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Feasibility of UV-Vis spectrophotometry for nitrite estimation in urine nitrification systems

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Photo on title page: Nitrification columns and sensor at Eawag (Angelika Hess)

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Abstract

At Eawag, source separated urine is stabilised with biological nitrification to recover nutrients. A sudden increase in the ammonia load to the nitrification reactor can cause an accumulation of the intermediate product nitrite. If not detected early enough, a nitrite accumulation can lead to permanent failure of the urine nitrification process. To prevent this, the nitrite concentration needs to be monitored. In this master thesis, the feasibility to estimate nitrite with UV-Vis spectrophotometry is investigated.

Three different sensor configurations are evaluated both in water and in treated urine spiked with nitrite and nitrate. All three spectrophotometers show saturation effects for the wavelengths where the primary nitrite and nitrate absorbance peaks are. However, clear secondary peaks, at around 300 nm for nitrate and at around 355 nm for nitrite, are not affected by saturation and allow to estimate nitrite based on the measured spectrum. Based on these findings, the UV-Vis spectrophotometer with an optical measurement path of 2 mm and a resolution of 2.5 nm is chosen for the experiments about the long-term effects.

For analysing the long-term effects from urine on the sensor, the sensor is exposed to urine for 3–4 days and the effect of cleaning on the absorbance measurements is evaluated. It is found that the urine causes biofilm formation on the sensor window influencing the absorbance measurements. Within three weeks of exposure, no scaling is detectable.

With a principal component regression (PCR), the nitrite concentrations in the urine samples are estimated based on the measured absorbances. The PCR model is able to correct the measured absorbances for the effects of biofilm on the sensor and to some extent for the changes in urine composition. The prediction error for nitrite is smaller than 20 mg NO_2^--N/L for different considered cases. The achieved accuracy and precision is sufficient to reliably detect nitrite accumulation in the urine nitrification reactor.

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Contents

1 Introduction

In the following sections, first the background and the motivation for this work are described. Afterwards, the measurement principle of spectrophotometry and previous work in this field are presented. In the last section, the scope and objectives of this thesis are elucidated.

1.1 Source separation of urine

Combined sewer systems as used for example in Switzerland are extremely inflexible and do not allow for significant innovation. Source separation allows for more flexible systems which can faster adapt to changes in technology (Larsen & Gujer, 2001). Furthermore, a paradigm shift is taking place from simply preventing pollution to a more sustainable view on wastewater treatment which includes the recovery of nutrients from the waste streams (Guest *et al.* , 2009). Urine contains most of the nutrients in domestic wastewater but only contributes less than one percent of the total wastewater volume (Maurer *et al.* , 2006). A highly concentrated waste stream facilitates the recovery of nutrients like nitrogen, phosphorus and potassium.

Since fresh urine is an unstable and odorous solution, it cannot be used in its fresh form as a fertiliser. Maurer *et al.* (2006) review different processes for the treatment of source-separated urine. They distinguish between seven purposes of treatment: hygienisation, volume reduction, stabilisation, P- and N-recovery, nutrient removal and handling of micropollutants. The review shows that none of the available processes can meet all seven demands.

At the Swiss Federal Institute of Aquatic Science and Technology (Eawag), the separately collected urine is treated with biological nitrification to stabilise the urine and therefore prevent high nitrogen losses and malodour. The pilot scale reactor in the Eawag main building Forum Chriesbach has a volume of 120 L and is operated as a continuous stirred tank reactor with biofilm carriers (Etter *et al.* , 2013). Udert & Wächter (2012) showed that biological nitrification followed by distillation can be a stable and efficient process for stabilisation and concentration of urine.

1.2 Biological nitrification

Nitrification is a microbial process in which reduced nitrogen (ammonia) is oxidised in two steps to nitrite and nitrate (Sedlak, 1991). In the first step, ammonia oxidising bacteria (AOB) oxidise ammonia to nitrite:

$$NH_3 + 1.5\,O_2 \rightarrow NO_2^- + H^+ + H_2O \tag{1.1}$$

In a second step (equation 1.2) nitrite oxidising bacteria (NOB) oxidise nitrite to nitrate.

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \tag{1.2}$$

Under normal conditions all the nitrite is oxidised immediately, so that very low concentrations of nitrite are measured. Imbalances between the activity of AOBs and NOBs, caused e.g.

by a sudden increase in ammonia load (Etter *et al.* , 2013) or an increase in pH (Udert & Wächter, 2012) can lead to an accumulation of nitrite in the reactor. Due to the higher availability of ammonia, the AOB activity increases immediately. The increased nitrite concentration inhibits the nitrite oxidisers leading to even higher nitrite concentrations until complete NOB inhibition occurs which corresponds to permanent failure of the reactor (Udert & Wächter, 2012). The inflow to the reactor at Eawag is controlled by the pH (Etter *et al.* , 2013). However, the pH is determined by the AOBs (Udert & Jenni, 2013) meaning that this control can fail to prevent nitrite accumulation in the reactor. An additional control step via nitrite estimation is necessary to guarantee stable operation of the reactor.

Typical nitrite concentrations measured in the nitrification reactors are around $2\,\mathrm{mg\,NO_2^-}$-N/L (see table 1). From concentrations from around $10\,\mathrm{mg\,NO_2^-}$-N/L it is assumed that nitrite starts to accumulate and the reactor is not in steady state any more. Up to concentrations of more than $50\,\mathrm{mg\,NO_2^-}$-N/L, the system can easily recover with appropriate measures (A. Fumasoli, personal communication, 2nd of March 2015). For nitrite concentrations higher than $200\,\mathrm{mg\,NO_2^-}$-N/L permanent failure will occur with a high probability (B. Sterkele, personal communication, 2nd of February 2015). Table 1 lists typical concentrations that can be expected in the nitrification reactor. For male urine the concentrations are around twice as high since the urine is less diluted due to the collection with waterless urinals.

Table 1: Typical concentrations measured in the reactor influent and effluent for female urine (Etter *et al.* , 2013).

		Influent	Reactor
NH_4^+	ammonium ($\mathrm{mg\,N\,L^{-1}}$)	1790 ± 180	899 ± 140
NO_3^-	nitrate ($\mathrm{mg\,N\,L^{-1}}$)	-	914 ± 203
NO_2^-	nitrite($\mathrm{mg\,N\,L^{-1}}$)	-	2 ± 1
Cl^-	chloride ($\mathrm{mg\,L^{-1}}$)	1830 ± 320	1780 ± 250
TIC	total inorganic carbon ($\mathrm{mg\,L^{-1}}$)	970 ± 109	< 4
TOC	total organic carbon ($\mathrm{mg\,L^{-1}}$)	863 ± 250	77 ± 42
COD	chemical oxygen demand ($\mathrm{mg\,L^{-1}}$)	2110 ± 390	217 ± 35

There are several reasons why nitrite is unwanted in high concentrations. Nitrite is toxic for fish (Gujer, 2007), therefore it has to be prevented from entering any waters. In human bodies, nitrite interferes with the oxygen transport in blood which is mainly of concern for infants (WHO, 2011). These two cases may not be as important if the urine fertiliser is not applied in excess on the fields. But nitrite is toxic for plants as well (Oke, 1966), so a fertiliser containing nitrite has no value. An additional concern is that nitrous oxide is prone to volatilise from solutions with high nitrite concentrations (Wunderlin *et al.* , 2013). This needs to be avoided since nitrous oxide is a strong green house gas (Foley *et al.* , 2010).

1.3 Nitrite measurement

To prevent permanent failure of the reactor due to nitrite accumulation, nitrite needs to be measured to detect such an accumulation early enough. At the moment, for the reactor operated at Eawag, nitrite is measured twice a week either with nitrite strips or with a lab analysis. On-line analysers which are able to measure nitrite are available. Since they need consumables and high maintenance they are not economically feasible for use in the urine nitrification reactor or other decentralised treatment units. No in-line sensor is available which can reliably measure nitrite in such high concentrated wastewater. There are different ideas how nitrite could be estimated: (*i*) spectrophotometry, (*ii*) soft sensors and (*iii*) electrochemical methods. In the soft sensing approach signals like oxygen and pH measurements are considered to contain indirect information about nitrite. With the help of process models, the nitrite concentrations are then predicted with measurements which are easily obtained (Mašić & Villez, 2014). Different authors showed that there is a linear relationship between the amperometric response and the nitrite concentration if a constant potential is applied at electrodes of different materials (Liu *et al.* , 2013, 2014; Palanisamy *et al.* , 2014). At Eawag, a linear relationship is found between the low nitrite concentrations and the voltage and one for nitrite concentrations up to 80 mg NO_2^--N/L with the current density in nitrified urine (H. Zöllig, personal communication, 4$^{\text{th}}$ of March 2015). The option to estimate nitrite via UV-Vis spectrophotometry is investigated in this work.

1.4 UV-Vis spectrophotometry

A UV-Vis spectrophotometer can be directly submerged into the medium. That brings the advantage that it requires no sampling, no sample preparation and no reagents (Gruber *et al.* , 2005). This can prevent a lot of errors due to sampling handling (Van den Broeke *et al.* , 2006).

The used sensor in this work (spectro::lyser, s::can) emits a light beam by a Xenon flash lamp and after its path through the medium its intensity is measured by a detector over a range of wavelengths. Substances in the liquid absorb the light beam at different wavelengths. For long-term stability of the produced signal, a split light beam design is used: one beam passes through the sample while the other travels along a parallel pathway in the instrument and acts as an internal reference (Van den Broeke *et al.* , 2006).

A linear relationship between the measured absorbance and the concentration of nitrite can be assumed according to the Beer-Lambert law (equation 1.3). This law states that for a given wavelength and a single component, the absorbance is proportional to that component's concentration. This only applies under ideal conditions, so deviations from the Beer-Lambert law have to be expected, for example for high concentrations in the liquid (Burgess, 2007).

$$A_\lambda = \varepsilon_\lambda \, b \, C \tag{1.3}$$

A_λ: Absorbance at wavelength λ (AU)
ε_λ: Molar absorptivity for wavelength λ (m^2/mol)
b: Path length (m)
C: Concentration (mol/m^3)

During the 1990s, the UV-Vis technology moved from the lab into the field. However, a lot of the available products are still relatively simple photometers with only one or two wavelengths at a time, e.g. the organics are often determined at 254 nm. Using the entire absorption spectrum though allows for a lot of additional information and reduced cross-sensitivity (Van den Broeke *et al.* , 2006). Table 2 lists the wavelengths for the primary and secondary absorbance peaks for nitrite and nitrate as reported in Spinelli *et al.* (2007).

Table 2: Absorbance peaks for nitrite and nitrate as reported in Spinelli *et al.* (2007).

	Nitrite	Nitrate
Primary absorbance peak (nm)	213	206
Secondary absorbance peak (nm)	354	302

Rieger *et al.* (2004) successfully estimated nitrite and nitrate in the effluent of a wastewater treatment plant for a limited working range with a UV sensor. The sensor showed a good long-term stability with only a minimal maintenance consisting of a monthly manual cleaning (Rieger *et al.* , 2008). Gruber *et al.* (2005) found that for COD-estimation in sewer, automatic cleaning with pressured air did a sufficient job. But as the cleaning system stopped working, a shift in the COD measurements was observed. Bouvier *et al.* (2008) showed good results for nitrite and nitrate estimation for industrial wastewater as well. Lemaire *et al.* (2011) used nitrite estimation via spectrophotometry for the process automation of a Sequencing Batch process called ANITA^{TM} Shunt to control the aeration length.

Prior work at Eawag showed spectroscopic methods together with chemometrics as a powerful tool for the estimation of nitrite concentrations in nitrified urine (Santos, 2014). Santos showed that saturation effects at the primary peaks have a substantial effect on nitrite estimation while particles in the urine have less impact. Further investigations indicated that there is additional information about nitrite and nitrate at wavelengths higher than the primary peaks which can be used for nitrite estimation.

1.5 Scope and objectives

The goal of this thesis is to further investigate the feasibility of UV-Vis spectrophotometry for nitrite estimation in urine nitrification systems.

As a first step, three different UV or UV-Vis spectrophotometers which are available at Eawag are tested to analyse their sensitivity at the secondary nitrite and nitrate absorbance peaks and how much they are affected by saturation at the primary peaks. The most suitable sensor is chosen for further experiments.

Afterwards, the long-term effects on the sensor from exposure to nitrified urine are examined. It is analysed if detectable disturbances due to biofilm formation or due to scaling caused by salt formations occur on the sensor window.

A principal component analysis (PCA) is conducted to better interpret the collected data. With a principal component regression (PCR) the nitrite concentrations are estimated based on the measured absorbances. The focus is placed on the model's ability to correct the nitrite estimation for changes in urine composition and the possible disturbances by biofilm formation and scaling.

The obtained results should contribute to the development of an automated control system for a urine nitrification reactor.

2 Material and Methods

In this chapter, first the used spectrophotometers are introduced. Afterwards the different experimental setups are explained. At the end, a section about the modelling with the obtained data follows.

2.1 UV and UV-Vis spectrophotometers

For the collection of absorbance measurements three different on-line spectrophotometers of the type spectro::lyser from s::can Messtechnik Gmbh (Vienna, Austria) are used. Table 3 shows the characteristics of the used sensors. The sensor with 0.5 mm measurement path measures only in the ultraviolet range, the other two both in ultraviolet (10–400 nm) and in the visible (400–800 nm) range (s::can, 2011).

Table 3: Specifications of the used sensors.

Type	Measurement path (mm)	Range (nm)	Resolution (nm)
Spectro::lyser	0.5	220–399	1
Spectro::lyser	2	200–735	2.5
Spectro::lyser	5	200–735	2.5

The used spectro::lyser is a compact submersible sensor which can measure directly in the liquid. If an in-situ installation is not possible, the absorbance can be measured ex-situ by means of circulation of the medium through a flow cell. Such a flow cell was used during the experiments in Forum Chriesbach (figure 1). In the experiments conducted for this work, a measurement is taken every minute. Such a high measurement frequency allows the detection of rapid changes in the liquid. No automatic cleaning was used in any of the conducted experiments.

Figure 1: Experimental setup at the nitrification reactor in the Eawag main building Forum Chriesbach.

2.2 Analysing alternative sensor configurations

Initial experiments were conducted to find out which of the sensor configurations are suitable for further experiments about the long-term behaviour and to gain information about their behaviour under controlled conditions in the lab.

2.2.1 Analysing alternative sensor configurations in nanopure water

This experiment aims to study the presence of additional information about nitrite and nitrate at wavelengths higher than the primary absorbance peaks. Two sensors are available to be tested against the sensor used in previous work to estimate nitrite under the same idealised conditions.

For all three sensors at least five spectra are collected consecutively (*i*) in nanopure water, (*ii*) in a solution with 125 mg NO_2^--N/L, (*iii*) in a solution with 2500 mg NO_3^--N/L and (*iv*) in a solution with both 125 mg NO_2^--N/L and 2500 mg NO_3^--N/L. For each measurement, the probe is inserted into 500 ml water in a 1000 ml measurement container. Continuous mixing is ensured with a magnet stirrer. Between the measurements, the measurement gap is cleaned with water, ethanol, HCl and NaOH as proposed by the manufacturer (s::can, 2011). The exact procedure is described in the experimental protocol in annex A.1.

2.2.2 Analysing alternative sensor configurations in nitrified urine

After analysing the different sensor configurations in water with added nitrite and nitrate, the next step is to analyse the sensors in urine. Measuring in urine adds a background spectrum, so higher overall absorbances are expected. The goal of this experiment is to see if saturation effects occur for the secondary peaks for the sensors with long measurement paths. A similar procedure as in the experiment with water (section 2.2.1) is used but this time urine is spiked with nitrite and nitrate. The urine is collected from the nitrification reactor and the biomass is settled for 60 minutes in an Imhoff cone. The exact procedure is described in annex A.2.

2.3 Analysing long-term effects on the UV-Vis measurements

This experiment aims to gain information about the long-term behaviour of the UV-Vis absorbance measurements in nitrified urine. Biofilm growth on the sensor window, scaling due to salt formation and deposition or blocking of the measurement gap are possible disturbances. In this work, biofilm and scaling are defined empirically as follows:

- Biofilm: biological growth or depositions of organics, particles and additional components which can be removed by mechanical cleaning.
- Scaling: deposits of e.g. salts which cannot be removed by mechanical cleaning but only by additional cleaning with acid or base.

The sensor is exposed to urine for 3–4 days. Since the sensor cannot be directly installed in the urine nitrification reactor, urine is pumped with a rate of approximately 0.5 L/min through the flow cell in which the sensor is mounted. After exposure, a cleaning sequence

is executed. Mechanical cleaning with water and a paper tissue is assumed to be able to remove a possible biofilm. The following cleaning step with hydrochloric acid can remove inorganic deposits like scaling. The third step with the base removes protein-based deposits. As a last step, cleaning with ethanol ensures that there are no more fats or organic materials on the sensor window which are not removed by the previous steps (Nopco, 2015).

Before and after every cleaning step, five absorbance measurements are collected consecutively in a solution with 500 mg NO_2^--N/L and 500 mg NO_3^--N/L. The difference in the measured absorbance before and after cleaning is an indicator for the cleaning effect of the considered cleaning step. A mechanical cleaning was carried out twice a week. The first complete cleaning sequence was done after three weeks of exposure to urine. These experiments are executed with the spectro::lyser with the 2 mm optical measurement path. The exact procedure is described in annex A.3.

2.4 Calibration of the UV-Vis sensor for nitrite measurement

2.4.1 Data collection

For the calibration of the UV-Vis sensor, the absorbance is measured in urine in a narrow measurement container. First, the nitrified urine is sedimented for 60 minutes in an Imhoff cone. Next, the urine is spiked with nitrite, so that six different concentrations ranging from below 2 mg NO_2^--N/L to more than 125 mg NO_2^--N/L are obtained (table 4). For that, a stock solution with a concentration of 12.875 g NO_2^--N/L is added in consecutive steps of 1 ml to a urine sample of 500 ml.

Table 4: Mixture for the different steps of the dilution series. The stock solution has a concentration of 12.875 g NO_2^--N/L.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Urine volume (ml)	500	500	500	500	500	500
Stock solution volume (ml)	0	1	2	3	4	5

The exact concentration depends on the nitrite concentration in the collected urine sample. For each concentration five spectra are collected. This procedure is done before and after the cleaning (section 2.3) on 7 different dates distributed over a period of four weeks (table 5). The exact procedure is described in the experimental protocol in annex A.3. Between the 2nd of February and the 5th of February, the sensor was not exposed to urine because of problems in the nitrification reactor. This explains the longer time span between the experimental days 2 and 3.

Table 5: Measured dilution series for the different days with experiments.

Measurements	Day 1 30 Jan	Day 2 02 Feb	Day 3 09 Feb	Day 4 12 Feb	Day 5 16 Feb	Day 6 20 Feb	Day 7 23 Feb
Before Cleaning	×	×	×	×	×	×	×
After mechanical cleaning	×	×	×	×	×	×	×
After complete cleaning					×		×

Before modelling, the absorbance data which are clearly determined as outliers are removed. For example in figure 19 in annex B.1 the measurements for a nitrite concentration of $52\,\mathrm{mg\,NO_2^-}$-N/L are excluded. It is assumed that for these outliers, the absorbance of an air bubble or a particle is measured instead of urine. It is assumed that an automatic detection algorithm could easily indicate these outliers. Only the wavelengths higher than 275 nm are considered for the model since Santos (2014) and preliminary experiments showed that the primary absorbance peaks are affected by saturation.

2.4.2 Modelling procedure

As a first step, the dataset is divided into two subsets, one used for calibration and validation (training) of the model and one used for testing the model at an independent subset. With the training set, the principal component regression (PCR) model is built and with a leave-one-out cross validation in the training data set, the best model is chosen. The final model is assessed with the test error, which is the prediction error in the independent test sample (Hastie *et al.* , 2009). In the following sections, the different steps are explained more in detail.

2.4.3 Principal component analysis

The central motivation behind a principal component analysis (PCA) is a reduction in dimensionality of a large data set while keeping as much as possible of the meaningful variation present in the data set. This is achieved by transforming the data to a new set of variables, the principal components, which are uncorrelated and organised such that the first components contain most of the variation. The principal components are a linear combination of the original variables (Jolliffe, 2002). The PCA analysis helps finding patterns in data of high dimensionality as well as facilitating interpretation.

For every sample, absorbance measurements for the wavelengths between λ_1 and λ_q are taken:

$$\boldsymbol{x} = \left(x_{\lambda_1}, ..., x_{\lambda_q}\right) \tag{2.1}$$

From the p samples, the mean for every wavelength is calculated:

$$\boldsymbol{\mu} = \frac{1}{p}\sum_{i=1}^{p} \boldsymbol{x}_i \tag{2.2}$$

The matrix with the centred absorbances $\bar{\boldsymbol{X}}$ is composed of the measurement vectors for the p samples:

$$\bar{\boldsymbol{X}} = \left(\boldsymbol{x}_1^\mathsf{T} - \boldsymbol{\mu}^\mathsf{T}, ..., \boldsymbol{x}_p^\mathsf{T} - \boldsymbol{\mu}^\mathsf{T}\right)^\mathsf{T} \in \mathbb{R}^{p \times q} \tag{2.3}$$

Central for the PCA is the singular value decomposition of $\bar{\boldsymbol{X}}$.

$$\bar{\boldsymbol{X}} = \boldsymbol{U} \cdot \boldsymbol{S} \cdot \boldsymbol{P}^\mathsf{T} = (\boldsymbol{u}_1, \ldots, \boldsymbol{u}_q) \cdot \begin{pmatrix} s_1 & & 0 \\ & \ddots & \\ 0 & & s_q \end{pmatrix} \cdot \left(\boldsymbol{p}_1, \ldots, \boldsymbol{p}_q\right)^\mathsf{T} \tag{2.4}$$

The matrix $\boldsymbol{U} \in \mathbb{R}^{p \times q}$ contains the left singular vectors $\boldsymbol{u}_j$, $\boldsymbol{P} \in \mathbb{R}^{q \times q}$ contains the right singular vectors $\boldsymbol{p}_j$ and $\boldsymbol{S} \in \mathbb{R}^{q \times q}$ is a diagonal matrix which contains the singular values s_j of $\bar{\boldsymbol{X}}$ ordered by their magnitude, $s_1 \geq s_2 \geq .... \geq s_q$ (Hastie *et al.* , 2009). In the context of PCA, the left singular vectors $\boldsymbol{p}_j$ are called principal components and the vectors $\boldsymbol{t}_i \in \mathbb{R}^q$,

$$\left(\boldsymbol{t}_1^\mathsf{T}, \ldots, \boldsymbol{t}_p^\mathsf{T}\right)^\mathsf{T} = \boldsymbol{U} \cdot \boldsymbol{S} \tag{2.5}$$

are called scores.

The singular values are the square roots of the eigenvalues and the principal components $\boldsymbol{p}_j$ are the eigenvectors of $\bar{\boldsymbol{X}}^\mathsf{T} \cdot \bar{\boldsymbol{X}}$. Since $\bar{\boldsymbol{X}}^\mathsf{T} \cdot \bar{\boldsymbol{X}}$ is proportional to the covariance matrix of the training data, truncating equation 2.4 can be interpreted as only considering the n directions with the largest variance in the training data (Jolliffe, 2002).

$$\bar{\boldsymbol{X}} \approx (\boldsymbol{u}_1, \ldots, \boldsymbol{u}_n) \cdot \begin{pmatrix} s_1 & & 0 \\ & \ddots & \\ 0 & & s_n \end{pmatrix} \cdot \begin{pmatrix} \boldsymbol{p}_1^\mathsf{T} \\ \vdots \\ \boldsymbol{p}_n^\mathsf{T} \end{pmatrix} = \begin{pmatrix} \boldsymbol{t}_1^{(n)} \\ \vdots \\ \boldsymbol{t}_p^{(n)} \end{pmatrix} \cdot \begin{pmatrix} \boldsymbol{p}_1^\mathsf{T} \\ \vdots \\ \boldsymbol{p}_n^\mathsf{T} \end{pmatrix} = \boldsymbol{T}^{(n)} \cdot \left(\boldsymbol{P}^{(n)}\right)^\mathsf{T} \tag{2.6}$$

The matrix $\boldsymbol{T}^{(n)} \in \mathbb{R}^{p \times n}$ is the truncated scores matrix and $\boldsymbol{P}^{(n)} \in \mathbb{R}^{q \times n}$ contains the first n principal components after the dimension is reduced.

2.4.4 Principal component regression

The principal component regression (PCR) performs a linear regression based on a PCA-model. Equation 2.7 shows how the estimated values for the intercept and the slope are obtained.

$$\boldsymbol{\beta} = \left(\boldsymbol{Z}^\mathsf{T} \cdot \boldsymbol{Z}\right)^{-1} \cdot \boldsymbol{Z}^\mathsf{T} \cdot \boldsymbol{Y} \qquad \text{with} \qquad \boldsymbol{Z} = \begin{bmatrix} \boldsymbol{1} & \boldsymbol{T}^{(n)} \end{bmatrix} \tag{2.7}$$

where $\boldsymbol{\beta} \in \mathbb{R}^{n+1}$ denotes the regression coefficients, $\boldsymbol{Z} \in \mathbb{R}^{p \times n+1}$ contains the scores and $\boldsymbol{Y} \in \mathbb{R}^p$ is the vector of known concentrations.

2.4.5 Predict nitrite concentration of new samples

The nitrite concentrations of a new sample are predicted based on the principal component regression performed for the training data set. The scores of the new data set with the centred data matrix $\bar{\boldsymbol{X}}_{\text{test}}$ are calculated:

$$\boldsymbol{T}_{\text{test}} = \bar{\boldsymbol{X}}_{\text{test}} \cdot \boldsymbol{P}^{(n)} \tag{2.8}$$

With the estimated $\boldsymbol{\beta}$ from the principal component regression on the training data, the concentrations can be estimated.

$$\hat{\boldsymbol{Y}} = \boldsymbol{Z}_{\text{test}} \cdot \boldsymbol{\beta} \qquad \text{with} \qquad \boldsymbol{Z}_{\text{test}} = [\mathbf{1} \quad \boldsymbol{T}_{\text{test}}] \tag{2.9}$$

To choose the best number of principal components, a leave-one-out cross validation is performed. From the training samples, one subset of data at a time is excluded from the calibration of the PCR-model but therefore used as the validation data set. The other subsets are meanwhile used for the calibration of the model. With this procedure, every datapoint is used once for validation. For every number of components n and every data set k as the validation data set, the root mean squared error $RMSR_k(n)$ is calculated.

$$RMSR_k(n) = \sqrt{\frac{\sum_{i=1}^{m_v} \left(\hat{Y}_{i,k}(n) - Y_{i,k}\right)^2}{m_v}} \tag{2.10}$$

with m_v the number of samples in the validation data set.

The mean error over all m_k cross validation data sets $\overline{RMSR}(n)$ and its standard deviation $\sigma_{RMSR(n)}$ are estimated as

$$\overline{RMSR}(n) = \frac{1}{m_k} \sum_{k=1}^{m_k} RMSR_k(n) \tag{2.11}$$

$$\sigma_{RMSR(n)} = \sqrt{\frac{1}{m_k - 1} \sum_{k=1}^{m_k} \left(RMSR_k(n) - \overline{RMSR}(n)\right)^2} \tag{2.12}$$

With this, the number of components which leads to the smallest mean error can be found.

$$n^* = \arg\min_{n \in \mathbb{N}} \overline{RMSR}(n) \tag{2.13}$$

To have a model which is as simple as possible, the number of components n_{pick} is chosen whose mean error lies within one standard deviation of the smallest one.

$$n_{\text{pick}} = \min\{n \in \mathbb{N} \mid \overline{RMSR}(n) \leq \overline{RMSR}(n^*) + \sigma_{RMSR(n^*)}\} \tag{2.14}$$

3 Results

In this section, first the results which led to the decision with which sensor the further experiments would be conducted are presented. Afterwards, the results about the long-term effects of urine on the sensor are shown. In the last two parts the PCR model is tested for different cases.

3.1 Analysing alternative UV and UV-Vis sensor configurations

Three sensors with different sensor configurations were tested in nanopure water spiked with nitrite and nitrate and in nitrified urine which was also spiked with nitrite and nitrate.

Figures 2 and 3 show the measured spectrum in treated urine measured with the UV-Vis sensor with a measurement path of 2 mm for different nitrite and nitrate concentrations. Clear weaker secondary peaks can be observed, around 302.5 nm for nitrate and around 355 nm for nitrite. These values correspond well with literature values for the secondary peaks of 301.6 nm for nitrate and 353.9 nm for nitrite (Spinelli *et al.* , 2007). These secondary peaks are also clearly visible for the sensor with the 5 mm measurement path, but not that clearly for the one with a measurement path of 0.5 mm (see figure 4). The UV-sensor with 0.5 mm measurement path shows an anomalous behaviour for high nitrite concentrations. So is the measured absorbance in water for some wavelengths higher for nitrite only than for both nitrite and nitrate (compare with figure 20 in annex B.2.1). This behaviour is not further investigated in this work since a different sensor was selected for future work.

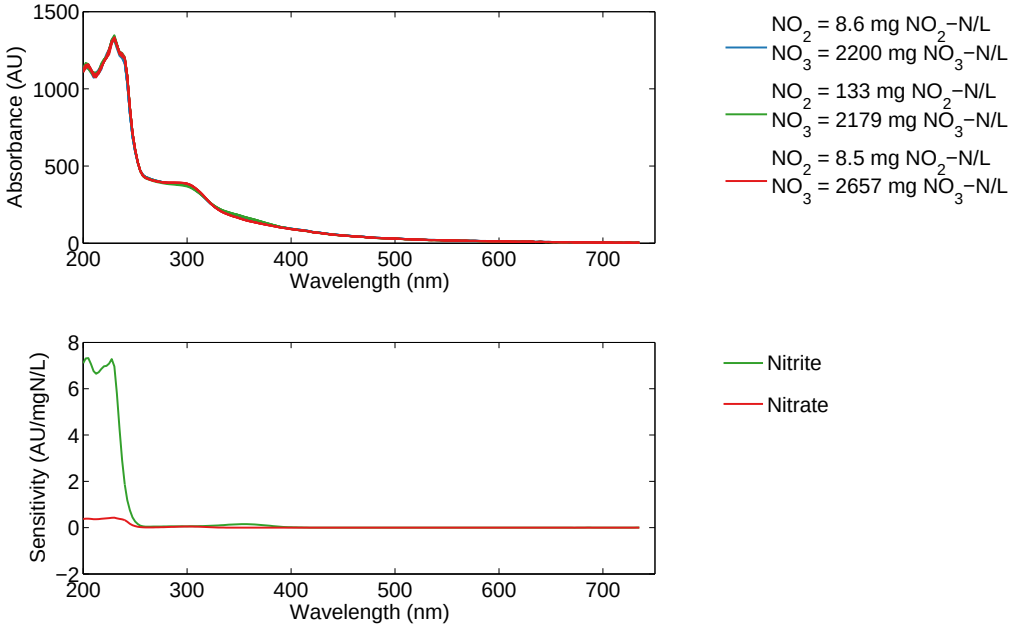


Figure 2: Spectrum and sensitivity for the UV-Vis sensor with 2 mm path.

All three sensors are sensitive to nitrite over the whole UV range, for nitrate the sensitivity

is around zero for longer wavelengths than 340 nm. The calculated sensitivities for the two peak secondary absorbances are listed in table 6. It shows that the sensitivities from the two UV-Vis sensors are similar, while the one for nitrite obtained with the UV-sensor is much higher. All three sensors are more sensitive for nitrite than for nitrate at the secondary peaks.

Table 6: Calculated sensitivity (AU/mg N/L) for the different sensor configurations.

	Measurement path (mm)		
	0.5	2	5
Nitrite (355 nm)	0.38	0.15	0.16
Nitrate (302.5 nm)	0.05	0.05	0.05

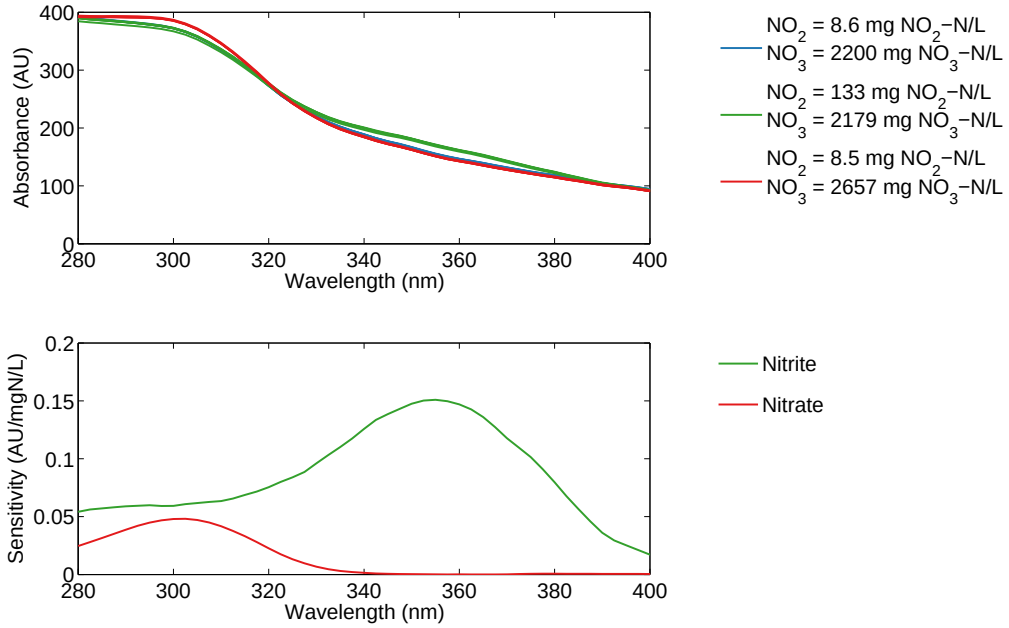


Figure 3: Close-up of the spectrum and the sensitivity for the UV-Vis sensor with 2 mm path.

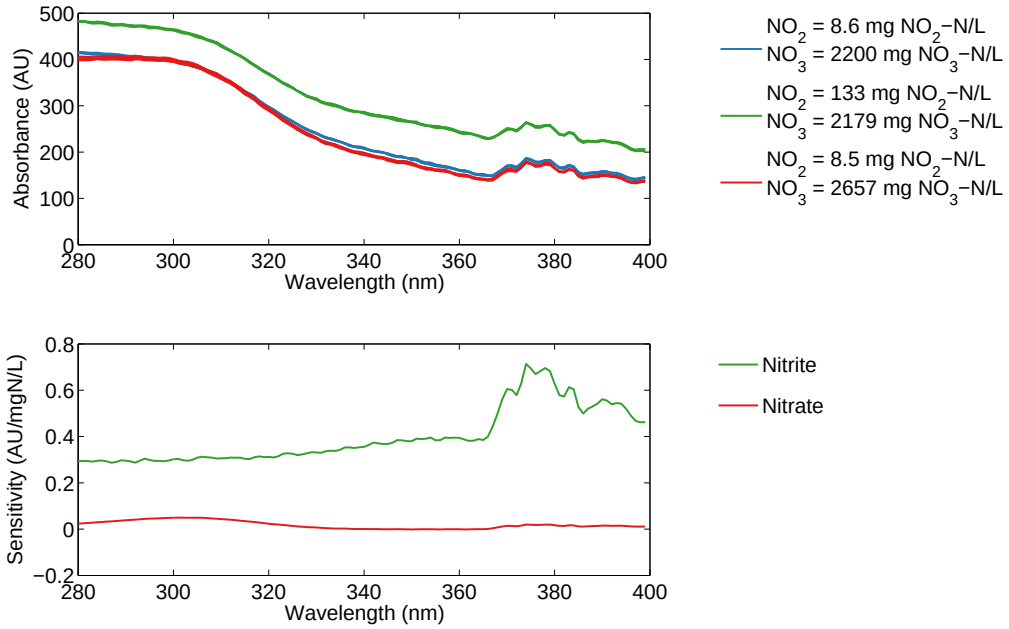


Figure 4: Close-up of the spectrum and the sensitivity for the UV-Vis sensor with 0.5 mm path.

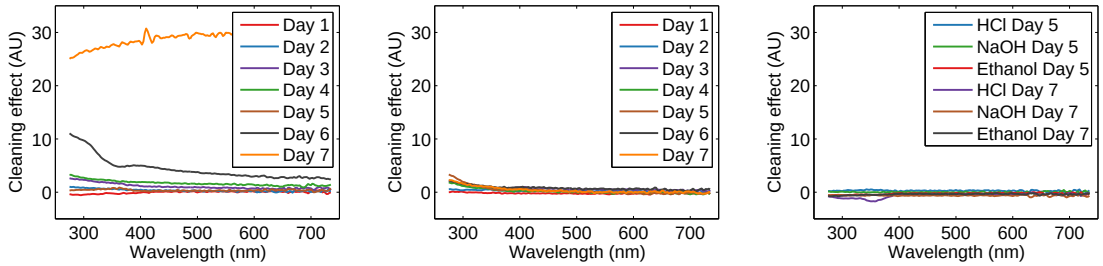
Measured in water, the sensor with 0.5 mm measurement path did not show saturation effects if only spiked with nitrite while the other two sensors did show saturation effects for the primary peaks. With the high nitrate concentrations, all three sensors show saturation effects for wavelengths below 240 nm both in nanopure water and in urine. With the added background due to urine, the secondary peaks are still not affected by saturation effects even for the sensor with the longest measurement path of 5 mm which is most prone to saturation. The measured absorbances in water for all three sensors and for urine for the two additional sensors are shown in annex B.2. Since the secondary peaks are around 50 nm apart from each other, a resolution of 2.5 nm seems to be sufficient to distinguish the peaks.

3.2 Analysing long-term influences from nitrified urine on absorbance measurements

Figure 5 depicts the measured cleaning effects for the different cleaning steps and for the different days on which experiments were conducted. The cleaning effect is specified as the mean difference between the measured absorbance before and after the respective cleaning step. The effect of cleaning the sensor with a spray bottle with water is the largest. The second cleaning step with a tissue has an additional cleaning effect for the low wavelengths but not for the ones higher than around 350 nm. Additional cleaning steps were applied for the first time after three weeks of exposure to the urine and they show no detectable effect. None of the three steps, cleaning with hydrochloric acid, with sodium hydroxide and with ethanol showed an effect. The uncertainty of the measurement for these cases is larger than the mean cleaning effect, so it can be stated that no effect is detectable within three weeks.

The cleaning effects with an uncertainty band are shown in annex, section B.3.

The cleaning effect of water is a lot larger for the last two days with up to 27 AU caused by biofilm at 355 nm. The effect was in the same range for all the first five days with a cleaning effect of around 2 AU at the secondary nitrite peak. On day 7, a counter-intuitive, negative cleaning effect is observed for hydrochloric acid. The uncertainty band shows a high uncertainty for this effect, so it is probably caused by an outlier and has no physical meaning. These experiments show that there is a detectable biofilm formation but no detectable effect of scaling within three weeks on the sensor window.



(a) Water (b) Tissue (c) Additional

Figure 5: Effect of cleaning the UV-Vis sensor for the different experimental days.

3.3 Calibration of the UV-Vis sensor

3.3.1 Model with data from cleaned sensor tested on data from the same day

Overall prediction performance As a first step, for every day with measurements, a PCR model is built with data obtained with the cleaned sensor and then tested on a test data set obtained on the same day but with the sensor before cleaning. The residuals between the predicted nitrite concentrations and the measured ones are shown in figure 6. The box plot shows the median; the box contains the range between the first and the third quartile and the whiskers include around three times the standard deviation (MathWorks, 2013). It can be seen that for all the days of experimentation, the model does predict nitrite concentrations with an error of less than 20 mg NO_2^--N/L for every case. For some days though, a much higher accuracy and also precision is reached than for others. For the first two days, the predictions are too high while for the other five days the nitrite concentrations are largely underestimated. The worst performance is achieved for the days 1 and 2 with a median residual of 7 mg NO_2^--N/L. These are also the days for which the model is least precise in the nitrite prediction.

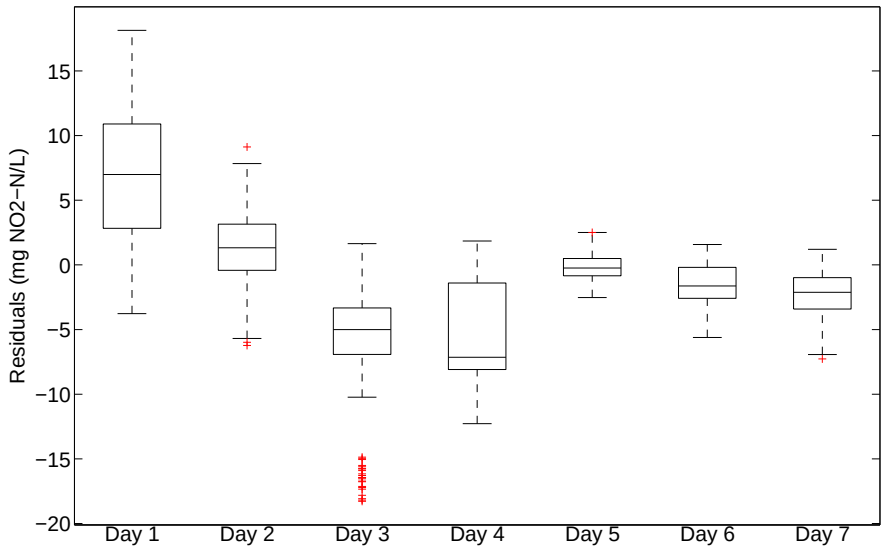
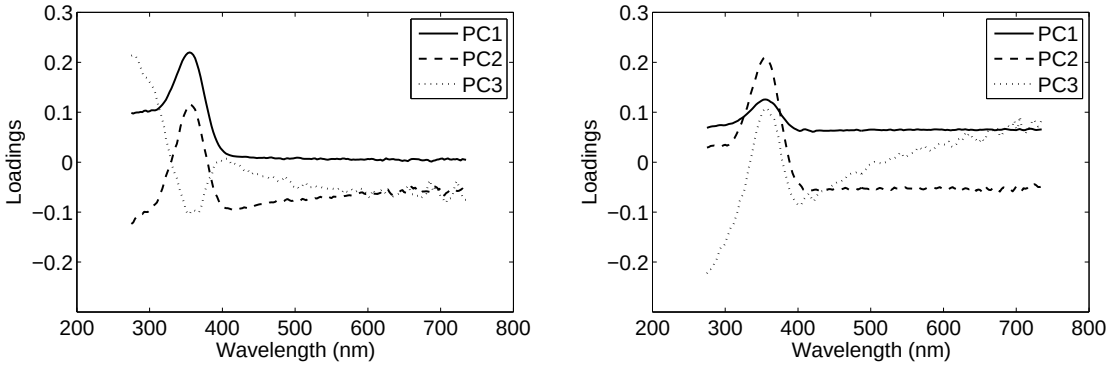


Figure 6: Prediction error for nitrite. The model is built with the cleaned sensor on a day and tested with data from the same day from the sensor before cleaning.

The principal components obtained for the different days do not always look the same. Figure 7 gives two examples which show the most typical results observed for all seven models. The first principal component from day 2 looks very similar to the absorption curve of nitrite with a peak around 355 nm, a sensitivity for the lower wavelengths and no sensitivity in the visible range as observed in figure 3 and found in literature (Spinelli *et al.* , 2007). The second component looks similar but with negative contributions of the wavelengths below and above the nitrite peak. It is assumed that these wavelengths can be related to organics and the turbidity, respectively (compare with Rieger *et al.* (2004)). The third component has negative contributions for the wavelengths at which nitrite absorbs. A possible explanation could be, that the model is forced to account for non-linear effects. The first component obtained for the data from day 3 is mainly related to an overall shift in the spectrum. Nitrite is only the second most important variation on this day, which can be seen in the second principal component. The third component behaves similar to the second on day 2 with positive contributions of nitrite and negative ones for the low and the high wavelengths which are possibly related to organics and turbidity. The principal components obtained for the five other days are shown in annex, section B.4.1. The components of higher numbers than three could not be interpreted unequivocally.



(a) Day 2 (b) Day 3

Figure 7: First three principal components for two example models.

Analysis of the number of selected principal components Three principal components are determined as the best number of components for four out of the seven days. For day 1, the automatic procedure chooses only two components. For day 5 and day 7 a high number of components is selected with eight and 14 principal components respectively. For these three days, a visual, subjective analysis of the validation error would decide for three principal components as well. Figure 8 shows the visual analysis for the example of day 7. The minimum error in the mean is achieved with 24 components (red circle). The smallest number which leads to an error which lies one standard deviation of the error of the 24^{th} component is with 14 components (green circle), the automatic determination chooses that to be the best number. A visual, subjective consideration suggests that for the first three components, the error decreases a lot and then stays more or less stable. Because of this, three components are considered as the best number of components (blue circle).

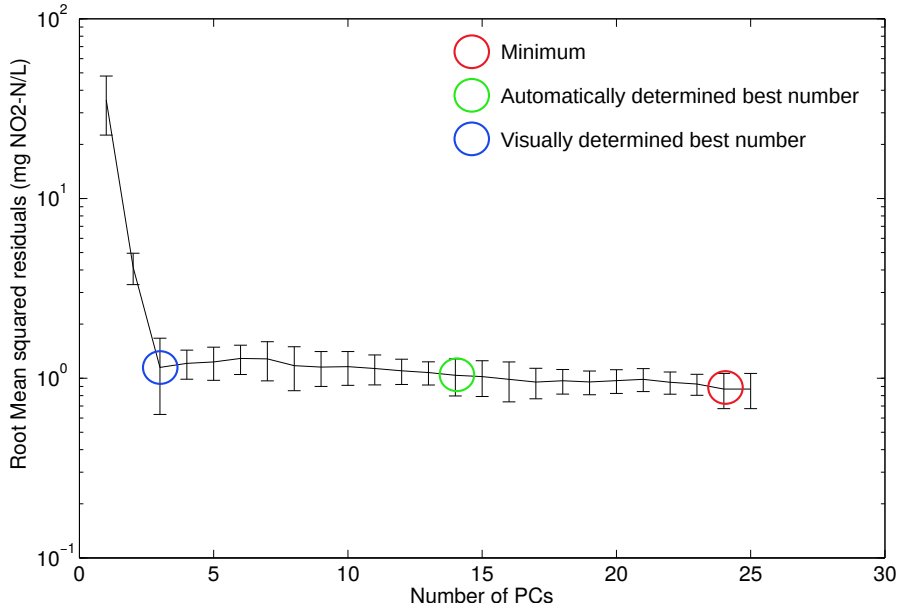
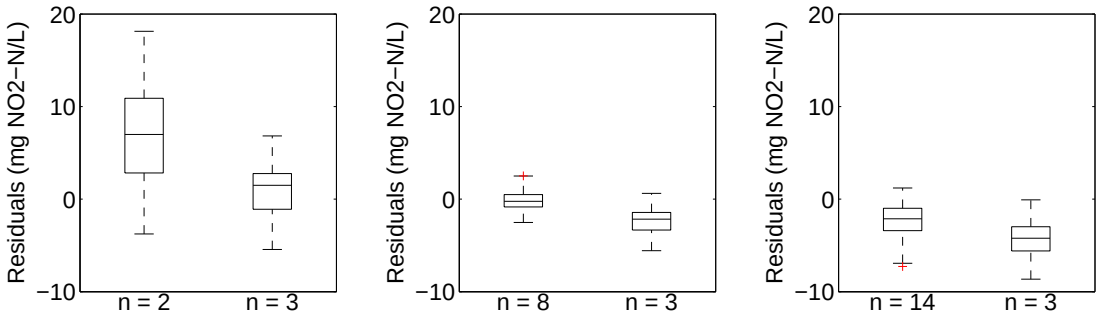


Figure 8: Error for the leave-one-out cross validation on the training data for the model on day 7.

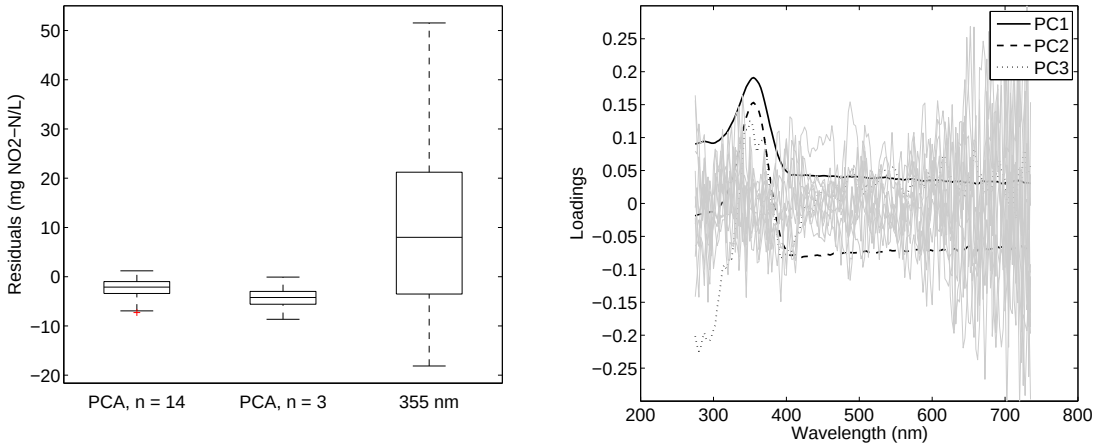
In figure 9 the prediction error for the different amounts of components are compared. For day 1, the additional component brings an increase both in accuracy and precision. The median residual decreases from 7 mg NO_2^--N/L to 1.5 mg NO_2^--N/L. For the other two days, the reduction by five respectively eleven principal components leads to a slightly worse performance of the model. The median error worsens from -0.2 mg NO_2^--N/L to -2 mg NO_2^--N/L for day 5 and from -2 to -4 mg NO_2^--N/L for day 7.



(a) Day 1 (b) Day 5 (c) Day 7

Figure 9: Prediction error on test data for nitrite for the three experiments where the automatic determination of the number of PCs does not match the subjective determination by the author.

Detailed analysis of the model for day 7 To give a sense for the quantitative performance of the resulting model, an example shows, how big the error would be for a very simple model on day 7. The simple model only takes one wavelength, 355 nm, into account and makes a linear regression with this information and the known nitrite concentrations. Already the variance in the absorbance measurement at 355 nm is expected to lead to a prediction uncertainty of around $\pm 12\,\mathrm{mg\,NO_2^-}$-N/L. At this day, the biofilm added an absorbance of around 5 AU at 355 nm which relates to an error in the nitrite prediction of around $35\,\mathrm{mg\,NO_2^-}$-N/L. These numbers explain the big prediction error we get with the simple model compared to both PCA model with 3 and 14 components (figure 10).

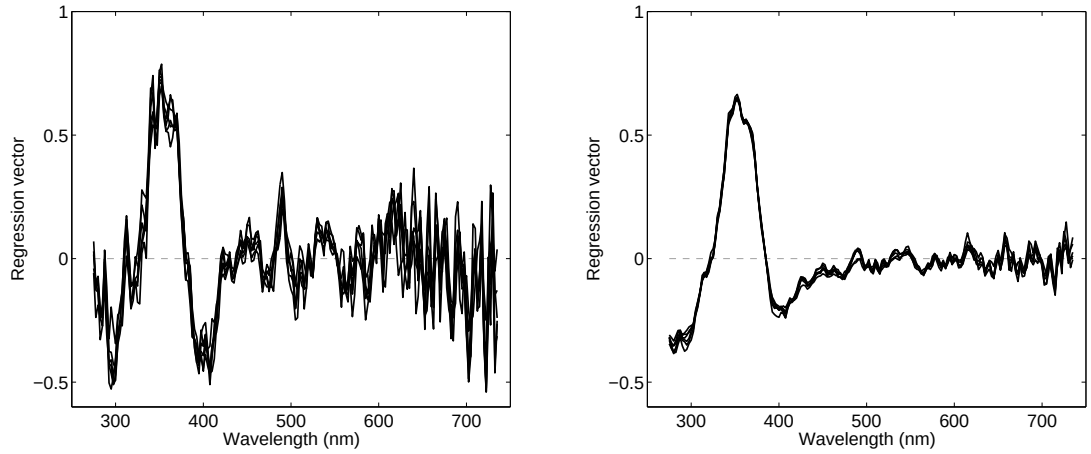


(a) Residuals

(b) Principal components

Figure 10: Residuals obtained with the PCA models and with a simple single wavelength model at 355 nm for the test data. For the PCA model the components are shown. The grey lines show the components of higher number than 3. Both with data measured on day 7.

Figure 11 shows the regression vector for the model built with the data from the cleaned sensor on day 7. The regression vector shows how the different wavelengths influence the nitrite estimation. Once only the first three components are considered and once 14. For the regression vector with only three components, a clear contribution of the wavelengths for the secondary peak for nitrite around 355 nm can be recognised. These wavelengths are corrected with the wavelengths below and after the nitrite peak. This can be interpreted that effects of organics and turbidity are taken into account. If 14 principal components are taken into account, the regression vector looks much more undefined. A nitrite peak can still be recognised, but the contributions of the lower and higher wavelengths can not be interpreted unequivocally.



(a) n = 14 (b) n = 3

Figure 11: Regression vectors obtained on day 7. Once three components and once 14 components are taken for the PCR model.

3.3.2 Model transferability to other days

Over the days, the urine composition in the reactor changes and therefore the measured background absorbance changes. Figure 12 shows the mean measured absorbance at 355 nm for the different days and the varying nitrite concentrations. The nitrate concentration varied only between 2420 mg NO_3^--N/L and 2510 mg NO_3^--N/L during this period and is therefore assumed as constant. For the first two days, a much higher absorbance is measured. For these two days, instead of treated urine from the reactor, treated urine from the storage tank was used for the dilution series. For the days 3 to 7, treated urine was collected directly from the nitrification column which led to a much lower background. For these days, the overall spectrum decreases with time, having the lowest background contributions at the days 6 and 7 of experiments. During the period of the experiments, the measured absorbance varied more than 50 AU at 355 nm.

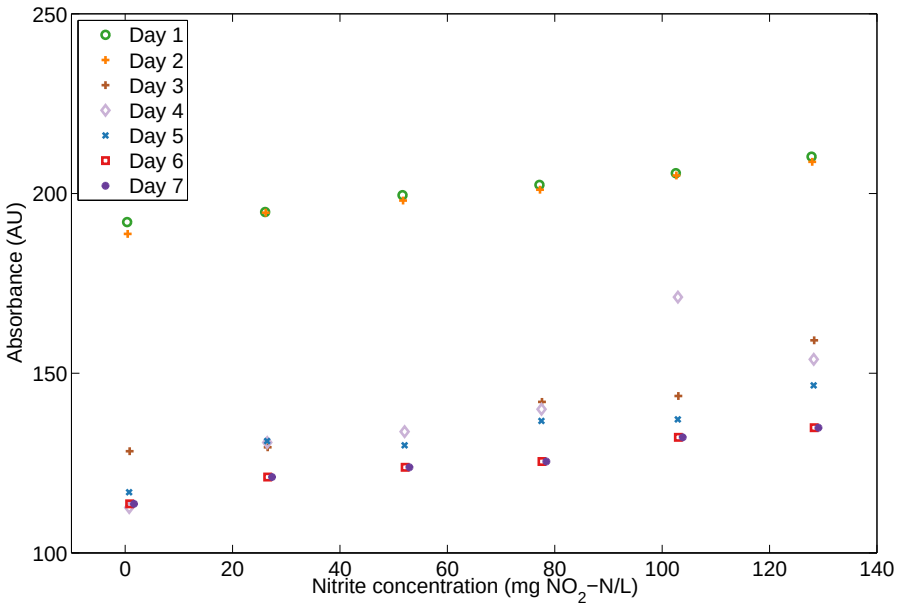


Figure 12: Mean absorbance at 355 nm measured with the mechanically cleaned sensor for the different days.

The results obtained with the model built with all the data from the first five days and tested on the last two days are presented in figure 13. It can be seen that the nitrite concentrations are over predicted for almost all the test cases. The linear regressions in the test data sets show, that the nitrite predictions are shifted compared to the ideal case (black line), but since the slope stays the same, no change in sensitivity occurred. The shift for test day 6 is with 9.9 mg NO_2^--N/L larger than for the second test day (day 7) with 4.6 mg NO_2^--N/L. For the entire test data set, no huge outlier results.

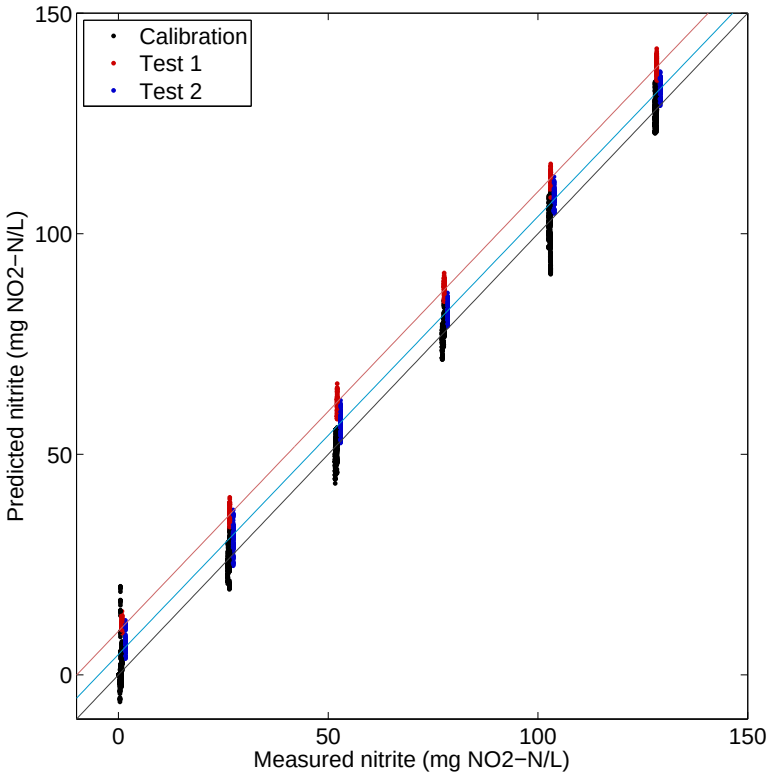
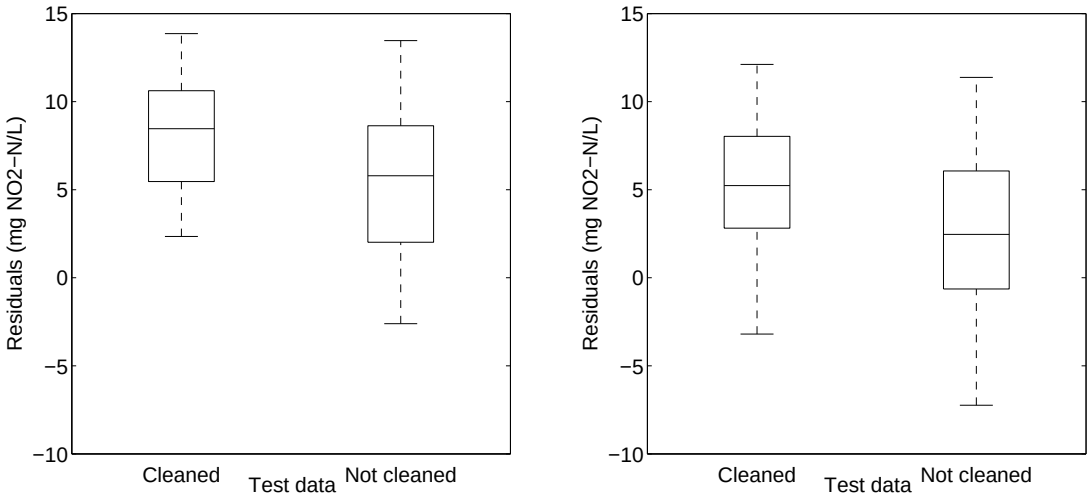


Figure 13: Predicted and measured nitrite concentrations. The model is built with data from day 1–5 and tested on day 6 (test 1) and day 7 (test 2).

Figure 14a shows the residuals between the measured and the predicted nitrite concentration for the last two days separately evaluated for the data obtained with the cleaned and the not cleaned sensor. For both test cases, the model shows a similar performance. For the data from the cleaned sensor, the accuracy is slightly worse but in return the precision is somewhat better.

A more challenging case is to only use data obtained with the cleaned sensor from the first five days to build the model and test it both with data from the cleaned and from the sensor with biofilm on it. Figure 14b shows the performance of that model. The model shows a similar performance for the data from the cleaned and from the not cleaned sensor. As for case (a), the accuracy is slightly worse with the cleaned sensor but a better precision is achieved.

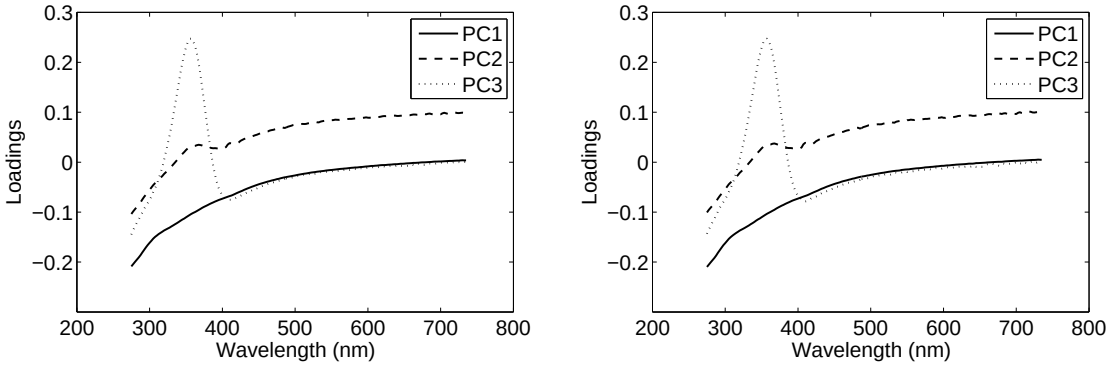


(a) Model with cleaned and not cleaned sensor (b) Model with cleaned sensor

Figure 14: Prediction error for nitrite. The model is built with the data from the first five experimental days and evaluated for the days 6 and 7.

A comparison of the two models, one with all the data from the first days for calibration-validation, the other only with the data from the cleaned sensor, shows that they achieve similar model performance. The model built only with data from the cleaned sensor shows a better accuracy but in return less precision. The median residual varies between 8.5 and 2.5 mg NO_2^--N/L. No case had an estimation error higher than 15 mg NO_2^--N/L.

For both cases, the dimension is reduced to three components which are depicted in figure 15. No matter if data only from the cleaned, or both from the cleaned and the not cleaned sensor is included in the model, the first three components look very similar. The first two components seem to correct for the different background caused by different urine compositions. The third component is related to the nitrite peak, with corrections at the lower and higher wavelengths which are assumed to be related to organics and turbidity.

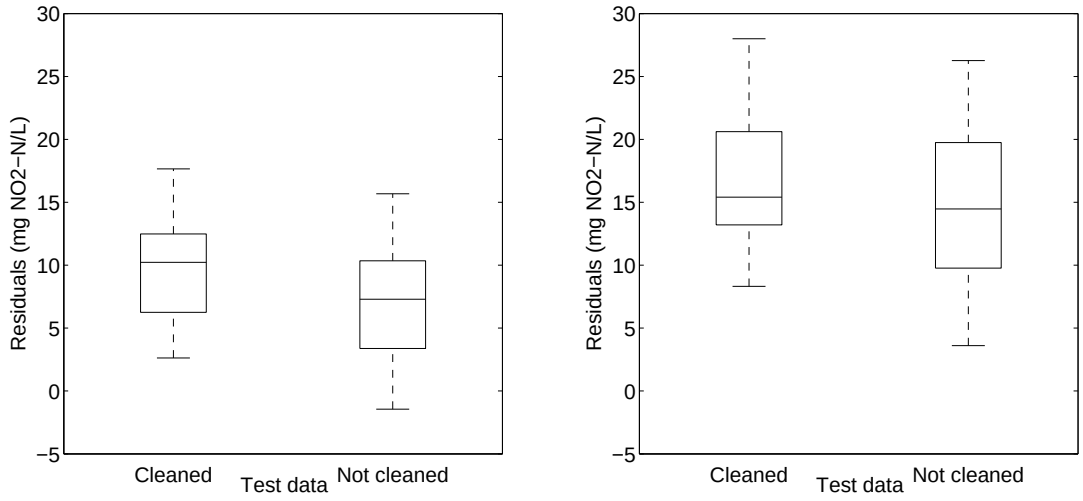


(a) Model with cleaned and not cleaned sensor (b) Model with cleaned sensor

Figure 15: Principal components for the models based on the first five days.

Next, a model is tested which only uses the data from day 3–5 to train the model, meaning that the data sets obtained with treated urine from the storage tank are not included. As before, once the model is built with all the data (figure 16a) and once with data from the cleaned sensor only (figure 16b). For each case, the performance is evaluated on the days 6 and 7 separately for the data from the cleaned and the not cleaned sensor. For three days, the model with all the data leads to a better performance in the test data set with a median residual of 10 and 7 mg NO_2^--N/L, respectively. The model obtained by using cleaned sensor data only strongly overestimates the nitrite concentrations in the median by 15 mg NO_2^--N/L.

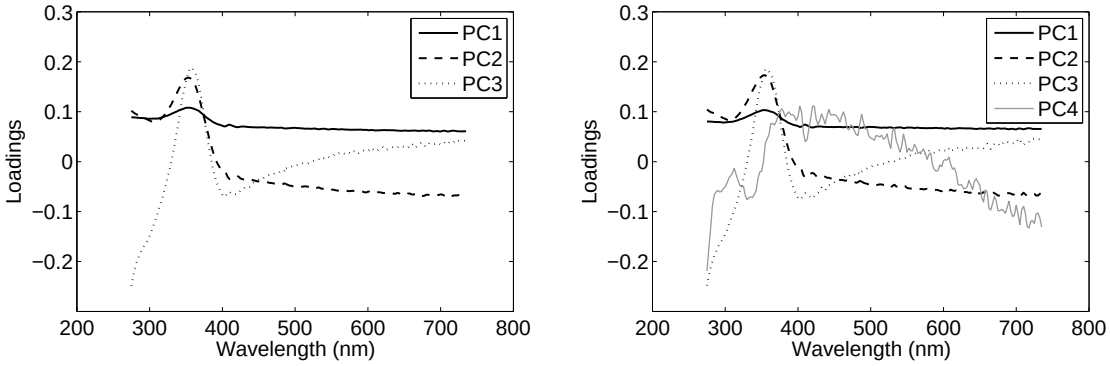
If all the data is used, the model including all five days and the one with only three days give a similar performance. If only the data from the cleaned data is considered, the model gets worse only using the days 3 to 5.



(a) Model with cleaned and not cleaned sensor (b) Model with cleaned sensor

Figure 16: Prediction error for nitrite. The model is built with the data from days 3–5 and tested for the days 6 and 7.

For the model with only three days of data, the best number of components is determined as 151 and 4 for the model with all the data and only considering the data from the cleaned sensor, respectively. In figure 17a only the first three components are depicted, in figure 17b all four. Again, for both cases, the first three components look very similar, but different compared to the ones obtained with the previous model (figure 15). The first component is related to an overall change in absorbance, e.g. caused by turbidity. The second and the third principal components are related to nitrite with different corrections which are interpreted as organics and turbidity.

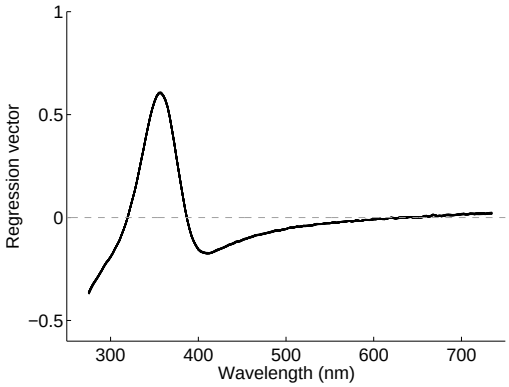


(a) Model with cleaned and not cleaned sensor (b) Model with cleaned sensor

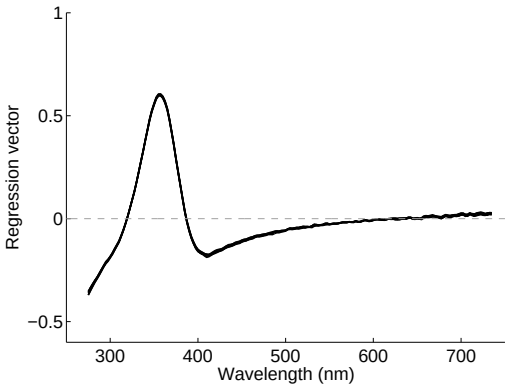
Figure 17: Principal components for the models based on three days.

The regression vectors for all four considered cases are depicted in figure 18. The ones for

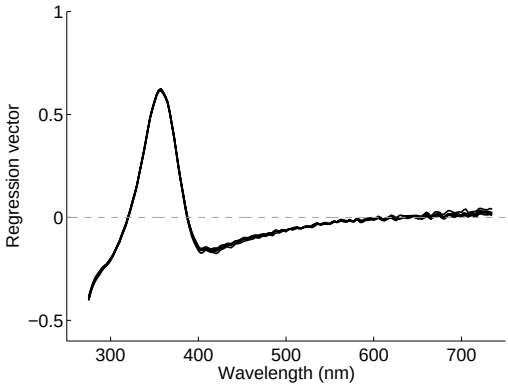
cases *(a)* to *(c)* look all very similar, but much more noisy for case *(d)* with the model for only three days with only the data from the cleaned sensor. All regression vectors show a clear nitrite peak around 355 nm. This peak is corrected with the wavelengths below 320 nm and with the ones above 400 nm which could be related to organics and turbidity, respectively. For case *(d)*, higher corrections for the lower wavelengths but less for the higher ones are made than for the three other cases which performed better.



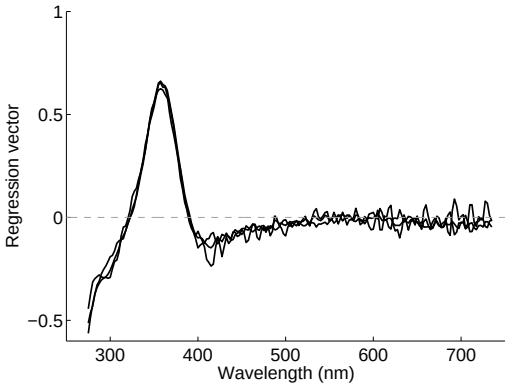
(a) Five days, all data



(b) Five days, cleaned



(c) Three days, all data



(d) Three days, cleaned

Figure 18: Regression vectors for the four different data sets for building the model.

4 Discussion

4.1 Selection of the sensor

The comparison of the three available spectrophotometers shows that all three of them show saturation effects for the primary absorbance peaks of nitrite and nitrate which are at 206 nm for nitrate respectively at 213 nm for nitrite (Spinelli *et al.* , 2007). None of them showed saturation for the secondary peaks which are at 302 nm for nitrate and 354 nm for nitrite (Spinelli *et al.* , 2007), if measured in nitrified urine. Based on these experiments, the UV-Vis sensor with a measurement path of 2 mm was chosen for the further experiments to be on the safe side concerning the saturation of the secondary peaks. The further experiments show that the measured absorbance at the secondary peaks varied more than 50 AU. Such an increase in absorbance would have led to saturation effects for the secondary nitrate peak for the 5 mm sensor since this sensor already was close to saturation in the experiment conducted in the lab (figure 25 in annex B.2). This can be of importance if in future studies nitrate is estimated as well or if the contributions of nitrite and nitrate should be differentiated. Since there have not been changes in nitrate, it cannot be affirmed that a resolution of 2.5 nm is sufficient to differentiate between nitrite and nitrate but nothing in the data conflicts with this assumption.

The use of the sensor with a 2 mm measurement path brings, compared to the sensor used for previous experiments with a 0.5 mm path, the advantage that it is assumed to be easier in maintenance. The larger measurement gap is likely less prone to blocking and is easier to clean.

The experiments show that the sensitivity for the secondary peaks is high enough to see the relevant changes in the nitrite concentration. The use of the secondary peaks for the nitrite estimation allows good predictions for high nitrite concentrations up to 130 mg NO_2^--N/L. Drolc & Vrtovšek (2010) for example defined a lower working range up to 13.7 mg NO_2^--N/L in their work which was done in wastewater.

4.2 Long-term effects influencing the nitrite measurements

The experiments about the long-term influences show that there is detectable biofilm growth on the sensor. For the last two experimental days, the effect of biofilm is much higher than for the previous five days. One possible explanation is, that the larger biofilm formation is due to the higher amount of the biofilm in the tubes. The tubes leading to the flow cell have not been cleaned over the whole period of exposure, meaning that biofilm could grow there undisturbed. This increasing biofilm in the tubes may has enhanced the biofilm formation on the sensor window.

Within three weeks no scaling on the sensor window is detected. But it is still possible that for a longer exposure time scaling would occur. This needs to be further investigated to define how often chemical cleaning is needed for a good sensor performance. Since mechanical cleaning is able to remove all detected disturbances, automatic cleaning as it is provided by the manufacturer seems to be sufficient to maintain the estimate quality. One option is to clean the sensor regularly with compressed air to remove the biofilm (s::can, 2011). If

automatic cleaning is sufficient, the maintenance of the sensor can be kept low.

An even larger effect on the absorbance measurements than from the biofilm comes from the change in the spectrum due to changes in urine compositions. The measured spectrum at 355 nm varied by up to 80 AU for constant nitrite concentrations. The highest absorbances are measured for the samples with nitrified urine from the storage tank. The samples from the storage tank are taken from the bottom of the tank. It is assumed that this urine contains a high amount of particles and probably also organics since the tank was not emptied for nearly half a year at the time when the samples were taken. This high amount of turbidity and organics could explain the high measured absorbances. The fact that the distiller had problems to treat this kind of urine supports the hypothesis that there was a high amount of particles in the stored urine at this time (B. Etter, personal communication, 23$^{\text{rd}}$ of February 2015).

At the beginning of the exposure, a nitrite accumulation occurred in the urine nitrification reactor with a maximum measured nitrite concentration of 168.5 mg NO_2^--N/L on the 29$^{\text{th}}$ of January. After this, an increased spectrum is measured which cannot be explained by increased nitrite concentrations. Santos (2014) observed high saturation effects for the samples with high nitrite concentrations due to accumulation as well, which leads to the assumption that it was not a unique coincidence of elevated nitrite concentrations in the sampled urine and the high overall absorbance measured. One possible explanation is that stress due to high nitrite concentrations leads to microbial processes like lysis or that the bacteria consume less organics, causing the increased absorbance measurements.

The long residence time in the nitrification reactor of more than 25 days (inflow was at maximum 0.2 L/h during the experiments) explains why the measured overall absorbance still decreased until the end of the four week period of experiments. For the measurements between the steps of the cleaning sequence in the solution with nitrite and nitrate no shift in the spectrum over time is observed. This supports the perception that the observed shift is not caused by the sensor itself. Such a shift by the sensor should be corrected by an internal light beam.

4.3 Model test and model calibration with data from the same day

The PCR model built with the data measured with the cleaned sensor is able to estimate the nitrite concentrations based on the absorbances measured with the sensor with biofilm on it with errors smaller than 20 mg NO_2^--N/L in every case. Results with such a high accuracy are only possible because the PCR-model is able to correct for the effects the biofilm has on the measured spectrum. In the training data set, the changing nitrite concentration is not the only variation in the measured absorbance which is observed. Additional variation probably comes from fluctuations in the turbidity and the organics and due to particles in the measured urine. It seems that the effect of these variations on the measured absorbance is similar to the effect the biofilm has on the measured absorbance and therefore the model is able to correct for the effects caused by the biofilm without having this effect in the training data set. The best performance is reached for the days where already in the training dataset a high variation occurred. For example, on day 6, the absorbance measurement at the nitrite peak was higher for a concentration of 103 mg NO_2^--N/L than for 128 mg NO_2^--N/L. For this

day the prediction error is for every case below 10 mg NO_2^--N/L.

For three models, the automatic determination of the number of chosen principal components does not agree with the subjective, visual determination. For day 1, the additional third component significantly increased the nitrite estimation performance in the test data set. In this case, the third component was not very important for the training data set but for the transferability of the model to the data obtained with the not cleaned sensor. For the other two cases, where a subjective identification of the best number would go for a smaller number of components, it can be stated that the automatic determination is not able to find the simplest model which gives reasonable results. The models with only three components only give slightly worse results with the number of components more than halved. For such small training data sets, another way to define the best number of components could be more stable.

4.4 Extrapolation in time

In general, it can be stated that the PCR model built with data obtained on some days is able to estimate the nitrite concentration on another day with a different urine composition. But the nitrite concentrations for the test data set (day 6 and 7) are overestimated. This error in the estimation is probably caused by the changed urine composition which led to a changed overall spectrum. A possible explanation for this over estimation could be an overcorrection of the nitrite contributions of the absorbance for organics and turbidity. This hypothesis of an overcompensation could also explain why the test data set from the sensor with biofilm delivered slightly better results than the test data set from the cleaned sensor. Meaning that the corrections for the nitrite estimation obtained for the urine with high turbidity and organics on the first days better reproduces the data where the biofilm contributes similar effects like observed in the urine of the training data set.

The fact that the estimated nitrite concentrations are only shifted compared to the real values, but no change in sensitivity occurred, facilitates an automated detection of a nitrite accumulation. If the estimated concentrations are only shifted compared to the real value, the change in concentration can be used to detect an accumulation even if the estimated concentrations do not correctly reproduce the reality. But even without such considerations, solely considering the estimated values, the obtained accuracy and precision is high enough to reliably detect a nitrite accumulation in the urine nitrification system. For the model built with data from the first five experimental days, the estimation error is always below 20 mg NO_2^--N/L. If the threshold for a warning signal for nitrite accumulation would be chosen as 30 mg NO_2^--N/L, in the worst cases the real value would be 10 mg NO_2^--N/L or 50 mg NO_2^--N/L. Both, the lower and the upper limit are in the range of nitrite concentration, where the reactor is definitely not in steady state any more and where the introduction of measures e.g. switching off the inflow pump, can prevent permanent failure of the reactor.

For the model which uses day 1–5 for calibration and validation, the performance is around the same, no matter if the training data set contains data from the sensor with biofilm on it or not. This finding brings the practical benefit that no data from a sensor with biofilm on it is necessary to calibrate a model. Nevertheless, the model is able to estimate both, nitrite concentration estimated with absorbance measurements from a cleaned sensor and also from

a sensor with biofilm on it.

If only day 3–5 are used to build the model, the results on the test data set on the days 6 and 7 are much worse if only data from the cleaned sensor is considered compared to the case considering both data from the cleaned and from the not cleaned sensor. For the days 3–5, the change in urine composition is less pronounced than if the data from the first two days is included as well. In this case, it seems to be necessary to take data obtained with the sensor with biofilm into account to have enough variation, besides the variation in the absorbance data due to nitrite, in the measurements for the training data set. This is not fulfilled if only the data from the cleaned sensor for these three days is considered. Another problem could also be that this training data set just did not contain enough measurements to reliably predict nitrite concentrations. This corresponds with the regression vector which looks quite noisy for this case.

4.5 Future work

It would be interesting to investigate if all measured wavelengths are necessary to estimate nitrite or if a model with only a few wavelengths could also deliver results accurate enough to detect nitrite accumulation. If only a few wavelengths are necessary, the option of using simpler sensors, which only measure at a few wavelengths, arises.

The urine samples in this work all origin from the same reactor, in future research it should be investigated if a model obtained with data from one reactor can be used for the estimation in another reactor under changed conditions, e.g. of pH and temperature. For further improving the prediction accuracy, it is important to consider as many different background spectra as possible in the training data set. A future challenge will be to sample as many different urine compositions from the nitrification reactor as possible to capture these background effects.

It is hypothesised in this work that automatic mechanical cleaning is sufficient to maintain the nitrite prediction quality. This hypothesis needs to be tested with an appropriate experiment. In this work, all the considered measurements are taken off-line in a measurement container. A next step is to use the continuous measurements obtained in the flow cell. These measurements contain a lot of outliers, probably caused by air bubbles. To get a good continuous prediction of the nitrite concentration, an automatic detection algorithm for outliers needs to be developed.

5 Conclusions

In this master thesis, the feasibility of UV-Vis spectrophotometry for nitrite estimation is investigated. First, it is evaluated which sensor configuration is most suitable for the experiments, using the additional information about nitrite at wavelengths higher than the primary absorbance peaks. With this sensor, the long-term effects of exposure to urine are examined. It is analysed if there is detectable biofilm formation and scaling occurring on the sensor window. Based on a principal component regression (PCR), it is studied if the contributions of biofilm, scaling and nitrite to the spectrum are differentiable and if the model can account for these effects.

All three analysed spectrophotometers show saturation effects for the wavelengths with the primary absorbance peaks of nitrite and nitrate if measured in urine. Two clear secondary peaks at around 300 nm for nitrate and at around 355 nm for nitrite, which do not show saturation for any of the sensors, are observed. Based on this preliminary experiments, the UV-Vis spectrophotometer with an optical measurement path of 2 mm and a resolution of 2.5 nm was chosen for the experiments about the long-term effects. The nitrite concentrations are estimated based on the secondary absorbance peaks.

The experiments about the long-term effects of the nitrified urine on the absorbance measurements show, that there is detectable biofilm formation on the sensor window. Within three weeks of exposure, scaling is not detected.

The PCR model is able to estimate nitrite concentrations based on the absorbance measurements with a prediction accuracy between 5–15 mg NO_2^--N/L if extrapolated in time and a prediction accuracy better than 5 mg NO_2^--N/L if tested on the same day. The extrapolation from the model in time leads to an over estimation of the nitrite concentrations, so the model is not able to correct for all the effects caused by a change in the urine composition. Yet, the estimation performance is still sufficient to detect a nitrite accumulation in the nitrification reactor. With the model, the measurements are corrected for the disturbances due to the biofilm formation on the sensor window without data obtained with the sensor with biofilm is included in the training data set.

Using the information from the secondary nitrite absorbance peak, a PCR model is able to estimate the nitrite concentrations in nitrified urine with an error smaller than 20 mg NO_2^--N/L for all the measured absorbances. Therefore, it can be stated that the achieved accuracy and precision are sufficient, even for measurements from the sensor with biofilm on it, to reliably detect a nitrite accumulation in the urine nitrification reactor.

References

Bouvier, J., Bekri, M., & Mazouni, D. 2008. On-Line Monitoring of Nitrate and Nitrite by UV Spectrophotometry in a SBR Process Used for the Treatment of Industrial Wastewaters. *International Journal of Chemical Reactor Engineering*, **6**(1), 1–19.

Burgess, C. 2007. The Basics of Spectrophotometric Measurement. *In:* Thomas, O., & Burgess, C. (eds), *UV-Visible Spectrophotometry of Water and Wastewater*. Amsterdam: Elsevier.

Drolc, Andreja, & Vrtovšek, Janez. 2010. Nitrate and nitrite nitrogen determination in waste water using on-line UV spectrometric method. *Bioresource Technology*, **101**(11), 4228 – 4233.

Etter, B., Hug, A., & Udert, K. 2013. Total Nutrient Recovery from Urine – Operation of a Pilot-Scale Nitrification Reactor. WEF/IWA International Conference on Nutrient Removal and Recovery 2013. 28-31 July, Vancouver, Canada.

Foley, Jeffrey, de Haas, David, Yuan, Zhiguo, & Lant, Paul. 2010. Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. *Water Research*, **44**(3), 831 – 844.

Gruber, G., Bertrand-Krajewski, J., De Beneditis, J., Hochedlinger, M., & Lettl, W. 2005. Practical aspects, experiences and strategies by using UV/VIS sensors for long-term sewer monitoring. 10th international conference on urban drainage. 21–26 August, Copenhagen, Denkmark.

Guest, Jeremy S., Skerlos, Steven J., Barnard, James L., Beck, M. Bruce, Daigger, Glen T., Hilger, Helene, Jackson, Steven J., Karvazy, Karen, Kelly, Linda, Macpherson, Linda, Mihelcic, James R., Pramanik, Amit, Raskin, Lutgarde, Van Loosdrecht, Mark C. M., Yeh, Daniel, & Love*, Nancy G. 2009. A New Planning and Design Paradigm to Achieve Sustainable Resource Recovery from Wastewater1. *Environmental Science & Technology*, **43**(16), 6126–6130. PMID: 19746702.

Gujer, W. 2007. *Siedlungswasserwirtschaft*. Springer-Verlag.

Hastie, T., Tibshirani, R., & Friedman, J. 2009. *The Elements of Statistical Learning*. Springer-Verlag.

Jolliffe, I.T. 2002. *Principal Component Analysis*. Springer-Verlag.

Larsen, T.A., & Gujer, W. 2001. Waste design and source control lead to flexibility in wastewater management. *Water Science & Technology*, **43**(5), 309–318.

Lemaire, R., Chauzy, J., Veuillet, F., Dimassimo, R., & Sorensen, K. 2011. Advanced control system to reduce N2O emission and improve performance of an SBR treating N-rich effluent via nitrite pathway. *Proceedings of the Water Environment Federation*, **2011**(9), 6480–6493.

Liu, Meiling, Wang, Linping, Meng, Yue, Chen, Qiong, Li, Haitao, Zhang, Youyu, & Yao, Shouzhuo. 2014. (4-Ferrocenylethyne) Phenylamine Functionalized Graphene Oxide Modified Electrode for Sensitive Nitrite Sensing. *Electrochimica Acta*, **116**(0), 504 – 511.

Liu, Yang, Zhou, Jie, Gong, Jin, Wu, Wei-Ping, Bao, Ning, Pan, Zhong-Qin, & Gu, Hai-Ying. 2013. The investigation of electrochemical properties for Fe3O4@Pt nanocomposites and an enhancement sensing for nitrite. *Electrochimica Acta*, **111**(0), 876 – 887.

MathWorks. 2013. *Matlab documentation, Box plot*.

Maurer, M., Pronk, W., & Larsen, T.A. 2006. Treatment processes for source-separated urine. *Water Research*, **40**(17), 3151 – 3166.

Mašić, A., & Villez, K. 2014. Model-based observers for monitoring of biological nitrification of urine in decentralized treatment. IWA International Conference Ecotechnologies for Wastewater Treatment. 23–25 June, Verona, Italy.

Nopco, Nopco.net, Advanced chemical solutions for the pulp and paper industry. 2015. *Removing dirt, grime and deposits*. http://www.nopco.net/Solutions/Cleaning-agents/, accessed 20 March, 2015.

Oke, O.L. 1966. Nitrite Toxicity to Plants. *Nature*.

Palanisamy, Selvakumar, Karuppiah, Chelladurai, Chen, Shen-Ming, & Periakaruppan, Prakash. 2014. Highly sensitive and selective amperometric nitrite sensor based on electrochemically activated graphite modified screen printed carbon electrode. *Journal of Electroanalytical Chemistry*, **727**(0), 34 – 38.

Rieger, L., Langergraber, G., Thomann, M., Fleischmann, N., & Siegrist, H. 2004. Spectral in-situ analysis of NO2, NO3, COD, DOC and TSS in the effluent of a WWTP. *Water Science & Technology*, **50**(11), 143–152.

Rieger, L., Langergraber, G., Kaelin, D., Siegrist, H., & Vanrolleghem, P.A. 2008. Long-term evaluation of a spectral sensor nitrite and nitrate. *Water Science & Technology*, **57**(10), 1563–1569.

Santos, Ana. 2014. *Evaluation of UV spectrophotometry for estimation of nitrite and nitrate in nitrified urine*. M.Phil. thesis, Universidade Nova de Lisboa, Portugal.

s::can, s:can Messtechnik GmbH. 2011. *Spectrometer Probe*. 2 edn. scan Messtechnik GmbH, Vienna, Austria.

Sedlak, R. 1991. *Phosphorus and nitrogen removal from municipal wastewater : principles and practice*. Soap and Detergent Assoc.

Spinelli, S., Gonzalez, C., & Thomas, O. 2007. UV Spectra Library. *In:* Thomas, O., & Burgess, C. (eds), *UV-Visible Spectrophotometry of Water and Wastewater*. Amsterdam: Elsevier.

Udert, K.M., & Jenni, S. 2013. Biological nitrogen conversion processes. *In:* Larsen, T.A., Udert, K.M., & Lienert, J. (eds), *Source Separation and Decentralization for Wastewater Management*. London: IWA Publishing.

Udert, K.M., & Wächter, M. 2012. Complete nutrient recovery from source-separated urine by nitrification and distillation. *Water Research*, **46**(2), 453 – 464.

Van den Broeke, J., Langergraber, G., & Weingartner, A. 2006. On-line and in-situ UV/vis spectroscopy for multi-parameter measurements: a brief review. *Spectroscopy Europe*, **18**(4).

WHO, World Health Organisation. 2011. *Nitrate and nitrite in drinking-water*.

Wunderlin, P., Siegrist, H, & Joss, A. 2013. Online N2O Measurement: The Next Standard for Controlling Biological Ammonia Oxidation? *Environmental Science & Technology*, **47**(17), 9567–9568.

A Experiment protocols

A.1 Analysing alternative UV and UV-Vis sensor configurations in nanopure water

Required products:

- Nanopure water
- Ethanol, HCl (7 %), NaOH (20 %) for cleaning
- Stock solution nitrite (goal: 125 mg NO_2^--N/L, 12.875 g NO_2-N/L solution) (See protocol A.1.1)
- Stock solution nitrate (goal: 2500 mg NO_3^--N/L, 2575 mg NO_3-N/L solution) (See protocol A.1.1)

Required hardware:

- Narrow measurement container (1000 mL)
- Standardized 500 mL flask
- Magnet stirrer with magnet
- Pipette (5 mL)
- s::can spectro::lyser UV spectrophotometer, 0.5 mm path length
- s::can spectro::lyser UV-Vis spectrophotometer, 2 mm path length
- s::can spectro::lyser UV-Vis spectrophotometer, 5 mm path length

Plan: Test three different UV /UV-Vis sensors under the same idealized conditions for estimating nitrite and nitrate.

Steps:

1. Prepare the UV sensors for measurement. If necessary: sprinkle water on the clamp holders that are holding the sensor to facilitate removal. Carefully and slowly open the clamps and remove the sensor.
2. Rinse the sensor with nanopure water, then with a small amount of ethanol, then with water again. Wet a tissue with HCl, fold it and clean the sensor gap thoroughly. Repeat with NaOH. Rinse with Nanopure water several times.
3. Measure 500 mL Nanopure water with the standardized flask and add it to the measurement container.

4. Add a stirring magnet and place the container on a magnetic stirrer. Let it stir at 1000 rpm.

5. Switch stirrer off to avoid bubbles.

6. Measure the absorbance with the three sensors.

    a) Insert the UV probe spectro::lyser with 0.5 mm measurement path until a volume on the measurement container reads about 900 mL.

        i) Start the stirrer again (1000 rpm).
        ii) Note the time and facts in the corresponding logbook.
        iii) Ensure that at least 5 measurements are recorded (5 minutes).
        iv) Switch stirrer off.
        v) Remove the sensor.
        vi) Clean the sensor with nanopure water, ethanol, HCl and NaOH and ensure that it is ready for use.

    b) Repeat step (a) with the cleaned (step 2) UV-Vis probe with 2 mm measurement path.

    c) Repeat step (a) with the cleaned UV-Vis probe with 5 mm measurement path.

7. Empty and clean the measurement container by rinsing thoroughly with water.

8. Repeat steps 3-7 with a solution containing 510 mL Nanopure water and 5 mL of nitrite stock solution. Make sure to wait with the measurements until the solution is completely mixed.

9. Repeat steps 3-7 with a solution containing 500 mL of nitrate stock solution and 15 mL Nanopure water. Make sure to wait with the measurements until the solution is completely mixed.

10. Repeat steps 3-7 with a solution containing 500 mL of nitrate stock solution, 10 mL Nanopure water and 5 mL nitrite stock solution. Make sure to wait with the measurements until the solution is completely mixed.

11. Fill the container with distilled water, submerge the spectro::lyser probe again and leave it be.

12. Make a PDF scan of the lab journal and update the digitized log file.

A.1.1 Preparation of stock solutions with nitrite and nitrate

Required products:

- Nanopure water
- $NaNO_2$ powder (Merck KGaA, S5674485 338)
- $NaNO_3$ powder (Merck KGaA, A0610237 342)

Required hardware:

- Funnel
- 100 mL flask
- 500 mL flask
- Magnet stirrer with magnet
- Pipette (10,5,1,0.2,0.1 mL)

Plan: Make stock solution for analysing three different UV-Vis sensor configurations.

Steps:

1. Nitrite stock solution (goal: 515 mL 125 mg NO_2^--N/L)
   a) Weigh 6.342 g of the $NaNO_2$ powder.
   b) Dissolve powder into water.
      i) Take 60 mL of Milipore water and add it to the standardized 100 mL flask.
      ii) Use a funnel to add the $NaNO_2$ powder to the container.
      iii) Flush the funnel with a small amount of water to collect all the powder remaining on the surface of the funnel.
      iv) Add stirring magnet to the flask.
      v) Place the flask onto a magnetic stirrer.
      vi) Switch the magnetic stirrer on and let it mix until all powder is dissolved.
      vii) Switch the magnetic stirrer off.
      viii) Remove the stirring magnet.
      ix) Fill the container with Milipore water until exactly 100 mL is reached Close the flask with a stopper, shake it slowly to mix the added water with the remaining solution.
   c) Take a sample to measure the nitrite concentration with a Hach-Lange cuvette test.
   d) Close the flask with a stopper.

e) Label the flask with your name and content description.

f) Store the flask in the fridge in B74.

g) Measure the nitrite sample with a Hach-Lange cuvette test (LCK 342)

   i Make a dilution of 1:5000 in two steps (1 mL of stock solution into a 50 mL flask from this solution 1 mL into a 100 mL flask).

   ii Follow the instructions on the product to measure the concentration.

h) Evaluate the quality of the solution (Expected concentration: 2.575 mg NO_2^--N/L). Repeat steps a-g if the obtained quality is insufficient.

2. Nitrate stock solution (goal: 515 mL 2500 mg NO_3^--N/L)

a) Weigh 7.813 g of the $NaNO_3$ powder.

b) Dissolve powder into water.

   i) Take 400 mL of Milipore water and add it to the standardized 500 mL flask.

   ii) Use a funnel to add the $NaNO_3$ powder to the container.

   iii) Flush the funnel with a small amount of water to collect all the powder remaining on the surface of the funnel.

   iv) Add stirring magnet to the flask.

   v) Place the flask onto a magnetic stirrer.

   vi) Switch the magnetic stirrer on and let it mix until all powder is dissolved.

   vii) Switch the magnetic stirrer off.

   viii) Remove the stirring magnet.

   ix) Fill the container with Milipore water until exactly 500 mL is reached Close the flask with a stopper, shake it slowly to mix the added water with the remaining solution.

c) Take a sample to measure the NO_3-N concentration with a Hach-Lange cuvette test.

d) Close the flask with a stopper.

e) Label the flask with your name and content description.

f) Store the flask in the fridge in B74.

g) Measure the nitrate sample with a Hach-Lange cuvette test (LCK 340)

   i Make a dilution of 1:100 (1 mL of stock solution into a 100 mL flask).

   ii Follow the instructions on the product to measure the concentration.

h) Evaluate the quality of the solution (Expected concentration: 25.75 mg NO_3^--N/L). Repeat steps a-g if the obtained quality is insufficient.

Calculations: Nitrite:

Desired concentration: 125 mg NO_2^--N/L

Amount of $NaNO_2$ for 100 mL stock solution (12.875 mg NO_2^--N/L):

$$M_{NaNO_2} = \frac{125\,\text{mgN/L} \cdot 515\,\text{mL}}{5\,\text{mL}} \cdot \frac{0.1\,\text{L}}{100\,\text{mL}} \cdot \frac{1\,\text{gN}}{1000\,\text{mgN}} \cdot \frac{68.995\,\text{gNaNO}_2\text{/mol}}{14.007\,\text{gN/mol}} \tag{A.1}$$

$$= 6.342\,\text{gNaNO}_2\text{/100 mL}$$

Nitrate:
Desired concentration: $2500\,\mathrm{mg\,NO_3^-}$-N/L
Amount of $NaNO_3$ for 500 mL stock solution ($2575\,\mathrm{mg\,NO_3^-}$-N/L):

$$\begin{aligned} M_{\mathrm{NaNO_3}} &= \frac{2500\,\mathrm{mgN/L} \cdot 515\,\mathrm{mL}}{500\,\mathrm{mL}} \cdot \frac{0.5\,\mathrm{L}}{500\,\mathrm{mL}} \cdot \frac{1\,\mathrm{gN}}{1000\,\mathrm{mgN}} \cdot \frac{84.994\,\mathrm{gNaNO_2/mol}}{14.007\,\mathrm{gN/mol}} \\ &= 7.813\,\mathrm{gNaNO_3}/500\,\mathrm{mL} \end{aligned} \tag{A.2}$$

A.2 Analysing the background spectrum of nitrified urine with three UV and UV-Vis sensors

Required products:

- 2 L treated urine
- Nanopure water
- Ethanol, HCl (7 %), NaOH (20 %) for cleaning
- Stock solution nitrite (goal: 125 mg NO_2^--N/L, 12.875 g NO_2-N/L solution) (See protocol A.1.1)
- Stock solution nitrate (goal: 500 mg NO_3^--N/L, 2.575 g NO_3-N/L solution) (See protocol A.1.1)
- Glass microfiber filter (Macherey-Nagel, MN GF-5, 0.4 μ m)

Required hardware:

- Narrow measurement container (1000 mL)
- Imhoff cone
- Standardized 500 mL flask
- Magnet stirrer with magnet
- Pipette (5 mL)
- s::can spectro::lyser UV spectrophotometer, 0.5 mm path length
- s::can spectro::lyser UV-Vis spectrophotometer, 2 mm path length
- s::can spectro::lyser UV-Vis spectrophotometer, 5 mm path length

Plan: Analyse the background spectrum of nitrified urine with three UV and UV-Vis sensor to see if there are saturation effects.

Steps:

1. Wastewater collection and preparation
    a) Take 2 L of treated urine from the tap on the nitrification reactor.
    b) Separate at least 1 litre of clear liquid from the sample as follows:
        i) Fill two 1 L Imhoff cones.
        ii) Let the biomass settle for 60 minutes.

iii) Collect the settled biomass by removing at least 150 mL from each cone by opening the bottom tap of the Imhoff cone. This liquid is wasted.
iv) Collect the remaining supernatant into a single container and ensure continuous mixing.
v) Repeat as necessary until at least 1 litre of bulk liquid is obtained.

2. Take a sample of the urine and measure nitrite and nitrate concentrations with Hach-Lange Cuvette tests.
   a) Filter the sample with a 0.4 µm glass microfiber filter to remove suspended particles.
   b) Nitrate: LCK 340 with a dilution of 1:100 (Expected concentration around 2000 mg NO_3^--N/L).
   c) Nitrite: LCK 342 without dilution (Expected concentration around 2 mg NO_2^--N/L).
3. Prepare the UV sensors for measurement. If necessary: sprinkle water on the clamp holders that are holding the sensor to facilitate removal. Carefully and slowly open the clamps and remove the sensor.
4. Rinse the sensor with nanopure water, then with a small amount of ethanol, then with water again. Wet a tissue with HCl, fold it and clean the sensor gap thoroughly. Repeat with NaOH. Rinse with nanopure water several times.
5. Measure 500 mL nanopure water with the standardized flask and add it to the measurement container.
6. Add a stirring magnet and place the container on a magnetic stirrer. Let it stir at 1000 rpm.
7. Switch stirrer off to avoid bubbles.
8. Measure the absorbance with the three sensors.
   a) Insert the UV probe spectro::lyser with the 0.5 mm measurement path until a volume on the measurement container reads about 900 mL.
      i) Start the stirrer again (1000 rpm).
      ii) Note the time and facts in the corresponding logbook.
      iii) Ensure that at least 5 measurements are recorded (5 minutes).
      iv) Switch stirrer off.
      v) Remove the sensor.
      vi) Clean the sensor with nanopure water, ethanol, HCl and NaOH and ensure that it is ready for use.
   b) Repeat step (a) with the cleaned (step 4) UV-Vis probe with 2 mm path.
   c) Repeat step (a) with the cleaned UV-Vis probe with 5 mm path.
9. Empty and clean the measurement container by rinsing thoroughly with water.

10. Repeat steps 5-8 with 515 mL of treated urine. Make sure to wait with the measurements until it is completely mixed.

11. Repeat steps 5-8 with 510 mL urine and 5 mL of the nitrite stock solution added. Make sure to wait with the measurements until the solution is completely mixed.

12. Repeat steps 5-8 with 505 mL urine and and 10 mL of the nitrate stock solution added. Make sure to wait with the measurements until the solution is completely mixed.

13. Repeat steps 5-8 with 500 mL urine and 10 mL of nitrate stock solution and 5 mL of nitrite stock solution added. Make sure to wait with the measurements until the solution is completely mixed.

14. Fill the container with distilled water, submerge the spectro::lyser probe again and leave it be.

15. Make a PDF scan of the lab journal and update the digitized log file.

A.3 Analysing long-term effects on the UV-Vis measurements

Required products:

- 1.5–2 L treated urine
- Nanopure water
- Ethanol, HCl (7 %), NaOH (20 %) for cleaning
- Stock solution nitrite (12.875 g NO_2-N/L) (See protocol A.1.1)
- Nitrite/nitrate stock solution (500 mg NO_2^--N/L, 500 mg NO_3^--N/L) (See protocol A.4)
- Glass microfiber filter (Macherey-Nagel, MN GF-5, 0.4 μm)
- Hach Lange cuvette test LCK 340 for measuring nitrate
- Hach Lange cuvette test LCK 341 for measuring nitrite

Required hardware:

- Narrow measurement container (1000 mL)
- Imhoff cones
- Standardized 500 mL flask
- Magnet stirrer with magnet
- Pipette (1 mL)
- s::can spectro::lyser UV-Vis spectrophotometer, 2 mm path length

Plan: Analysing the effect of cleaning on the absorbance measurements.

Steps:

1. Urine collection and preparation
    a) Take 2 L of treated urine from the tap on the nitrification reactor.
    b) Separate at least 1 litre of clear liquid from the sample as follows:
        i) Fill two 1 L Imhoff cones.
        ii) Let the biomass settle for 60 minutes.
        iii) Collect the settled biomass by removing at least 150 mL from each cone by opening the bottom tap of the Imhoff cone. This liquid is wasted.
        iv) Collect the remaining supernatant into a single container and ensure continuous mixing.

v) Repeat as necessary until at least 1 litre of bulk liquid is obtained.

2. Take a sample of the urine and measure nitrite and nitrate concentrations with Hach-Lange Cuvette tests.
   a) Filter the sample with a 0.4 µm glass microfiber filter to remove suspended particles.
   b) Nitrate: LCK 340 with a dilution of 1:100 (Expected concentration around 2000 mg NO_3^--N/L).
   c) Nitrite: LCK 341 with a dilution of 1:10 to avoid disturbance by the high nitrate concentration (Expected concentration around 0.5 mg NO_2^--N/L).
3. Measure absorbance in a dilution series.
   a) Measure 500 mL of the sedimented urine by means of a standardized flask and add it to the measurement container.
   b) Measure at least 5 spectra in the urine.
   c) Remove the sensor from the urine and add 1 mL of nitrite stock solution (12.875 g NO_2^--N/L) to the urine.
   d) Let it stir for 3–5 minutes to ensure complete mixing.
   e) Measure again at least 5 spectra in the urine.
   f) Repeat steps c–e until 5 mL of the nitrite stock solution have been added and a nitrite concentration above 120 mg NO_2^--N/L is reached in the measurement container.
4. Apply a sequence of mechanical cleaning.
   a) Wipe the urine from the sensor, but not in the measurement gap.
   b) Rinse the sensor and the measurement container with the nitrite/nitrate stock solution.
   c) Add 500 mL of the nitrite/nitrate stock solution to the narrow measurement container.
   d) Measure at least 5 spectra in the stock solution.
   e) Rinse the sensor with nanopure water from the spray bottle. Rinse the sensor afterwards with the nitrite/nitrate solution.
   f) Measure again at least 5 spectra.
   g) Clean the sensor window with a tissue wetted with water and rinse the sensor with nitrite/nitrate solution afterwards.
   h) Measure again at least 5 spectra.
5. Measure the absorbance in a dilution series with the mechanically cleaned sensor (repeat step 3). Rinse the sensor gap with urine before measuring.
6. Apply a complete cleaning sequence (only for the days with complete cleaning!)

a) Add 500 mL of the nitrite/nitrate stock solution to the measurement container.
b) Wipe the urine from the sensor, but not in the measurement gap.
c) Rinse the sensor and the measurement container with the nitrite/nitrate stock solution.
d) Add 500 mL of the nitrite/nitrate stock solution to the narrow measurement container.
e) Measure at least 5 spectra in the stock solution.
f) Rinse the sensor with nanopure water from the spray bottle. Rinse the sensor afterwards with nitrite/nitrate solution.
g) Measure again at least 5 spectra.
h) Clean the sensor window with a tissue wetted with water and rinse the sensor with nitrite/nitrate solution afterwards.
i) Measure again at least 5 spectra.
j) Clean the sensor with hydrochloric acid. Wet a tissue with HCl, fold it and clean the sensor gap thoroughly. Rinse the sensor first with water and then with the nitrite/nitrate solution.
k) Measure again at least 5 spectra.
l) Clean the sensor with sodium hydroxide. Wet a tissue with NaOH, fold it and clean the sensor gap thoroughly. Rinse the sensor thoroughly with water and then with the nitrite/nitrate solution.
m) Measure again at least 5 spectra.
n) Rinse the sensor with ethanol, afterwards thoroughly with water and then with the nitrite/nitrate solution.
o) Measure again at least 5 spectra.

7. Measure the absorbance a dilution series with the cleaned sensor (repeat step 3). Rinse the sensor gap with urine before measuring.

8. Make a PDF scan of the lab journal and update the digitized log file.

Schedule:

Thu, 30.01.2015: Mechanical cleaning

Mon, 02.02.2015: Mechanical cleaning

Mon, 09.02.2015: Mechanical cleaning

Thu, 12.02.2015: Mechanical cleaning

Mon, 16.02.2015: Complete cleaning

Fri, 20.02.2015: Mechanical cleaning

Mon, 23.02.2015: Mechanical cleaning

A.4 Nitrite/nitrate stock solution

4 Litres of the solution with 500 mg NO_2^--N/L and 500 mg NO_3^--N/L are produced at once. For this, the same procedure as described in A.2 is used but with 9.852 g $NaNO_2$ and 12.136 g $NaNO_3$.

B Additional results

B.1 Measured absorbance with outliers

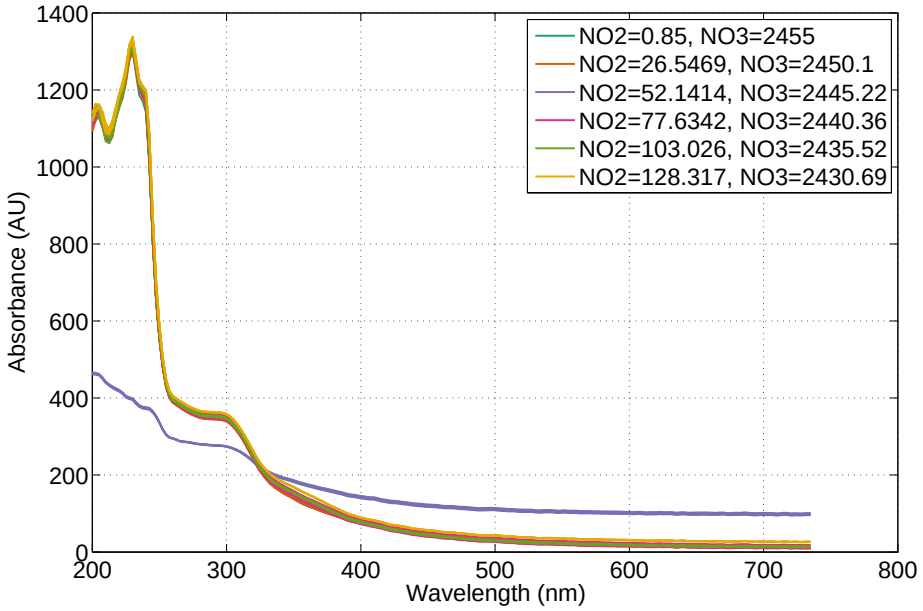
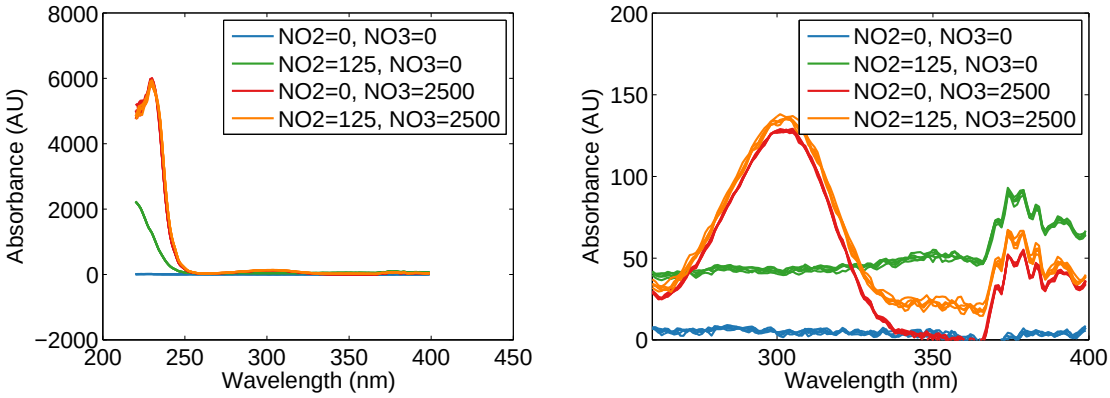


Figure 19: Measured absorbances for the dilution experiment on Day 3. The concentrations are given in mg N/L.

B.2 Analysing alternative UV and UV-Vis sensor configurations

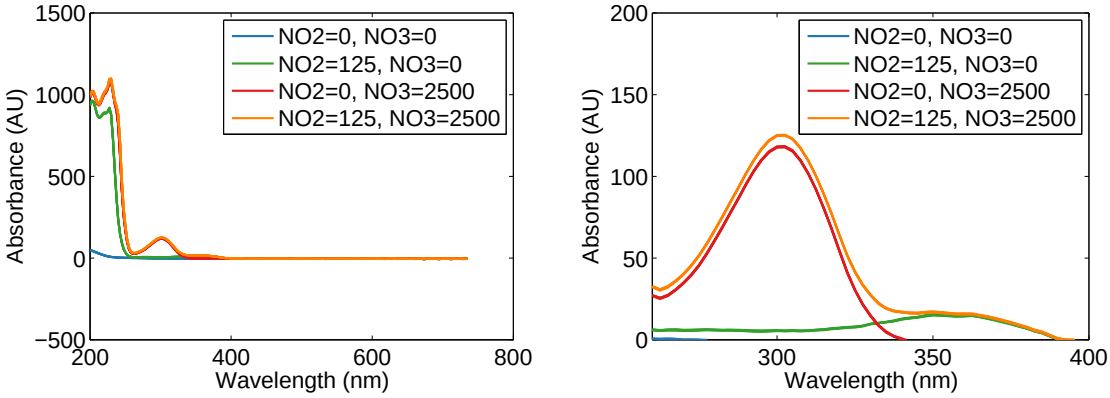
B.2.1 Water



(a) Full fingerprint

(b) Zoom to the nitrite and the nitrate peaks

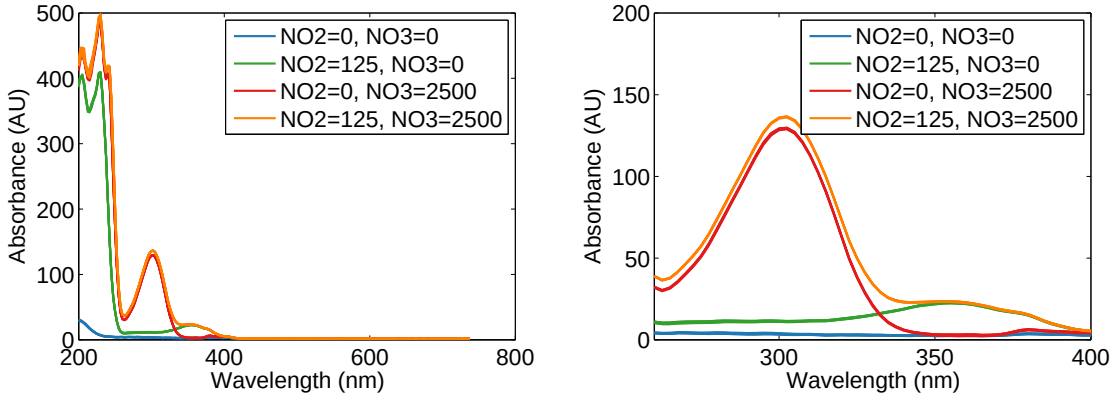
Figure 20: Measured spectra in water spiked with nitrite and nitrate. The nitrite and nitrate concentrations are given in mg N/L. Measured with the spectro::lyser with 0.5 mm measurement path.



(a) Full fingerprint

(b) Zoom to the nitrite and the nitrate peaks

Figure 21: Measured spectra in water spiked with nitrite and nitrate. The nitrite and nitrate concentrations are given in mg N/L. Measured with the spectro::lyser with 2 mm measurement path.



(a) Full fingerprint

(b) Zoom to the nitrite and the nitrate peaks

Figure 22: Measured spectra in water spiked with nitrite and nitrate. The nitrite and nitrate concentrations are given in mg N/L. Measured with the spectro::lyser with 5 mm measurement path.

B.2.2 Urine

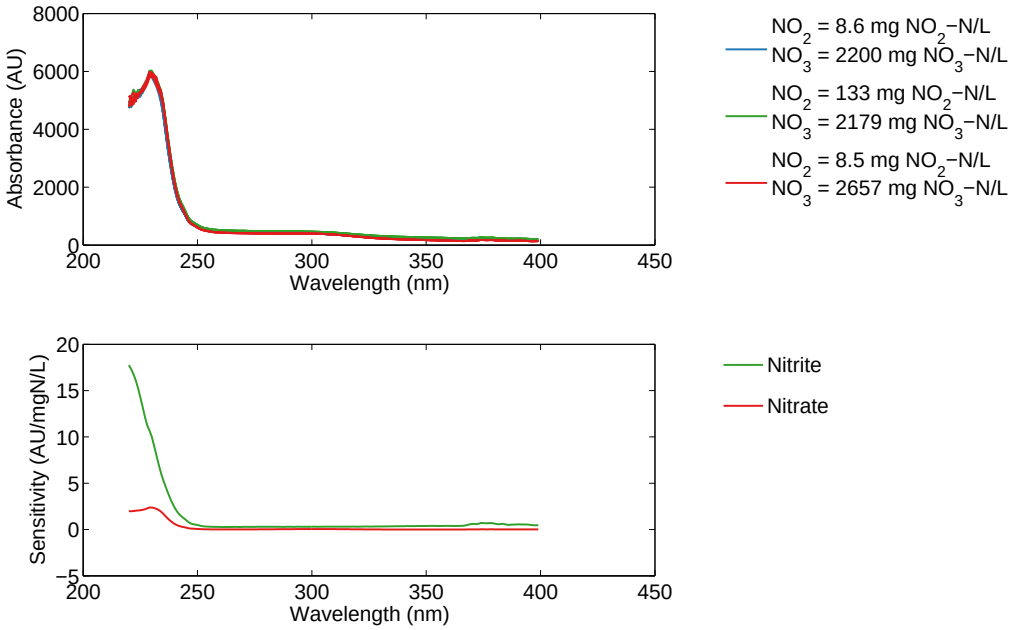


Figure 23: Fingerprint and sensitivity for the UV-Vis sensor with 0.5 mm path.

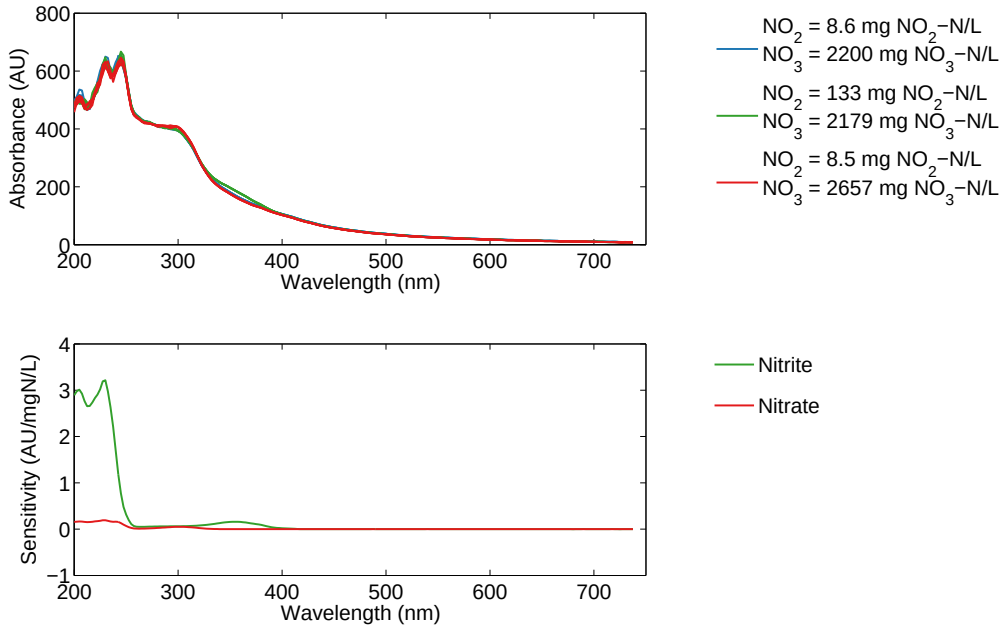


Figure 24: Fingerprint and sensitivity for the UV-Vis sensor with 5 mm path.

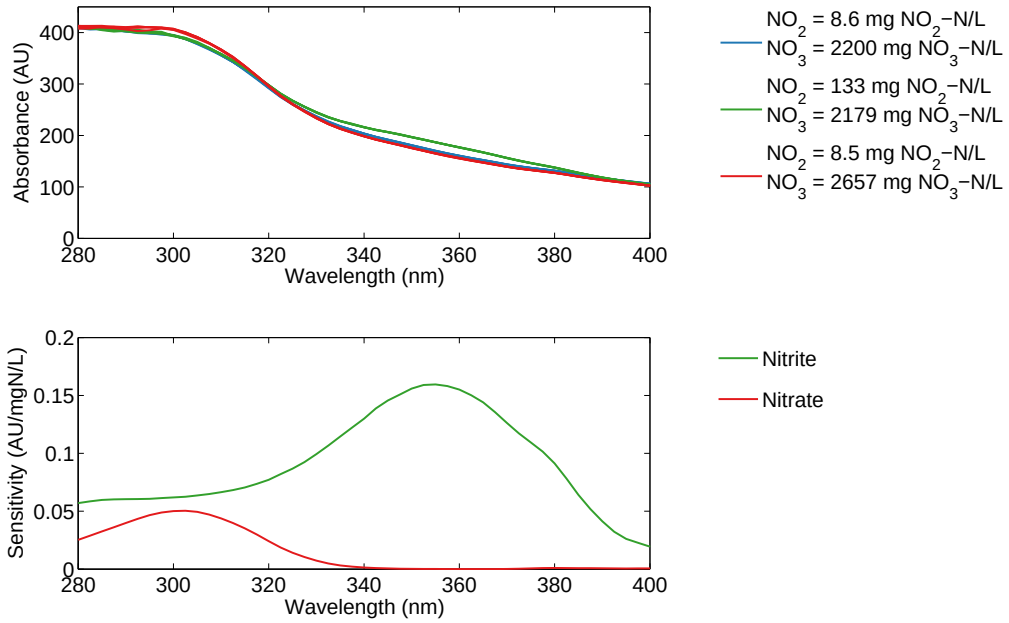


Figure 25: Zoom for the fingerprint and sensitivity for the UV-Vis sensor with 5 mm path.

B.3 Analysing long-term influences from nitrified urine on nitrite measurements with UV-Vis sensors

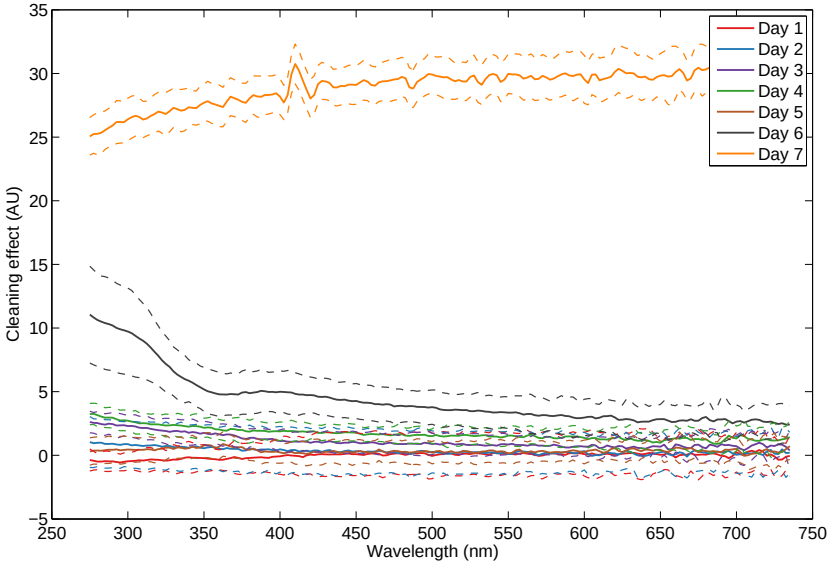


Figure 26: Effect of cleaning with water for the different days with the experiment, measured with the sensor with 2 mm measurement path. Solid lines are the mean of the five measurements and the dashed lines are 3 standard deviations added and subtracted.

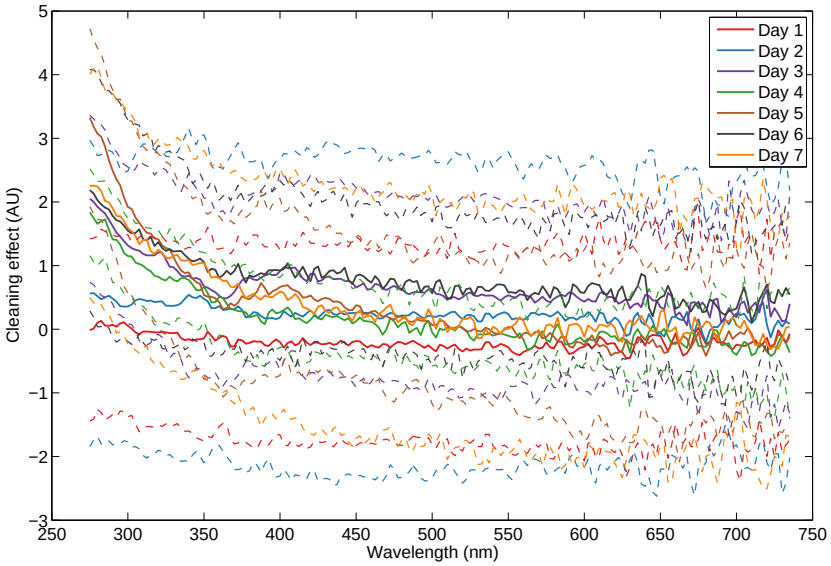


Figure 27: Effect of cleaning with a tissue wetted with water for the different days with the experiment, measured with the sensor with 2 mm measurement path. Solid lines are the mean of the five measurements and the dashed lines are 3 standard deviations added and subtracted.

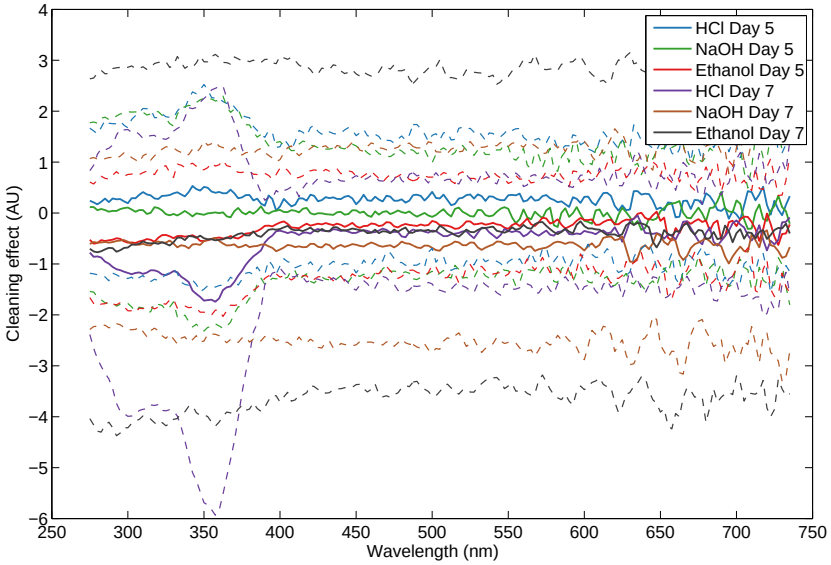
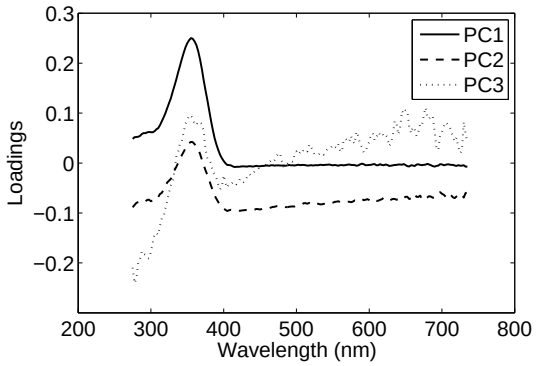


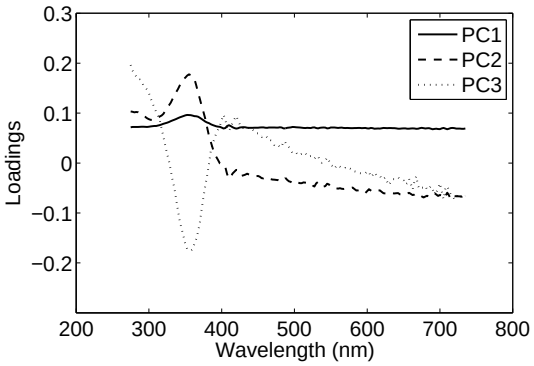
Figure 28: Effect of the additional cleaning steps for the different days with the experiment, measured with the sensor with 2 mm measurement path. Solid lines are the mean of the five measurements and the dashed lines are 3 standard deviations added and subtracted.

B.4 Calibration of the UV-Vis sensor

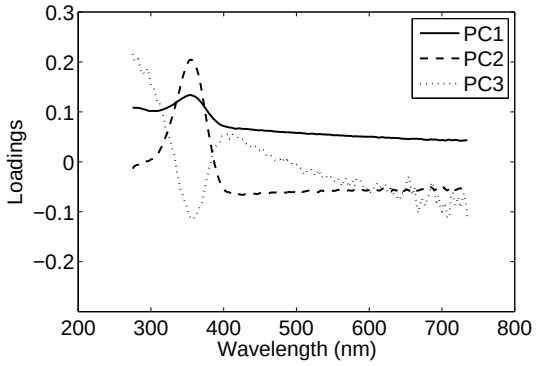
B.4.1 Model with data from cleaned sensor tested on the data from the same day



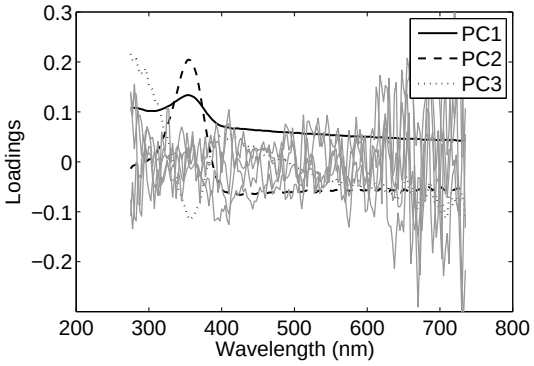
(a) Day 1



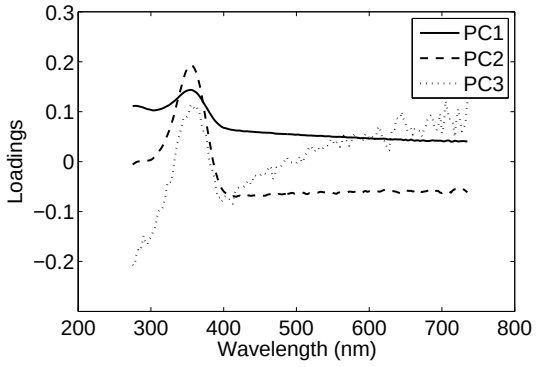
(b) Day 4



(c) Day 5



(d) Day 5



(e) Day 6

Figure 29: Principal components obtained for different days with data with the cleaned sensor. The grey lines show the principal components higher than 3.