Measure Nitrite with UV-Vis Spectrophotometry in a Urine Nitrification System

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Project Work

Measure Nitrite with UV-Vis Spectrophotometry
**Abstract**

Nutrients can be recovered from source-separated urine through biological nitrification. It promotes a more sustainable future of wastewater collection and treatment. However, accumulation of nitrite during biological nitrification, caused by undesirable conditions such as varying ammonia loads and increase of pH, will lead to an irreversible failure of the nutrients recovery system if no appropriate and timely measures are taken. Therefore, it is essential to measure the nitrite concentration in reactor to provide relevant information for operators. This project is set up to estimate nitrite concentration online and automatically with a combination of a commercially available in-situ UV-Vis spectrophotometer and PCR modelling method.

In this project, two different experimental phases are covered. Nanopure phase is designed to test the sensitivity of the UV-Vis sensor in measuring nitrite and nitrate via artificial addition of nitrite to nitrate stock solution, which is prepared with nanopure water. Relevant results suggest that the UV-Vis sensor responds well to the concentration changes of nitrite and nitrate. Treated urine phase aims at modelling a linear relationship between nitrite concentration and absorbance via PCA, PCR and LOOCV modelling methods. The PCR model with 37 principal components is applied to predict nitrite concentration and the prediction error is smaller than 9.7 mg NO$_2$-N/L.

**Keywords:** nitrification, nitrite accumulation, UV-Vis Spectrophotometry, nitrite concentration measurement, PCA, LOOCV, PCR
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1 Introduction

The background, motivation and objectives of this project are described in this chapter.

1.1 Background

Despite its undeniable success in treating wastewater, for most cities in the world, centralized sewer-based wastewater treatment is too costly and not easy to adapt to new challenges, e.g., population growth or new treatment standards (Larsen et al., 2013). An attractive alternative consisting of a network of small-scale reactors can be reliable in treating wastewater, especially when the wastewater streams are separated according to their properties (Fumasoli et al., 2016).

Urine, with the highest nutrient content among all common wastewater streams, has been studied and tested during several processes such as ammonia stripping, struvite precipitation, and nitrification/distillation. Due to its high nutrient content, nutrients recovery from urine is promising. Separate treatment of urine provides such an opportunity (Larsen and Gujer, 1996).

Nutrient recovery from source-separated urine is a promising improvement for the wastewater collection and treatment. By recovering nutrients from source-separated urine, a dry and affordable sanitation system, which produces a valuable fertilizer and promotes economic entrepreneurship (Udert et al., 2015), is developed (Etter et al., 2015). In addition, separated collection of human excrement and urine at the source significantly reduces the organic loading of inflow to wastewater treatment plant (WWTP). Nevertheless, challenges such as destabilisation due to nitrite accumulation still exist (Fumasoli et al., 2016). This project is undertaken to explore a new strategy to estimate nitrite concentration during biological nitrification.

1.1.1 Biological Nitrification

Biological nitrification can be applied to stabilize nitrogen content during nutrients recovery process (Udert and Wächter, 2012). During the nitrification process, ammonia is oxidized to nitrate, a more stable form of nitrogen, in two steps, as shown in eq. 1.1 and eq. 1.2. In the first step, ammonia oxidizing bacteria (AOB) oxidize ammonia into nitrite. After that, nitrite is oxidizing into nitrate by nitrite oxidizing bacteria (NOB) in the second step. It is clear that AOB and NOB utilize $NH_3$ and $HNO_2$ to metabolize, respectively. However, $NH_3$ and $HNO_2$ are not only limiting but also inhibiting compounds (Anthonisen et al., 1976 and Wiesmann, 1994). If the NOB performs well, almost all the nitrite will be transferred into nitrate. If the activity imbalance between AOB and NOB happens, nitrite will start to accumulate. For instance, if a sudden raise of ammonia load occurs (Etter et al., 2013), the metabolism of the AOB will increase immediately due to an increased ammonia availability, generating more nitrite in the reactor. Both AOB and NOB will be inhibited by $HNO_2$, but activity of the NOB will be influenced most, which results in an even higher nitrite concentration. This causes a positive feedback loop that the increased nitrite concentration reduced the activity of NOB, in return leading to a further increase of the nitrite concentration where no NOB activity occurs at all. What’s more, a sudden raise of pH can lead to nitrite accumulation as well (Udert and Wächter, 2012). Because when pH increases, hydroxide ion ($OH^-$) will react with the hydrogen ion ($H^+$) in eq. 1.1, promoting the ammonia oxidizing reaction, which means higher pH value benefits the first
step of nitrification to produce more nitrite. Therefore, to some degree, nitrite accumulates if NOB cannot consume enough nitrite. Besides, the concentrations of NH$_3$ and HNO$_2$ are in a pH dependent equilibrium with their acid NH$_4^+$ and base NO$_2^-$ as shown in eq. 1.3 and eq. 1.4, respectively. If there is a sudden increase of pH, the concentrations of ammonia and nitrite will increase suddenly. Consequently, more nitrite will be generated while the growth rate of NOB will decrease so that accumulation of nitrite happens.

\[ \begin{align*} 
NH_3 + 1.5 O_2 & \rightarrow NO_2^- + H^+ + H_2O \\
NO_2^- + 0.5 O_2 & \rightarrow NO_3^- \\
NH_3 + H^+ & \rightarrow NH_4^+ \\
HNO_2 + OH^- & \rightarrow NO_2^- + H_2O 
\end{align*} \]

In conclusion, it is essential to have control steps to guarantee stable operation of the reactor. These controllers can be based on online measurements of pH and nitrite concentration in order to adjust ammonia load and pH. Among these measurements, the estimation of nitrite concentration should be the best one. After all, increase of pH will finally lead to nitrite accumulation.

When the nitrite concentration in the nitrifying reactor exceeds 10 mg NO$_2^-$-N/L, a self-accelerating nitrite accumulation starts. Consequently, the reactor is no longer in steady state. The system can be easily recovered with appropriate actions even the nitrite accumulates to a concentration of 50 mg NO$_2^-$-N/L (Hess, 2015). However, if the nitrite continues to accumulate to a concentration above 200 mg NO$_2^-$-N/L, an irreversible failure of the reactor may occur (Mašić et al., 2015).

### 1.1.2 Previous Nitrite Measurements

In order to prevent the system failure caused by nitrite accumulation with appropriate measurements, it is essential to estimate the concentration of nitrite during the nitrifying process. There are several methods of determining the nitrite concentration. For instance, Hach-Lange nitrite cuvette, which is based on the diazotization process (Hach-Lange GmbH, 2015), is applied to measure nitrite concentration. However, it is indirect because it is based on color assessment. In addition, it is offline due to requirement of sample preparation processes (Van den Broeke et al., 2006). On the contrary, application of UV spectrophotometry in estimating nitrite concentration has been demonstrated as a reliable approach (Moorcroft et al., 2001). It requires no sampling, preparation of sample or reagents (Gruber et al., 2005) due to the submersible sensor. In addition, absorbance spectra can be collected from the connected computer. Nevertheless, challenges of spectrophotometry in nitrite concentration estimation in high strength wastewater still exist due to several factors (Mašić et al., 2015). First of all, there is an obvious overlap between the absorbance wavelengths of nitrate and nitrite on account of their close peak absorbance wavelengths as Table 1.1 illustrates. In addition, the nitrified urine wastewater contains many other compounds, e.g., organics, that also absorb light. Besides, the primary absorbance peaks are affected by saturation (Santos, 2014). Finally, the existing suspended particles will cause backscatter and influence the absorbance measurements in a nonlinear way (Lourenco et al., 2012). However, influence of suspended particles has been proved to be much smaller than the saturation effects at the first absorbance peak (Santos, 2014). The organics, other salts and suspended particles are usually described as background compounds.
Table 1.1: Absorbance peaks for nitrite and nitrate as reported in Spinelli et al. (2007).

<table>
<thead>
<tr>
<th></th>
<th>Nitrite</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st absorbance peak (nm)</td>
<td>213</td>
<td>206</td>
</tr>
<tr>
<td>2nd absorbance peak (nm)</td>
<td>354</td>
<td>302</td>
</tr>
</tbody>
</table>

1.2 Motivations & Objectives

Considering the changing organic content discussed in section 1.1.3, the UV-Vis spectrophotometry in this project was chosen to account for the variations in the organic compound. Compared with a UV spectrophotometer, the UV-Vis sensor has a much wider range of wavelengths which is from approx. 200 nm to 750 nm. The secondary absorbance peaks of nitrate and nitrite occur at 302 nm and 354 nm respectively. Despite the fact that absorbance peaks of nitrite and nitrate occur within the UV light range (between 200 nm and 400 nm), this wavelength range is also sensitive to some other compounds. For the visible light range, around 400 nm to 750 nm, there is no absorbance of nitrate and nitrite but sensitive and specific to some other background compounds. By modelling the absorbance spectra data of the whole visible light range, the contributions of the background compounds are taken into consideration. In addition, considering that the primary absorbance peaks are affected by saturation, it is required to use partial spectra of the UV light range.

The aim of the work in this report is to establish a relationship between the nitrite concentration and the absorbance spectra to estimate the nitrite concentration online and automatically so that the operator can decide when and how to take appropriate actions to prevent system failure. This relationship is derived by applying PCA to reduce dimension, PCR to obtain the model and LOOCV to assess the model.

Compared to previous work about nitrite estimation, it is new in this project to combine application of a UV-Vis spectrometer with a measuring path of 2 mm and PCR modelling method. Besides, it is different in nitrified urine samples and sample preparation procedures.

To be mentioned, after this project, I hope that I am able to answer these four questions listed as follows.

1. How does the UV-Vis sensor correspond to nitrate and nitrite?
2. What is the PCR model used to estimate nitrite concentration?
3. What is predicted error of the model with 99% confidence? Can it be controlled within ± 10 mgN/L? Can it be as precise as possible by developing a better model?
4. Is the model sufficient enough to be applied to prevent system failure?
2 Materials

The chemicals and instruments used in this project are listed in Table 2.1. In addition, interpretations of some important instruments and compounds are discussed.

<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration/ Category</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrified urine</td>
<td>---</td>
<td>Eawag</td>
</tr>
<tr>
<td>Sodium Nitrate</td>
<td>---</td>
<td>EMD Millipore Corporation</td>
</tr>
<tr>
<td>Sodium Nitrite</td>
<td>---</td>
<td>EMD Millipore Corporation</td>
</tr>
<tr>
<td>Hach-Lange Cuvette</td>
<td>LCK 399</td>
<td>Hach</td>
</tr>
<tr>
<td></td>
<td>LCK 340</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LCK 341</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LCK 342</td>
<td></td>
</tr>
<tr>
<td>Spectrophotometer</td>
<td>DR 2800</td>
<td>Hach</td>
</tr>
<tr>
<td>UV-Vis Spectrometer</td>
<td>Spectro::lyser™ UV-Vis</td>
<td>S::can</td>
</tr>
<tr>
<td>Analytical Balance</td>
<td>XS 204</td>
<td>Mettler Toledo</td>
</tr>
<tr>
<td>Magnetic Stirring Apparatus</td>
<td>RCT standard</td>
<td>IKA®</td>
</tr>
</tbody>
</table>

2.1 Source-Separated Urine

Urine used in this project was collected from urine-diverting flush toilets (NoMix toilets, Roediger Vacuum, Hanau, Germany) through a separate piping system at the main building of Eawag (Dübendorf, Switzerland). For each weekday, an average of 100 L urine is collected. What’s more, prior to nitrification, the collected women’s urine and men’s urine are stored separately. Only urine of males was nitrified during the period of this project and the composition of men’s urine can be found in Fumasoli (2016).

![Fig 2.2: S::can spectrometer probe - measuring section](image)
The urine samples for this project were collected from the SoDAN nitrification reactor at the Experimental Hall at Eawag. Fig 2.1 exhibits the reactor. As shown, it is a continuous stirred tank reactor (CSTR) with suspended biomass.

### 2.2 Hach-Lange Cuvette

Hach-Lange cuvette tests based on colour assessment are applied to measure the reference concentrations of nitrite and nitrate. The barcoded cuvette is inserted into a Hach DR 2800 spectrophotometer (Fig 8.1 in Appendix) to identify the concentration which is showed on the screen in mg/L. Four different Hach-Lange cuvettes were used in this project. They are LCK 399, LCK 340, LCK 341 and LCK 342.

### 2.3 UV-Vis Spectrophotometer

The used in-situ spectrophotometer is a spectro::lyser™ UV-Vis sensor manufactured by s::can. The spectrometer provides absorbance spectra in a light range between 200 nm and 750 nm with a resolution of 2.5 nm.

Fig 2.2 shows a picture of the applied sensor. The sensor consists of three main components: the emitter, measuring cell and receiving unit. The measuring section includes ① optical measuring path, ② cleaning nozzles and ③ fixtures for the measuring path.
3 Methods

In order to achieve the goals demonstrated in Section 1.2, two experimental phases are implemented. They are the nanopure phase (Phase 1) and the treated urine phase (Phase 2). Phase 1 is set up to assess how sensitive the UV-Vis sensor is to variations of the nitrate and nitrite concentrations. Phase 2 is set up to develop the relationship between nitrite concentration and absorbance spectra by means of modelling. The research strategies for Phase 1 and Phase 2 are shown in Fig 3.1 and Fig 3.2, respectively.

![Fig 3.1: Research strategy for Phase 1: spectra and concentration data are collected to compute sensitivity.](image1)

![Fig 3.2: Research strategy for Phase 2: spectra and concentration data are collected to be modelled in Matlab. Within the LOOCV procedure, MSR and residuals are calculated. Outliers are removed based on residuals and LOOCV is repeated for the remaining data; the optimal NPCs corresponds to the minimal value of MSR. After that, PCA is applied according to the optimal NPCs and a PCR model is built finally.](image2)
3.1 Measure Absorbance

Protocol A1, A2, A4, A5 and A6 in Appendix 8.1 explain how to prepare stock solutions of sodium nitrate and sodium nitrite for Phase 1 and Phase 2. Besides, the particle effects are minimized due to application of centrifuge during experimental Phase 2. Subsequently, the UV-Vis spectrophotometer is submerged into a cylinder filled with 500 mL NaNO₃ stock solution to measure the absorbance as presented in Fig 3.3. Protocol A3 and A7 describe the detailed operation steps for Phase 1 and Phase 2, respectively. In order to collect the absorbance data at different nitrite concentration levels, the addition of nitrite into corresponding nitrate stock solution is executed according to Table 8.2 for Phase 1 and Table 8.4 for Phase 2 in Appendix 8.1, respectively. For each sample, six observations (spectra) are recorded.

![Fig 3.3: Experiment installation](image)

3.2 Sensitivity Calculation

The plots of absorbance spectra give us a direct impression about how the sensor corresponds to concentration changes of nitrate and nitrite. In addition, it can be read from such plots where the secondary absorbance peaks occur. Besides, specific calculations about sensitivity can be used to interpret more about the first question demonstrated in section 1.2. According to the Beer-Lambert law (eq. 3.1), a linear relationship between nitrite concentration and absorbance can be assumed. Therefore, as eq. 3.2 shows, the sensitivity of the sensor is equal to the slope of the linearly fitted curve obtained from the absorbance and concentration data.

\[
Abs_x = ε_s * L * C \quad eq. 3.1
\]

\[
Abs_y = S * C + I \quad eq. 3.2
\]
Absorption at wavelength \( \lambda \) (AU)

\( \varepsilon \) : Molar absorptivity for wavelength \( \lambda \) (m\(^2\)/mol)

\( L \) : Path length (m)

\( C \) : Concentration

\( S \) : Sensitivity (AU/mgN/L)

\( I \) : Interception (AU)

### 3.3 Modelling

#### 3.3.1 Data Pre-Processing

The absorbance spectra data is collected in form of a \( m \times n \) matrix, where \( m \) rows stand for the observations (absorbance), and \( n \) columns represent the variables (wavelengths).

\[
X = \begin{bmatrix}
Abs_{s1} & \ldots & Abs_{sn} \\
\ldots & \ldots & \ldots \\
Abs_{sm1} & \ldots & Abs_{smn}
\end{bmatrix}
\]

Prior to dimension reduction of data, the absorbance spectra data must be pre-processed. In general, mean centring is applied for the multivariate calibration model (eq. 3.3). The mean-centred transformation ensures that the results will be interpretable in terms of deviation around the mean (Maesschalck et al., 1999).

\[
X_{\text{center}} = X_i - \text{mean}(X_i)
\]  

Where \( X_{\text{center}} \), \( X_i \) and \( \text{mean}(X_i) \) are the mean centring results, original absorbance data and mean absorbance data of the \( i^{th} \) column, respectively.

#### 3.3.2 Singular Value Decomposition (SVD)

Singular value decomposition (SVD) of data is required before eigenvalue analysis and dimension reduction. The SVD can be rewritten as follows:

\[
X_{\text{center}} = U \times S \times V^T
\]  

Where \( U \) (\( m \times r \)) and \( V \) (\( n \times r \)) are orthogonal matrices, and \( S \) (\( r \times r \)) is a diagonal matrix containing the variance described by each singular value and \( r \) is the rank of \( X_{\text{center}} \) (Jolliffe, 2002). The principal components of \( X_{\text{center}} \) are presented in the columns of \( U \times S \) (Trevor et al., 2009). Elements in matrix \( V \) are the loadings, the weights by which each standardized original variable should be multiplied to get the component scores.

#### 3.3.3 Eigenvalue Analysis

In this project, eigenvalue analysis only contributes to have a more critical analysis of results in the discussion chapter. The eigenvalues of the covariance matrix of \( X_{\text{center}} \) are calculated as eq. 3.5 illustrates, which are equal to the singular values of the matrix \( X_{\text{center}} \). When the cumulative percentage of eigenvalues is greatly approaching 100%, it is common to figure out the corresponding number to be the number of principal components (NPCs).

\[
\text{Eigenvalue} = \text{diagonal}(S) \times \text{diagonal}(S)
\]  

---

- 8 -
3.3.4 Reduce Dimensionality: PCA

PCA is a statistic analysis method that helps reduce the dimensionality of data to avoid overfitting. Meanwhile, the information of original data can be retained as much as possible (Jolliffe, 2002). In this project, PCA is achieved by means of SVD. Following the SVD one can compute the principal scores. The principal scores are the representations of absorbance spectra on the principal component space, which is determined by the loading vector (columns of matrix V). Eq.3.6 shows how to convert the measured observations ($X_{center}$) into principal scores. Through such conversion, dimension is reduced. As an example, if the optimal NPCs is determined to be N, the score matrix T should be $[T_1 \ T_2 \ \ldots \ T_N]$. Consequently, the dimension is reduced to N.

$$T = U * S = X_{center} * V$$  \hspace{1cm} \text{eq.3.6}$$

Where T ($m \times r$) is the score matrix containing the principal components.

3.3.5 Principal Component Regression (PCR)

After reducing dimension, PCR is utilized to develop a relationship between nitrite concentration and absorbance data. The relationship can be rewritten as follows:

$$\hat{y} = Z * \begin{bmatrix} \bar{b} \\ a \end{bmatrix}$$  \hspace{1cm} \text{eq.3.7}$$

$$\begin{bmatrix} \bar{b} \\ a \end{bmatrix} = (Z^T * Z)^{-1} * Z^T * y$$  \hspace{1cm} \text{eq.3.8}$$

$$Z = [1 \ T_1 \ \ldots \ T_N]$$  \hspace{1cm} \text{eq.3.9}$$

Where $\hat{y}$ and $y$ are the predicted and measured nitrite concentration, respectively. Z is a matrix containing a vector composed of ones and the scores of principal components which are mentioned in eq.3.6. For example, if the significant NPCs is decided to be two, then the following calculations are executed to compute the nitrite concentration of the new (testing) absorbance spectra.

$$\begin{bmatrix} \bar{b} \\ a \end{bmatrix} = \begin{bmatrix} b \\ a_1 \\ a_2 \end{bmatrix}$$

$$Z = [1 \ T_1 \ T_2]$$

$$\hat{y} = Z * \begin{bmatrix} \bar{b} \\ a \end{bmatrix} = [1 \ T_1 \ T_2] * \begin{bmatrix} b \\ a_1 \\ a_2 \end{bmatrix} = b + T_1 * a_1 + T_1 * a_2$$

3.3.6 Leave-one-out of Cross-Validation (LOOCV)

Cross-validation is used to assess the performance of the model generated by a statistic analysis by means of applying the model to an independent data set. Leave-one-out of cross-validation (LOOCV) is chosen as a method applied in this project to assess the model automatically. Relevant calculations are demonstrated by eq.3.10 to eq.3.14.

$$R_{i,j} = \hat{y}_{i,j}(j) - y_{i,j}$$  \hspace{1cm} \text{eq.3.10}$$

$$MR_j = \frac{1}{m} \sum_{i=1}^{m} R_{i,j}$$  \hspace{1cm} \text{eq.3.11}$$
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\[ MSR_j = \frac{1}{m} \sum_{i=1}^{m} \sum_{j=1}^{x} R_{i,j}^2 \]  \hspace{1cm} eq. 3.12

\[ STD_j = \left( \frac{1}{m-1} \sum (R_{i,j} - MR_j)^2 \right)^{0.5} \]  \hspace{1cm} eq. 3.13

Where \( \hat{y}_{i,j} \) is the predicted elements of the testing data set with \( j \) principal components, and \( y_{i,j} \) is the measured nitrite concentration. \( R_{i,j} \) is the residuals of the \( i^{th} \) model with \( j \) principal components. \( m \) represents the number of analysed spectra. \( MR_j \) is the mean of residuals of the \( m \) models with \( j \) principal components. \( MSR_j \) and \( STD_j \) are the mean of squared residuals and standard deviation of the \( m \) models with \( j \) principal components, respectively. \( x \) is the maximal NPCs determined by eq. 3.14.

\[ x = \min(m - 1, n) - 1 \]  \hspace{1cm} eq. 3.14

Where \( m \) and \( n \) is the row and column of matrix \( X \).

As mentioned in section 3.3.1, there will be \( m \) observations (spectra). Accordingly, \( m - 1 \) observations are used for calibration to build a PCR model, while the remaining one observation serves as the validation set to assess the performance of the PCR model. Fig 3.4 illustrates how the training and testing observations are defined in LOOCV procedure. For each \( i \) within this loop, \( x \) PCR models are built according to the training observations and NPCs defined in another loop discussed in the next paragraph, and the residuals between predicted values and measured values are computed as well. After this loop, the mean of squared residuals (MSR) and standard deviation (STD) of the residuals are calculated. Fig 3.5 shows how to define the values of NPCs for each \( i \) within the previously described loop (Fig 3.4). Consequently, MSR and STD for different NPCs can be obtained after LOOCV procedure.

In this project, the optimal NPCs applied to reduce dimensionality is defined as the number corresponding to the minimal value of MSR.

\[
\begin{array}{cccccccc}
\text{i=1} & \text{testing} & \text{training} & \text{training} & \ldots & \text{training} & \text{training} \\
\text{i=2} & \text{training} & \text{testing} & \text{training} & \ldots & \text{training} & \text{training} \\
\text{i=3} & \text{training} & \text{training} & \text{testing} & \ldots & \text{training} & \text{training} \\
\vdots & & & & & & \\
\text{i=m-1} & \text{training} & \text{training} & \text{training} & \ldots & \text{testing} & \text{training} \\
\text{i=m} & \text{training} & \text{training} & \text{training} & \ldots & \text{training} & \text{testing} \\
\end{array}
\]

Fig 3.4: Sketch of LOOCV procedure
3.3.7 Remove Outliers Based on Residuals

Prior to determining final PCR model, it requires to check whether there contains any outliers in the data. Since outliers can influence the accuracy of data significantly. In order to figure out these outliers, residuals are computed within the LOOCV procedure. In this project, as demonstrated in section 1.2, I want to control the prediction error within ± 10mgN/L. Therefore, if the residuals of validation data exceed the range between −10mgN/L and +10mgN/L, then the corresponding observations are defined as outliers. Subsequently, these observations are removed from the modelled data and LOOCV is repeated for the remaining data. Once more, check the residuals computed based on the remaining data. If there are any residuals which are smaller than −10mgN/L or larger than +10mgN/L, these corresponding observations are removed from the remaining data. Besides, LOOCV procedure is repeated again in order to observe the values of residuals. Such calculations are stopped when all the residuals are within +10mgN/L.
4 Results

In this section, first the sensitivity results of experimental Phase 1 are presented. Afterwards, the results about the outliers for experimental Phase 2 are shown. Besides, eigenvalue analysis is executed in this chapter as well. In the last part, different PCR models are listed.

4.1 Sensitivity Test

The spectro:lyser™ UV-Vis sensor was tested in nanopure water spiked with nitrite and nitrate to assess the sensitivity of the sensor. Fig 4.1 shows the absorbance spectra for different nitrite and nitrate concentrations. The secondary absorbance peaks can be observed clearly, around 302.5 nm for nitrate and around 357.5 nm for nitrite both of which correspond well to the literature values listed in Table 1.1. What’s more, as presented in Fig 4.1 a), the changes of absorbance peaks correspond to the concentration changes of nitrate. Similarly, the peak values of absorbance (Fig 4.1 b) increase with the increase of nitrite concentration. In other words, the sensor responds to concentration changes of nitrate and nitrite. Besides, the results of computed sensitivity of nitrate and nitrite are presented in Table 4.1. It is easy to see that the sensor responds well to nitrite with a sensitivity of approx. 0.13 AU/mgN/L. For measuring nitrate, the sensor also responds but with a smaller sensitivity of 0.04 AU/mgN/L. In conclusion, the spectro:lyser™ UV-Vis sensor is sensitive in measuring nitrite and nitrate in the UV range. What’s more, the value of absorbance never decreased to zero within the visible light range.

![Fig 4.1: Spectra for different nitrate and nitrite concentrations collected from the UV-Vis sensor](image_url)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sensitivity (AU/mgN/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>0.04</td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.13</td>
</tr>
</tbody>
</table>
4.2 Outliers

As shown in Fig 4.2 a) (next page), 18 observations are defined as outliers indicated by red circles. To be mentioned, the residuals displayed in Fig 4.2 a) are computed based all observations (96 observations). After removing the 18 outliers shown in Fig 4.2 a), LOOCV procedure is repeated to calculate residuals of validation data for the remaining 78 observations. Results are presented in Fig 4.2 b). It is observed that there are still 4 residuals of observations whose values exceed 10 mgN/L. As a result, these 4 observations are figured out to be outliers. Consequently, it requires to remove these 4 observations from the remaining 78 observations. Thereafter, only 74 observations are utilized to repeat LOOCV once more. Relevant results demonstrated in Fig 4.2 c) shows that all the residuals of validation data are smaller than 10 mgN/L or larger than -10 mgN/L. In conclusion, there are 22 observations defined as outliers which should be removed from modelled data.

4.3 Eigenvalue Analysis

The cumulative percentage of singular values of the covariance matrix ($X_{center}$) is displayed in Fig 4.3. The results suggest that only a single principal component can dominate most of the information of data (approx. 96.5%) while almost all the information (around 100%) is included within about the first ten principal components. In addition, the first three principal components contribute more than 99.9% to the variance of data, while the remaining principal components only occupy a very small part of data (less than 0.1%), which indicates that the first three principal components are the most important principal components.

![Fig 4.3: Cumulative percentage of eigenvalues corresponding to the first 15 principal](image)
Fig 4.2: Residuals vs. Sample _index: a) Based on all observations; b) Based on all observations without 18 observations indicated by red circles in a); c) Based on all observations without 22 observations
4.4 Nitrite Prediction Using Partial Spectra

In order to avoid the saturation effect occurring at the short wavelengths and the ‘NAN’ data measured at the long wavelengths, only a part of the whole spectra is used to model. It is clear that ‘NAN’ data begins at 737.5 nm. Therefore, the cut-off point at the long wavelengths is 735nm. Several PCR models are built based on whole spectra and partial spectra to find an appropriate range of modelling wavelengths. Relevant results are summarized in Fig 4.4 in a similar manner to facilitate comparison between them. The 1st row corresponds to the whole spectra while the last four rows display the results of using partial spectra whose cut-off points at short wavelengths are 260nm, 275nm, 280nm and 290nm, respectively. It is observed that a complex model with 42 principal components can predict nitrite concentrations of validation observations with a fit of $R^2=0.9705$ when modelling with the whole spectra. In addition, the STD value of this model is 5.1 mgN/L. Compared to the 1st model, four less complex models are obtained based on partial spectra as presented in the rows 2 to 5 in Fig 4.4. However, the difference in complexity is small. The least complex model is the 5th model which is composed of 36 principal components. Besides, the smallest MSR value is obtained when modelling with partial spectra between 260n and 735nm. What’s more, the minimal STD value and best fit are expected with the 2nd model. While if the cut-off point is set to be 275nm, the values of MSR and STD increase and the fit decreases. After that, if the cut-off point at the short wavelengths is increased, as presented in the last two rows, the MSR and STD values are increased as well, while the values of $R^2$ are decreasing, which means a worse model compared to the 2nd model. Overall, the performance of model is changing with the selection of different cut-off points at the short wavelengths.

In the ideal case, the predicted nitrite concentrations should be exactly equal to the measured nitrite concentrations. However, the ideal case is not observed. Despite that, all three models deviate from the ideal case slightly, especially the second one.
Project Work

Measure Nitrite with UV-Vis Spectrophotometry

Fig 4.4: Nitrite prediction using whole and partial spectra.
5 Discussions

The executed experiments and subsequent data analysis enable to answer the sensitivity of the UV-Vis sensor in measuring nitrate and nitrite and enable to obtain the relationship between nitrite concentration and absorbance spectra as well. In addition, detailed and critical discussions in this chapter will illustrate more about this project.

5.1 Reasons for Outliers

As Fig 4.2 presents, 22 observations are removed as outliers. During the experiments, some errors may occur, resulting in outliers. The concentrations of prepared nitrate and nitrite stock solutions are slightly deviating from expectations (Fig 8.2 in Appendix). Therefore, it is assumed that the absorbance of an air bubble or a particle is measured instead of urine for these outliers.

5.2 Determination of Final PCR Model

From relevant results displayed in Fig 4.4, the complexity of model can be reduced by using partial spectra. What’s more, according to Fig 4.4, it seems that the best model is obtained when modelling with partial spectra between 260 nm and 735 nm. However, it is reported from Santos (2014) and preliminary experiments that the primary absorbance peaks are affected by saturation. Therefore, only the wavelengths higher than 275 nm are considered for building a model (Hess, 2015). Consequently, despite the fact that the best fit and smallest STD and MSR values are observed in the 2nd model in Fig 4.4, it cannot be referred as the best model for predicting concentration of nitrite. From the report, we know that wavelengths higher than 275 nm should be applied to build a PCR model, but we do not know that at which wavelength the best model can be obtained. Therefore, further studies about the selection of partial spectra was executed and relevant results are listed in Figure 4.4. It is observed that if the cut-off point at the short wavelengths is increased from 275 nm to 290 nm, the model firstly becomes better at 280nm and then becomes worse at 290 nm. The worse model based on cutting off at 290nm is caused by loss of information. In other words, when cutting-off at 290 nm, it is definite that the saturation effects are avoided, however, some information within 275 nm and 290 nm is lost, thereafter, a worse model is obtained. The performance of model is optimized by using partial spectra between 280 nm and 735 nm instead of partial spectra between 275 nm and 735 nm.

In conclusion, in order to avoid saturation effects, ‘NAN’ measurements and retain information as much as possible, the partial spectra between 280nm and 735nm are applied to build the final PCR model.

5.3 Critical Analysis for Determination of NPCs

As shown in Fig 4.3 and Fig 4.4 c2), the NPCs determined by PCA and LOOCV are quite different. Actually, it is definite that the concentrations of nitrite, nitrate and sodium varied dramatically due to artificial addition during experiment, which means they contribute to significant variation of absorbance. Therefore, at least three principal components are expected, which is corresponding to the results of eigenvalue analysis. However, after LOOCV assessment (Fig 4.4 d2)), compared to the minimal MSR, which is 14 (mgN/L)^2 and corresponding to 37 principal components, it is observed that three principal components have a much greater MSR value, which is 190 (mgN/L)^2. For one and ten
principal components, the MSR values are $850 \text{ (mgN/L)}^2$ and $39 \text{ (mgN/L)}^2$, respectively. Accordingly, smaller MSR value means smaller deviation between predicted and measured nitrite concentrations. In other words, the smaller the MSR value is, the more precise the model is. From this perspective, a model with 37 principal components should be applied in estimation of nitrite concentration. Furthermore, considering that one can never find the NPCs corresponding to the minimal MSR value from eigenvalue analysis, I stick to use the NPCs resulted from LOOCV procedure to reduce dimension of data. Despite that, eigenvalue analysis contributes to have a better interpretation of principal components.
6 Conclusions and Outlook

6.1 Conclusions

From the project, the following conclusions can be obtained:

- The spectro::lyserTM UV-Vis sensor produced by s::can is sensitive in measuring nitrite and nitrate.
- The prediction model with 37 principal components can be used to estimate nitrite concentrations of the testing data with a fit of $R^2=0.9842$ and a STD of 3.77 mgN/L.
- With 99% confidence, the error between predictions and measurements is $\pm 9.7$ mgN/L, which is sufficient to be applied to predict nitrite concentration in nitrification reactors to prevent system failure.

6.2 Outlook

In the presented experiments, the relationship between predicted nitrite concentration and absorbance spectra is obtained based on biomass free reactor liquid. However, in practice, in order to online and automatically measure nitrite concentration in the nitrification system, no additional sample preparation procedures will be done, which means biomass will affect the measurements. Therefore, modelling with biomass content contained should be studied future.
7 References


URL http://uk.hach.com/nitrite-cuvette-test-0-6-6-0-mg-l-no2-n/product?id=26370291441


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8 Appendix

8.1 Protocols

8.1.1 Protocols for Phase 1

- **Protocol A1: Preparation of 1 L nitrate solution**

  **Aim:**
  Make 1000 mL nitrate solutions for \( X = 2 \) or 2.25 or 2.5 or 2.75 or 3 gNO\(_3\)-N/L

  **Required products:**
  1) NaNO\(_3\) powder
  2) Nanopure water

  **Required hardware:**
  1) Funnel
  2) 1 L glass container
  3) 1000 mL standardized flask
  4) 2 x 100mL standardized flasks
  5) Magnet stirrer with magnet
  6) Pipette (10, 5, 1, 0.2, 0.1 mL)

  **Table 8.1 Information for preparing nitrate stock solution**

<table>
<thead>
<tr>
<th>Nitrate Conc. (mg NO(_3)-N/L)</th>
<th>NaNO(_3) weight (g)</th>
<th>Expected value of LCK 399</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>12.1360</td>
<td>2.00</td>
</tr>
<tr>
<td>2250</td>
<td>13.6530</td>
<td>2.25</td>
</tr>
<tr>
<td>2500</td>
<td>15.1700</td>
<td>2.50</td>
</tr>
<tr>
<td>2750</td>
<td>16.6870</td>
<td>2.75</td>
</tr>
<tr>
<td>3000</td>
<td>18.2040</td>
<td>3.00</td>
</tr>
</tbody>
</table>

  **Steps:**
  1) Weigh \( X \) [g] of the NaNO\(_3\) powder
  2) Dissolve powder into water
  3) LCK 399 measurements with a dilution of 1:1000 (2 mL stock solution into a 100 mL flask (shake the flask), from this solution 5 mL into 100 mL flask (shake the flask)).
  4) Proceed with Lange Test LCK 342 to check whether nitrite contained
  5) Evaluate the quality of the solution. Repeat steps a-h if the obtained quality is insufficient.

  **Calculations:**
  Nitrate:
  Desired concentration: \( X \) g NO\(_3\)-N/L
  Desired volume 1 L

  \[
  M_{NaNO_3} = X \frac{gN}{L} \cdot 1 \text{ L} \cdot \frac{84.994 \frac{gNaNO_3}{mol}}{14.007 \frac{gN}{mol}} = X \cdot 6.068 \frac{gNaNO_3}{L}
  \]

- **Protocol A2: Preparation of nitrite stock solution with nitrate.**

  **Aim:**
  Make 100mL nitrite stock solution with constant nitrate solution (goal: 505 mL nanopure with \( X \) mg NO\(_3\)-N/L and 7575mg NO\(_2\)-N/L).
Project Work

Measure Nitrite with UV-Vis Spectrophotometry

Required products:
1) NaNO₂ powder
2) NO₃⁻ stock solutions: 2000, 2250, 2500, 2750, 3000 mg NO₃⁻N /L (depending on the experiment)
   See protocol A1!
3) Nanopure water

Required hardware:
1) Funnel
2) 2x 100 mL flasks
3) 500 mL flask
4) Magnet stirrer with magnet
5) Pipette (10, 5, 1, 0.2, 0.1 mL)
6) LCK 342 Nitrite Lange Tests

Steps:
1) Weigh 3.7313 g of the NaNO₂ powder
2) Dissolve powder into water
3) LCK 342 measurements with a dilution of 1:5000 (1 mL stock solution into a 100 mL flask (shake the flask), from this solution 2 mL into 100 mL flask (shake the flask)).
4) Evaluate the quality of the solution (Expected concentration: 1.51 mg NO₂⁻N/L). Repeat steps a-g if the obtained quality is insufficient

Calculations:
Nitrite:
Desired concentration: 75 mg NO₂⁻N/L in 505 mL cylinder solution
Amount of NaNO₂ for 100 mL stock solution:

\[ M_{NaNO_2} = \frac{75 \text{ mgN} \cdot 505 \text{ mL}}{0.1 \text{ L} \cdot 1000 \text{ mgN} \cdot \frac{68.995 \text{ gNaNO}_2}{14.007 \text{ gN mol}^1}} = 3.7313 \text{ gNaNO}_2 \cdot \frac{100\text{ mL}}{100\text{ mL}} \]

- Protocol A3: Testing simultaneous nitrate and nitrite estimation with UV-Vis sensor -

Aim:
Test UV-Vis sensor under the same idealized conditions for estimating nitrite and nitrate.

Required products:
1) Nanopure water
2) Stock solution Nitrite (goal: 7575mgN/L, 7.575gN/L solution) Protocol A2
3) Solution Nitrate (X mgN /L) Protocol A1

Required hardware:
1) 2 narrow measurement cylinder (1000 mL)
2) 2 standardized 500 mL flask
3) Magnet stirrer with magnet
4) Pipette (2 mL)
5) s::can Sensor “blue”
6) Laptop

Experimental Plans
Project Work

Measure Nitrite with UV-Vis Spectrophotometry

0-5 nitrate concentration 0-3000mgNO₃-N/L
R-W nitrite concentration 0-75 mgNO₂-N/L

Example: Experimental row 3R-3W nanopure water with 3000mgNO₃-N/L and 0 to 75mgNO₂-N/L

Table 8.2 Information for absorbance spectra measurements

<table>
<thead>
<tr>
<th>Nitrate mgN/L / Nitrite mgN/L</th>
<th>R</th>
<th>S</th>
<th>T</th>
<th>U</th>
<th>V</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3000/0</td>
<td>3000/15</td>
<td>3000/30</td>
<td>3000/45</td>
<td>3000/60</td>
<td>3000/75</td>
</tr>
<tr>
<td>4</td>
<td>2750/0</td>
<td>2750/15</td>
<td>2750/30</td>
<td>2750/45</td>
<td>2750/60</td>
<td>2750/75</td>
</tr>
<tr>
<td>3</td>
<td>2500/0</td>
<td>2500/15</td>
<td>2500/30</td>
<td>2500/45</td>
<td>2500/60</td>
<td>2500/75</td>
</tr>
<tr>
<td>2</td>
<td>2250/0</td>
<td>2250/15</td>
<td>2250/30</td>
<td>2250/45</td>
<td>2250/60</td>
<td>2250/75</td>
</tr>
<tr>
<td>0</td>
<td>0/0</td>
<td>0/15</td>
<td>0/30</td>
<td>0/45</td>
<td>0/60</td>
<td>0/75</td>
</tr>
</tbody>
</table>

Steps:

Prepare the sensors for measurement. Clean it with nanopure water and dry it.

1) Fill 500 mL nanopure water in the first measurement cylinder: measure the nanopure absorbance with the sensors.
   a) Insert the sensor until a volume on the measurement container reads about 830 mL.
   b) Start the stirrer again (1000 rpm)
   c) Note the time and facts in the corresponding Logbook
   d) Ensure that at least 6 measurements are recorded (7-10 minutes)

2) Measure the absorbance spectra with the sensor for rows 0-5
   a) Prepare the UV sensor for measurement. Dry it.
   b) For all experiments: Take 500 mL of the nitrate solution by means of a standardized flask and add it to the narrow measurement cylinder.
   c) Add a stirring magnet and place the container on a magnetic stirrer. Let it stir.
   d) Switch stirrer off to avoid bubbles, insert the UV probe until the volume on the measurement container reads around 900 mL, start the stirrer again.
   e) Check that no bubbles are caught under the sensor.
   f) Note the time, start the measurements. Ensure at least 5 measurements (approximately 6-7 minutes in total) are recorded.
   g) Lift the sensor from the measurement container.
   h) Add 1mL of nitrite stock solution.
   i) Insert the probe again (check for bubbles) and record at least 5 measurements.
   j) Remove the sensor from the measurement container.
   k) Add four more times 1mL of the nitrite stock solution and measure every time at least 6 spectra.
   l) Remove the container from the stirrer and empty the solution into the sink, without losing the magnet.

3) Dry the probe. Tidy up the lab place or start new experimental series.

4) Make a PDF scan of the lab journal and update the digitized log file.
8.1.2 Protocols for Phase 2

➢ Protocol A4: Preparation of urine samples

Aim:
Approximately 2 L of SoDAN Reactor content (nitrified urine).

Required products:
1) Approximately 2 L of SoDAN Reactor content (nitrified urine)

Required hardware:
1) 2 1L Plastic buckets with lids
2) Centrifuge
3) 2x 1000mL Schott glass
4) Scale (d= +/- 0.1g)

Steps:
1) Take approx 0.2L of nitrified urine from the tap on the SoDAN nitrification reactor, to pre wash one Schott glass bottle and the sample nozzle at the reactor.
2) Pour back the 0.2L in the reactor.
3) Take 1.5L of nitrified urine from the tap on the SoDAN nitrification reactor.
4) Write down the time and task in the SoDAN logbook.
5) Separate at least 1.1 L of clear liquid from 1.5L of sample as follows:
   a) Distribute the sample evenly so that both plastic buckets have the same weight +/- 0.1g including the bucket and the lid.
   b) Place both buckets opposite to each other into the centrifuge.
   c) Start the centrifuge for 4min at 2500 RPM
6) Decant supernatant into 2000mL Schott glass bottle. Make sure to have at least 1.500L in order to execute the experiment.
7) Clean the plastic bottle and waste the biomass.
8) If you do not have 1.5L of particle free urine repeated all steps from step c) with the approximate volume you need plus 25%

➢ Protocol A5: UV/Vis-measurements in urine spiked with nitrate and nitrite

Aim:
Prepare two 600mL urine samples spiked with two different nitrate concentrations.

Required products:
1) around 1.5L prepared urine (see protocol A4)
2) Nanopure water
3) NaNO3 salt
4) 2x Hach Lange Cuvette test LCK 340 (nitrate)
5) Hach Lange Cuvette test LCK 341 (nitrite)

Required hardware:
1) funnel
2) 500mL standardized flask
3) 5x 100mL standardized flask
4) 1x 1000mL Schott glass bottle
5) pipette (0.2mL, 1mL, 10mL)
6) stirrer und stirring magnet

Steps:
1) Take a sample of the prepared urine and measure nitrite and nitrate concentration with Hach-Lange Cuvette tests
   a) Filter the sample with a 0.4 μm glass microfiber filter to remove suspended particles.
   b) Dilute with a dilution of 1:100.
   c) LCK 340 measurements for nitrate: expected concentration around 2300 mgNO₃⁻-N/L
   d) LCK 341 measurements for nitrite: expected concentration around 2 mgNO₂⁻-N/L
2) Spike the filtered urine with nitrate, according to the experiment row.
   a) Weight Xg of NaNO₃ salt (see table below).
   b) Add the powder into the 500mL standardized flask via a funnel.
   c) Add the pure urine via funnel to the 500mL flask and shake.
   d) Fill 100mL of unspiked urine into a 100mL standardized flask
   e) Fill both the 500mL spike and 100mL unspiked urine into the 1000mL Schott Glass. Shake well!
   f) LCK 340 measurements for nitrate with a dilution of 1:250 (pipette 1mL of spiked urine into 250mL standardized flask, fill up with Nanopure water).
   g) If the result is insufficient, repeat from step 1.

Table 8.3 Information for preparing nitrate stock solution with nitrified urine samples

<table>
<thead>
<tr>
<th>Target concentration</th>
<th>≈2300 mgN/L</th>
<th>≈2600 mgN/L</th>
<th>≈2900 mgN/L</th>
<th>≈3200 mgN/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition of nitrate</td>
<td>0 mg/L</td>
<td>300 mg/L</td>
<td>600 mg/L</td>
<td>900 mg/L</td>
</tr>
<tr>
<td>NaNO₃ weight (g)</td>
<td>0</td>
<td>1.0922</td>
<td>2.1844</td>
<td>3.2767</td>
</tr>
<tr>
<td>Dilution for LCK 340</td>
<td>1:100</td>
<td>1:100</td>
<td>1:100</td>
<td>1:100</td>
</tr>
<tr>
<td>Expected LCK340 values</td>
<td>23.0</td>
<td>26.0</td>
<td>29.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

- Protocol A6: Preparation of nitrite stock solution based on urine spiked with nitrate

Aim:
Make 50mL nitrite stock solution based on urine with constant nitrate concentration (goal: 503 mL urine sample with X mg NO₃⁻-N/L and 75mg NO₂⁻-N/L consisting of 500mL of nitrite free urine and 3mL of nitrite stock solution consisting of urine).

Required products:
1) NaNO₂ powder
2) urine spiked with NO₃⁻, NO₂⁻-N concentrations of around 2300, 2600, 2900, 3200 mg/l NO₂⁻-N (depending on the experiment) See protocol A5!
3) Nanopure water

Required hardware:
1) Funnel
2) 1x 50 mL standardized flask
3) 2x 100 mL standardized flask
4) Magnet stirrer with magnet
5) Pipette (0.2, 0.1 mL)
6) LCK 342 Nitrite Lange Tests

Steps:
1) Weigh 3.0971 of the NaNO₂ powder
2) Dissolve powder into urine spiked with nitrate
3) LCK 342 measurements for nitrite with a dilution of 1:5000 (1 mL stock solution into a 100 mL flask (shake the flask), from this solution 2 mL).

Evaluate the quality of the solution (Expected concentration: 2.52 mgN /L, effect of nitrite in urine negligible). Repeat steps a-g if the obtained quality is insufficient

Calculations:

Nitrite:
Desired concentration: 75 mg NO₂⁻/L
Amount of NaNO₂ for 50 mL stock solution:

\[
M_{NaNO_2} = \frac{75 \text{ mgN}}{L} \cdot \frac{503 \text{ mL}}{3 \text{ mL}} \cdot \frac{0.05L}{1000 \text{ mL}} \cdot \frac{1 \text{ gN}}{68.995 \text{ gNaNO}_2} \cdot \frac{14.007 \text{ gN}}{\text{mol}} \cdot \frac{50 \text{ mL}}{1 \text{ mol}}
\]

\[
= 3.0970662 \text{ gNaNO}_2
\]

- **Protocol A7: Calibration data for simultaneous nitrate and nitrite estimation with UV-Vis sensor in urine with changing background**

Aim:
Test UV-Vis sensor with a constant urine background conditions for estimating nitrite and nitrate.

Required products:
1) Stock solution Nitrite (goal: 75 mgN/L, 9.4500 gN/L solution ) Protocol A6
2) Urine spiked with nitrate (X mgN /L) Protocol A5

Required hardware:
1) narrow measurement cylinder (1000 mL)
2) standardized 500 mL flask
3) Magnet stirrer with magnet
4) Pipette (1 mL)
5) s::can Sensor with 5mm gap
6) Memory Stick

Experimental plan:
2-5 nitrate concentration 0, 25, 50, 75 mgNO₂-N/L
A-D nitrite concentration: 2300, 2600, 2900, 3200 mgNO₃-N/L

<table>
<thead>
<tr>
<th>NO₂-N/NO₃-N</th>
<th>A (0)</th>
<th>B (1.0922g)</th>
<th>C (2.1845g)</th>
<th>D (3.2767g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (0)</td>
<td>S4.4</td>
<td>S2.2</td>
<td>S3.1</td>
<td>S1.3</td>
</tr>
<tr>
<td>3 (25)</td>
<td>S1.1</td>
<td>S3.3</td>
<td>S2.4</td>
<td>S4.2</td>
</tr>
<tr>
<td>2 (50)</td>
<td>S3.2</td>
<td>S4.1</td>
<td>S1.2</td>
<td>S2.1</td>
</tr>
<tr>
<td>1 (75)</td>
<td>S2.3</td>
<td>S1.4</td>
<td>S4.3</td>
<td>S3.4</td>
</tr>
</tbody>
</table>

Table 8.4 Information for measuring absorbance spectra considering different backgrounds
**Steps:**

1) Prepare the sensor for measurement. Clean it with nanopure water and dry it.
2) Take 500 mL of the urine spiked with nitrate solution by means of a standardized flask and add it to the narrow measurement cylinder.
3) Add a stirring magnet and place the container on a magnetic stirrer. Let it stir.
4) Switch stirrer off to avoid bubbles, insert the UV probe until the volume on the measurement container reads around 830 mL, start the stirrer again.
5) Check that no bubbles are caught under the sensor.
6) Note the time, start the measurements. Ensure at least 6 measurements (approximately 7-8 minutes in total) are recorded.
7) Lift the sensor out of the measurement container but keep it above the container to collect any dripping solution.
8) Add 1mL of nitrite stock solution, let it stir for 2min.
9) Insert the probe again (check for bubbles) and record at least 6 measurements.
10) Add two more times 1mL of the nitrite stock solution and measure every time at least 6 spectra.
11) The data exactly corresponds to the sample listed in Table 8.4 are used for modelling.

**8.2 Additional Information of Material**

Fig 8.1 shows the graphs of Hach-Lange tests. Table 8.5 describes the composition of urine. Table 8.5 shows on which day the urine samples were taken and measured, including some comments.

---

*Fig 8.1: Images of Hach-Lange cuvette and Hach DR 2800 spectrophotometer*
8.3 Additional Information of Phase 2

The concentrations of nitrate and nitrite stock solutions were measured by LCK 340 and LCK 342, respectively. Results are displayed in Fig 8.2.

![Fig 8.2: Results of Hach-Lange cuvette tests vs. expected concentrations](image-url)
8.4 Matlab Code

The used Matlab code during this project are listed in this section.

8.4.1 Codes for Phase 1

clear all

% basic selecting of data

wavelengths=[200:2.5:750];
start_time_in='18.10.2016 12:00:00';
end_time_in='25.10.2016 18:00:00';
labjournal_filename='labjournal_spectra.csv';

[NO2_conc_out,NO3_conc_out,NO3_volu_out,NO2_volu_out,spectra_out,spectra_timestamps_out, csv_filename] = ...
    get_spectra(start_time_in,end_time_in,labjournal_filename);
X=spectra_out(:,21:215); % to remove the NaN data
wavelengths_cut=wavelengths(:,21:215);
y_m=NO2_conc_out'; % define matrix of measured NO2-N conc.

M2250=mean(X(180:185,:)); %compute the average absorbance of 6 repetitions fro nitrate
M0=mean(X(144:149,:));
M2750=mean(X(108:113,:));
M2500=mean(X(72:77,:));
M2000=mean(X(36:41,:));
M3000=mean(X(1:5,:));
figure,plot(wavelengths_cut,M3000,wavelengths_cut,M2750,wavelengths_cut,M2500,wavelengths_cut,M2250,wavelengths_cut,M2000,wavelengths_cut,M0);
ylim([0,200]);
set(gca,'YTick',[0:50:200]);
xlim([250,700]);
set(gca,'XTick',[250:50:700]);
xlabel('Wavelength(nm)');
ylabel('Absorbance(AU)');
legend({'3000 mgN/L : 0 mgN/L' '2750 mgN/L : 0 mgN/L' '2500 mgN/L : 0 mgN/L' '2250 mgN/L : 0 mgN/L' '2000 mgN/L : 0 mgN/L' '0 mgN/L : 0 mgN/L'},'location', 'NW');%above is the plot of increase of NO3-N conc.

% plot spectra with constant nitrate conc.

- 30 -
M00=mean(X(144:149,:));%compute the average absorbance of 6 repetitions for nitrite
M15=mean(X(150:155,:));
plot(wavelengths_cut, X(150:155,:));
M30=mean(X(156:161,:));
M45=mean(X(162:167,:));
M60=mean(X(168:173,:));
M75=mean(X(174:179,:));
figure,plot(wavelengths_cut,M00,wavelengths_cut,M30,wavelengths_cut,M45,wavelengths_cut,M60,wavelengths_cut,M75);
xlabel('Wavelength(nm)');
ylabel('Absorbance(AU)');
legend({'0 mgN/L : 0 mgN/L' '0 mgN/L : 30 mgN/L' '0 mgN/L : 45 mgN/L' '0 mgN/L : 60 mgN/L' '0 mgN/L : 75 mgN/L'},'location', 'NW');
ylim([0,20]);
set(gca,'YTick',[0:5:20]);
xlim([250,700]);
set(gca,'XTick',[250:50:700]); %above is the plot of increase of NO2-N conc.

8.4.2 Codes for Phase 2

clear all
%------------------------
% basic selecting of data
%------------------------

wavelengths=[200:2.5:750];
start_time_in='02.11.2016 10:00:00';
end_time_in='23.11.2016 14:00:00';
labjournal_filename='labjournal_spectra_remove3.csv';
%labjournal_filename='labjournal_spectra_original.csv';

[NO2_conc_out,NO3_conc_out,NO3_volu_out,NO2_volu_out,spectra_out,spectra_timestamps_out, csv_filename] = ...
    get_spectra(start_time_in,end_time_in,labjournal_filename);

cutoff=33; %cutoff at 280 nm
wavelength_cut=wavelengths(:,cutoff:215);
X=spectra_out(:,cutoff:215);
y_m=NO2_conc_out'; % define matrix of measured NO2-N conc.
[N, M]=size(X);
%-------------------------------------------------------------------
% leave-one-out cross validation analysis
%-------------------------------------------------------------------
PCs=min(N-1,M)-1;

index=1:N; % create the sample_index to refer to the samples

for i=1:length(index)
    i;
    cal_temp=index~=i;
    cal=cal_temp~=0;
    val=index==i;
    Xval=X(val,:);
    Xcal=X(cal,:);
    Ym_cal=y_m(cal);
    Ym_val=y_m(val);
    %-------------------------------------------------------------
    % centering the validation and calculation set
    %-------------------------------------------------------------
    [m, n]=size(Xcal);
    mn=mean(Xcal);
    Xcal_center=Xcal-repmat(mn,m,1);
    Xval_center=Xval-repmat(mn,1,1);
    %in order to compute the center of Xval, the repmat should be
    % repmat(ab,a,1) or not?
    %-------------------------------------------------------------
    % singular validation decomposition (PCA analysis)
    %-------------------------------------------------------------
    [Ucal,Scal,Vcal]=svd(Xcal_center,'econ');
    Tcal=Xcal_center*Vcal;
    Tval=Xval_center*Vcal;
    %-------------------------------------------------------------
    % regression for the calculation set
    %-------------------------------------------------------------
    for d=1:PCs
        d;
        Tscal=Tcal(:,1:d); % define the score matrix of calculation set when PCs equals d
        Tsval=Tval(:,1:d); % define the score matrix of validation set when PCs equals d
        [p, q]=size(Tscal);
        Zcal=[ones(p,1) Tscal];
        [r, s]=size(Tsval);
        Zval=[ones(r,1) Tsval];
        P_cal=((Zcal'*Zcal)^-1)*Zcal'*Ym_cal;
        %-------------------------------------------------------------
        % use the P_cal to predict the predicted NO2-N conc.
        %-------------------------------------------------------------
        Yp_val(i,d)=Zval*P_cal;
        error_val(i,d)=(Ym_val-Yp_val(i,d)).^2;
    end
end
residuals(i,d)=Yp_val(i,d)-Ym_val;

end

SSR=sum(error_val(:,1:PCs)); % calculate the sum of squared residuals of different PC
ASR=sum(error_val(:,1:PCs))/N; % compute the average squared residuals of different PC
STD=std(residuals(:,1:PCs));
[w, v]=min(ASR); % decide the corresponding PCs numbers when ASR min
STD_v=std(residuals(:,v));
CV(:,1)=y_m;
CV(:,2)=Yp_val(:,v); % define a matrix to store the measured and predicted conc. in CV procedure
TSS=sum((y_m-mean(y_m)).^2);
RSS=sum((CV(:,2)-CV(:,1)).^2);
R_square=1-RSS/TSS;

%-----------------------------
% final PCR model after determining the number of PCs
%-------------------------------

mean_X=mean(X); %mean_X will be applied finally
X_center=X-repmat(mean_X,N,1);
[U,S,V]=svd(X_center,'econ'); %V will be applied finally
T=X_center*V;
Ts=T(:,1:v);
[row_T, column_T]=size(Ts);
Z=[ones(row_T,1) Ts];
P=((Z'*Z)^-1)*Z'*y_m; % P is the coefficient, which will be applied finally
Yp=Z*P;
R=Yp-y_m; % R is the residuals

%-----------------------------
% eigenvalue anlysis
%-----------------------------

var=diag(S).*diag(S);
var_p=100*cumsum(var)/sum(var);
figure,plot(1:15,var_p(1:15,:),'r^-');
xlim([0,15]);
set(gca,'XTick',[0:3:15]);
xlabel('Number of Principal Components')
ylabel('Percent Explained in Y (%)')

% plots

f=fit(CV(:,1),CV(:,2),'poly1');
figure,plot(f,CV(:,1),CV(:,2),'.');
xlabel('Measured Nitrite Conc.(mgN/L)');
ylabel('Predicted Nitrite Conc.(mgN/L)'); % plots "Predicted vs. measured"

plot(wavelength_cut,X);
xlim([200,750]);
set(gca,'XTick',[200:110:750]);
xlabel('Wavelength (nm)');
ylabel('Absorbance (AU)'); % plots of spectra

figure,plot(1:PCs,ASR);
xlabel('Number of Principal Components');
ylabel('Mean of Squared Residuals ((mgN/L)^2)');
ylim([0,10]);
set(gca,'YTick',[0:2:10]);
xlim([0,60]);
set(gca,'XTick',[0:10:60]); % plots 'ASR vs. NPCs'

%for j=1:N/6
  % sample_index((1:6)+(j-1)*6)=j;
%end

figure,scatter(sample_index,R,'.');
xlabel('Sample Index');
ylabel('Residuals (mgN/L)');
title('final PCR');
figure,scatter(sample_index,residuals(:,v),'.');
xlabel('Sample Index');
ylabel('Residuals (mgN/L)');
title('Cross validation');