Diel variations of ammonium in surface water of lakes determined with on-line monitoring technique

Lingfeng International Summer Research School
(VVRF05)

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Abstract
Ammonium exists naturally in water and is a vital compound for the marine ecosystem. Elevated amounts, caused by anthropogenic emissions, is however an increasing, worldwide problem. It has effects such as eutrophication, toxicity to marine organisms and damage of drinking water. In water, ammonium is present in very low concentrations due to its involvement in many different processes and hence monitoring methods must be very precise. Using an on-line monitoring instrument, the method of fluorometry determination and flow-injection analysis (FIA), the ammonium concentration in the surface water of two lakes and a reservoir in Xiamen, were studied. In the lab, a highly linear calibration curve ($R^2=0.9993$) with the linearity range of 0~10.0μmol/L was produced, good repeatability (RSD=0.77%) was found and the influence of reaction temperature and flow speed of the reagents were studied. In field, the concentration of ammonium was monitored during one day. The result was compared to data of sunlight intensity, water temperature (T) and dissolve oxygen (DO). Results indicate that the ammonium concentration show a reverse pattern to all of these factors. The ammonium levels were higher and the levels of the light intensity, the DO and the T were lower in the morning, and the other way around in the afternoon. Explanations to the observed pattern include variations in microbial activity and changes of chemical equilibriums.

Keywords: ammonium, on-line monitoring, flow-injection analysis, FIA, fluorometry, surface water, diel variations
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1. Introduction

Ammonium is an important link in the nitrogen cycle of aquatic ecosystems and a vital nutrient for aquatic biological life. An elevated amount of ammonium in lakes and oceans, caused by human activities, is an increasing, worldwide problem. An excess of ammonium can contribute to eutrophication, it can be toxic to many marine organisms (Amornthammarong et al., 2008) and it can affect the odour, taste and disinfection efficiency of drinking water (Fawell et al., 1996).

It is important to monitor ammonium concentrations; however this still remains a difficult task. The main reasons for the complexity of accurate determination of ammonium concentrations is it’s susceptibility to contamination, that the methods traditionally used to perform these measurements are difficult to control in the lab (Aminot et al., 2001) and that ammonium is frequently present in such small concentrations such as 1 µM (Watson et al., 2004). Sensitive and accurate device are necessary to overcome the difficulties of producing accurate measurements of ammonium. The last decades methods have been refined and since the combination of using orthophenol-sulfite and flow injection analysis (FIA) was discovered, measurements have improved dramatically. The method offers many advantages compared to other methods and it can easily be adapted to in situ measurement (Aminot et al., 2001) which is favorable considering the instability of ammonium.

This report will present a system using the method of fluorometry determination and flow-injection analysis (FIA) for on-line monitoring of ammonium. The device has been assembled at the State Key Laboratory of Marine Environmental Science, Environmental Science Research Center, Xiamen University, China. The design of the system, as well as the regents used, has been adapted to suit the local research. Experiments, aiming to optimize the device, are performed in the lab. In-situ measurements are performed in the surface water of a lake in Xiamen mainland.

1.1 Aim

The aim of this study is to understand the methods, and learn how to operate the device, for ammonium detection. Measurements of the ammonium concentration in the surface water of lakes are to be performed. Results of these measurements should be studied to see how the ammonium concentration varies along the day. Fluctuations in the ammonium concentrations should be compared to variations of several abiotic factors and correlations between the changes should be analyzed.

1.2 Limitations

The research time of the study was limited to four weeks. Despite that literature research had been conducted before this four week period, the time was too short to fully learn how to manoeuvre the monitoring apparatus. In-situ monitoring was only performed once. To receive reliable data, the in-situ monitoring should be performed several times in able to make sure that temporal circumstances, such as for example the weather, does not affect the result. The results of this study should not be used as a reliable source of information, but rather as a small part of a bigger study. It shall also be kept in mind that all preparations and experiments have been

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1 In situ means the natural habitat/location of the discussed object (www.ne.se)
conducted by students. The human factor, leading to mistakes, misunderstandings and similar errors is especially important to consider when experiments are performed by unskilled people.

2. Background on ammonium

2.1 Ammonium in the nitrogen cycle

Nitrogen is the most abundant element in the atmosphere (about 78%) and it is also a crucially important component of all biological life since it constitutes the building block for proteins and amino acids. The natural biogeochemical nitrogen cycle is complex and starts with that nitrogen is converted from its inert, atmospheric form (N₂) into forms which can be used in biological processes. Once nitrogen (N₂) reaches the soils and surface waters, mainly through deposition, the two nitrogen atoms are split and combine with hydrogen (H) to create ammonium, NH₄⁺. (Environmental Literacy Council, 2002). Ammonium is involved in the biological processes nitrogen fixation, mineralization and nitrification. Another reaction of importance is that of when ammonia, NH₃, is dissolved in water; the ammonium cat ion, NH₄⁺, is formed along with hydroxyl ions, OH⁻. The degree of ionization depends on the temperature, the pH and the concentration of salts in the water (Fawell et al., 1996).

Ammonium is involved in many of the biogeochemical processes in the nitrogen cycle, both in terrestrial and aquatic environments. In aquatic systems it is for example produced by heterotrophic bacteria, it constitutes an essential nutrient serving as energy source for phytoplankton and it is involved in nitrification and denitrification processes. Due to its involvement in many different processes, ammonium is often present in very low concentrations in aquatic systems (often lower than 1 µmol L⁻¹) (Watson et al., 2004).

2.2 Ammonium and water related, environmental problems

Apart from natural processes, nitrogen is added to nature by anthropogenic influence. Ammonia and ammonium are both frequently used by man in e.g. in commercial fertilizers, animal feed production, the manufacture of fibers, plastics, explosives, paper and rubber. They are also used in coolants, in metal processing and as a starting product for many nitrogen-containing compounds. Ammonia and ammonium salts are used in cleaning agents and as food additives (Fawell et al., 1996).

Although ammonium is a vital compound for many biological processes, high concentrations are toxic to many marine organisms. Fish, shrimps, abalone and sea urchin, especially juveniles of these species have been pointed out to be sensitive (Amornthammarong et al., 2008). Several studies have showed toxicity symptoms in different types of plants when cultured on ammonium as the exclusive N source. Sensitivity to ammonium has even been seen in higher animals, including humans, where neurological and insulin disorders have been observed (Britto et al., 2002). Since ammonium is a nutrient, an excess amount will contribute to eutrophication. In China, the problem of eutrophication is serious; disrupting the normal supply of drinking water in shore cities and causing huge economic losses. A predominant part of this problem can be traced back upon anthropogenic emissions (Le et al., 2002). High ammonium levels in drinking water have, in addition to bad taste and smell, other negative effects. Chloramines are often added in drinking water to give it disinfectious qualities. The disinfection efficiency of the water might be decreased since the chlorine may react with the ammonium, making the chlorine
unavailable for disinfection. If high levels of ammonium is present in raw water, this may also interfere with the removal of manganese since the ammonium interfere with the filters due to that high amounts of oxygen are consumed when nitrification occur. This will result in earthy-tasting water. Ammonium in raw water might also result in water containing nitrite as a result of catalysis or ammonium-oxidizing bacteria colonies (Fawell et al., 1996). Nitrite is directly toxic to human beings.

2.3 Methods used for measurement of ammonium
Traditionally, measurements of ammonium in aquatic systems have been conducted using the Berthelot’s reaction in which ammonium is detected by coloring the ammonium with indophenol blue. Disadvantages of this method are that it has a low sensitivity and a high reagent blank (Amornthammarong et al., 2008). In the early 1970s, methods creating a fluorescent compound by allowing ammonium react with o-phthaldialdehyde (OPA) developed (Amornthammarong et al., 2006). This method was later on refined and the OPA-sulfite-NH$_3^+$ reaction which exhibit higher sensitivity and greater selectivity for ammonium over amino acids became popular. When detecting ammonium in water using this method the results suffered from interferences of salt effect and low sample throughput. Refinement of this method has however led to that it is possible to overcome these disturbances and today it is suitable for examining the ammonium concentration in aquatic systems (Amornthammarong et al., 2008).

3. Methodology
Collecting precise data of ammonium at µM level requires accurate and fast detection methods. The instruments must be sufficiently sensitive to measure low ammonium concentrations, measurements must be rapid to manage the quick concentration shifts and constructed to minimize the effect of interference species (e.g. amines, amino acids) and salt effect (Amornthammarong et al., 2008). Using on-line monitoring technique offers the possibility of receiving results of measurements directly.

3.1 Apparatus and flasks
The technique used to detect ammonium is Flow Injection Analysis (FIA) and fluorometry determination. The basic principle of a FIA system is to inject a sample into a flowing carrier stream of reagent. The sample solution disperses into the reagent, causing the product to form. Downstream, the flow passes a detector (FIAlab Instruments Co.) which records the desired physical parameter. The device used in this study is shown in figure 1 (photo) and a schematic overview is presented in figure 2.

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$^2$ NH$_3$ and NH$_4^+$ often refer to the same compound in scientific contexts.
Figure 1. A photograph of the FIA system used in this study.

Figure 2. A schematic overview of the FIA system used in this study. R1: OPA-solution, R2: Sodium sulfite solution, R3: buffer (Na₃B₃O₇), P1 and P2: pumps.

A peristaltic pump, P1, (LEAD-1, Baoding Lange Pump Co.) was used to pump the reagents (R1, R2), the buffer (R3) and the carrier through the system. R1, R2 and R3 mix according to figure 2. Fresh MilliQ was used as a carrier. The carrier and the sample flow through the six-port injection
valve (VICI Valco Instruments Co. Inc). The six-port injection valve has two positions which create an intermittently injection of the sample. These positions, labeled A (injection) and B (fill) are described more closely in figure 3. In position A, when the sample and the carrier meet this blend continues to the reaction chamber which consists of a water bath of temperature 65°C. The chemical reaction, merging the ammonium with the OPA-solution and creating the fluorescent compound, occurs in the reaction chamber. The final product is detected by the fluorescence detector and the data is recorded on the computer using the software LABview 8.2. All tubes used in the device are of the material PTFE.

In figure 3 the two different positions (A and B) of the six-port injection valve are presented.

![Diagram of six-port injection valve positions A and B](image)

**Figure 3.** The two different positions (A and B) of the six-port injection valve.

In position B the injection loop (red) is filled with the sample (5-1) whiles in position A the sample is pumped by a second pump, P2, (BT01-100, Baoding Lange Pump Co.) to waste (5-6). In position B the carrier will continue to the detector without mixing with the sample (2-3). The recorded intensity will result in the baseline of the graph. In position A the carrier is injected to the injection loop (2-1) and mix with the sample from the previous cycle. The mixture will leave the injection loop and meet with the reagents (4-3). The injection of position A will result in a peak on the screen, indicating the light intensity of the fluorescent compound. The time interval of the positions can be manipulated using the software and is normally set to A: 60 seconds and B: 150 seconds.

All bottles used in the experiments were plastic bottles (high density or polypropylene). Glass or polyethylene terephthalate (PET) material should be avoided since it might absorb ammonium.

### 3.2 Reagents

The water used in all experiments was fresh MilliQ water. All flasks and bottles were cleaned at least three times between each step of the experiment. The tubes in the experimental setup were
cleaned using acetone (C₆H₁₂OH) to remove organic compounds and hydrochloric acid (HCl) and sodium hydroxide (NaOH) to remove inorganic compounds. The tubes were subsequently flushed with MilliQ water. Since the experiment was performed every day it sufficed to use the HCl and the MilliQ on a daily basis. The other detergents were used when the device had been out of use for a couple of days. An injection filled with silica gel was used to protect the tubes from air contamination when the device was not in use. The regents used in the experiment were prepared as described in table 1.

<table>
<thead>
<tr>
<th>Reagent/Solution</th>
<th>Components</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.8375 g OPA 50 ml methanol dilute with 250 ml water</td>
<td>This reagent is stable for about a month</td>
</tr>
<tr>
<td>R2</td>
<td>1.260 g 5mM formaldehyde dilute with 1 l water</td>
<td>This reagent is stable for about a month</td>
</tr>
<tr>
<td>R3 (buffer)</td>
<td>15 g dilute in 1 l water</td>
<td>This reagent is stable for about a month</td>
</tr>
<tr>
<td>Ammonium stock solution</td>
<td>0.6607 g (heated in 110°C 2 hours) dilute in 1 l water (cool the solution to 4°C)</td>
<td>The ammonium concentration in the stock solution will be 10 mM. The working solution is diluted from this.</td>
</tr>
<tr>
<td>Acidized silica gel</td>
<td>300 ml (3mM) 500 g silica gel</td>
<td></td>
</tr>
</tbody>
</table>

The OPA is provided from Alfa Aesar, A. Johnson Matthey Company. The sodium sulfite (AR) and the Na₃B₃O₃ (GR) were provided from Sinopharm Chemical Reagent Co. Ltd.

3.3 The experiment

3.3.1 Preparations for the experiment

A calibration curve, in which the intensity is plotted as a function of the ammonium concentration, was made in the lab. In the experimental section this curve is used to find the ammonium concentration in a water sample by measuring it’s intensity. When preparing the solutions used to create the calibration curve the ammonium stock solution (10 mM) is used. 1 ml of the stock solution is diluted to 100 ml, using MilliQ water. The ammonium concentration hence become 100 µM. 1 ml of this solution is diluted to 100 ml, using MilliQ water. The ammonium concentration of the final solution is 1 µM (working solution). Samples with different ammonium concentrations are prepared by mixing different amounts of the working solution with MilliQ water.

Apart from preparing the calibration curve, several parameters should be adjusted in the experimental setup, in aim to optimize the system. The temperature in the reaction chamber as
well as the flow speed of the fluids, will affect the reaction output. The repeatability should be controlled to guarantee the stability of the system.

3.3.2 The in-situ experiment
The device is adapted to in-situ measurements and the experimental setup used in field is very similar to the one in the lab. The water samples are pumped through a tube which is placed in the water. A de-bubbler, to remove some of the bubbles from the sample, is connected to the tube. The sample is collected in a bottle and manually moved to the experimental device. Before investigating the water it is filtered to remove large particles. Reagents used in the field studies were prepared in the lab.

4. Results
A great part of the experimental section aimed to optimize the analytical process and the device used for investigating the ammonium concentration in water. Secondary, experiments to determine the ammonium concentration of different fresh waters was performed.

4.1 Results in the lab
The major part of the work was performed in the lab. In chapter 4.1 the repeatability, the calibration curve and the result of the control of the calibration curve are presented. Further, the analysis of how the flow speed and the temperature in the reaction chamber will affect the detected ammonium concentration and the results from investigating the ammonium concentration in tap water and in 2 lakes are presented.

4.1.1 Repeatability
The repeatability was evaluated by detecting 7 peaks of the same sample (2 µM). The peaks are shown in figure 4.

![Figure 4. Recorded peaks when investigating the repeatability.](image)

The standard deviation was 0.44 and the RSD was 0.77%. If the RSD is less than 2% the result is considered satisfactory. Samples which were run the same day were generally good. Results of samples which were run different days however often gave different results. The working
solution was prepared every morning. The stock solution and the reagents were changed when too old or when they ran out. Small differences in the preparation of these might explain the differences in the results.

4.1.2 Calibration curve
The calibration curve was created in the lab by running samples containing different ammonium concentrations in the FIA process. For each concentration two or three intensity peaks were detected and the mean value of these was calculated. The measured intensity was plotted as a function of the known concentration. The standard deviation (R²) of the curve indicates the accuracy of the data, were R²=1 is a perfect result. The concentrations used when creating the calibration curve are presented in table 2.

Table 2. The ammonium concentration of the samples used to create the calibration curve.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µM)</td>
<td>0</td>
<td>0.50</td>
<td>1.00</td>
<td>2.00</td>
<td>3.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

The intensity for each of the samples was found using the peaks shown in figure 5.

![Figure 5. Recorded peaks which show the light intensity of the investigated samples.](image)

Recording 2 peaks for some of the concentrations and 3 peaks for others might seem strange, and as can be seen in figure 5 peaks for the same concentrations show somewhat different intensity. Due to the short time of this study, a limited number of calibrations curves could be created. The one presented in the report is the one which proved to have the best R² value. Also, the repeatability showed a good result the same day (figure 4). The calibration curve is presented in figure 6.
R² = 0.9993 which indicates a good result. The equation of the line is

\[ y = 16.9751x + 0.7694 \]

where y is the intensity and x is the ammonium concentration. This equation can be used to calculate the ammonium concentration after detecting the intensity of a water sample.

### 4.1.3 Control of calibration curve

Every day the intensity of a sample with the concentration 2 µM was recorded. If the standard deviation was less than 2% when comparing the result with the same point in the calibration curve, the calibration curve could be used. Differences of the baseline level are assumed to be so small that they can be neglected. In most of the experiments the calibration curve in figure 6 was used. However, a couple of times the result of the control was insufficient. A temporal calibration curve was created at these occasions. If nothing else is mentioned, the calibration curve in figure 6 is the one referred to in this report.

### 4.1.4 Temperature

To investigate how the result of the detected ammonium concentration changes at different reaction temperatures a sample (2 µM) is run at different temperatures. The purpose of this experiment is to find the ideal reaction temperature in able to maximize the chemical reaction creating the fluorescent compound. The temperatures investigated, and the detected intensity, is presented in table 3.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>75</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recorded intensity (au)</td>
<td>1.187</td>
<td>4.576</td>
<td>8.937</td>
<td>16.57</td>
<td>22.64</td>
<td>29.35</td>
<td>34.91</td>
<td>38.60</td>
<td>42.33</td>
</tr>
</tbody>
</table>

Using the values in table 3, the intensity has been plotted as a function of the temperature. The result is shown in figure 7.
As can be seen in figure 7 the intensity increases as the reaction temperature increases. This indicates an increased reaction output. However, energy consumption is an important parameter when developing a system that should be adapted for in-situ measurements. Hence it can’t directly be concluded that the highest temperature will be optimal for the system.

4.1.5 Flow speed

The day of this experiment the results of the control did not agree with the calibration curve and hence a temporal calibration curve was produced. To investigate how the flow speed affects the result, the intensity is detected when a sample (2 µM) is run at different pump speeds. The injection time was changed for different flow speeds since the time for the sample to reach the detector varies. The pump speeds investigated and their corresponding injection times are presented in table 4. Normally, the pump speed 5 rpm is used and hence it was not investigated in this experiment. The values for the pump speed 5 rpm in table 4 are taken from the data used when creating the temporal calibration curve.

<table>
<thead>
<tr>
<th>Pump speed (rpm)</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of position A (s)</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Time of position B (s)</td>
<td>300</td>
<td>225</td>
<td>150</td>
<td>150</td>
<td>130</td>
</tr>
</tbody>
</table>

In figure 8 the recorded intensity is plotted as a function of the flow speed.
In figure 8 it can be observed that the intensity peaks at 5 rpm meaning that the reaction output is optimal at this flow speed.

4.1.6 Investigating tap water and water samples from 2 lakes

The day of this experiment the results of the control did not agree with the calibration curve and hence a temporal calibration curve was produced. The ammonium concentration of tap water and of the water of two fresh water lakes, one in Xiamen University campus (Lake A) and one outside campus (Lake B), was examined to see if their concentrations were within the detection range. The lake water was filtered before investigated to remove large particles. 5 intensity peaks were recorded for the tap water and 4 peaks were recorded for Lake A and Lake B respectively. The average intensity was calculated and compared to the calibration curve to find the ammonium concentration. The average intensity and the concentration are presented in table 5.

Table 5. Average intensity (rounded off to 4 significant numbers) and ammonium concentration (rounded off to 4 significant numbers), of tap water, Lake A and Lake B.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tap water</th>
<th>Lake A</th>
<th>Lake B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average intensity (au)</td>
<td>17.27</td>
<td>60.08</td>
<td>38.51</td>
</tr>
<tr>
<td>Concentration (µM)</td>
<td>0.03998</td>
<td>3.095</td>
<td>1.556</td>
</tr>
</tbody>
</table>

As can be seen in table 5 the ammonium concentration was highest in Lake A, followed by Lake B. Tap water contained least ammonium according to this analysis.
In connection to this experiment the recovery rate for lake B was investigated. A small volume sample of the working solution, with a concentration very close to that of the sample, was added to the sample. The concentration of this solution was investigated and found to be more or less twice as high as the original concentration giving us a good recovery rate (93.64%). The recovery rate was within the range 80~120% showing that this method is well adapted for fresh water lakes.

4.2 Results from in-situ measurements
Field studies were performed one day (7th of July, 2010) at a lake in Xiamen mainland. The lake served as a reservoir for drinking water. The FIA system, as well as other necessary material, was brought to the sampling site and measurements were preformed in situ. The ammonium concentration of the water, the dissolved oxygen (DO), the water temperature (T) and the light intensity were recorded and the results are presented in chapter 4.2.

4.2.1 The ammonium concentration along the day
The ammonium concentration was measured every 20 minutes. In figure 9 the ammonium concentration is plotted as a function of time.

![Figure 9. The ammonium concentration along the day (7th July 2010) at a lake in Xiamen mainland.](image)

The ammonium concentration varies quite a lot during the day, altering from over 1.6 µM to below 0.3 µM. The highest and lowest concentrations are measured before noon and the fluctuations seem to level out during the afternoon.

The light intensity, the DO and the T were measured at approximately the same time interval as the ammonium concentration.

4.2.2 Light intensity
In figure 10, the light intensity (the y-axis to the left) and the ammonium concentration (the y-axis to the right) are plotted as function of time.
Both the ammonium concentration and the light intensity fluctuate a lot along the day. One relation that can be observed is that the ammonium concentration peak when the light intensity dips, and the other way around.

### 4.2.3 Dissolved oxygen

In figure 11, DO (the y-axis to the left) and the ammonium concentration (the y-axis to the right) are plotted as function of time.

The observed relationship between the DO and ammonium concentration is similar to the relationship between light intensity and ammonium concentration.
4.2.4 Water temperature
In figure 12, T (the y-axis to the left) and the ammonium concentration (the y-axis to the right) are plotted as function of time.

![Graph showing T and Ammonium concentration over time](image)

Figure 12. T (green) and ammonium concentration (blue) as a function of time.

The observed relationship between T and ammonium concentration is similar to the relationship between light intensity and ammonium concentration.

5. Discussion

5.1 Repeatability, calibration curve and control of calibration curve
The repeatability of the device was often very good at one measuring occasion. However, comparing the repeatability of two different measuring occasions, the peaks did not always show a reiterate level. The working solution was prepared every day and that this work was performed by different people and in different bottles, might have lead to somewhat different concentrations in the samples, explaining this variation.

Constructing the calibration curve is a delicate task since it must be very precise to suffice as a basis of comparison when investigating samples. To determine the quality of the calibration curve the standard deviation ($R^2$) and the relative standard deviation (RSD) are used. Due to the short time available, a limited number of calibration curves were constructed. The one with the best $R^2$ and RSD values was chosen in this report.

Every day the calibration curve is checked as described in chapter 4.1.3. This daily control is important since many circumstances can change between the experimental occasions. Small differences in the procedure of preparing the solutions and the amount of pollutants in the tubes are 2 examples of factors that might affect the result significantly. If the result of the control differs too much from the calibration curve, a temporal calibration curve should be made. Even
though the $R^2$ and the RSD of the temporal curve are poor compared to the original curve, it is preferable to use a curve which take the circumstances of the day into account.

5.2 Temperature and flow speed

When the temperature is raised the intensity increases indicating that the chemical reaction in which the ammonium combines with the fluorogenic reagent has been more fully accomplished. Normally the experiment is performed at 65 °C. In the figure it can be seen that the intensity increase even at higher temperatures. That 65 °C is considered the optimal temperature despite this is motivated by that the slope is very flat after this temperature meaning that for a certain temperature change the increase of the reaction will be small. Since energy consumption is a factor that need to be considered when performing the experiment, a temperature increase above 65 °C cannot be motivated due to the small increase in reaction output. Amornthammarrong et al. (2008) have, in studies of the same topic, found the same optimal reaction temperature. At higher temperature they found that bubbles might form inside the system causing erroneous signals.

An increased flow speed will increase the sample throughput but according to experiments performed by Watson et al. (2004) the sensitivity of the system will decrease at increased flow speed. One of their explanations for the decreased sensitivity is the decreased reaction time. According to figure 8 the best result is observed when the flow speed is 5 rpm. When the flow speed was investigated a 2 µM ammonium sample was used. In creating the temporal calibration curve used at the day of this experiment the flow speed was 5 rpm. The intensity value for the flow speed 5 rpm in figure 8 is taken from the 2 µM point of the calibration curve. The big difference observed between the intensity for the flow speed 4 and 5 (and 6 and 5) (figure 8) might be explained by that the intensity for the flow speed 5 rpm was not received in direct connection to the flow speed experiment. When creating the original calibration curve (figure 6) a pump speed of 5 rpm was used. In this curve, at the 2 µM point, the intensity is ~35 (rather than ~45). Since the $R^2$ value of this curve is better compared to the temporal calibration curve, this value is more reliable. It can be assumed that there was a problem of the system this day. However, 35 is higher than the intensity recorded for the flow speeds 3, 4, 6 and 7 (figure 8). From the information at hand it can conclusively be said that the optimal flow speed seems to be 5 rpm.

5.3 Tap water and water of 2 lakes

As can be seen in table 5 the ammonium concentration is significantly lower in tap water compared to in the lakes. The concentration in lake A is the highest; almost twice as high as in lake B. Before sent to residents, tap water is treated to remove excess amounts of ammonium to meet the criteria of drinking water. Further, drinking water is generally chlorated to give it disinfection qualities. One of the substances which can react with chlorine is ammonium. This reaction is undesirable since it will lead to lower efficiency of the disinfectious ability of the water. In the case of high ammonium levels in raw water, more chlorine needs to be added in the drinking water to reach the same disinfection level. This might have negative health effects and add to the costs of the drinking water treatment procedure (Sawyer, 2008). The low ammonium amount in the tap water might be explained both by the pre-treatment and by that chlorine has formed a complex with ammonium and hence it can’t be detected. Concerning tap water it would be more interesting to investigate the chlorine concentrations to assure that they don’t reach
toxic levels. Lake A is a natural lake in Xiamen University campus while lake B is a natural lake outside the campus which is used as a reservoir for drinking water. The surrounding environment, the biological condition in the water as well as several abiotic factors affects the ammonium concentration. Since no of these aspects or parameters were investigated at the sampling sites it is difficult to draw any conclusions which might explain the concentration difference in lake A and lake B.

5.4 In-situ measurements

The day of the field study the weather was hot but quite cloudy. As these circumstances might affect the results, this should be kept in mind when reading the analysis below. Also it should be mentioned that the device used originally was developed for ammonium detection in the ocean, i.e. salt water. However, the recovery test (chapter 4.1.6) proved that the system is applicable for fresh water measurements.

In figure 9 it can be seen that the ammonium concentration varies a lot along the day. The fluctuations are not so surprising since ammonium is sensitive to many factors. Light intensity might not have any direct effects on the ammonium concentration, but rather indirect since it will affect the dissolved oxygen (DO) and the water temperature (T). By comparing figure, 10, 11 and 12 it is observed that the light intensity, DO and T prove to have similar curves.

According to Aminot et al. (2001) DO will affect the experiment by eliminating the fluorescence. However, it is stated that experiments show no eliminating effect when concentration which are much higher than those measured in our study, are investigated. Nitrification is a oxygen demanding process. At oxygen deficiency the nitrification process will be pushed to the left meaning ammonium in produced. The opposite relationship between the curves in figure 11 could partly be explained by this.

Aminot et al. (2001) state that no differences in the result will be observed when investigating water samples with different temperatures but with the same concentration. The method will withstand great variations in the sample temperature. However, all chemical equilibriums are dependant of the temperature and hence changes in temperature will affect the chemical compositions in water. In figure 12 it is clear that the ammonium concentration decreases as the water temperature increases. The solubility of ammonia in water decreases as the temperature increases which partly could explain the pattern. However, not only the ammonia – ammonium equilibrium could be considered since ammonium is an intermediate in many chemical processes, and not solely dependent on dissolved ammonia.

As light intensity increases, T rises. Photosynthesis increases creating more DO in the water. These conditions are favorable for microbial activity and as ammonium is used as an energy source by many microorganisms it is natural that its concentration decreases. These facts agree with the pattern observed in figure 10, 11 and 12.

The decrease of fluctuations that can be observed after noon (figure 9) might partly be explained by that our operating of the device was not systematically performed during the morning. Later
on in the day we had developed a routine for the experiment and it was performed more smoothly.

Several other aspects such as for example pH and salinity will affect the ammonium concentration in water. The system produce optimal results at a certain pH and because of this the buffer solution (R3) is used to counteract pH-variations in the sample. How ammonium concentrations vary with pH was not investigated in this study and hence no conclusion about this can be drawn. The salinity effect was not either considered in this study. However, all samples investigated are from fresh water lakes and hence the effect of salinity is less important than if the water samples had been collected from the ocean. Factors such as interference species will affect the detected result. Amornthammarong et al. (2008) investigated how several interference species (methylamine, ethanolamine, glycine –alanine, and L-phenylalanine) might affect the signal. Even thou the FIA system proves to be good in minimizing their effect, the result might be somewhat disturbed. When performing field studies we did not consider this factor and it should hence be kept in mind that this might have affected our results.

6. Conclusion
This project aimed to develop an understanding for the device used for ammonium detection and to investigate surface waters of lakes using this device. Parameters of the system were supposed to be adjusted in aim to optimize the result of the detection. The optimal reaction temperature was found to be 65 °C which agree with previous studies. Even though the results of the pump speed experiment were not perfect, it was concluded that the optimal speed is 5 rpm. A calibration curve of high precision was produced and used as a basis of comparison when investigating water samples. The repeatability of the system was proved to be good and the recovery rate of an investigated sample was satisfactory.

In field studies the correlation between ammonium concentration and light intensity, water temperature and dissolved oxygen respectively was investigated. The original aim was investigate diel variations, i.e. variations along a 24 hour period; however experiment were only performed during daytime. The results found prove that as the mentioned abiotic factors increase, the ammonium concentration decreases, and the other way around. The results are primarily explained by that microbial activity increase when these abiotic factors show high levels, and thus consumption of ammonium is observed.

All experiments in this report were performed in less than 4 weeks. Due to this short time most experiments were only performed once. The project will be continued by some of the group members for at least one year. Hopefully, the results presented in this report can be used as a basis for continuous research within the area.

7. Acknowledgement
The author would like to thank Prof. Dongxing Yuan and Min Zhang for their help when writing this report. A special thanks to Yong Zhu and Wenfang Lu for good cooperation and for patience explanations. Further, thank you to Linus Zhang and Rolf Larsson for organizing the cooperation with Xiamen University and thank you to SWECO and ITT Water & Wastewater who partly sponsored Lingfeng summer research school 2010.
8. References

8.1 Articles


8.2 Websites


8.3 Other documents