**9th IWA Symposium on Systems Analysis and Integrated Assessment**
**14-17 June 2015, Gold Coast, Australia**

# Nitrite estimation in nitrified urine by UV spectroscopy of weak nitrite/nitrate absorbance peaks

**Mašić, A.*, Santos, A.T.L.**, Hess, A.***, Thürlimann, C.*, Etter, B.*, Udert, K.M.* and Villez, K.***

* Eawag: Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland
** Faculty of Science and Technology, Universidade Nova de Lisboa, Portugal
*** Department of Civil, Environmental and Geomatic Engineering, ETH Zurich, Switzerland

**Keywords:** nitrite estimation; source-separated urine; UV spectrophotometry

## Summary of key findings

In this study, nitrite estimation with ultraviolet (UV) spectrophotometry in undiluted, unfiltered, nitrified urine is investigated. Early detection of nitrite accumulation in a nitrification reactor is essential for preventing its failure as temporary accumulations can quickly lead to permanent loss of reaction performance. Current results suggest that secondary nitrite and nitrate peaks in the UV absorbance spectrum, which are weaker than those typically targeted, are useful in our case because of the much higher nitrite and nitrate concentrations found in nitrified urine than in conventional wastewater systems. This is expected to permit deployment of commercially available instruments even when saturating effects in the lower UV range result from high nitrate concentrations. The absorbance spectra are used in combination with a classic chemometric model, principal component regression (PCR), to obtain estimates of nitrite. Moreover, a UV-Vis sensor with a lower wavelength resolution and a longer measurement path is presented as a preferable alternative to a UV sensor.

## Background and relevance

Decentralized wastewater treatment has emerged as a suitable option for resource recovery from urine, with the aim of producing fertilizers (Larsen et al., 2013). The treatment of source-separated urine is faced with challenges due to loss of valuable nutrients during its storage. To overcome this problem, urine is subjected to nitrification – a biological process in which bacteria convert ammonia to nitrate via nitrite – after which the urine is safely stored until further processing (Udert&Wächter, 2012).

Nitrification is relatively stable, but it can be severely affected by sudden changes in influent load, in some cases leading to an irrecoverable failure of the reactor (Rieger et al., 2008). Under normal operation, nitrite in the reactor remains near zero since it is as quickly consumed as it is produced. However, when ammonia suddenly increases, the ammonia oxidizing bacteria convert more ammonia to nitrite, thereby increasing the load on the nitrite oxidizing bacteria (NOB). Since both species compete for oxygen, nitrite begins accumulating in the reactor, which in turn further inhibits the NOB, leading to a buildup of nitrite. If the buildup is not quickly mitigated, the reactor risks a complete failure. Thus, monitoring of nitrite in the reactor is essential. Currently, there is no direct and affordable online method available and operators instead have to resort to frequent sample analyses.

In our previous work (Mašić et al., in prep.) we studied the potential use of a UV sensor as a tool in combination with chemometrics to estimate nitrite in nitrified source-separated urine. Both nitrite and nitrate absorb light in the UV range, with peak absorbances at around 200 nm, making it theoretically possible to determine the concentration of nitrite (Sun et al., 2012). In the study, effects of suspended particles and high concentrations of nitrite and nitrate that can interfere with the absorbance, potentially leading to unreliable measurements, were investigated. Chemometrics allows constructing mathematical models, establishing empirical relationships between input signals, such as absorbance spectra, and output signals, such as compound concentrations (Drolc&Vrtovsek, 2001). Assuming a linear relationship, a linear regression technique (PCR) based on principal component analysis (PCA) (Haimi et al., 2003) for dimension reduction of the high-dimensional spectral data was applied. It was shown that a submersible in-situ UV sensor can be used in offline urine samples for determination of nitrite. The effect of suspended particles was negligible and the saturation effect could be mitigated by a reduction of the absorbance spectra through removal of the saturated, lower end of the UV range. A simple chemometric model could be used to obtain a credible estimation of nitrite, even in saturated samples.

In this work, we study nitrite estimation with UV spectrophotometry in undiluted, unfiltered offline urine samples, with and without background nitrate variation. Moreover, we investigate the reasons why it suffices to consider spectra with removed short wavelengths to obtain good estimates of nitrite and look into the possibility of using a standard UV-Visible sensor with a lower resolution.

**Results and discussion**

Evaluation of spectral data, collected over 11 weeks with a submersible in-situ UV sensor (s::can spectro::lyser, 0.5 mm path length, 1 nm resolution), shows a good fit (Fig. 1a) between estimated and measured nitrite concentrations with a model consisting of 2 principal components (PCs). The nitrified urine was only subjected to sedimentation before the measurements, without filtering or dilution. During the sampling time, nitrate concentration remained high and almost constant. The UV sensor records spectra in the range 220-399 nm (Fig. 1b). The signal is saturated in the first part of the range, which can be interpreted as a loss of signal, where also the absorbance peaks of nitrite and nitrate are found. In this case, the model removes the first 94 wavelengths and considers the spectra in a range 314-399 nm, marked by a dashed vertical line in Fig. 1b. This indicates that the second half of the spectra contains sufficient information about nitrite. One way of visualizing this is by looking at the regression vectors obtained through PCR. In Fig.1c we see the regression vectors that were multiplied by the spectra in Fig. 1b to make the estimation in Fig. 1a. The regression vectors show a positive peak around 355 nm, emphasizing this part of the spectra that leads to a good estimate of nitrite.
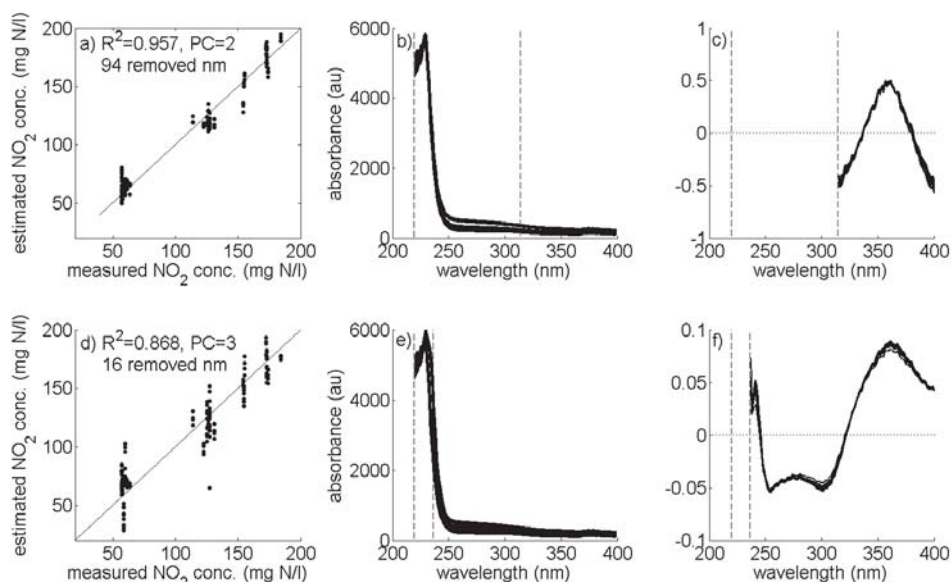


**Figure 1. (a),(d): Estimation of nitrite (mg N/L); (b),(e): absorbance spectra; (c),(f): regression vectors.**

When nitrate variation is present in the reactor (Fig. 1d) it influences the model performance by slightly weakening the estimation. Here, a model consisting of 3 PCs was used, removing the first 16 wavelengths and considering the range 236-399 nm (Fig. 1e). In this case, only the completely saturated part of the signal is removed. The regression vectors in Fig. 1f show a similar peak around 355 nm as in Fig. 1c, but with another pronounced negative peak around 300 nm. These two peaks recur in other data sets that have been modeled (data not shown). Upon further investigation we have found that both nitrite and nitrate have additional weaker peaks in the UV range: nitrite at 354 nm and nitrate at 302 nm (Spinelli et al., 2007). These weaker peaks are typically not visible in a standard wastewater application, but due to the very high concentrations in a failing nitrification reactor with source-separated urine, this suggests that these secondary, weaker peaks absorb enough light to emit a good signal. Since these peaks are well separated from each other, there is no need to use a high-resolution sensor. Instead, a UV-Vis sensor with a resolution of 2.5 nm could also be used.

To confirm the above finding, a preliminary test with a UV-Vis sensor (s::can spectro::lyser, 2 mm path length, 2.5 nm resolution) was used to measure the absorbance in nitrified urine with addition of nitrite and nitrate stock solutions. In Fig.2a we see the spectra from 3 samples, each with 5 replicate measurements of the same sample. The spectrum from the urine is denoted by a black solid line, with

added nitrite by a green dashed line and with added nitrate by a blue line with small markers. The first part of the spectrum is once again saturated and resembles the spectra seen in Figs. 1b & 1e. However, the nitrate peak at 302 nm can now clearly be seen as well as the nitrite peak. Fig.2b shows a close-up of the peaks and we see that they are formed due to differences in nitrate and nitrite concentrations, respectively. These measurements suggest that a UV-Vis sensor records sufficient data accurately and more pronounced than the UV sensor. Moreover, the UV sensor has a measuring path length of 0.5 mm while the UV-Vis has a path length of 2 mm, making the latter much easier to maintain and clean. As such, it seems to be the optimal sensor for a future development towards online implementation.
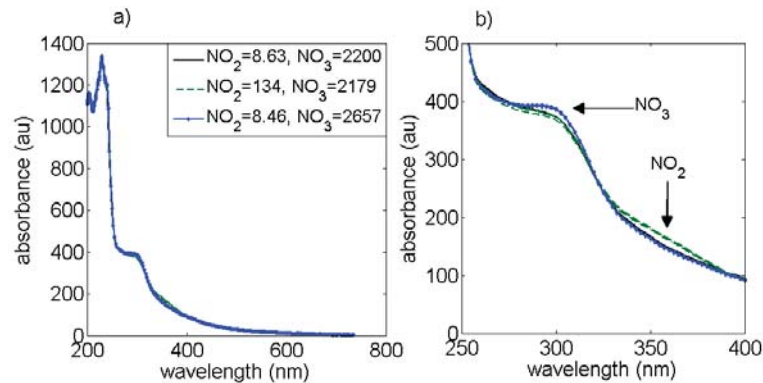


**Figure 2. UV absorbance spectra with focus on nitrite and nitrate peaks.**

### References

Drolc, A. and Vrtovsek, J. (2010) Nitrate and nitrite nitrogen determination in waste water using on-line UV spectrometric method, *Bioresource Technology*, 101, 4228–4233

Haimi, H., Mulas, M., Corona, F., Vahala, R. (2013) Data-derived soft-sensors for biological wastewater treatment plants: An overview, *Environmental Modelling & Software,* 47, 88–107

Larsen, T.A., Udert, K.M., Lienert, J. (2013) Source separation and decentralization for wastewater management, IWA Publishing, London, UK.

Mašić, A., Santos, A.T.L., Etter, B., Udert, K.M., Villez, K. (in preparation) Estimation of nitrite in source-separated nitrified urine with UV spectrophotometry

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

Sun, F.Y., Dong, W.Y., Shao, M.F., Li, J., Peng, L.Y. (2012) Stabilization of source-separated urine by biological nitrification process: treatment performance and nitrite accumulation, *Water Science & Technology,* 66(7), 1491–1497

Spinelli, S., Gonzalez, C., Thomas, O. (2007) UV spectra library, In: O. Thomas, C. Burgess (Eds.), *UV-Visible spectrophotometry of water and wastewater,* Elsevier, Amsterdam.

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