sample from Nolato MediTor AB "Daily sample 20161208", *report* [internal communication].

Álvarez, E. A., Mochon, M. C., Sanchez, J. C. J. and Rodríguez, M. T. (2002) Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere,* 47**,** 765-775.

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S. and Srinath, E. G. (1976) Inhibition of Nitrification by Ammonia and Nitrous Acid. *Journal (Water Pollution Control Federation),* 48**,** 835-852.

BASF SE (2014) Safety data sheet Tamol NN 9104. 3.0 ed. Germany/Sweden.

Birkved, F. K., Christensen, A. M., Ingerslev, F. and Halling-Sorensen, B. (2004) Toxicity of Mixtures. (Blandingers Toksikologi). Lægemiddelforskning 2004, ISSN 0905-0051. (In Danish).

Blum, D. J. W. and Speece, R. E. (1991) A Database of Chemical Toxicity to Environmental Bacteria and Its Use in Interspecies Comparisons and Correlations. *Research Journal of the Water Pollution Control Federation,* 63**,** 198-207.

Brenntag Nordic AB (2015) Safety data sheet Zinc Transparent. 4.1 ed. Malmö, Sweden.

BTC (2013) Safety data sheet Emulan OC Loesung. 3.0 ed. Göteborg, Sweden.

Çeçen, F., Semerci, N. and Geyic, A. G. (2010) Inhibitory effects of Cu, Zn, Ni and Co on nitrification and relevance of speciation. *Journal of Chemical Technology and Biotechnology,* 85**,** 520-528.

ECETOC – European Centre for Ecotoxicology and Toxicology of chemicals (2001) Aquatic Toxicity of Mixtures. Technical Report No. 80, ISSN: 0773-8072-80.

ECHA - European Chemical Agency (2016) Carbonic acid, zinc salt, basic. 2016-12-20 ed.

Erteco Rubber and Plastics AB (2014) Safety data sheet Darvan SMO. 1.2 ed. Stockholm, Sweden.

Cheng, M. H., Patterson, J. W. and Minear, R. A. (1975) Heavy Metals Uptake by Activated Sludge. *Journal (Water Pollution Control Federation),* 47**,** 362-376.

Dalzell, D. J. B., Alte, S., Aspichueta, E., de la Sota, A., Etxebarria, J., Gutierrez, M., Hoffmann, C. C., Sales, D., Obst, U. and Christofi, N. (2002) A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. *Chemosphere,* 47**,** 535-545.

Gomez, J., Mendez, R. and Lema, J. M. (2000) Kinetic study of addition of volatile organic compounds to a nitrifying sludge. *Applied Biochemistry and Biotechnology,* 87**,** 189-202.

Gray, N. F. (2004) *Biology of Wastewater Treatment*. 2nd ed. Imperial College Press, ISBN 978-1-86094-328-7.

Halling-Sørensen, B. and Jørgensen, S. E. (1993) *The Removal of Nitrogen Compounds from Wastewater*. Elsevier Science Publishers B.V. ISBN 0-444-89152-8.

Hammer, M. and Hammer, Jr. M. (2011) Water and wastewater technology. 7th ed. Prentice Hall, London: Pearson Education, Upper Saddle River, N.J. ISBN 1-292-02104-7.

HERA (2009) Alcohol Ethoxylates. 2.0 ed.: Human and Environmental Risk Assessment on ingredients of European household cleaning products.

ISO 9509 (1989) Water quality - Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms. *European Committee for Standardization (CEN)/International Organization for Standardization (ISO),* Ref. No. EN ISO 9509:2006 E.

ISO 11923 (1997) Water quality - Determination of suspended solids - Method by filtration through glass fibre filters. *European Committee for Standardization (CEN)/International Organization for Standardization (ISO),* Ref. No. EN ISO 9509:1997 E.

Jin, RC., Zheng, P., Mahmood, Q. and Hu, BL. (2007) Osmotic stress on nitrification in an airlift bioreactor. *Journal of Hazardous Materials*, 146, 148-154.

Juliastuti, S. R., Baeyens, J., Creemers, C., Bixio, D. and Lodewyckx, E. (2003a) The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge. *Journal of Hazardous Materials,* 100**,** 271-283.

Juliastuti, S. R., Baeyens, J. and Creemers, C. (2003b) Inhibition of Nitrification by Heavy Metals and Organic Compounds: The ISO 9509 Test. *Environmental Engineering Science,* 20**,** 79-90.

Jönsson, K. (2001) Inhibition of nitrification in municipal wastewater - sources, effects, evaluation and remedies. Bloms i Lund Tryckeri AB. ISSN 1650-5050, ISBN 91-7874-127-0.

Jönsson, K., Pottier, A., Dimitrova, I. and Nyberg, U. (2008) Utilising laboratory experiments as a first step to introduce primary sludge hydrolysis in full-scale. *Water Science and Technology,* 57**,** 1397-1403.

Jönsson, K., Aspichueta, E., de la Sota, A. and Jansen, J. la C. (2001) Evaluation of nitrfication-inhibition measurements. *Wat. Sci. Tech.,* 43**,** 201-208.

Kelly 2nd, R. T., Henriques, I. D. and Love, N. G. (2004) Chemical inhibition of nitrification in activated sludge. *Biotechnology and bioengineering,* 85**,** 683-694.

Univar (2016) Safety data sheet calcium nitrate tetrahydrate. 7.12 ed. Darmstadt, Germany.

Li, X., Kapoor, V., Impelliteri, C., Chandran, K. and Domingo, J. W. S. (2015) Measuring nitrification inhibition by metals in wastewater treatment systems: Current state of science and fundamental research needs. *Critical Reviews in Environmental Science and Technology,* 46**,** 249-289.

Lu, Q. and Shim, H. (2015) Comparison of chloride effect between A2O and SBR processes treating domestic wastewater. *Desalination and Water Treatment,* 53**,** 2549-2554.

Madoni, P., Davoli, D. and Guglielmi, L. (1999) Response of sOUR and AUR to heavy metal contamination in activated sludge. *Water Research,* 33**,** 2459-2464.

Malmqvist, Å. (2013) Evaluation of complementing analyses regarding inhibition of nitrification in industrial wastewater from Nolato in Torekov. (Utvärdering av kompletterande analyser beträffande nitrifikationshämning i avloppsvatten från Nolato i Torekov). Embreco AB, Sweden. (In Swedish).

Cheng, MH., Patterson, JW. and Minear, RA. (1975) Heavy metals uptake by activated sludge. *Journal (Water Pollution Control Federation)*, 47, 362-376.

Nilsson, J. (2017) *Conversation with Jennifer Ekholm and Vidar Aspelin during study visit at Nolato MediTor*, Torekov, 14 February.

Nolato MediTor (2017a) *Email to Jennifer Ekholm and Vidar Aspelin*, Dipping section process scheme, 2 February.

Nolato MediTor (2017b) *Email to Jennifer Ekholm and Vidar Aspelin*, Summary of measurements [internal material], 18 January.

Nolato MediTor (2017c) *Email to Jennifer Ekholm and Vidar Aspelin*, Ingoing chemicals [internal material], 26 January.

NSVA (2011) General regulations for usage of Scania Northwest municipalities' general water- and wastewater plants. *In:* NSVA (ed.).

Petersson, JE. (2017) *Email to Jennifer Ekholm and Vidar Aspelin*, 26 April.

Philips, S., Laanbroek, H. J. and Verstraete, W. (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science and Biotechnology,* 1**,** 115-141.

Roslander, C. (2017) *Conversation with Jennifer Ekholm and Vidar Aspelin during study visit at Torekov WWTP*, Torekov, 14 February.

Safic Alcan (2015) Safety data sheet FAXAN WAQ. Revision No. 1 ed. France.

Sandberg, K. (2016) *Initial meeting with Jennifer Ekholm and Vidar Aspelin at Nolato MediTor,* Torekov, 14 December.

Sandström, D. (2014) Report Nolato MediTor Reduction of inhibition of nitrification, Investigation, Final report (Rapport Nolato MediTor Reduktion av nitrifikationshämning, Utredning, Slutrapport). WSP Sverige AB, Sweden.

SFS (1998) Constitution of prohibition etcetera in some cases in relation to management, import and export of chemical products. Department of energy. (Förordning om förbud m.m. i vissa fall i samband med hantering, införsel och utförsel av kemiska produkter.) *In:* ENERGIDEPARTEMENTET, M.-O. (ed.) *1998:944.*

Shammas, N. K. (1986) Interactions of Temperature, pH, and Biomass on the Nitrification Process. *Journal (Water Pollution Control Federation),* 58**,** 52-59.

Stenström, M. K. and Song, S. S. (1991) Effects of Oxygen Transport Limitation on Nitrification in the Activated Sludge Process. *Research Journal of the Water Pollution Control Federation,* 63**,** 208-219.

Svenskt Vatten (2009) *Recommendations regarding receival of wastewater from industry and other activity (Råd vid mottagande av avloppsvatten från industri och annan verksamhet)* [Online]. Available: http://www.gryaab.se/wp-content/uploads/2015/08/P95_2010_rev.pdf [Accessed 2017-01-31].

Svenskt Vatten (2016) *Nitrogen- and phosphorus reduction - goals, requests and rules for seas (Kväve- och fosforreduktion – mål, krav och regler för havet)* [Online]. Available: http://www.svensktvatten.se/vattentjanster/avlopp-och-miljo/utslapp-och-recipient/kvave--och-fosforreduktion/ [Accessed 2017-01-25 2017].

Swedish EPA (1995) Screening method for estimation of inhibition of nitrification at municipal wastewater treatment plants. (Screeningmetod för bestämning av nitrifikationshämning - vid drift av kommunala avloppsreningsverk). SKARV-projektet. Naturvårdsverket. Report No. 4547, ISBN 91-620-4424-9, ISSN 0282-7298. (In Swedish)

Swedish EPA (2016a). *Environmental goals, No eutrophication, Infusion of nitrogen to the coast - Kattegatt (Miljömålen, Ingen övergödning, Tillförsel av kväve till kusten - Kattegatt)* [Online]. Naturvårdsverket.
Available: http://www.miljomal.se/Miljomalen/Alla-indikatorer/Indikatorsida/?iid=130&l=SEKA&t=Havsbass&pl=2 [Accessed 2017-01-25 2017].

Swedish EPA (2016b) *Environmental quality goals (Miljökvalitetsmålen)* [Online]. Naturvårdsverket.
Available: http://www.naturvardsverket.se/Miljoarbete-i-samhallet/Sveriges-miljomal/Miljokvalitetsmalen/ [Accessed 2017-01-17 2017].

Texier, A.-C. and Gomez, J. (2002) Tolerance of nitrifying sludge to p-cresol. *Biotechnology Letters,* 24**,** 321-324.

VA SYD (2014) Environmental report - Klagshamn wastewater treatment plant 2014 (Miljörapport). 2 ed. Malmö, Sweden.

VKI (1997) Analytical Quality Assurance and Control - A Training Guide for the Improvement and Maintenance of Good Laboratory Performance.

Wild, H. E., Sawyer, C. N. and McMahon, T. C. (1971) Factors Affecting Nitrification Kinetics. *Journal (Water Pollution Control Federation),* 43**,** 1845-1854.

Wilén, BM. (2010) Variation in dissolved oxygen concentration and its effect on the activated sludge properties studied at a full scale wastewater treatment plant. *In the proceedings of IWA World Water Congress and Exhibition, September 2010 in Montreal.*

Appendix I

Control samples – ammonium consumption rate during the screening tests

Here the ammonium-nitrogen concentration as a function of the time of nitrification during the screening tests is presented. Good linear fits (an R^2 close to 1) indicate constant ammonium consumption rate and a well-performing test. Also, sets of data points (start, middle and end at the same reaction time) lying close to each other indicate that the consumption rates during the start, the middle and the end of the test were similar, which indicates that the ammonium consumption was even over the whole time of the test. In the first test, the control samples in the end of the test (see figure A1) is deviating from the other two sets of control samples (start and middle) which indicates that something happened during the end of the test that increased the ammonium consumption. This makes samples close to the end of this screening test unreliable and they should be taken more critical. Hence, we have excluded the results from those samples from the result part. All the rest of the tests showed similarity between the three different sets of control samples and good linear fits, indicating well-performing tests (see figures A2-A5). As a conclusion, the results from test 2-5 are all reliable.

Figure A1. The ammonium concentration as a function of the time of nitrification among the three sets of control samples (start, middle and end) run in the first screening test.

Figure A2. The ammonium concentration as a function of the time of nitrification among the three sets of control samples (start, middle and end) run in the second screening test.

Figure A3. The ammonium concentration as a function of the time of nitrification among the three sets of control samples (start, middle and end) run in the third screening test.
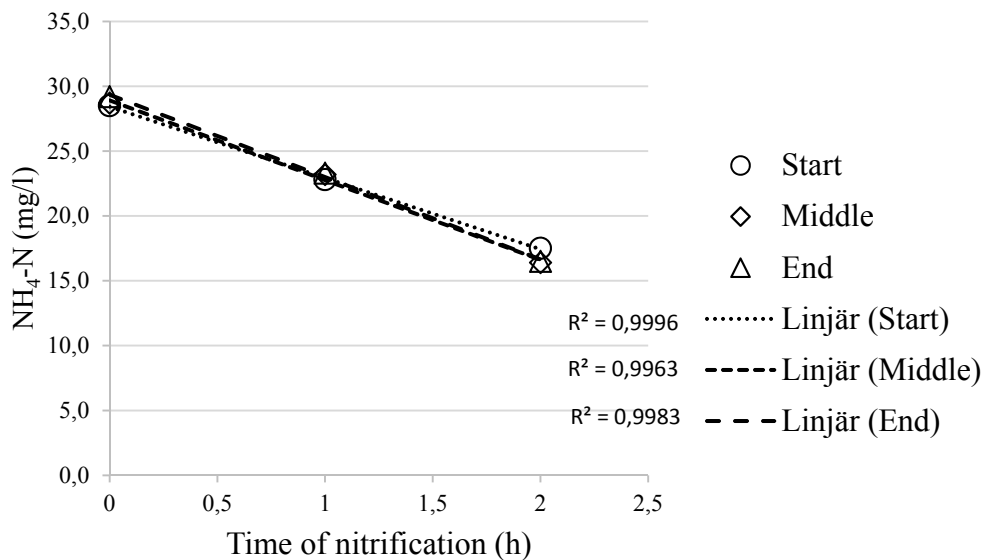
Figure A4. The ammonium concentration as a function of the time of nitrification among the three sets of control samples (start, middle and end) run in the fourth screening test.
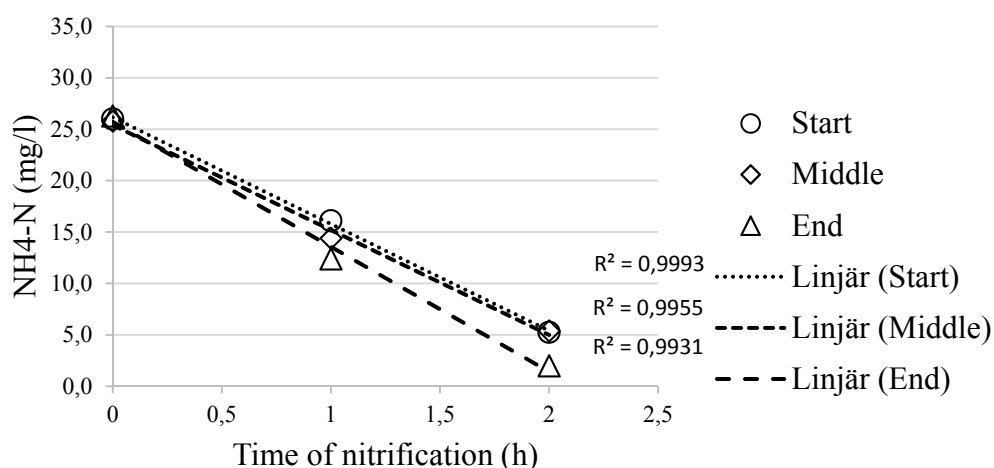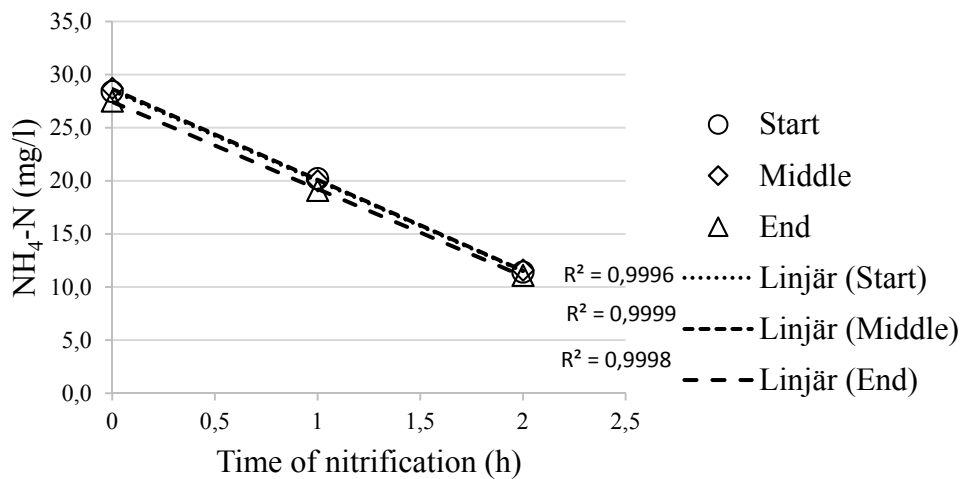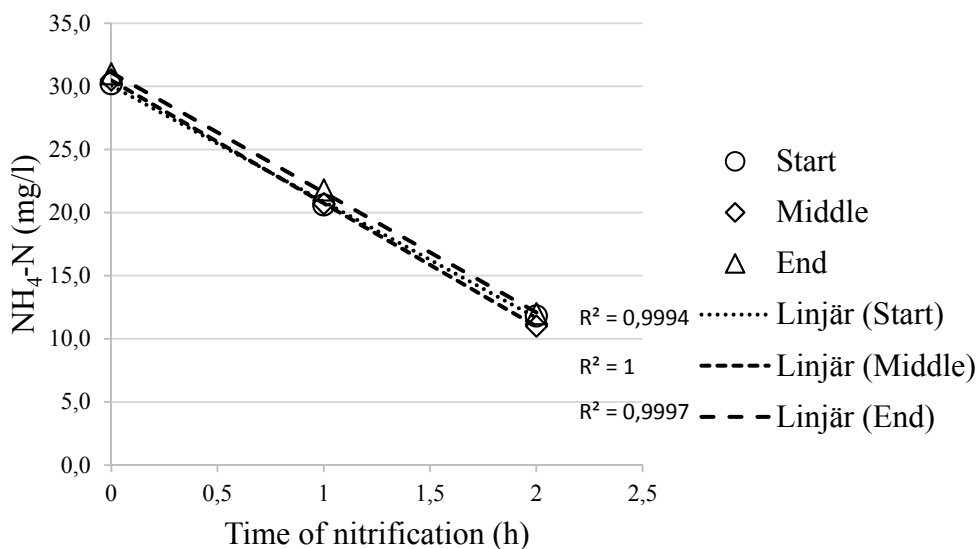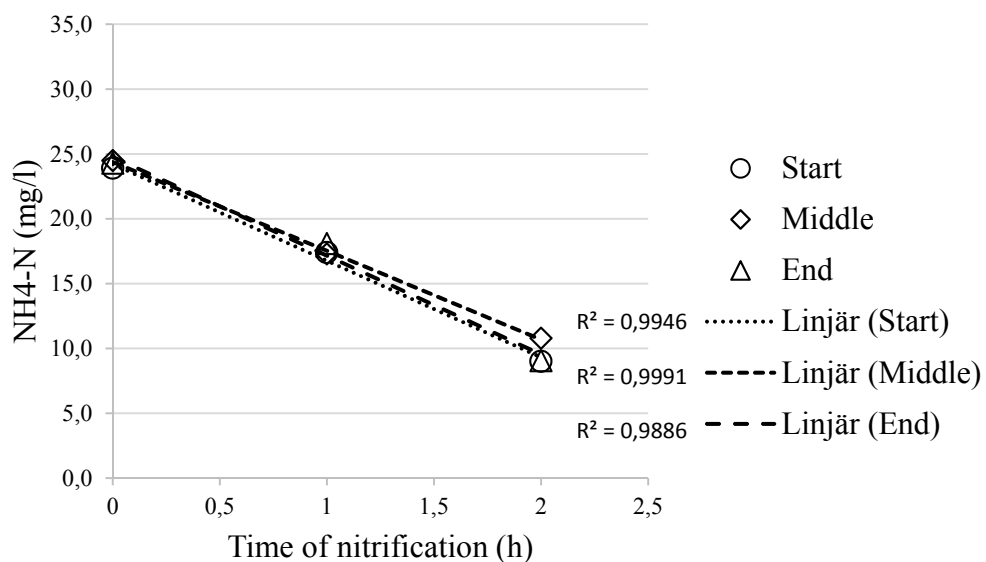

Figure A5. The ammonium concentration as a function of the time of nitrification among the three sets of control samples (start, middle and end) run in the fifth screening test.

Appendix II

Conductivity – verifying that dilutions were prepared as planned

Below, figures are shown where the conductivity is plotted against concentration of NO_3-N, NO_2-N and NO_x. This was done in order to check that the dilutions made to generate nitrate and nitrite curves and mixtures of both nitrate and nitrite were prepared as aimed for, and to point out potential dilutions that were deviating from the expected trend. All the data points, corresponding to different dilutions, follow the expected trend and hence there are no major dilution errors found among the dilutions tested.
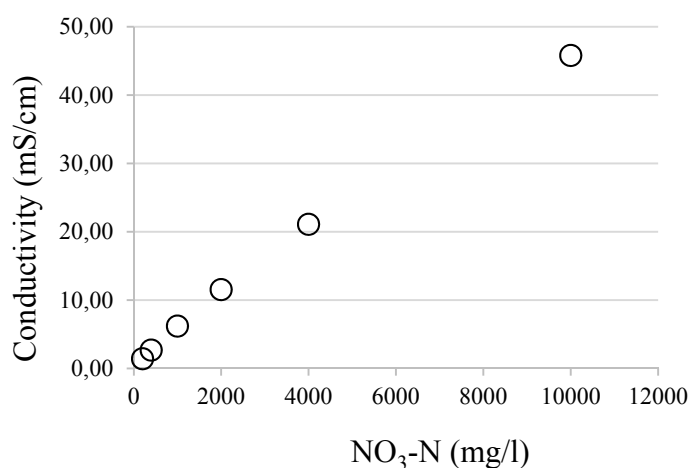
Figure A6. The measured conductivity as a function of the concentration of NO_3-N aimed for when preparing pure calcium nitrate tetrahydrate dilutions.
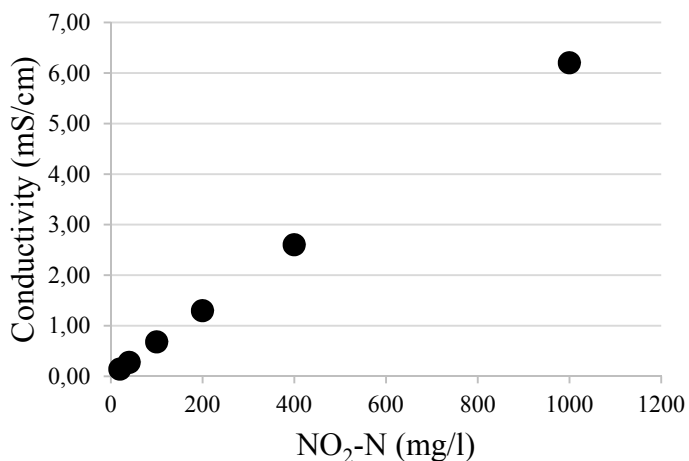
Figure A7. The measured conductivity as a function of the concentration of NO_2-N aimed for when preparing pure sodium nitrite dilutions.
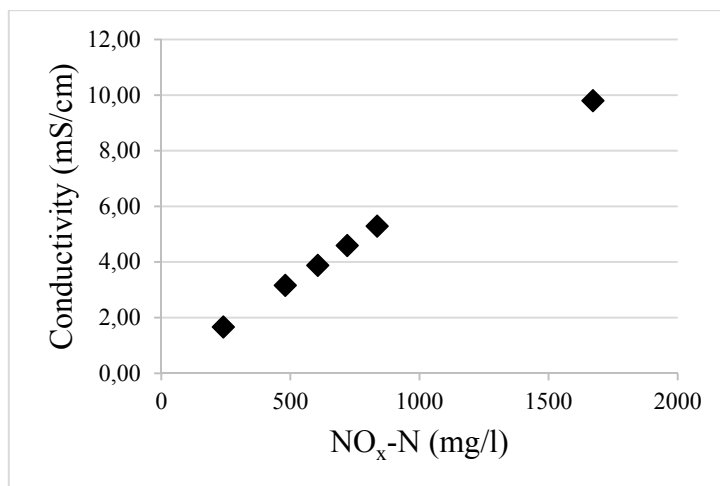
Figure A8. The measured conductivity as a function of the concentration of NO_x-N aimed for when preparing mixtures of sodium nitrite and calcium nitrate tetrahydrate.

Appendix III

Additivity test – calculations

In Table A1 the values used for calculations of interactive indices for the mixtures are presented. The values of $I_{nitr, i}$ are the measured inhibitions from the mixtures, while d's are the concentrations of each compound in the mixtures. The equation describing the interactive index is presented in chapter 3 (Equation 7).

Table A1. Results obtained and calculated from the test of additive inhibition, with mixtures and dilution of mixtures, their corresponding inhibition of nitrification ($I_{nitri, i}$), their composition of nitrate and nitrite (d's) and the calculated interactive index (II).

Mixture, dilution	$I_{nitri, i}$ (%)	d_{NO3-N} (mg/l)	d_{NO2-N} (mg/l)	II
A	65	300	60	1.10
A/1.5	48	200	40	1.23
A/3	38	100	20	1.05
B	70	800	36	1.19
B/2	49	400	18	1.05
B/2.8	40	290	13	1.00

Example of how the values of the estimated inhibitions from nitrate and nitrite alone, in mixture *A/3*, were determined:
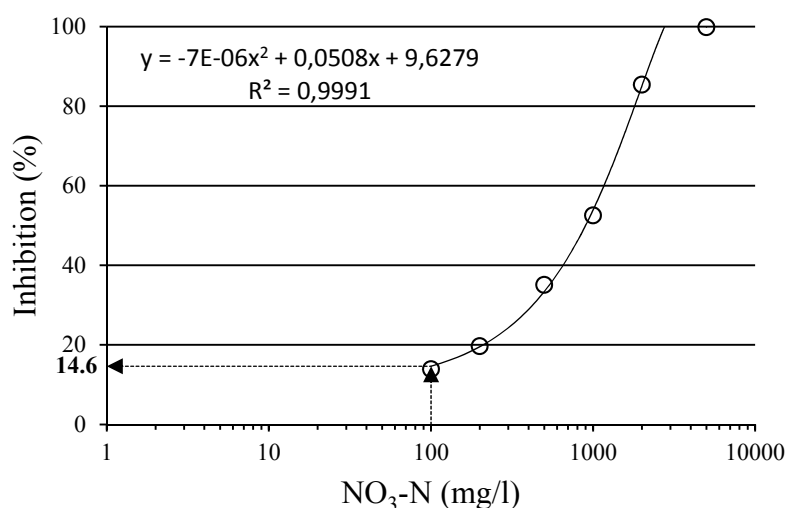
Figure A9. Example of how the estimated inhibition from nitrate alone, in mixture A/3, was determined.
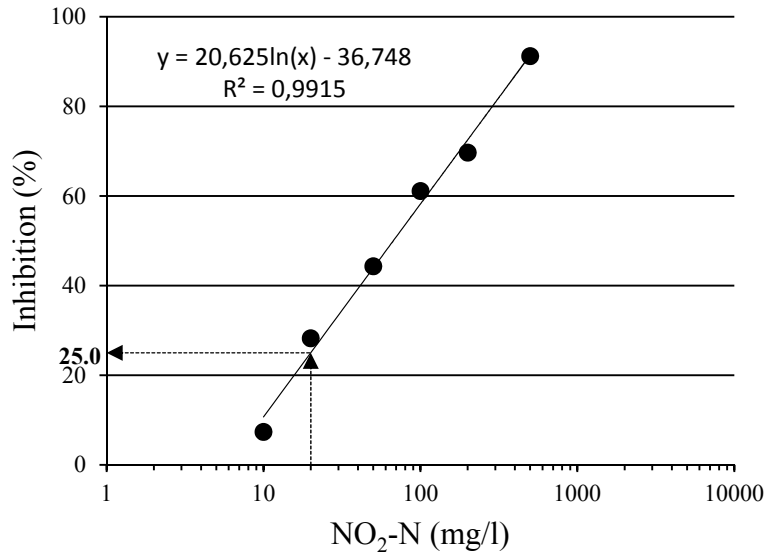
Figure A10. Example of how the estimate inhibition from nitrite alone, in mixture A/3, was determined.

Corresponding calculation of the interactive index in mixture *A/3*:

$$II_{A/3} =$$

$$= \frac{-7 \cdot 10^{-6} d^2_{NO_3-N,\frac{A}{3}} + 0.0508 d_{NO_3-N,A/3} + 9.6279}{38} + \frac{20.625 \cdot \ln(d_{NO_2-N,A/3}) - 36.748}{38}$$

$$= \frac{-7 * 10^{-6} \cdot 100^2 + 0.0508 \cdot 100 + 9.6279}{38} + \frac{20.625 \cdot \ln(20) - 36.748}{38}$$

$$= \frac{14.6}{38} + \frac{25.0}{38} = 1.05$$

Appendix IV

COD in leaching wastewaters and tested solutions of production chemicals

The measured concentrations of COD are presented in Table A2. The worst-case concentrations also presented in Table A3 are to be compared with the tested concentrations. The tested concentrations of WAQ and Tamol are relative similar to the worst-case concentrations that could be present in leaching WW in 20 % dilution. The COD in the leaching WW and the COD in WAQ and Tamol are roughly in the same magnitude.

Table A2. Concentrations of COD in leaching WWs from different days.The COD is given as the concentration in the fraction 20% by volume in the leaching WWs and in the production chemicals at 200mg/l of each chemical.

Test liquid	COD (mg/l)
Leaching WW 17/2 - 20%	276
Leaching WW 14/2 - 20%	173
Leaching WW 28/2 - 20%	211
Leaching WW 1/3 - 20%	158
Leaching WW 2/3 - 20%	246
Leaching WW 3/3 - 20%	222
Emulan - 200 mg/l	76
SMO - 200 mg/l	159
WAQ - 200 mg/l	160
Tamol - 200 mg/l	253

Table A3. Concentrations tested compared with the estimated worst-case concentrations for the production chemicals.

Chemical components	Concentration in test tube (mg/l)	Worst-case concentration in leaching WW diluted to 20% by volume (mg/l)	Estimated COD for worst-case concentration (mg/l)
Tamol	200	140	53
WAQ	200	116	92
Emulan	200	220	176
SMO	200	236	298
All	-	-	620

The COD for the worst-case concentrations were estimated by dividing the worst-case concentration with the actual concentration (200 mg/l) and multiply with the COD measured for the 200 mg/l concentration. It can be seen that the sum of the COD for the worst-case concentrations (620 mg/l) is much bigger than any measurement of COD in the leaching WW (158-276 mg/l).

Appendix V

Raw data from screening tests 1-5

The test samples, dilution, measured concentrations of NH_4-N before and after reaction, calculated ammonium oxidation rate and calculated inhibition of nitrification are presented in Table A4.

Table A4. Raw data from screening tests 1-5.

	Sample	Dilution (%)	NH_4-N, "C_x" (mg/l)	"C_0" (mg/l)	NH_4-N-rate (mgNH_4-N/(gVSS*h))	Inhibition (%)
Test 1						
	Control	-	5.32	26	9.40	-
	Leaching WW 14/2	20	12.90	26.23	6.05	36
	Leaching WW 14/2	50	19.40	26.52	3.23	66
	Nitrate 650	50	12.20	26.03	6.28	33
	Nitrate 600	50	12.20	26.03	6.28	33
	Nitrate 550	50	12.20	26.03	6.28	33
	Nitrate 300	50	8.28	26.03	8.06	14
	Nitrate 200	50	8.49	26.03	7.96	15
	Nitrate 150	50	8.09	26.03	8.15	13
	Nitrate 100	50	4.63	26.03	9.71	-3
	Nitrate 40	50	2.89	26.03	10.51	-12
Test 2						
	Control	-	11.37	28.20	11.05	-
	Leaching WW 17/2	20	18.55	28.88	6.78	39
	Leaching WW 17/2	30	21.15	29.21	5.29	52
	Leaching WW 14/2	20	18.10	28.59	6.89	38
	Leaching WW 14/2	30	20.20	28.79	5.64	49
	Heat exchanger	50	19.60	28.79	6.03	45
	Heat exchanger	20	15.25	28.43	8.65	22
	Rinsing bath	20	13.35	28.20	9.75	12
	Rinsing bath	10	11.85	28.20	10.73	3
	Emulan	50	16.65	28.20	7.58	31
	Emulan	20	14.25	28.20	9.16	17
	SMO	50	13.85	28.20	9.42	15
	SMO	20	12.10	28.20	10.57	4
	WAQ	50	23.85	28.20	2.86	74

WAQ	20	18.10	28.20	6.63	40
Tamol	30	13.95	28.20	9.35	15
Tamol	20	15.30	28.20	8.47	23
Nitrate 242 mg/l	50	13.85	28.20	9.42	15
Nitrate 310 mg/l	50	14.95	28.20	8.70	21
Test 3					
Control	-	11.60	30.57	13.23	-
Leaching WW 28/2	20	20.50	30.90	7.26	45
Leaching WW 1/3	20	20.60	30.97	7.24	45
Leaching WW 2/3	20	21.05	31.00	6.94	48
Leaching WW 3/3	20	20.00	31.00	7.68	42
Outgoing WW 27/2	20	14.10	31.29	11.99	9
Outgoing WW 27/2	50	17.40	32.37	10.45	21
Outgoing WW 28/2	20	14.35	30.98	11.60	12
Outgoing WW 28/2	50	19.55	31.59	8.40	37
Outgoing WW 1/3	20	16.85	32.21	10.72	19
Outgoing WW 1/3	50	25.80	34.67	6.19	53
Outgoing WW 2/3	20	20.55	32.31	8.21	38
Outgoing WW 2/3	50	31.65	34.94	2.29	83
Outgoing WW 3/3	20	16.90	30.94	9.80	26
Outgoing WW 3/3	50	24.55	31.51	4.86	63
Nitrate 45 mg/l	50	12.75	30.57	12.43	6
Nitrate 70 mg/l	50	15.75	30.57	10.34	22
Nitrate110 mg/l	50	15.70	30.57	10.37	22
Test 4					
Control	-	16.80	28.80	9.70	-
Polymer 2/3*	20	16.55	28.80	9.91	-2
Polymer 3/3*	20	16.90	28.80	9.62	1
Dipping 2/3*	20	16.15	28.80	10.23	-5
Dipping 3/3*	20	16.50	28.80	9.95	-3
Nitrite 2.69 mg/l	50	16.75	28.80	9.74	0
Nitrite 22.8 mg/l	50	17.95	28.80	8.77	10
Nitrite 10.9 mg/l	50	16.80	28.80	9.70	0
Nitrite 44.9 mg/l	50	20.95	28.80	6.35	35
Nitrite 13.9 mg/l	50	17.75	28.80	8.94	8
Nitrite + nitrate 27/2	50	17.75	28.80	8.94	8

Nitrite + nitrate 28/2	50	19.00	28.80	7.92	18
Nitrite + nitrate 1/3	50	18.85	28.80	8.05	17
Nitrite + nitrate 2/3	50	21.55	28.80	5.86	40
Nitrite + nitrate 3/3	50	19.40	28.80	7.60	22

Test 5

Control	-	9.62	24.23	8.64	-
Dipping 5/4	20	13.55	24.34	6.38	26
Dipping 6/4	20	15.00	24.36	5.54	36
Dipping 7/4	20	14.85	24.35	5.62	35
Polymer 5/4	20	35.45	48.23	7.56	13
Polymer 6/4	20	24.00	37.55	8.02	7
Polymer 7/4	20	24.60	34.11	5.63	35
Outgoing WW 5/4	20	15.05	24.66	5.69	34
Outgoing WW 6/4	20	14.8	24.84	5.94	31
Outgoing WW 7/4	20	16.00	24.48	5.02	42
Cold wash 12/4	20	11.25	24.26	7.70	11
Nitrate 5000 mg/l	50	24.65	24.23	-0.25	100
Nitrate 2000 mg/l	50	22.10	24.23	1.26	85
Nitrate 1000 mg/l	50	17.30	24.23	4.10	53
Nitrate 500 mg/l	50	14.75	24.23	5.61	35
Nitrate 200 mg/l	50	12.50	24.23	6.94	20
Nitrate 100 mg/l	50	11.65	24.23	7.45	14
Nitrite 500 mg/l	50	22.95	24.23	0.76	91
Nitrite 200 mg/l	50	19.80	24.23	2.62	70
Nitrite 100 mg/l	50	18.55	24.23	3.36	61
Nitrite 50 mg/l	50	16.10	24.23	4.81	44
Nitrite 20 mg/l	50	13.75	24.23	6.20	28
Nitrite 10 mg/l	50	10.70	24.23	8.01	7
A/3	50	15.15	24.23	5.37	38
A/1.5	50	16.60	24.23	4.52	48
A	50	19.15	24.23	3.01	65
B/2.8	50	15.45	24.23	5.20	40
B/2	50	16.85	24.23	4.37	49
B	50	19.80	24.23	2.62	70

*The samples were after the test discovered to be samples of stormwater, taken by mistake in the wrong sewers

Appendix VI

The screening method

Hereunder follows a description of the screening method ("Screening Method for Determination of Inhibition of Nitrification of Activated Sludge"), an exact copy of pages 145-153 in the appendix of Jönsson (2001).

Screening Method for Determination of Inhibition of Nitrification of Activated Sludge

1. Introduction

This instruction specifies a rapid screening method for determination of short-term inhibitory effects of test substances (e.g. wastewater) on nitrifying bacteria in activated sludge. The inhibitory effects are estimated over an exposure period of two hours (the exposure time may be shortened or prolonged, see 10.1).

This method is suitable for tests with nitrifying activated sludge derived from domestic sewage, but it is possible to use any nitrifying activated sludge, for example activated sludge derived from industrial wastewater or synthetic sewage, provided that the nitrification rate of the sludge is suitable (see 9 and 10.1).

The method is applicable to wastewaters and chemical substances that are soluble in water, but insoluble substances may also be tested, if care is taken to ensure as much homogeneity as possible. Even volatile substances may be tested in this method, as the reaction takes place in capped test tubes. Initial controls to ascertain that the oxygen and ammonium contents in the test tubes are sufficient shall be performed (see 9).

The inhibitory effect of a test substance may be different for different activated sludges. For example, an industrial wastewater may cause less inhibition to an activated sludge adapted to this particular industrial wastewater than to a domestic activated sludge.

2. Definitions

2.1 Activated sludge: Accumulated biological mass (floc) produced in the treatment of wastewater by the growth of bacteria and other microorganisms in the presence of dissolved oxygen (ISO 6107-1, 1996). Activated sludge contains both active microorganisms and inert organic and inorganic material.

2.2 Total Suspended Solids (SS): The concentration of particles, suspended solids, expressed as grams of dry matter per litre, which is retained at a filter of specified pore size when a known volume of suspension is filtered (SS 02 81 12, 1983).

2.3 Volatile Suspended Solids (VSS): The concentration of volatile suspended solids is the organic fraction of SS analysed according to (SS 02 81 12, 1983).

2.4 Substrates: Ammonia, carbonate and phosphate.

2.5 Activated sludge suspension: A mixture of activated sludge, substrates and tap water (see 5.6)

2.6 Test substances: wastewaters, pure chemicals, mixtures and chemical products.

2.7 Oxidised nitrogen: $NO_2 + NO_3 = NO_X$

2.8 Nitrification: The oxidation of ammonium salts by bacteria. Usually, the end product of such an oxidation is nitrate (ISO 6107-1, 1996).

2.9 Nitrification rate: Biological oxidation of ammonium per unit of time expressed as mg N/(g VSS·h), mg N/(g SS·h) or mg N/(l·h) where N is NH_4-N or NO_X-N.

2.10 Inhibition of nitrification: The decrease in nitrification rate in a test tube containing activated sludge, substrates and test substance compared to the nitrification rate in a reference test tube where tap water is added instead of test substance. The decrease or degree of inhibition is expressed as a percentage of the nitrification rate in the reference test tubes.

3. Principle

The test is performed at a constant temperature, usually between 20°C and 25°C, in an atmosphere free from dust and toxic vapours. The nitrification reaction takes place in capped test tubes in which nitrifying sludge suspension (including substrates) and either tap water or test substances are added. In order to ensure that the concentration of ammonium will not be rate limiting, the concentration of activated sludge is, based on an estimated nitrification rate, adjusted so that the ammonium concentration in the test tubes will be more than 3 mg NH_4-N/l at the end of the test. Aeration and mixing take place by shaking of the tubes, the oxygen being supplied by the air head space in the tubes. Of the total volume of the tubes, 1/3 is liquid and 2/3 is air (or pure oxygen, see 10.2). Parallel aeration of a nitrifying sludge in the presence and absence of test substances is made during two hours. (The exposure time may be shortened or prolonged, see 10.1.) The nitrification rate is calculated either as the increase of oxidised nitrogen related to sludge concentration and time (g NO_X-N/(g VSS·h)) or as the decrease of ammonia related to sludge concentration and time (g NH_4-N/(g VSS·h)). The inhibition caused by the test substance is then calculated by comparison of the nitrification rates obtained in the test tubes containing test substance and tap water respectively. Note that the nitrification rates based on the decrease in ammonia and on the increase in oxidised nitrogen are not directly comparable, as ammonia for example may be used for cell synthesis instead of being converted to nitrite or nitrate.

4. Interferences

The interferences of the method are the same that apply for the determination of oxidised nitrogen and are dependent on the method used for this determination.

High organic matter concentrations in the test tubes of the screening test may result in lower nitrification activity because of heterotrophic consumption of oxygen. If the COD concentration in the test tubes exceeds 400 mg COD/l and the heterotrophic oxygen consumption rate ($R_{HETR.}$·VSS_{TT}) exceeds 90 mg O_2/(l·h), the method can be modified according to 10.2. ($R_{HETR.}$ = heterotrophic respiration rate expressed in mg O_2/(g VSS·h), VSS_{TT} = the concentration of suspended solids in the test tubes (g VSS/l).)

High amounts of ammonia/ammonium may result in inhibition of nitrification. If the samples to be tested contain high ammonium concentrations, the method can be modified according to 10.3.

5. Materials and Reagents

5.1 Nitrifying activated sludge

It is possible to use any type of activated sludge in this method, provided that it is nitrifying. If the nitrification rate of the sludge is unknown, a preliminary test to find the nitrification rate is performed (see 10.1).

Analyses of SS and VSS concentrations of the nitrifying activated sludge should be performed if the concentrations are unknown. If needed, the sludge may be concentrated by for example sedimentation or centrifugation. Activated sludge should be used within 24 hours of collection and it should be maintained under aerobic conditions during storage.

5.2 Substrates

Ammonium sulfate [$(NH_4)_2SO_4$] of analytical quality, e. g. Merck 1217.

Sodium bicarbonate [$NaHCO_3$] of analytical quality, e. g. Merck 6329.

KH_2PO_4 of analytical quality, e. g. Merck 5108.

5.3 Acid / Base

HCl or NaOH is used for pH adjustments.

5.4 Tap water

The sludge suspension is prepared using tap water and tap water is also added to the reference tubes instead of test substances. If the tap water is chlorinated, it must be dechlorinated by aeration at room temperature overnight before using it in the test.

Note that if the tap water contains oxidised nitrogen, the calculation of the nitrification rate has to be adjusted according to 10.4.

5.5 Reagent-grade water

Distilled or deionised water is used to prepare dilutions of the test substances.

5.6 Activated sludge suspension

The nitrifying activated sludge is concentrated to a suitable volatile suspended solids concentration, see below. The concentration may be carried out by sedimentation or centrifugation.

Initial control according to section 9 shall be performed in order to ensure that the ammonium content in the test tubes is sufficient for a successful test.

Preparation of activated sludge suspension

The prepared activated sludge suspension shall have a volatile suspended solids concentration of 1.5-6 g VSS/l, an ammonia concentration of 50 mg N/l, bicarbonate concentration of 8 mM and a phosphate concentration of 10 mg PO_4-P/l. pH is adjusted to 7.5±0.5.

♦ Dissolve 0.236 g ammonium sulfate $[(NH_4)_2SO_4]$, 0.672 g sodium bicarbonate $[NaHCO_3]$ and 0,044 g KH_2PO_4 in some tap water in a one-litre beaker. (If the samples to be tested contain high amounts of ammonia, the ammonium sulfate should be excluded from the activated sludge suspension, see 10.3.)

♦ Ensure that the oxygen concentration of the nitrifying activated sludge is less than 0.5 mg O_2/l before it is mixed with the substrates.

♦ Add activated sludge so that the final VSS concentration of the sludge suspension will be between 1.5-6 g VSS/l. It shall be ascertained that the chosen VSS concentration does not lead to total ammonium depletion in the test period (see 9).

♦ pH is adjusted to 7.5±0.5 with HCl or NaOH. Fill up with tap water to 1000 ml.

♦ The VSS concentration of the activated sludge suspension is determined by triple analysis.

5.7 Test substances

Stock solution or suspension of the test substances may be prepared.

6 Equipment (Apparatus)

a. *Test tubes with caps and a capacity of 30 ml*
b. *Rack for the test tubes*
c. *Shaker for the test tubes*
d. *Wide-bore pipettes*
e. *pH-meter (The probe must be suitable for fast determinations of pH in the test tubes after the incubation.)*
f. *Thermometer, range 0-50°C*
h. *Timer*
i. *Magnetic stirrer with stirring bar*
j. *Beaker, 1000 ml*
k. *Filters and funnels (filters of glass fibre or paper which does not release nitrogen)*
l. *Apparatus necessary for analytical determination of ammonia and oxidised nitrogen in solution*
m. *Glass fibre filter disks of 0.45μm pore size*
n. *Filtration apparatus, i.e. a membrane filter funnel*
o. *Suction flask, of sufficient capacity for sample size selected*
p. *Drying oven, for operation at 105±3°C*
q. *Muffle furnace for operation at 550°C*
r. *Analytical balance, capable of weighing to 0.1 mg*

7 Procedure

7.1:   Prepare the activated sludge suspension according to 5.6. Place the suspension in a beaker on a magnetic stirrer and provide gently mixing.

7.2:   Ensure that the sludge suspension and test substances are of the same temperature, equal to the ambient temperature.

7.3:   Check the pH and temperature of the test substances just before the start of the test. If pH of the test substances deviates more than 0.5 pH units from 7.5, pH shall be adjusted with HCl or NaOH.

7.4:   Prepare a set of test tubes for the test. Use two test tubes for each concentration of test substance and nine test tubes for references with tap water.

7.5:   Add 5 ml of tap water (5.4) to each test tube used as reference. Add 5 ml of test substance (or diluted test substance) to the test tubes used as samples.

7.6:   Add to each test tube 5 ml of activated sludge suspension (5.6) with a wide-bore pipette. Close the test tube, place it on the shaker and start shaking. The incubation time begins when the sludge suspension is added. It is important to add the sludge suspension to the test tubes with fixed time intervals in such a way that the incubation time is the same (2 hours) for all test tubes.

Start one set of reference test tubes (with 0, 1 and 2 hours incubation time respectively) in the beginning of the test, one set in the middle and one in the end.

Test tubes used as references with incubation time 0 hours do not need to be placed on the shaker. Mix the content and filter it to stop the reaction. Analyse the oxidised nitrogen concentration and the ammonium concentration. The test tubes used as references with incubation time 1 hour are used to check the linearity in oxidised nitrogen production during the test.

7.7:   Incubate all test tubes for 2 hours at a constant temperature and aerate by shaking.

7.8:   After 2 hours, measure pH and temperature in at least one of the duplicate test tubes and in all reference test tubes, and then stop the nitrification reaction by filtering the samples. Analyse the oxidised nitrogen concentration of the filtrates. For references, the ammonium concentration should be analysed too. To assure the same incubation time for all test tubes, stop the reactions using the same time intervals as when the test was started.

8 Calculations of results

The initial concentrations of oxidised nitrogen and ammonium in the test tubes are calculated from measured N concentrations in the test substance and in the controls with incubation time 0 hours. Note that if the tap water contains oxidised nitrogen, the initial concentration of oxidised nitrogen in the test tubes ($N_{TT,0}$) has to be calculated according to the modified equation found in 10.4.

$$N_{TT,0} = N_{C,0} + N_{TS} \cdot \frac{V_{TS}}{V_{TT}}$$

where

$N_{TT,0} =$ the initial concentration of oxidised nitrogen in the test tubes (mg N/l),
$N_{C,0} =$ average concentration of oxidised nitrogen in the three controls with incubation time 0 hour (mg N/l),
$N_{TS} =$ concentration of oxidised nitrogen in the test substance (mg N/l),
$V_{TS} =$ the volume of test substance added to the test tube (ml),
$V_{TT} =$ the total volume of liquids added to the test tube (= 10 ml).

The concentration of volatile suspended solids in the test tubes (VSS_{TT}) is calculated from the analysis of volatile suspended solids in the sludge suspension (VSS_{SS}).

$$VSS_{TT} = VSS_{SS} \cdot \frac{V_{SS}}{V_{TT}}$$

where

$VSS_{TT} =$ the concentration of suspended solids in the test tube (g VSS/l),
$VSS_{SS} =$ the concentration of suspended solids in the sludge suspension (g VSS/l),
$V_{SS} =$ the volume of sludge suspension added to the test tube (ml).
$V_{TT} =$ the total volume of liquids added to the test tube (= 10 ml).

The nitrification rate ($R_{NITR.}$) is calculated as follows:

$$R_{NITR.} = \frac{N_{TT,end} - N_{TT,0}}{t \cdot VSS_{TT}}$$

where

$R_{NITR.} =$ nitrification rate (mg N/(g VSS·h))
$N_{TT,end} =$ the final concentration of oxidised nitrogen in the test tubes (mg N/l),
$N_{TT,0} =$ the initial concentration of oxidised nitrogen in the test tubes (mg N/l),
$t =$ the incubation time (h),
$VSS_{TT} =$ the concentration of suspended solids in the test tube (g VSS/l).

The percentage inhibition of production of oxidised nitrogen is calculated as follows:

$$I = \frac{R_{NITR,C} - R_{NITR,S}}{R_{NITR,C}} \cdot 100$$

where

I = inhibition of nitrification (%)
$R_{NITR,C}$ = average nitrification rate of the test tubes containing tap water
(mg N/(g VSS·h)),
$R_{NITR,S}$ = average nitrification rate of the test tubes containing sample
(mg N/(g VSS·h)).

9 Initial control to perform before starting a screening test

The decrease in ammonium concentration in the test tubes during a test (Δ_{NH4}) should be between 8 and 22 mg NH_4-N/l. In practise, it is convenient to aim at ca. 15 mg NH_4-N/l in order to ensure a successful test. Suitable combinations of nitrification rate and VSS concentrations can be estimated by the following equation:

$$\Delta_{NH4} = R_{NITR.} \cdot VSS_{TT} \cdot t$$

Δ_{NH4} = The decrease in the ammonium concentration during the test (mg N/l)
$R_{NITR.}$ = Nitrification rate of the activated sludge (mg NH_4-N/g·VSS·h)
VSS_{TT} = Volatile suspended solids concentration in the test tubes (g VSS/l)
t = Incubation time (2 hours)

10 Modifications

10.1 Nitrification rate

If the nitrification rate of the nitrifying activated sludge is unknown, a preliminary test with tap water is performed. For example 10 test tubes can be prepared so that five duplicate samples are incubated 0, 30, 60, 120 and 180 minutes, respectively.

If the nitrification rate is outside the suitable range, the incubation time of the test should be adjusted (i. e. shortened if the nitrification rate is too high and prolonged if the nitrification rate is too low). Compare section 9.

10.2 Organic matter

If the oxygen concentration in the test tubes decrease too much, the nitrification activity will be limited. High amounts of easily biodegradable organic matter in the samples to be tested may result in low dissolved oxygen concentrations due to oxygen consumption of heterotrophic microorganisms in the activated sludge. The dissolved oxygen concentration of the test tubes should not be allowed to drop below 4 mg O_2/l.

If the COD concentration in the test tubes exceeds 400 mg COD/l and the heterotrophic oxygen consumption rate ($R_{HETR.} \cdot VSS_{TT}$) exceeds 90 mg O_2/(l·h), it may be necessary to

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introduce pure oxygen in the head space of the test tubes. Follow the normal procedure (see section 7) and before closing the tubes, add pure oxygen to the gas phase, for example with a Pasteur pipette, for 30 seconds. This modification of the method will allow investigations of samples without any limit of the concentration of organic material.

Note: All organic material has been regarded as easily biodegradable when the limit of organic material content in the test tubes has been set to 400 mg COD$_{Cr}$/l. In practice, it may be possible to test solutions with a far higher content of organic material using air in the head space of the test tubes, provided that the biodegradability of the organic material and the heterotrophic respiration rate of the activated sludge used are low.

10.3 Ammonium

High concentrations of ammonium may result in inhibition of nitrification. If the samples to be tested contain high ammonium concentrations, the ammonia should be excluded from the sludge suspension. Instead, an ammonium solution is prepared from which suitable amounts are put in the different test tubes immediately before the sludge suspension is added. The volume of ammonia solution put into each test tube is adjusted so that the initial ammonium concentration in each test tube does not exceed 100 mg NH$_4$-N/l.

10.4 Tap water contains oxidised nitrogen

If the tap water contains oxidised nitrogen, the initial concentration of oxidised nitrogen in the test tubes (N$_{TT,0}$) is calculated as follows:

$$N_{TT,0} = N_{SS} \cdot \frac{V_{SS}}{V_{TT}} + N_{TS} \cdot \frac{V_{TS}}{V_{TT}} + N_W \cdot \frac{V_W}{V_{TT}}$$

where

N$_{TT,0}$ = the initial concentration of oxidised nitrogen in the test tubes (mg N/l),

N$_{SS}$ = average concentration of oxidised nitrogen in three samples from the sludge suspension (mg N/l),

V$_{SS}$ = the volume of sludge suspension added to the test tube (ml),

V$_{TT}$ = the total volume of liquids added to the test tube (= 10 ml),

N$_{TS}$ = concentration of oxidised nitrogen in the test substance (mg N/l),

V$_{TS}$ = the volume of test substance added to the test tube (ml),

N$_W$ = concentration of oxidised nitrogen in the tap water (mg N/l),

V$_W$ = the volume of tap water added to the test tube (ml).

11 Report of analysis

The test report shall include in particular:

a)   all the data needed for the identification of the samples tested;

b)   the specific nitrification rate of the activated sludge;

c)   the method of preparation of the samples:

- for wastewaters, the manner and duration of storage of the samples and, if necessary, the conditions in which sampling, decantation, filtration and thawing of the samples were carried out;

- for pure chemicals, the method of preparation of the stock solutions and the test solutions;

d)   the source, concentration and pretreatment method of the activated sludge;

e)   the results of the test in the form of percentage inhibition of each concentration of test substance;

f)   in the case of chemical analyses of the substances, the method used;

g)   the test temperature with limits;

h)   the pH of the test with limits;

i)   any operating detail not specified in this description of the method and incidents which may have affected the results.

12 References

Arvin, E., Dyreborg, S., Menck, C. and Olsen, J. (1994) A mini-nitrification test for toxicity screening, MINNTOX. *Wat. Res.*, Vol. 28, No. 9, 2029-2031.

ISO 6107-1 (1996) Water quality – Vocabulary. Part 1. *International Organization for Standardization (ISO).*

ISO 9509 (1989) Water quality - Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste water. *European Committee for Standardization (CEN)/International Organization for Standardization (ISO), Ref. No. EN ISO 9509:1995 E.*

SS 02 81 12 (1983) Determination of suspended solids in wastewater and their residue on ignition. (Vattenundersökningar – Bestämning av i avloppsvatten suspenderad substans och dess glödgningsrest.) Svensk standard, *SIS – Standardiseringskommissionen i Sverige.* (In Swedish.)

Swedish EPA (1995) Screening method for estimation of inhibition of nitrification at municipal wastewater treatment plants. (Screeningmetod för bestämning av nitrifikationshämning - vid drift av kommunala avloppsreningsverk). SKARV-projektet. Naturvårdsverket. Report No. 4424, ISBN 91-620-4424-9, ISSN 0282-7298. (In Swedish).

Appendix VII

Nitrogen load from Nolato MediTor

Table A5 presents the calculations of the contribution from Nolato MediTor to Torekov WWTP regarding the flow, the total nitrogen and nitrate-nitrogen.

Table A5. Calculations of nitrogen load from Nolato MediTor.

Torekov WWTP incoming WW. Yearly report 2015	
Flow (m^3/y)	1508986
Flow (m^3/d)	4134
N-tot kg/y	38162
N-tot kg/d	105
Raw data. average 2015-2016 (mg/l)	
NO_3-N in (g/l)=kg/m^3	0.0029
NO_3-N kg/d	12.11
Zn kg/d	0.256
Nolato MediTor outgoing WW. Yearly report 2015	
Flow (m^3/d)	75
N-tot (mg/l)	165
N-tot kg/m^3	0.165
N-tot kg/d	12.38
NO_3-N (g/l)=kg/m^3	0.09
NO_3-N kg/d	6.79
Zn g/l	0.00078
Zn kg/d	0.059
Fraction from Nolato MediTor to Torekov WWTP (%)	
Flow	2
N-tot	12
NO_3-N	56
Zn	23

Populärvetenskaplig artikel

På jakt efter orsaken till hämningen av kvävereningsbakterierna

Med helikopter och förstoringsglas har vi undersökt avloppsvattnet på företaget Nolato MediTor. Reningsverkets krav är inte uppfyllt! Vad finns i vattnet?

Under 20 veckor har vi arbetat med att analysera ett kemiskt avloppsvatten från ett latexföretag. Det giftiga avloppsvattnet har orsakat problem på det kommunala reningsverket, där bakterierna inte mått så bra. Giftigheten har undersökts genom så kallad nitrifikationshämning, som betyder avloppsvattnets effekt på kväverening. Då vi blandat slam (bakterier) med avloppsvattnet så fungerar inte bakteriernas nitrifikationsprocess som den ska. Kväverening fungerar långsammare när nitrifikationsbakterierna utsätts för höjda koncentrationer av ämnen som på ett eller annat vis hamnat i avloppsvattnet. Till exempel utsläpp från industriföretag. Avloppsvattnets hämning varierade mycket i våra mätningar och vi har undersökt varför avloppsvattnet från företaget är hämmande. Höga koncentrationer av nitrat visade sig vara en orsak. Även nitrit var hämmande, men i lägre koncentrationer. Båda dessa komponenter återfinns i avloppsvattnet i de koncentrationer som gav hämning i våra tester.

För att bättre förstå hämning av nitrat och nitrit, de ämnen vars koncentrationer och hämning är kända, satte vi upp ett additivitetstest. Det fick goda resultat. När den totala hämningen i vattnet inte är stor, kan hämningen av nitrat och nitrit adderas med varandra och den sammanlagda hämningen kan alltså räknas ut. Vi har också utfört tester med kemikalier som används i latexproduktionen, som visade sig hämmande, men dessa vet vi inte koncentrationerna av i avloppsvattnet. Därför är det svårt att säga om de utgör en del av hämningen i avloppsvattnet.

Att använda bakterier som reningsmetod är både vanligt och smart, men då måste man se till att bakterierna mår bra. Det problem som uppstår då hämmande ämnen släpps ut till reningsverk är att verket kanske inte kan hålla sina utsläppskrav, av till exempel kväve. Många bäckar små, blir en stor å! För mycket kväve som kommer till vattendrag orsakar övergödning, algblomning och syrefria bottnar, vilket i sin tur försämrar våra ekosystem. I Sverige har vi ett miljömål som heter *Levande sjöar och vattendrag*. Vi vill kunna lämna efter oss ett samhälle till nästa generation, som är hållbart och minst lika bra som när vi växte upp. Därför är det viktigt att ta miljömässigt ansvar.

Företaget behöver komma till rätta med vad som finns i avloppsvattnet för att hitta en reningsmetod att avgifta avloppsvattnet med. Då kan de med gott samvete fortsätta producera latex och de viktiga produkter de tillverkar – de är faktiskt världsledande inom medicinska andningsblåsor! Nu har de en pusselbit på plats av alla de kemikalier som orsakar toxiciteten, och det bästa vore om de la krut på att göra fler analyser för att sedan välja rätt reningsmetod. Vi har även hittat en del pusselbitar som inte alls tycks passa i pusslet; höga koncentrationer av nitrit vi inte kan hitta källan till, och knepiga förhållanden mellan giftighet i delströmmar på fabriken; det krävs alltså mer detektivarbete.