Master thesis

DO and pH sensor characterization for the soft sensing of urine nitrification systems

Author: Elisabeth Grimon
elisabeth.grimon@yahoo.com

Head: Prof. Dr. Eberhard Morgenroth

Supervisors: Dr. Kris Villez
Supervisors: Christian Thürlimann

Zurich, 19 Mai 2015

Photo on title page: pH step change test in pH 4 and pH 7 adjusted samples, with two pH sensors. The current experiment is with cleaned sensors and reactor medium that has been previously filtered and autoclaved (Elisabeth Grimon).

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Declaration of originality

The signed declaration of originality is a component of every semester paper, Bachelor's thesis, Master's thesis and any other degree paper undertaken during the course of studies, including the respective electronic versions.

Lecturers may also require a declaration of originality for other written papers compiled for their courses.

I hereby confirm that I am the sole author of the written work here enclosed and that I have compiled it in my own words. Parts excepted are corrections of form and content by the supervisor.

Title of work (in block letters):

DO and pH sensor characterization for the soft sensing of urine nitrification systems

Authored by (in block letters):
For papers written by groups the names of all authors are required.

Name(s):	First name(s):
Grimon	Elisabeth Françoise Florence

With my signature I confirm that

- I have committed none of the forms of plagiarism described in the '*Citation etiquette*' information sheet.
- I have documented all methods, data and processes truthfully.
- I have not manipulated any data.
- I have mentioned all persons who were significant facilitators of the work.

I am aware that the work may be screened electronically for plagiarism.

Place, date	Signature(s)
Zurich, 19/05/2015	

For papers written by groups the names of all authors are required. Their signatures collectively guarantee the entire content of the written paper.

Abstract

At the Eawag, source-separated urine is stabilized to produce a value added fertilizer. Nitrite accumulation is one of the main threats to the reactors, and so estimating nitrite is essential. Soft sensing with pH and DO (dissolved oxygen) sensors is a promising solution, but these sensors are not ideal. This master thesis aims to evaluate if soft-sensing is still of value when considering a more realistic sensor behavior.

First, the DO and pH sensors present in the reactor were characterized in terms of their response time, precision, and trueness. This was undertaken both in field and standard conditions, and with dirty or clean sensors. Sensor models were set up. The UKF was then run on simulated data, with and without realistic sensor behavior.

Sensor cleaning or field conditions had little influence on the data. Measurement direction made the biggest difference on the response time of the DO sensor. DO and pH sensor dynamics were found to fit a modeled 2nd order and 1st order response, respectively. While the nitrite increase could be detected, the UKF performance was severely affected by realistic sensor behavior. A number of further evaluations or approaches are suggested to improve the performance of this soft sensor.

Acknowledgements

Table of Contents

1. Introduction

Source-separation and treatment of urine is a promising decentralized treatment option for developing countries. Udert & Wachter (2012) suggested a system with biological nitrification to further recover nutrients and produce a value-added fertilizer. This was implemented in the VUNA project in a moving bed biofilm reactor MBBR (Etter *et al.*, 2015). One of the main challenges here is the accumulation of nitrite, which can rapidly lead to process failure. For long-term operation under varying loads, estimating nitrite is essential.

Soft sensors have been widely used in the process industry to estimate unmeasured quantities. In their review of the wastewater treatment field, Bourgeois *et al.* (2001) emphasize on the need for a shift from laboratory techniques, which estimate load parameters, to real-time and in-line techniques. Previous work from Masic and Villez (2014) showed soft sensor potential for the estimation of nitrite in nitrification reactors.

Modeling sensor behavior is important when Spanjers and Olsson (1992) improved their calculation of the respiration rate by modeling DO sensor response. Alex *et al.* (2003) further demonstrate that using realistic sensor models becomes necessary when simulation is used to determine the control structure and parameters.

Sensor uncertainty can be characterized by its response time, precision and trueness, as defined in the ISO standards (ISO, 1994; ISO, 2003). Furthermore, Rieger *et al.* (2005) emphasize the importance of measuring sensor uncertainty under field conditions, such as in the reactor itself, where the water matrix will be different and varying.

While this master thesis has a smaller scope, the ultimate goal would be to have a soft sensor that estimates nitrite sufficiently well with an operational reactor. The idea would be to install a control system based on such estimates. Such an ultimate goal would require both a good model performance as well as a good soft sensor performance. This master thesis aims to characterize and model of DO and pH sensors for the soft-sensing of urine nitrification systems.
Does sensor behavior affect soft sensor performance?

In a first step, the nitrification system and model will be described. Then, soft-sensing and the UKF will be introduced. Finally, sensor characteristics will be defined, and realistic sensor modeling reviewed, especially for DO and pH sensors.

1.1.   Nitrification system and ongoing nitrite challenge

VUNA project and nitrification system
The VUNA (Valorization of Urine Nutrients in Africa) project aims to "harvest" (south African "vuna") a value-added fertilizer from urine using small, affordable, and resilient decentralized reactors. In fact, nutrients such as nitrogen, phosphorous, and potassium are excreted almost only through urine. Figure 1 explains the two-step nitrification process driven by the activity of AOBs (ammonia oxidizing bacteria) and NOBs (nitrite oxidizing bacteria). Stabilization of the urine, through a nitrification process, is necessary to avoid high nitrogen losses due to NH_3 volatilization and unpleasant odors.

The two urine reactors in the Forum Chriesbach at the Eawag have been (sometimes intermittently) in operation since 2011. This includes two buffer tanks and a distiller to concentrate the end product. Further information about the reactor setup is available in Etter *et al.* (2013), and further information about the broader scope and research is available in the final VUNA report (Etter *et al.*, 2015).
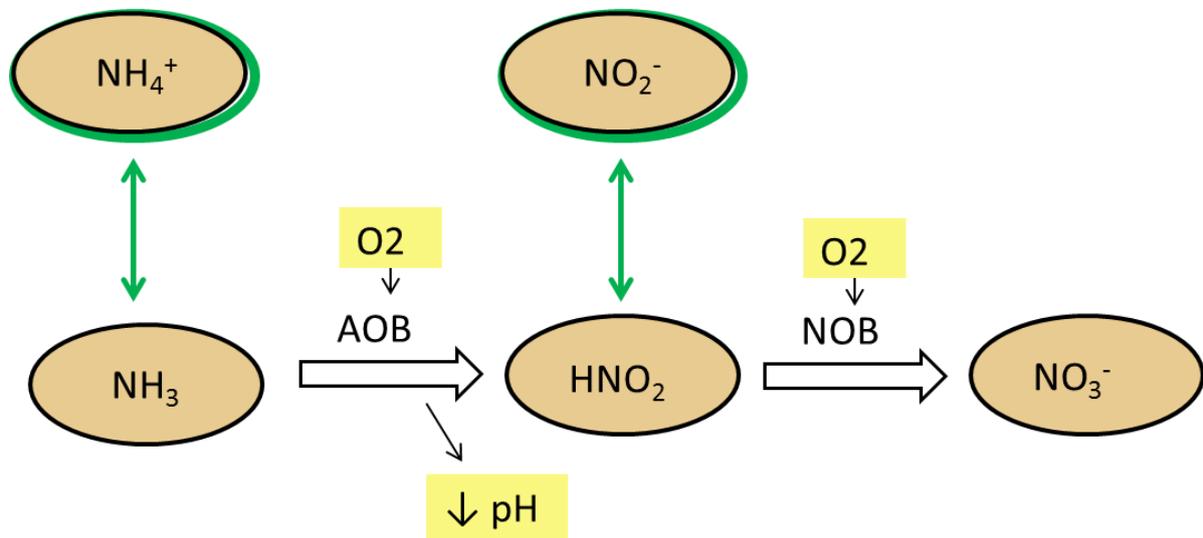
Figure 1. Nitrification process as modeled. Acid-Base equilibria in green with main components in green circles. (AOB: Ammonia Oxidizing Bacteria ; NOB: Nitrite Oxidizing Bacteria)

Nitrification model

The original urine nitrification system model was developed by Fumasoli and Garbani, and described in Garbani (2014). This is a dynamic, explicit, and deterministic model taking into account the urine buffer tank, urine reactor, and distiller. The Nitrification is modeled as two-step process, driven by AOBs and NOB activity. The list of the 37 states modeled can be found in Appendix A.1. The kinetics and parameters of the process matrix can be found in Appendix A.2. The pH inhibition of the AOBs was specifically developed by Fumasoli *et al.* (2015). NOBs were inhibited by HNO_2. No heterotrophs were added. Biological equations involve growth and decay of NOBs and AOBs. Chemical reactions included Acid-Base reactions, including the carbonate system and the formation of phosphate and sulfate complexes. Gas exchange is important for the O_2 concentration, and CO_2 stripping. Calibration and validation of the parameters of the model is still ongoing.

Nitrite estimation challenge

One of the greatest challenges for reactor resilience and stability is the accumulation of nitrite, which inhibits nitrite oxidation by NOBs and may lead to failure of the system above 50 mgN/L. Currently, nitrite is only measured only offline: once a week with Dr. Lange tests and more often with nitrite strips. The inflow rate can be varied to control the ammonia oxidation rate, but action may need to be taken within a couple hours. A continuous estimation of the actual nitrite concentrations is necessary. No online sensors are currently able to measure nitrite continuously and reliably under such potentially high concentrations and high salinity levels.

The oxidation of nitrite to nitrate is a key process for the safety and effectiveness of the end-product and for reactor stability. The WHO (2011) reviewed the human health hazards from nitrite and nitrate, and set drinking water guidelines at 3 mg/L and 50 mg/L, respectively. Nitrite exposure is especially dangerous for infants (blue baby syndrome / Methaemoglobinaemia). Moreover, nitrate is a more stable compound. With ammonium and nitrite in the final end-product, the nitrogen content could be lost as nitrogen gas by an anammox process. The stability can further be observed as nitrate is widespread in the environment. On the contrary, nitrite is only found under reduced conditions. Finally, the accumulation of nitrite and thus nitrous acid will also lead to inhibition of the AOBs, and still lead to process failure.

Different methods are currently being examined for the *in-situ* monitoring of nitrite in the nitrification reactor. Hess (2015) showed concluding results with UV-Vis sensors, but additional costs remain important. Soft-sensing with ammonia and nitrate measurements was evaluated by Masic and Villez (2014) for simulated data, but such sensors would also be expensive to install. Both of these would impose significant limitations for further development as a decentralized technology.

pH and DO measurements may also provide information for nitrite estimation (Figure 1). Assuming endogenous respiration as negligible, DO measurements can be directly linked to AOBs and NOBs and their activity. pH measurements can then help determine the percentage of O2, which is used for the oxidation of ammonia. Soft-sensing would be a much cheaper alternative as DO and pH sensors are already present in the urine reactor. This master thesis focuses on this option: soft sensing with pH and DO measurements.

1.2.   Soft sensing and the UKF

Soft sensing concept

Soft-sensing is the simultaneous processing of measured quantities in a computer software to obtain unmeasured quantities of interest. Typically, a soft sensor uses a model to relate to the unmeasured quantities. A model within the soft sensor relates the unmeasured quantities with measured variables. Soft sensors are sometimes also refered to as inferential sensors, virtual on-line analysers and observer-based sensors (Kadlec *et al.,* 2009).

Gonzales (1999) and Kadlec *et al.* (2009) review some of some soft sensor research and applications, with a focus on process industries. Soft sensors can be of assistance for:

- On-line Prediction
- Process Fault Detection
- Process Monitoring and more optimized control strategies
- Sensor Fault Detection
- Sensor backups when these become unavailable or when measurements are insufficient (too sparse in time and space, too much delay)
- Cheaper alternatives to expensive sensors.

They further refer to a number of different soft-sensors.

Unscented Kalman Filter (UKF)

The Unscented Kalman Filter is recursive algorithm for state estimation, which uses a physically interpretable model. The unobserved states are defined over time by estimates in their mean and covariance matrices. Measurements are used to correct these estimates. The Unscented Kalman Filter is an improvement of the Kalman Filter, where the mean and covariance are still captured when undergoing a nonlinear transformation. Julier and Ulhmann (2004) review the motivations, algorithms, and implications of the UKF. As further illustrated in Figure 2 the UKF is composed of two steps:

- A prediction / time update step, where the state estimates are obtained thanks to a model, and are based on the updated previous time step.
- A measurement update state, where the inputs from sensor measurements are used to correct the predicted states. The Kalman gain represents the relative importance of the error with respect to the prior estimate. These updated states are then fed back into the model.

In the UKF, state distribution is defined by a discrete number of chosen sample points, which capture the true mean and covariance of the state estimates. For this, $1+2*(n_x + n_v)$ samples are necessary, where n_x is the number of states and n_v the number of inputs. Thus, increasing the number of states leads to a linear increase in the computation time.
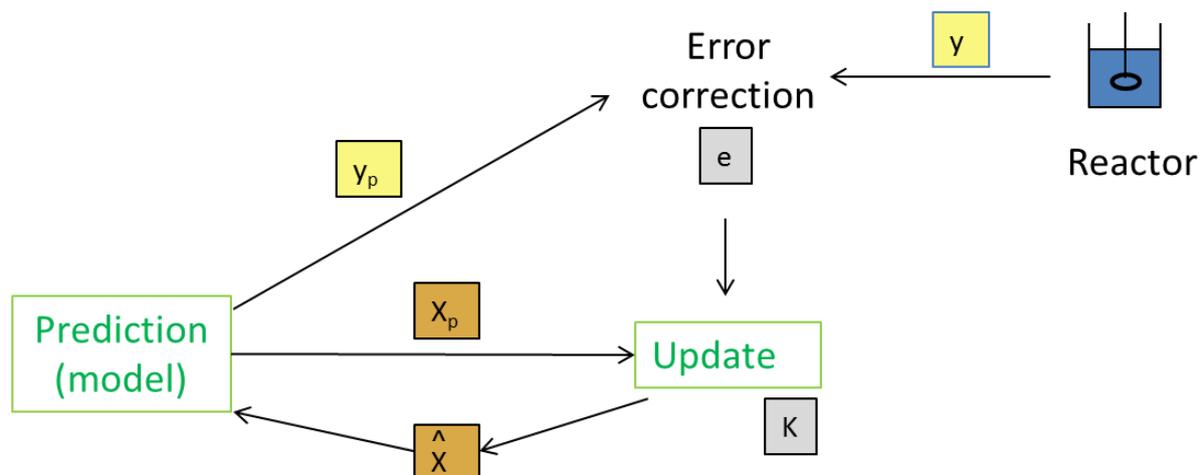
Figure 2. Functioning of the UKF, with y the measured quantities and their predictions, X the predicted and estimated states, e the error correction, K the Kalman gain used to update the states.

In the nitrification system explained in part 1.1 above, the UKF is of particular interest as a soft sensor. In fact, a physically-interpretable model exists. The UKF can capture the nonlinearities imposed by this model. When developing a control by simulation, though, Alex *et al.* (2003) showed that modeling realistic sensor behavior is necessary.

1.3. Characterizing sensor uncertainty and sensor models

Standards for the characterization of sensor uncertainty

An example of the difference between ideal and realistic sensors is illustrated with DO measurements in Figure 3. When regarding only the measuring equipment, sensors can be characterized by their precision, trueness, and response time. Standard definitions for these three characteristics are shown in Table 1, and an extended version with ISO notes can be found in Appendix A.3.

To measure the response time, an instantaneous change in conditions is necessary. While more complicated for sensors requiring sample preparation, this is generally perfomed by changing the sensor between different buckets containing different concentrations (Rieger *et al.*, 2003). Philichi and Stenstrom (1989) examined the response time error of DO probes with different membrane thicknesses in both DO directions, from high to low DO concentrations and vice versa. The instantaneous change was obtained, for example, by having the DO in small beaker of low DO, itself being partly immersed in a much larger aerated tank. The small beaker was rapidly lowered into the tank while the DO sensor stayed fixed, now immersed in the tank medium. Nitrogen gas was used to obtain low DO concentrations.
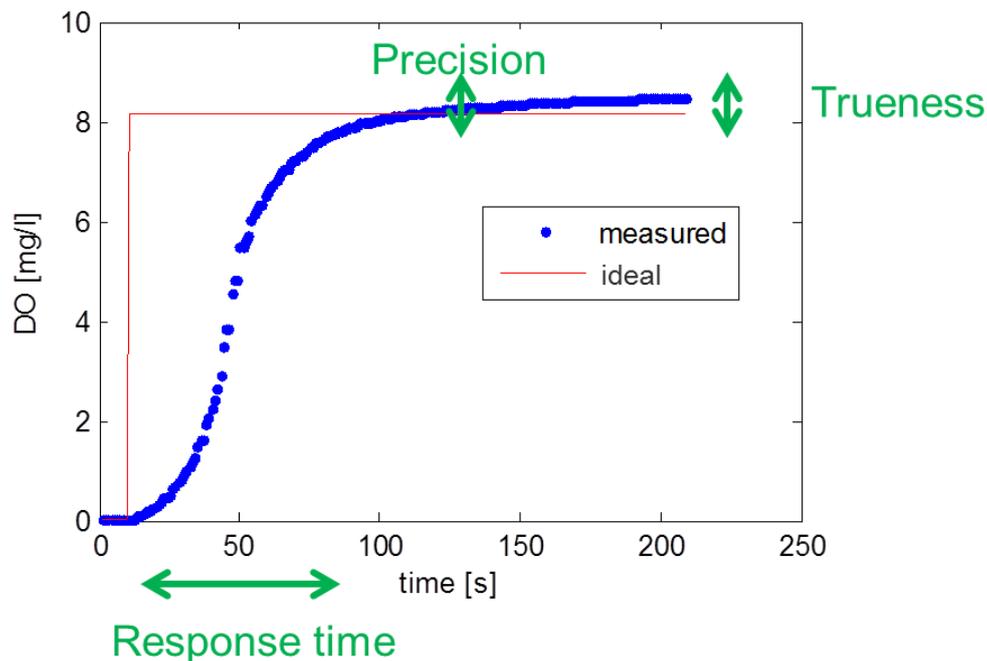
Figure 3. Ideal and realistic DO sensor for a step change in nanopure water with a clean sensor at 23°C. The ideal sensor curve was obtained by a step change from a beaker with no oxygen to a 2nd beaker at saturation. The saturation value was obtained from the sensor operating instructions (Endress + Hauser, 2012)

Realistic sensor models

Rieger *et al.* (2003) suggested two groups of sensor models for use in testing control strategies within the COST simulation benchmark environment: sensor specific and for different classes of sensors.

First, when sensor response time, measurement range, trueness and precision are known, Rieger *et al.* (2003) suggest that models describing specific sensors could be set up to optimize control strategies. The precision was calculated with different standard deviations at 20% and 80 % of the measuring range, assuming a linear response over the measuring range. A series of Laplace transfer functions was used to model the response time. A continuous drift effect was also modeled. No attenuation or systematic errors were taken into account. Calibration and cleaning routines were also modeled with a pulse generator and reset the drift error to zero.

Second, when sensor-specific information was not available, Rieger *et al.* (2003) suggested six classes of sensors to help with the design of control strategies, where sensors are defined by their response time and measuring interval. A system order was suggested for each continuously measuring sensor class. For discontinuously measuring sensors, an additional sample time was used. Noise was set as constant for all the classes as 2.5 % of the maximum boundary of the measuring range.

For electrochemical DO sensors, the sensor response has also been modeled as 1st order. Philichi and Stenstrom (1989) noted a first order lag, and that low-end data truncation at 20% of the final concentration improved the quality of the results. To calculate the respiration rate for activated sludge, Spanjers and Olsson (1992) also note having experimentally verified that DO sensors can be modeled by a first-order response model.

Table 1. ISO standard definitions from ISO 5725-1:1994 (*) and ISO 15839:2003 (**)

Precision*	The closeness of agreement between independent test results obtained under stipulated conditions.
Trueness*	The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.
Response time**	Time interval between the instant when the on-line sensor/analysing equipment is subjected to an abrupt change in determinand value and the instant when the readings cross the limits of (and remain inside) a band defined by 90 % and 110 % of the difference between the initial and final value of the abrupt change.

Field conditions

Field conditions refers to testing the sensor in the water matrix where it will be used. This is especially important when choosing a sensor for a specific application, but is yet poorly documented topic. Two different approaches have been documented in ISO 15839 (ISO, 2003; *cited in* Beaupré, 2010):

- Testing the sensors directly in the field. As the system in the field cannot be considered as at steady state, the characteristics obtained are time and location specific.
- Testing the sensor with grab samples from the field. Biological activity and stripping may alter the sample matrix.

Defining sensor characteristics over time is more difficult due to the additional variability of the matrix. Rieger *et al.* (2005) defined field conditions as directly in wastewater treatment plants. Sensor characterization in field conditions was undertaken by comparing the measurements to a reference. Linear regression was used to differentiate between precision and trueness. The response time as well as reference quality was evaluated and accounted for beforehand. Their definition of "precision" had to extended compared to the ISO norm in Table 1 to account for the changes in the matrix.

As part of his master thesis on the characterization of on-line sensors, Beaupré (2010) describes the need for standardized field conditions. This would allow the reproducibility of the tests, comparison between sensors, and the evaluation of disturbances separately. He tested four different sensors (spectrometers, and turbidity and nitrate sensors)under a reproducible experiment with bubble aeration. Another sensor was tested for the affect of increased turbidity. The indicators used to characterize the effects included : number of NaN values, standard deviation , and bias..

2. Objectives and Hypotheses

The end goal of the project would be to develop a control system for urine nitrification based on a soft-sensor using dissolved oxygen (DO) and pH measurements. So far the project has assumed ideal sensor behavior. This master thesis aims to evaluate if the soft-sensor is still of value when considering a more realistic sensor behavior. The objectives and hypothesis of this master thesis are:

1. Characterize DO and pH sensor behavior.

    a) Field-specific media effects

    Hypothesis 1
    ➔ The accuracy, precision, and response time are not significantly different between measurements undertaken in water or with field-specific media.

    b) Cleaning effects
    Hypothesis 2
    ➔ The accuracy, precision, and response time are not significantly different before and after cleaning of the sensor.

    c) Adjust the experiments according to conclusions
2. Model DO and pH sensor behavior.

3. Evaluate the performance of the soft-sensor after addition of realistic sensor behavior.

    Hypothesis 3
    ➔ The soft-sensor performance is not significantly affected by the addition of realistic sensor behavior.

3. Methods

First, the DO and then pH sensor characterization experiments will be explained, and then the testing of the soft sensor performance will be described.

3.1.   DO sensor characterization experiment

The experiment was undertaken to determine the response time, precision and trueness of the DO sensor in the nitrification reactor, and evaluate if sensor cleaning and sample medium significantly affected the results.

3.1.1. General experimental setup

The general experimental setup is further shown in Figure 4 and the relevant protocol can be found in Appendix A.4. The DO sensor was rapidly changed between different beakers: oxygen saturated (about 8.2 mg/L) and oxygen-free (0 mg/L) , in both directions. At each beaker change, the sensor was left about 15min to stabilize. The beaker step changes were determined for three different conditions:
- Field conditions and dirty sensor
- Nanopure water and clean sensor
- Field conditions and clean sensor

Each step direction in each condition was repeated once. The significance of the differences was determined from an engineering rather than a purely statistical perspective. What is meant here by "field conditions" is explained in part 3.1.2 below. The theoretical oxygen saturation at the given temperature and calibration procedure in saturated air were also determined, to relate the results to the calibration procedure.
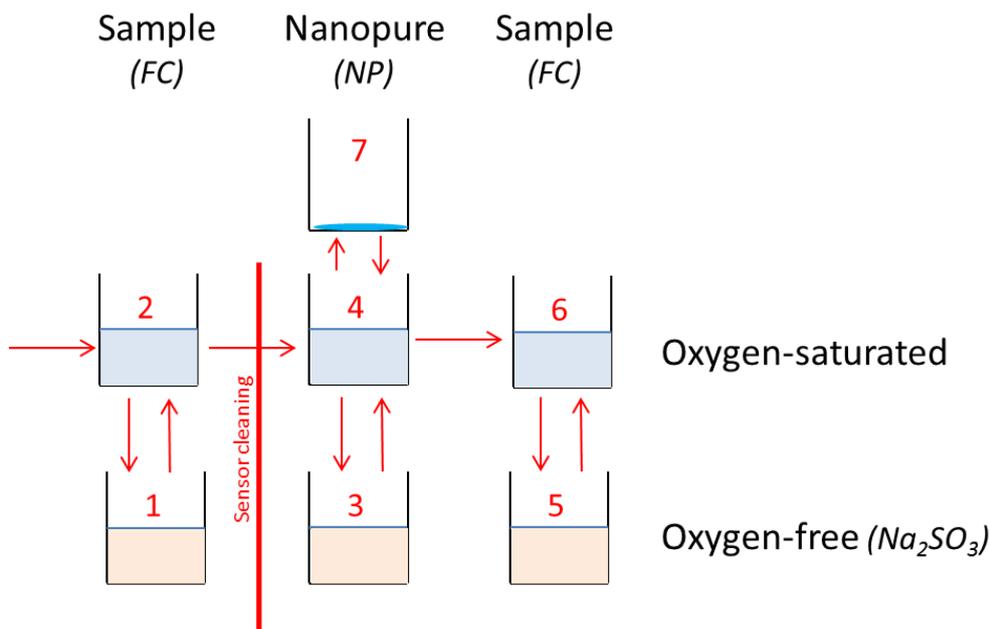

Figure 4. Measurement procedure of the DO sensor changes into beakers of different sensor cleaning and medium conditions, and beaker numbering. The red arrows represent the respective changes of the DO sensor.

Table 2 shows the definitions and methods used in this study to characterize sensors by their precision, trueness, and response time. The code for the determination of all three of these characteristics with 1 s experimental data can be found in Appendix A.5. The model was of the form:

$$\frac{dy_1}{dt} = \frac{y_{final} - y_1}{\tau_1} \quad (1) \qquad\qquad \frac{dy_2}{dt} = \frac{y_1 - y_2}{\tau_2} \quad (2)$$

Table 2. Definitions and quantification methods used for the DO sensor in this study

Precision	ISO 5725-1:1994 The noise of the signal obtained from 3 min of measurements (1 s measurement interval), expressed as a standard deviation. This was determined for both oxygen saturated beakers (about 8.2 mg/L) and oxygen-free beakers (0 mg/L) conditions. Only the first signal in each beaker was used there were doubts on the independence of test results.
Trueness	ISO 5725-1:1994 The average value obtained from 3 min of data (1 s measurement interval) in each oxygen saturated beaker, and the reference was oxygen saturated nanopure water (same procedure used). This was determined for oxygen saturated beakers (about 8.2 mg/L) conditions. Only the first signal in each beaker was used there were doubts on the independence of test results.
Response time	Not quantified as in ISO 15839:2003. Time constants were calculated for the 2nd order sensor model by fitting of a second order curve with a least squares methods in Matlab. Initial and final values were fixed, and calculated as the average from 3 min of stable signal (1 s measurement interval) before and after the step change. The objective function that was minimized with fminsearch for this fitting can be found in Appendix A.5. Two values were obtained for each condition and step direction as the experiment was repeated.

3.1.2. Beaker preparations

Collection of the field samples

For the experiment, about 2.5 L of treated urine from the Forum Chriesbach reactor (Eawag main building) needed to be collected. For every subsequent experiment, a similar treated urine composition could not be ensured from the nitrification reactor in the Forum Chriesbach. Furthermore, while particulate matter might also affect the sensors, biological activity would alter stored samples. The sample collection protocol here was developed to allow for the repetition of sensors tests. Thus, the 2.5 L from the reactor were sedimented in Imhoff cones, filtered at a 0.7μm level, and then autoclaved (120°C, 1.2 bar). The protocol set up for this initial collection can be found in Appendix A.4 (Protocol 1).

After undertaking any experiments, this sample was once again filtered (0.7μm) and autoclaved.

Oxygen saturated beakers
Aeration overnight with the same humidified air used in the reactor was used to accelerate oxygen saturation and avoid excessive evaporation. To prevent potential over-saturation, aeration was stopped at least 2 hours before undertaking the first tests (See Protocols 2 and 5 in Appendix A.4).

Oxygen-free beakers
Oxygen was depleted by the addition of sodium sulfite (Na_2SO_3) with a security factor of 5.5 compared to the theoretical value, thus dosing 215 mg in each 500 mL beaker. 16 mg of Cobalt(II)chlorid-Hexahydrat ($CoCl.H_2O$) was also added to catalyze the reaction in all three oxygen-free beakers. This corresponds to a factor 15 of the value mentioned in the ATV M 20E (GFA 1996). There were no tests to check for complexation, but some complexation was expected to occur. Both of these were added shortly before the sensor was added. The following oxidation process then takes place:

$$Na_2SO_3 + 0.5 \; O_2 \rightarrow Na_2SO_4$$

The detailed protocols can be found in Appendix A.4 (Protocols 3, 4, and 5).

3.1.3. Sensor management

Figure 5. DO sensor setup with efficient mixing, here for saturated nanopure water.

DO sensor type
As the development of the soft sensor is be first tested on the nitrification system in the Forum Chriesbach reactors, the sensor used for the DO experiment was the one present in the reactor (Figure 5). This was a Oxymax COS61D sensor from Endress + Hauser. The oxygen measurement of this sensor is based on the principle of fluorescence quenching.

Calibration procedure
Calibration of the DO sensor was undertaken after the experiments if necessary. The sensor was placed upright in a beaker above nanopure water and left to adapt for more than 10 min. Assuming 100% relative humidity, this value is then compared to the theoretical value in the sensor manual (Endress + Hauser, 2012) that should be obtained at that temperature. Calibration was undertaken for difference of more than 0.2 mg/L, and was expected to be necessary or tested for every month.

Sensor cleaning
The sensor dynamics were tested before and after cleaning. The sensor membrane was cleaned with nanopure, a hand glove and tissue. In future measurements, the removal and cleaning of the sensor cap with nanopure could have limited the release of particles accumulated within the cracks. When undertaking the experiment, the sensor had been cleaned a week before.

3.2. pH sensor characterization experiment

The experiment was undertaken to determine the response time and precision of the pH sensor in the reactor, and evaluate if sensor cleaning and sample medium significantly affected the results.

3.2.1. General experimental setup

The general setup is illustrated in Figure 6 and the relevant protocol can be found in Appendix A.4. The pH sensor experiment was somewhat similar to that of the DO sensor described above, in the sense that it also involved a number of step changes for different conditions. pH 7 and pH 4 solutions were obtained either from standard buffer solutions or from pH-adjusted reactor samples. The beaker changes from pH 7 to pH 4 were also undertaken in three different conditions:
- Field conditions and dirty sensor
- Nanopure water and clean sensor
- Field conditions and clean sensor

Each step direction in each condition was repeated twice, and two sensors were compared. The significance of the differences was determined from an engineering rather than a purely statistical perspective.

As the response time was very short (couple seconds) for these sensors, care was taken to transfer them quickly and at the same time from one beaker to the next. At each beaker change, the sensors were left about 5 min to stabilize. As a second pH sensor could not be added in the Forum Chriesbach reactor, the sensors were installed in the smaller scale nitrification reactors present in the laboratory. These were taken out of the laboratory reactor and cleaning only for the experiment.
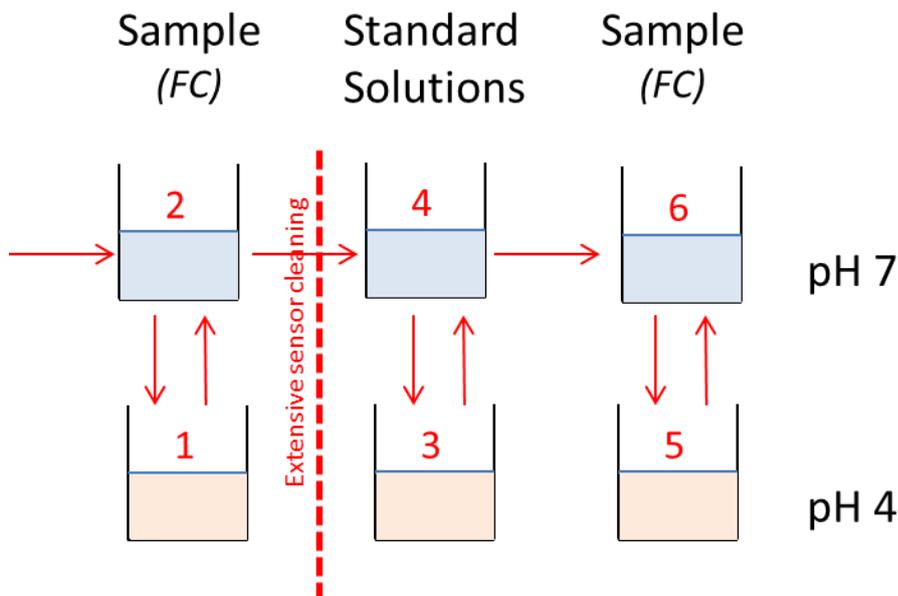

Figure 6. Measurement procedure of the pH sensor changes into beakers of different sensor cleaning and medium conditions, and beaker numbering. The red arrows represent the respective changes of the pH sensors.

Table 3 shows the definitions and methods used in this study to characterize the pH sensors by their precision and response time. Only two characteristics were tested: response time and precision. The trueness of the sensors could not be tested, because the field samples were adjusted using these pH sensors. The code for the determination of these two characteristics with 1 s experimental data can be found in Appendix A.6. The model used to fit the time constants was of the form:

$$\frac{dy}{dt} = \frac{y_{final} - y}{\tau} \qquad (3)$$

Table 3. Definitions and quantification methods used in this study

Precision	ISO 5725-1:1994 "independent measurements" defined in two ways: - the noise of the signal as in Table 2 with 2.3 min of measurements (1 s measurement interval), expressed as a standard deviation. - the standard deviation of the average stable signal for each condition (each step change was repeated three times, and could be considered as independent)
Trueness	NA as the beakers were pH-adjusted
Response time	Not quantified as in ISO 15839:2003. Time constants were calculated for the 1st order sensor model by fitting with a least squares methods in Matlab. Initial and final values were fixed, and calculated as the average from 2.3 min of stable signal (1 s measurement interval) before and after the step change. The objective function that was minimized with fminsearch for this fitting can be found in Appendix A.6.

3.2.2. Beaker preparations

Collection of the field samples
This was the same as in section … above, and the protocol can be found in Appendix A.4 (Protocol 1). After undertaking any experiments, the sample medium was once again filtered (0.7μm) and autoclaved.

Beakers at pH 7
After previous dosage experiments, a 2 mol/L NaOH solution was used to adjust the pH, from pH 6.2 to pH 7. The pH and mV signals were first obtained with a cleaned pH sensor in a pH 7 buffer solution. Then, the field sample was adjusted drop by drop until these values were reached. With this stock solution, less than 2 mL needed to be added for a 1 L stabilized urine solution. One micropipette drop of this solution corresponded to a change of about 0.006 pH. The protocol can be found in Appendix A.4 (Protocol 8).

Beakers at pH 4
A similar procedure was used for adjustment of the field sample to pH 4. Two different solutions available in the laboratory were used. First, about 0.2 mL of a HCl 32% solution [10.17 M; density 1.159 g/mL] was added until the pH was below 4.6. Then, about 3.5 mL of a 0.1 M HCl solution was added for pH and mV signals to correspond to that of the buffer solution pH 4.The detailed protocol can be found in Appendix A.4 (Protocol 7).

3.2.3. Sensor management

pH sensor type

The same type of sensors as in the Forum Chriesbach reactors were used, Orbisint CPS11 (Figure 7) from Endress + Hauser. Both were 3 months old. These pH sensors have an Ag-AgCl reference electrode, a large dirt-repellent PTFE diaphragm, and a contactless inductive signal transmission through Memosens technology. The Nernst equation is used to convert the electric potential formed at the electrochemical boundary layer of the membrane into the corresponding pH value (Endress + Hauser, 2008).

Figure 7. Experimental setup with 2 pH sensors for pH 7 and pH 4 buffer solutions.

Calibration procedure

Calibration of the pH sensors was undertaken after the experiments if the measurement obtained in the standard solutions was more than 0.2 pH off the theoretical value. The instructions on the measurement transformer were followed by placing the sensor in a solution of pH 7 and pH 4 (2-step calibration). Calibration was expected to be necessary or tested for every 2 weeks.

Sensor cleaning

As for the DO sensor, the pH sensor dynamics were tested before and after cleaning. During that extensive cleaning procedure, the sensors were first cleaned with tap water to remove light biological coatings, fibers, and suspended substances. Then soaking them 10 min in 10% ethanol was to remove any grease and oil. Finally, these were also soaked no longer than 2 min in 3% hydrochloric acid to remove any scaling. After such strong cleaning, the sensor was left at least 20 min in the next solution for the signal to adjust.

3.3. Testing the UKF performance with realistic sensor behavior

3.3.1. UKF and testing the UKF performance

The UKF was already introduced in part 1.2. The equations used for the implementation of the UKF can be found in Wan & van der Merwe (2001). The Matlab files concerning the modeling with realistic sensors can be found in Appendix A.7. The UKF was implemented with previously simulated data.

The end goal, as mentioned in the introduction, would be to obtain nitrite estimates that correspond to reality. This requires both a good model and a good soft-sensor. It is important to differentiate between:

- Model performance: the mismatch between the modeled states and those present in the reactor.
- Soft-sensor performance: how good the soft sensor can estimate the unmeasured quantity based on measurement input.

This master thesis focuses just on soft sensor performance only, and how it is affected by sensor dynamics. Thus, to rule out model-reality mismatch, the same model as that present in the soft-sensor was used for the UKF input values, and the sensor model was also present in

both the simulated inputs and in the UKF. Figure 8 illustrates the setup used to test if sensor behavior affects soft sensor performance.

Additonally, using the model as input and comparison also has further advantages:

- Continuous nitrite "measurements" are available for comparison.
- The experiments are repeatable.
- A broader range of conditions can be tested
- System failure can occur without having to re-start the reactor
- A couple days of data can be simulated within a couple hours

The performance of the soft sensor is determined when comparing the nitrite estimate to the nitrite obtained by the simplified nitrification model for the same simulation. For the VUNA reactors, such a soft-sensor and model would be of value if it could detect nitrite concentrations above 20 mg/l within 3 hours (K. M. Udert, personal communication, April 2015). Increases in the nitrite should especially be detected in time. While the model does not correspond to reality, these criteria will be used for general evaluation of the soft-sensor performance.
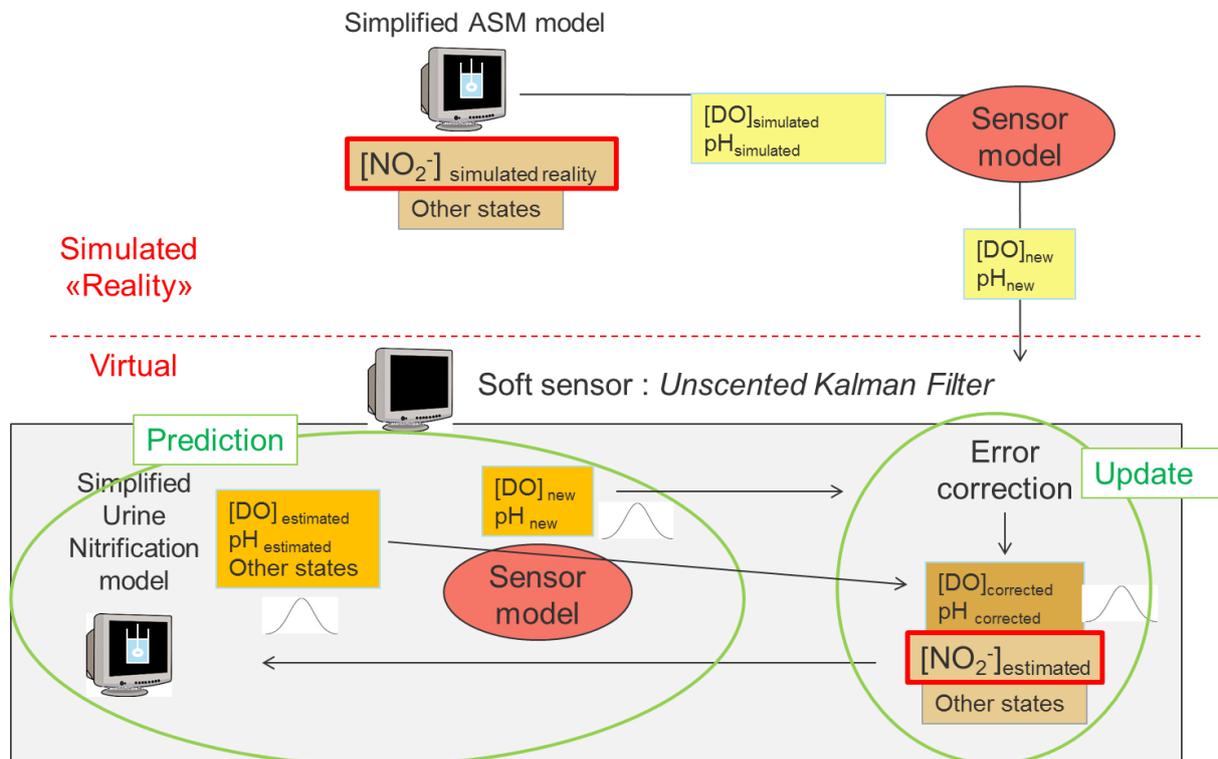
Figure 8. Simulation setup to test if realistic sensor behavior affects soft sensor performance.

3.3.2. Reduced nitrification model and nitrification model simulation

The original nitrification model was introduced in part 1.1. The list of the 37 states modeled, the kinetics and the parameters of the process matrix can be found in Appendices A.1 and A.2. The reduced model was simplified to just the urine reactor, and 13 varying states, as shown in Appendix A.1. As the salt concentration was not expected to change at steady state, the formation of complexes was removed. This significantly decreased the computational time of the soft sensor. Inputs included the inflow and outflow pump rates, and the gas flow rate. Disturbances from the urine and gas pumps were set at zero.

First steady state values were first obtained for an inflow of 24 L/d. Then, a change in the inflow and outflow rates from 24 L/d to three times that amount (72L/d) was simulated for a period of about 11 days. Three times was considered as a significant change and extreme loading rates, but remains possible. Considering a full buffer tank of 1000 L, 7 days with 72 L/d would empty the buffer tank by half. With a reactor size of 120 L, this corresponded to a change of the hydraulic residence time from 5 days to 1.7 days. No control system was operated. The gas flow rate was constant throughout the simulation.

3.3.3. Sensor model

The sensor models for pH and DO sensors were based on the experiments in parts 3.1 and 3.2. The Matlab codes and functions concerning the modeling with realistic sensors can be found in Appendix A.7.

Sensor precision was incorporated within the UKF as an additional function using the measured standard deviations.

Sensor trueness was not included as this could not be measured for the pH.

Sensor dynamics were integrated within the reduced nitrification model so that the states would be calculated simultaneously. The sensor dynamics were added as a set of ordinary differential equations: 2nd order response for the realistic DO sensor and 1st order for the pH sensor, as previously shown in the equations (1), (2) and (3) above. Thus, three extra states were added to the simplified nitrification model: two states for the DO sensor and one state for pH sensor. Modeling different responses depending on the measurement direction (eg. Increasing or decreasing DO concentrations) was not possible due to the stiffness of the UKF.

4. Results

4.1. DO sensor characterization

4.1.1. Raw data

Figure 9 shows DO raw data obtained during the DO experiment. The color shading represents specific medium and sensor cleaning conditions. The experiment for each condition was repeated once. When looking at the measured saturation values within each color shade, though, there seems to be a systematic decrease in the oxygen concentration each time. Within each shading, though, the measurements were always obtained from the same beakers: one oxygen-saturated and the other oxygen-free. With such a systematic concentration decrease, it was decided that these could not be considered as independent samples. The 1st measurement time step for each color shading was selected when further determining the trueness and precision of the data.
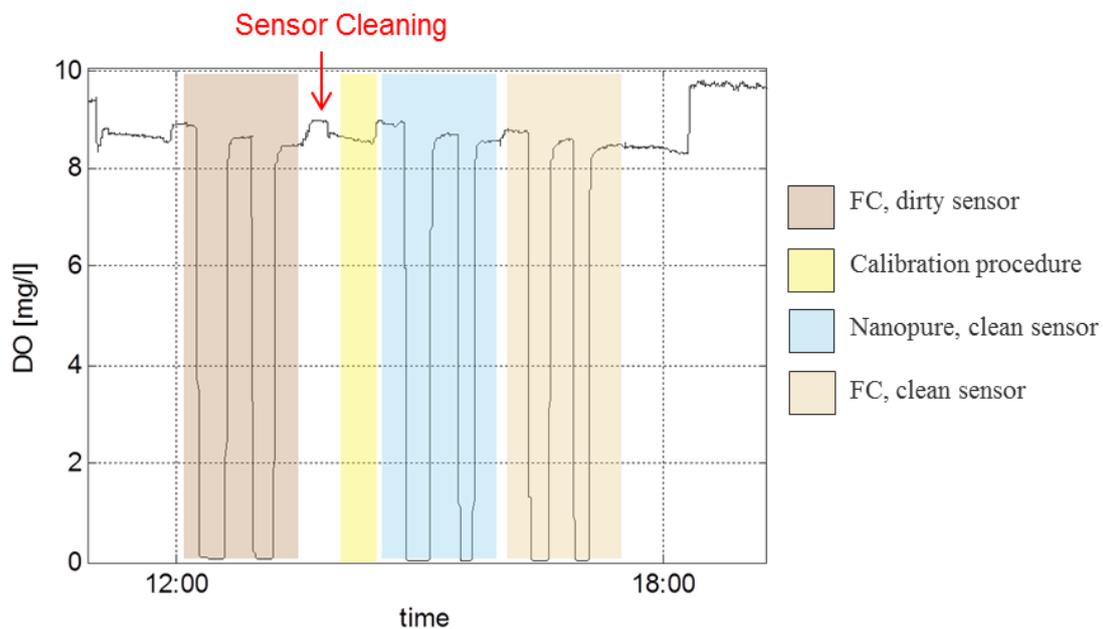
Figure 9. DO sensor raw data for the experiment, with color shading for the different conditions (FC: field conditions).

4.1.2. Trueness

From the raw data, only the 1st stable sensor signal was used for each beaker, as independence could not be guaranteed with multiple step changes. The trueness was not investigated for the oxygen-free samples.

For oxygen saturated samples, Figure 10 shows the mean values for different medium and sensor cleaning conditions. Taking an engineering perspective, there was no differences between dirty and clean sensors. In terms of medium (nanopure or sample medium), a 0.2 mg/L difference was detected in the trueness.
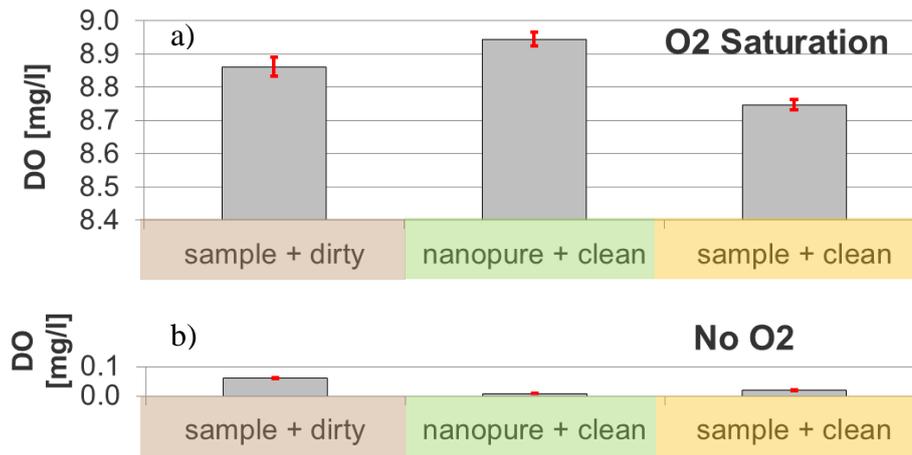
Figure 10. Average value of the first stable signal for each condition and a) at oxygen saturation and b) in an oxygen-free medium. The red error bars correspond to two times the standard deviation. From about 3 min of the sensor signal with a 1 s measurement interval

Other findings in terms of trueness may also be relevant to this study. There seems to be either oversaturation of the aerated beakers, or an error in the calibration procedure air value. In Figure 9 the measurement in humidified air (yellow) and in saturated nanopure (blue) were 8.9 and 8.5 mg/L, respectively. This difference was greater than between the conditions themselves. Furthermore, while this is not important when looking at relative differences, calibration would have been necessary. The theoretical value obtained at the room temperature of 23°C was 8.14 mg/L.

4.1.3. Precision

Similarly, only the 1st stable sensor signal was used when looking at the precision. The red error bars in Figure 10 represent twice the standard deviation calculated from the noise of the signal during 3 min. First, all these standard deviations are very small. A difference in precision can nonetheless be detected between low (σ_{max}= 0.001 mg/L) and high (σ_{max}= 0.014 mg/L) oxygen concentrations, with a better precision at low concentrations.

4.1.4. Response time

The response time was defined as a set of time constants for the different conditions and step directions.

Figure 11 shows the DO measurements for the two step directions and the models fitted by least squares, as explained in part 3.1.1. The response was found to be 2nd order. Models of higher order did not lead to significant improvements. The response is faster for decreasing DO concentrations.

Figure 12 shows the average (two sets of measurements) 2nd order time constants for the different experimental conditions. First, a clear difference in the step direction can be generally confirmed, with faster responses when the DO concentration decreases. Comparing groups 1 and 3, there seems to be no significant difference due to cleaning. Comparing these with group 2, the average time constants seems lower for nanopure water than for sample medium for decreasing DO concentrations ("down"). No difference was visible for increasing DO concentrations ("up").

17
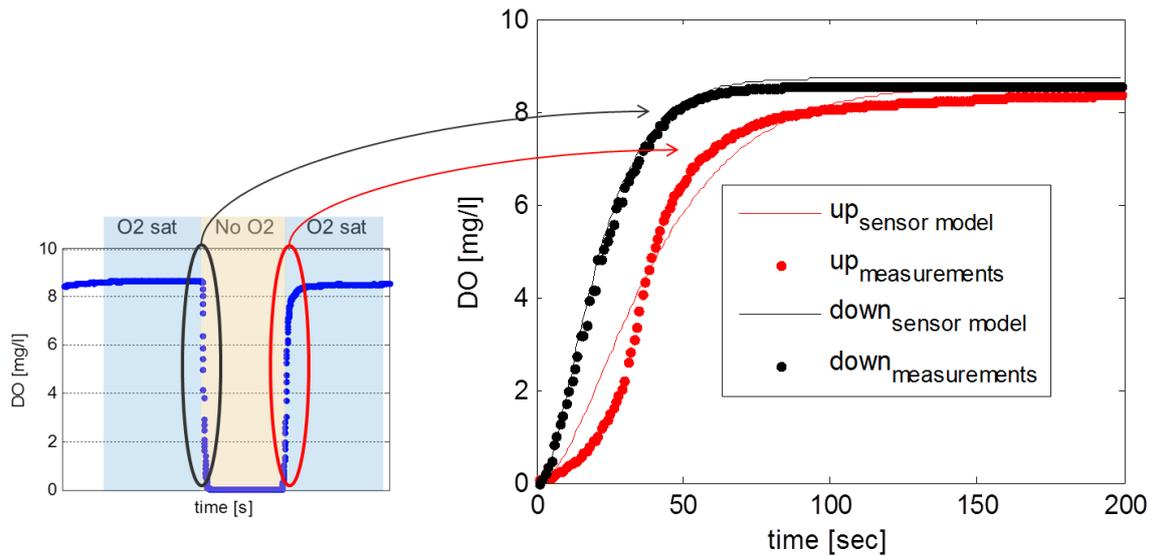
Figure 11. Example results for a clean sensor in sample medium; DO raw data for two step changes (left), plotted together (right). Note that the black curve/data have been inverted. The time axis corresponds to the initial beaker change. Dots are measurement points. Lines are the fitted 2nd order sensor dynamics model (down: τ1= τ2= 11 s; up: τ1= τ2= 21 s).
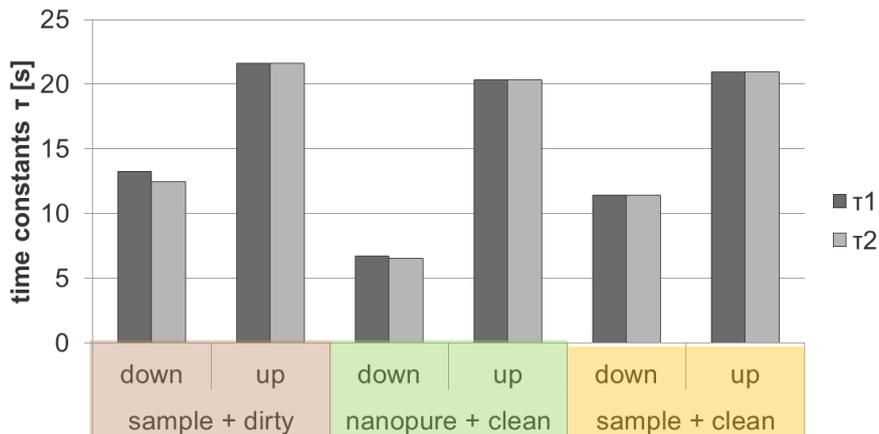
Figure 12. Average DO sensor 2nd order time constants for the different experimental conditions. Average of two values. Down relates to step changes with decreasing DO concentrations from saturation to oxygen-free beakers. Up vice versa.

4.1.5. Sensor model

Considering the worst case scenario, the DO sensor can be characterized and modeled with the time constants, standard deviations, and bias found in Table 4.

Table 4. Worst characteristics measured in terms of sensor response time, precision, and trueness. (*expected a bias of 1 mg/L considering a salinity of 20‰).

	Quantified as:	DO
Response (↑DO)	τ1 / τ2	22s / 22s
(↓DO)	τ1 / τ2	14s /14s
Precision (high DO)	σ	0.014 mg/L
(low DO)	σ	0.001 mg/L
Trueness	bias	0.2 mg/L *

4.2. pH sensor characterization

4.2.1. Raw data

Figure 13 shows DO raw data obtained during the pH experiment. These were similar for sensors 1 and 2. Each condition was repeated twice.
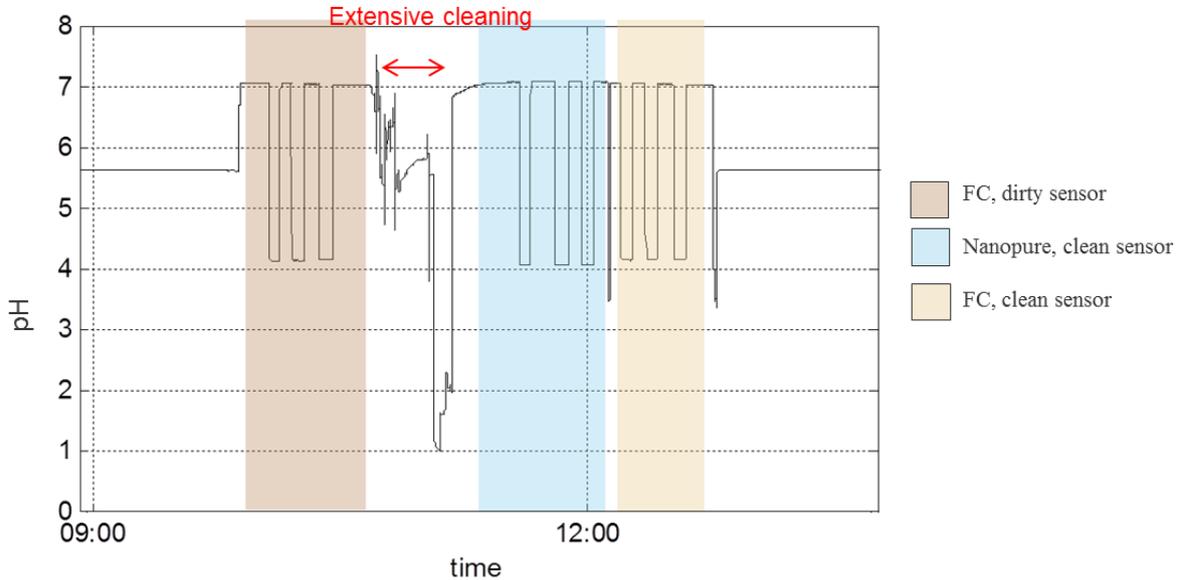
Figure 13. pH sensor 1 raw data, with color shading for the different conditions (FC: field conditions).

4.2.2. Trueness

The pH sensor 2, had been used to adjusted the field samples at pH 7 and pH 4 the day before. Thus, trueness could not be examined in this experiment.

4.2.3. Precision

Figure 13 shows good repeatability of the step changes. Thus, precision was calculated in two different ways: with the sensor noise (as for the DO experiment), and with the average of repeated tests. The stable signal used was always 2.3 min long. Each condition was repeated 3 times.
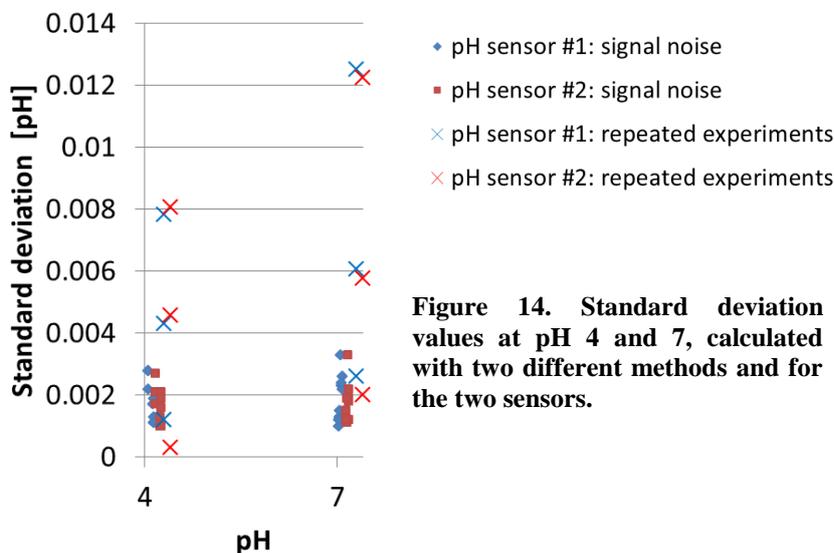
Figure 14. Standard deviation values at pH 4 and 7, calculated with two different methods and for the two sensors.

Figure 14 and Figure 15 show standard deviation values obtained from the individual signals and from the repetition of the step changes for the two pH levels, and for sensor and medium conditions. When looking at the sensor signal noise, the standard deviation of the signal was similar and small in all the conditions and pH levels. A maximum of 0.0033 pH

19

was detected, with both pH sensors showing higher values for that event. When looking at the final values obtained when repeating the experiments, differences were detected when looking at the different conditions For the standard solutions, looking at repeated samples led to similar if not smaller standard deviations than the signal noise. In both beakers with field conditions, the standard deviations obtained were 2 to 7 times as large. Both pH sensors reacted in the same way.
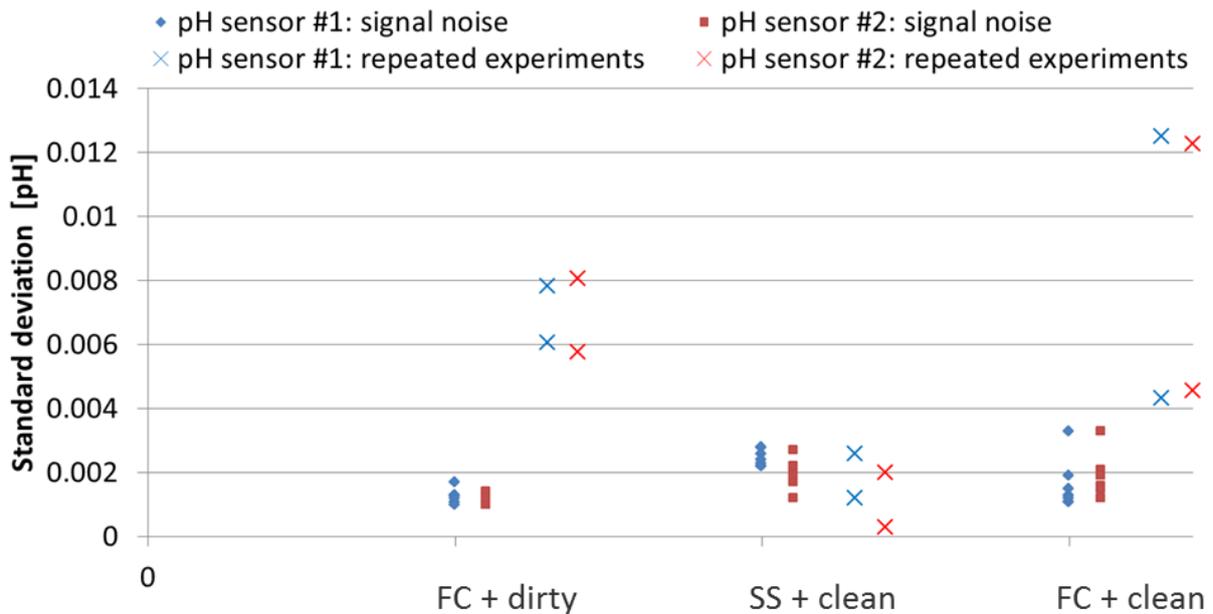
Figure 15. Standard deviation values for the different conditions. of the signal at each new step for different conditions and different pH sensors. The signal used was always 2.3 min long. The experiment was repeated 3 times. (FC: Field conditions ; SS: Standard Solutions).

4.2.4. Response time

The response time was defined as a time constant for the different conditions and step directions.
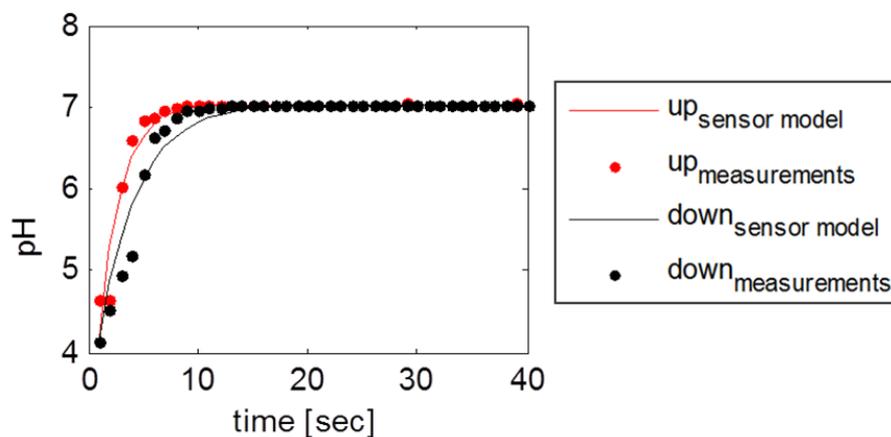
Figure 16. pH measurements for two step directions over time for a dirty sensor in field conditions, and the fitted model. Note that the black curve/data have been inverted in terms of pH axis. The time axis corresponds to the time from the initial beaker change. Dots are measurement points. The fitted 1st order sensor dynamics model have τ values of 2 s and 3.5 s for increasing pH (up) and decreasing pH (down), respectively.

20

Figure 16 shows the pH measurements for two step directions and the sensor models fitted. The response was found to be 1st order. The black curve/data was inverted for comparison of these two step directions.

Figure 17 shows the 1st order time constants for the different sensor and medium conditions, repeated 3 times. Here, no differences can be made in the step direction, medium, or sensor cleaning. Again, both sensors are similar and their time constants are low. pH sensor 2 had been used and cleaned to do the pH adjustment of the samples the day before the experiment. pH 1 had not been cleaned for 17 days. The maximum time constant was 3.5 s, which corresponds to 10.5 s to get to 99% of the final value. The measurement data and model for this step change up and down are shown in Figure 16. Over all the experiments, the average tau is 1.5 and 1.2 s for the sensors 1 and 2, respectively. This corresponds to reaching 99% of the final value in 4.5 s and 3.6 s, respectively.
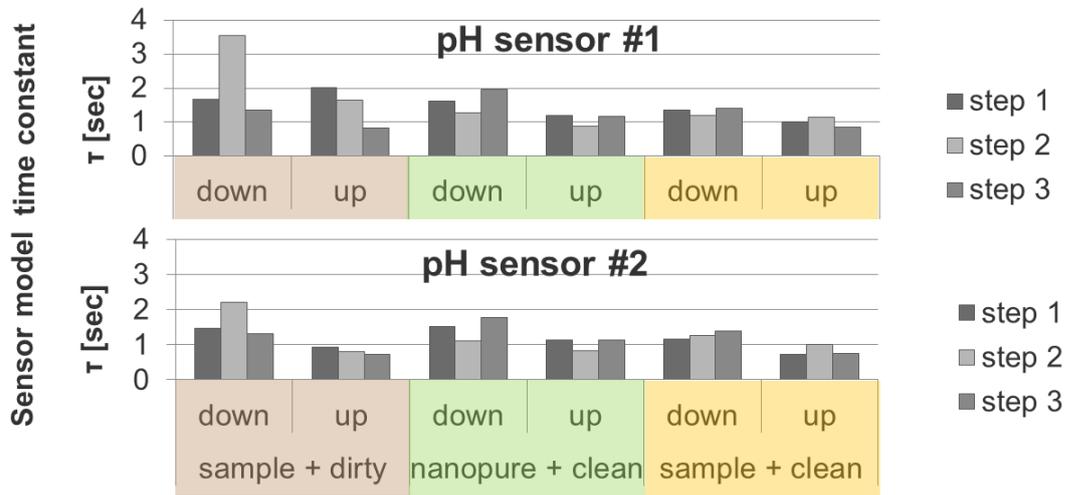
Figure 17. 1st order time constants for the different sensor and medium conditions, repeated 3 times.

4.2.5. Sensor model

Considering the worst case scenario, the pH sensor can be characterized and modeled with the time constants, standard deviations, and bias found in Table 5.

Table 5. Worst characteristics measured in terms of sensor response time, precision, and trueness.

	Quantified as:	pH
Response	τ	3.5 s
Precision	σ	0.0033 pH
Trueness	bias	NA

4.3. Soft sensor estimations with ideal and realistic sensors

4.3.1. Model simulation
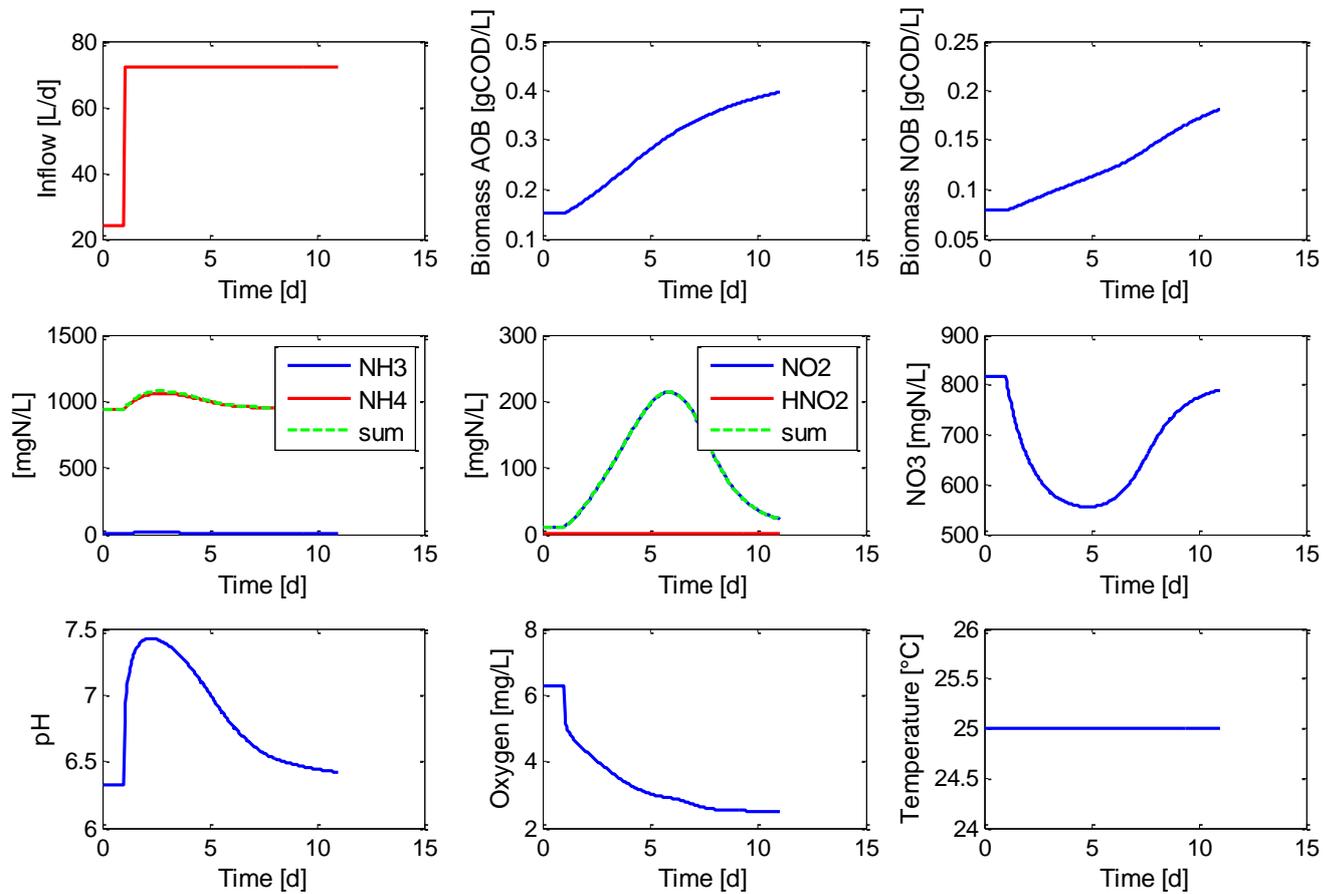
Figure 18. changes in different states for an inflow step change from 24 L/d to 72 L/d.

A step change in the inflow rate from 24 L/d to three times that amount (72L/d) was simulated without any control strategies. The nitrification process was illustrated in Figure 1. Nitrification process as modeled. Acid-Base equilibria in green with main components in green circles. (AOB: Ammonia Oxidizing Bacteria ; NOB: Nitrite Oxidizing Bacteria)
Figure 18 shows the important nitrification state changes that occur.

- NH_4^+ and NH_3 concentrations peak on day 3, with an increase by about 130 mgN/L and 15 mgN/L, respectively. For NH_3, this corresponds to a factor 14. These concentrations then decrease and stabilize again after about 8 days.
- At the same time, the pH increases sharply up to 7.4 due to the influent urine, which has a pH of about 8. The pH then decreases slowly.
- This slow decrease in the pH can be explained by the increase in AOB growth and activity.
- The $[NO_2^-]$ increases due to AOB activity as more NH_3 is converted into HNO_2 and NO_2^-. After about 6 days, the $[NO_2^-]$ reaches a peak and starts to decrease. $[HNO_2]$ on the other hand peaks a bit before day 6. While $[HNO_2]$ do not exceed 0.18 mgN/L, the simulation led to an increase by a factor 23.

- NOB growth and activity also increases.
- This decrease in the $[NO_2^-]$ corresponds to the increase in the $[NO_3^-]$. The initial $[NO_3^-]$ decrease is due to reactor hydraulics as the residence time is decreased from 5 to 1.7 days.
- The oxygen concentration decreases due to increased biomass growth and activity, reaching a stable value at the end of the 10 days.
- No temperature variation was included in the model.

Such high $[NO_2^-]$ would lead to process failure in the FC reactor. In the model, though, the system manages to recuperate from such high $[NO_2^-]$. This model-reality mismatch is beyond the scope of this master thesis, but further emphasizes the need to use the model as input for the soft-sensor in the performance evaluations.

4.3.2. Sensor model

Table 6. Sensor characteristics added in the sensor model.

	Quantified as:	DO	pH
Response	τ	22 s / 22 s	3.5 s
Precision	σ	0.014 mg/L	0.004 pH

Table 6 shows the sensor characteristics that were used to add sensor realism. These corresponded to the worst values in Table 4 and Table 5. The signal noise was used to characterize the pH sensor precision to stay consistent. Similarly, no bias was included as this could not be calculated for the pH sensors.

The precision was a parameter of the UKF. The DO and pH sensor responses were modeled with 2nd order and 1st order equations, respectively, as explained in parts 3.1.1 and 3.2.1. Modeling differences for $[O2]$ changes going up and down led to numerical issues (stiff model). The pH and DO differences caused by the sensor model were not visible without zooming in at the hourly time step.

4.3.3. Ideal sensors –UKF results

Here the standard deviation parameter was still present in the UKF, as in Table 6, but the sensor dynamics (response) were not modeled. Figure 19 shows the nitrite estimation results compared to the model results for these pseudo-ideal sensors. The confidence interval is plotted as three times the standard deviation around the estimated value. The estimation is very good, with a confidence interval which reaches a maximum at 12.7 mg NO_2^--N/L on day 2. The UKF estimate is off from the model by a maximum of 1.7 mg NO_2^--N/L.

4.3.4. Realistic sensor –UKF results

Figure 20 shows the nitrite estimation results compared to the model results for realistic sensors (precision and sensor dynamics included). The UKF estimation is severely affected by sensor dynamics, but the $[NO_2^-]$ increase is still detected. The UKF estimation is first slightly overestimated, and then drastically underestimated by 100 mg NO_2^--N/L. Furthermore, the peak is earlier than in the model. The uncertainty interval that reaches a maximum at 225 mg NO_2^--N/L just before day six ($\sigma = 38$ mgN/L), which corresponds to this UKF peak.
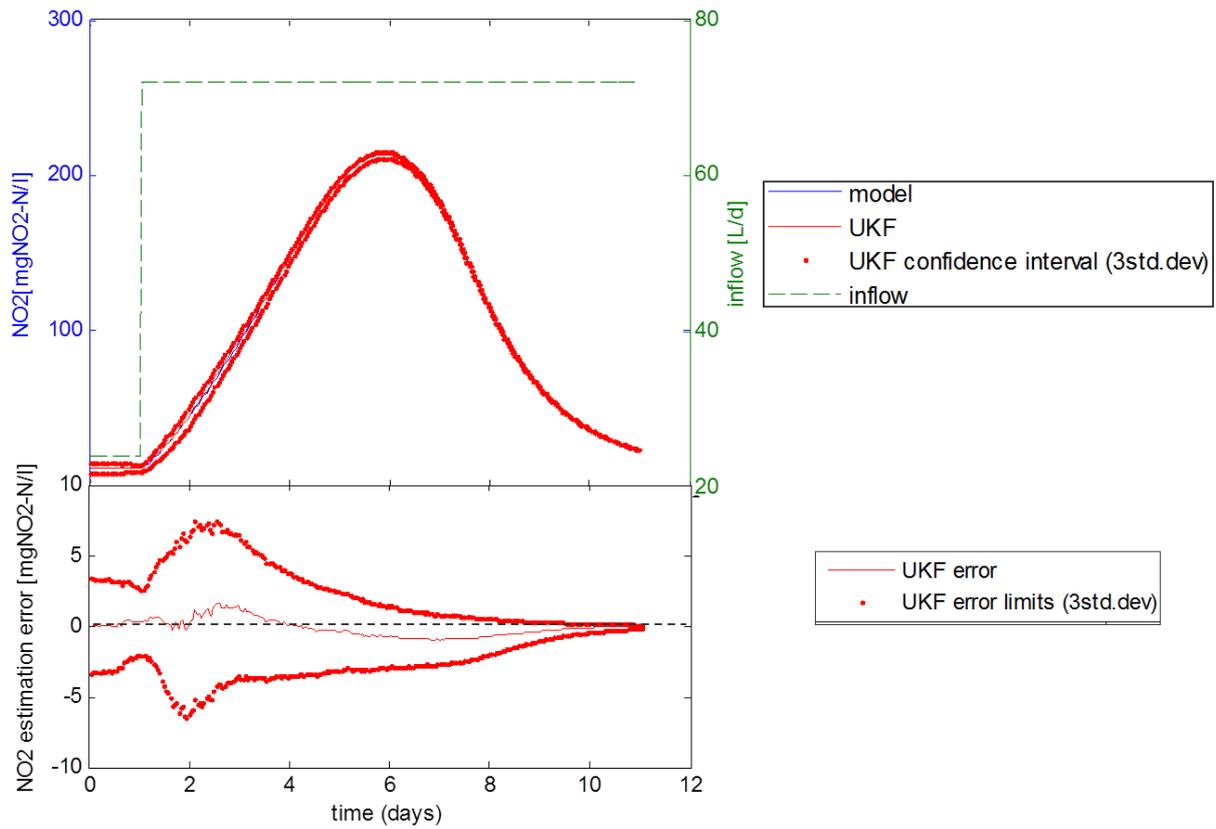
Figure 19. With pseudo-ideal sensors (precision included, but no sensor dynamics). Nitrite from the deterministic model and estimated from the UKF. The estimation error is calculated by subtracting the UKF estimation and confidence interval by the simulated reality (model) curve in the graph above.
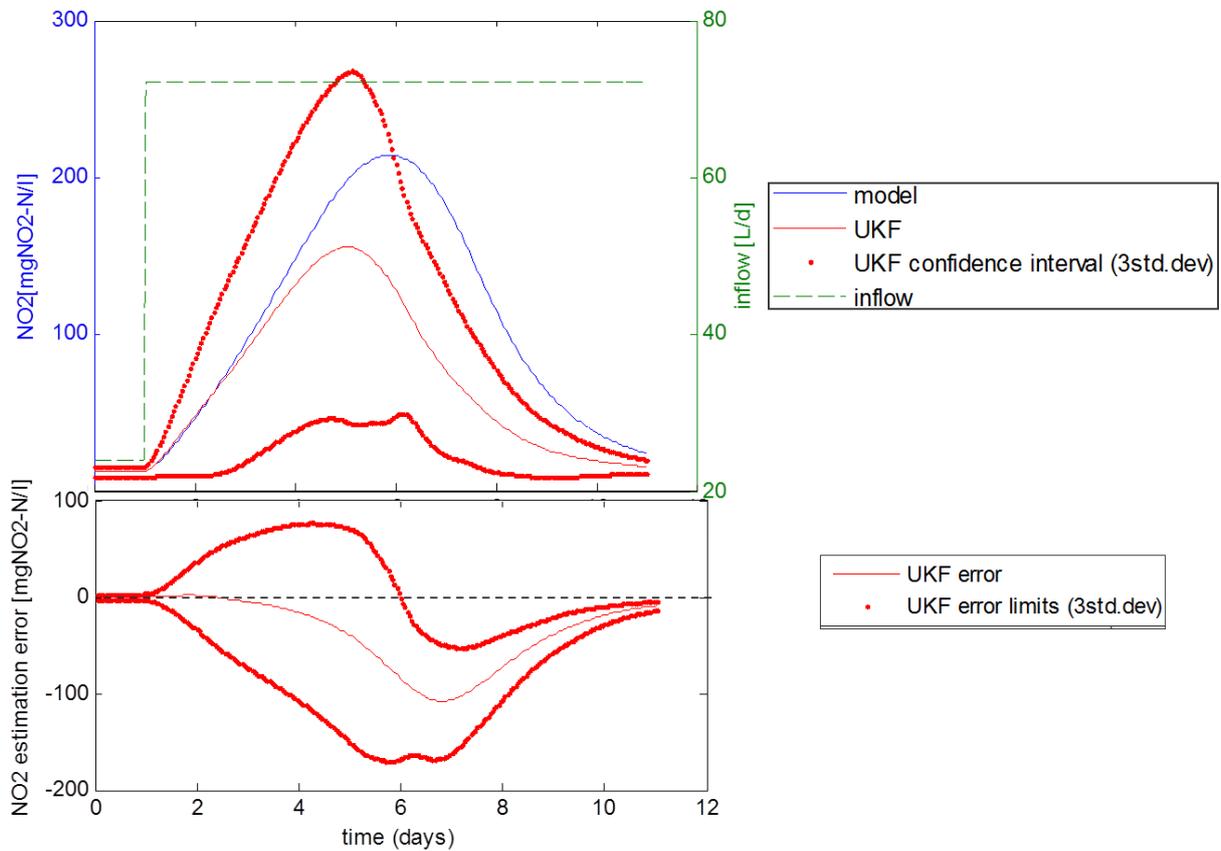
Figure 20. With realistic sensors (with precision and sensor dynamics). Nitrite from the deterministic model and estimated from the UKF. The estimation error is calculated by subtracting the UKF estimation and confidence interval by the simulated reality (model) curve in the graph above.

The nitrification process was illustrated in the Introduction in Figure 1. For realistic sensors, Figure 21 shows the UKF observer estimations compared to the simulated reality results for six important process states. The pH is underestimated by the observer, and an increase in the AOB activity would lead to higher $[NO_2^-]$ in the UKF. The $[O_2]$ is also underestimated, which would be due to an increased AOB/ NOB activity and growth. In fact, both AOB and NOB biomass increase in the simulated reality and in the UKF estimate. The NOB biomass is especially overestimated. After day six, the real value is no longer within the confidence interval set. When looking at the $[NO_3^-]$, it is clear that NOB activity is overestimated also. Thus, the NO_3^- discrepancy originates from the fact that much more NO_2^- is being converted to NO_3^- than predicted in the simulated reality.
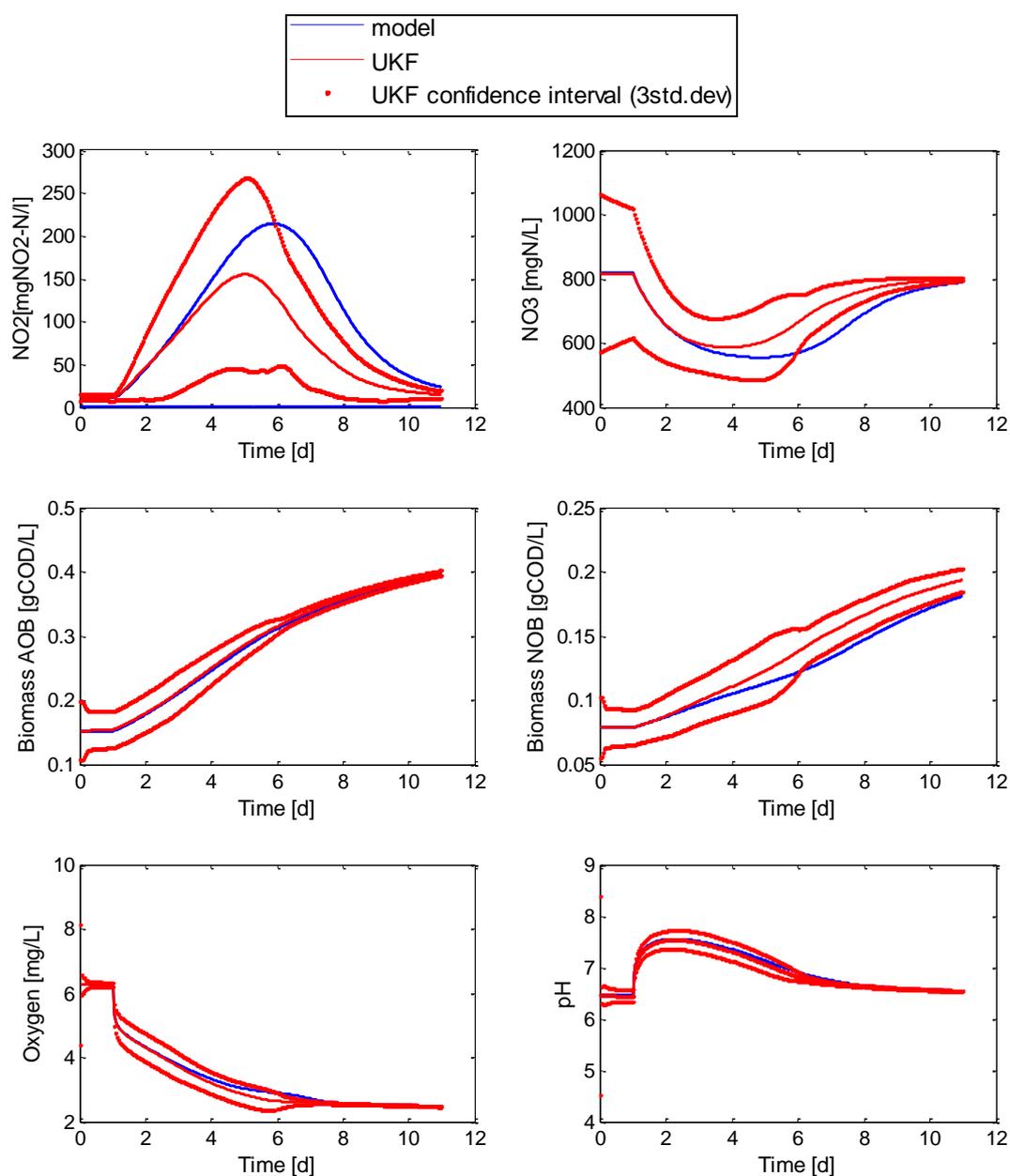
Figure 21. With realistic sensors (with precision and sensor dynamics): Different states from the deterministic model and estimated from the UKF with a confidence interval of three standard deviations.

25

Figure 22 shows the pH and DO estimation errors. For the DO, the estimated value is a maximum of 0.3 mg/L lower than the model value. The confidence interval is maximum about 1 mg/L wide. For the pH, the estimated value is a maximum of 0.1 pH lower than the model value. The confidence interval is maximum about 0.5 pH wide.

Figure 22. With realistic sensors (with precision and sensor dynamics). Estimation error of the pH and DO concentrations, obtained from subtracting the UKF estimation and confidence interval by the simulated reality curve.

5. Discussion and Outlook

5.1. Experimental setup

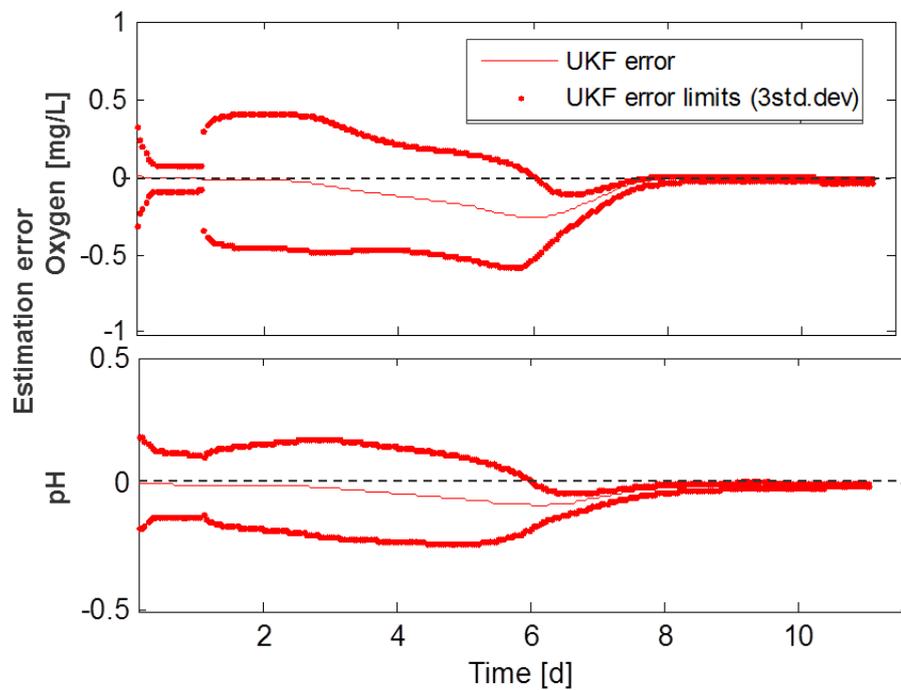Testing and adapting the protocols have been an important part of this master thesis, especially for the DO sensor. In fact, before obtaining the above experimental procedure, a number of nanopure tests were performed and protocols changed. Mixing proved to be decisive for the response time. Oxygen depletion was originally planned with nitrogen gas, as in Philichi and Stenstrom (1989), to avoid potential changes to the water matrix, but the mixing due to nitrogen bubbles could not be considered as equivalent in the different beakers. These previous tests and the lessons learned are summarized in Appendix A.8.

Nonetheless, the experimental setup still seems to show some weak points.

First, step changes from oxygen saturation to no oxygen free medium were necessary to obtain reproducible experiments. Step changes at different concentrations may have led to different results. The reactor DO concentration was often at saturation or even over-saturation, so oxygen-free conditions are of little interest.

Then, the measurement of pH in two different ways, in Figure 14 and Figure 15, with repeated measurements and signal noise shows that the signal noise may misrepresent the actual pH sensor precision. It would be interesting to see if the standard deviation changed with the measurement interval.

With this in mind, finding the cause of the systematic decrease in the DO concentration measured at saturation becomes important. While the sensor membrane had been thoroughly cleaned the last two conditions in Figure 9, particles stuck inside the sensor cap found their way into the solution. The sensor cap should maybe have been removed for more thorough cleaning. Another reason could be that the beakers were oversaturated. In fact, the calibration protocol in humidified air was 0.4 mg/L lower than the saturated DO concentration in nanopure water. The theoretical value at that temperature was 0.7 mg/L lower. The beakers should probably not be aerated overnight, but left overnight to settle.

A further concern in terms of the experimental setup is the underestimation of the difference between saturated field conditions and nanopure water for the DO sensor in Figure 10. While a 1 mg/L bias was expected, due to about 20 ‰ salinity content present in the nitrified urine (Metcalf & Eddy, 2004), only a maximum of 0.2 mg/L was detected. Different salts may have a different valency, but it would be worth calculating the salinity of both the initial conditioned sample stored and the same sample used for the experiments. Salt precipitation or the formation of salt complexes may also have occurred. Cobalt was used as a catalyst to the reaction of sodium sulfite with oxygen for the oxygen-free medium. To test the oxygen transfer in activated sludge aeration tanks, the GFA (1996) suggest performing initial tests with cobalt to see if the cobalt is complexed.

5.2. Field conditions and standardized field conditions

In this study, field conditions were defined as filtered (0.7 μm) and autoclaved samples from the reactor, and not directly in the reactor. This allowed the reproducibility and so comparability of the experiments over time. The effects of the field conditions and the extent of the uncertainty due to field conditions, though, may have been severely underestimated as:

- The salinity and the sample matrix may have been altered, as discussed above.
- The particles in suspension were neglected. The solution was always filtered and autoclaved.
- The mixing in the experiment is expected to be different than in the reactor. In the previous protocols tested (Appendix A.8), mixing was found to be especially important. The reactor was expected to be very well mixed by intense aeration to keep the carriers in suspension, but the extent of this mixing was not quantified.

In this study, the worst measured case was used for the time constants and the precision in the sensor models, but further variation of these time constants and standard deviations should be undertaken in the soft sensor.

Furthermore, the present results should be compared with experiments undertaken with reactor samples that have not been filtered or autoclaved and the measurement signal obtained from the reactor itself. Alternatively, standardized field conditions like those conducted by Beaupré (2010) would also help determine the most important factors which could influence the uncertainty.

5.3. Modeling as only a part of sensor management

Dealing with sensor uncertainty is more complex than suggested by the simplified sensor model. Sensor realism should be addressed by: (1) better initial planning, (2) solving errors at the source, (3) predicting and preventing sensor failure, and as a last step (4) evaluating and accounting for data quality losses. While this study focuses on step 4, it is important to bear in mind the other three.

Sensor (and actuator) measurement errors may originate from a wide range of potential sources. ISO (1994) standards note variability due to: the operator, the equipment used, the calibration of the equipment, the environment (temperature, humidity, air pollution, etc.), and the time elapsed between measurements. Rieger *et al.* (2010) produced a detailed list of potential error sources for flow measurements, sampling, sample analysis, and for online sensors. In some cases, an additional lag or uncertainty can be deliberately added by the producers. In fact, the pH precision may be better at low concentrations because the sensors do not have a real zero to avoid negative values. A broad distinction can be made between measurement errors and equipment failures. Preventing both of these is primordial for good data quality of the DO and pH sensors.

First, initial planning is necessary. Lynggaard-Jensen (cited in Bourgeois *et al.,* 2001) determined 8 different sensor/analyzer properties that should be taken into account when choosing sensors: cost of ownership, ease of use, placement of the sensors, response time, reliability, accuracy, detection limit, and measurement type. The choice of sensor will depend on the application. Rieger *et al.* (2003) note that monitoring will need high accuracy, whereas control may need have higher demands on the time scale.

When errors cannot be solved at the source, they should then be predicted and prevented. Thomann *et al.* (2002) suggested the use of Shewhart control charts with a two-step warning and alarm system to detect sensor failure, improve data quality, and quantify sensor uncertainty for on-line sensors.

Finally, data quality losses should be accounted for as a last step. The full range of the errors, instead of just the DO and pH measurement equipment, may be better quantified by other methods, such as mass balances, error propagation or stochastic approaches (Rieger *et al.*, 2005), even though the current approach is simpler to implement as part of the soft sensor. It also allows the comparison of different conditions to understand the causes of this sensor uncertainty.

To extend the current characterization and modeling of the sensor, a first step would be to detect sensor changes over time. Repeating the experiments over time is important to take into account sensor drift. The experimental protocols have been set up to allow the reproducibility of the experiments. Sensor failure analysis would have to occur at a larger time scale and more sensors to test.

5.4. UKF applications for control and optimization.

The transferability of this nitrite observer to other processes is limited. The nitrification system is observable with DO and pH sensors because endogenous respiration can be considered as negligible. This would not be the case in activated sludge systems.

The ultimate goal would be to have a soft sensor that estimates nitrite sufficiently well with an operational reactor. The idea would be to install a control system based on such estimates. The soft sensor tested for a simulated environment tells us when to react, but cannot provide a solution. In this sense, it would currently probably be more useful as a warning system than as a control system per se. Regular manual nitrite measurements would still be necessary.

Furthermore, the ultimate goal would require both a good model performance as well as a good soft sensor performance.

5.5. UKF performance outlook

Soft sensor performance may be able to be improved by:
- Increasing the run-up time. While NH_4^+ and NH_3 do not seem to play an important role in the NO_2^- estimation, they may play a role in the uncertainty width. The NH_4^+ uncertainty is especially high at the beginning due to the initial default value set by the UKF.
- Changing the Kalman gain parameter in the UKF. The Kalman gain is a UKF parameter which represents the relative importance of the error with respect to the prior estimate.
- Adding a 2-point oxygen controller or other controller system. When aeration is turned off, the uncertainty bands might decrease.
- Changing the model form or model parameters. In the simulation, the HNO_2 peaked at the same time as the nitrite in the UKF estimation. Kämpf (2015) found in his master thesis that the HNO_2 concentration was critical to most of the processes. The goal of his master thesis was to calibrate and validate NOB parameters, especially the inhibition term.
- The nitrification model could be further simplified. Only AOB and NOB activity and not growth and decay could be used, for example. Masic and Villez (2014) had used a three state model with just NH_3, HNO_2, and NO_3^-.

6. Conclusions

In this master thesis, DO and pH sensors were characterized, by a set of beaker changes, in terms of their time constants, precision and trueness. Differences between field and standard conditions and between dirty or clean sensors were examined. A model of the sensor dynamics was set up. The UKF was then run on simulated data, with and without realistic sensor dynamics.

Sensor cleaning effects were small for both pH and DO sensors. Field-specific media effects were also small and less important than expected in terms of the trueness for the DO sensor. For the pH sensor, the effects of field conditions on the precision became apparent when using repeated measurements instead of sensor noise to calculate a standard deviation. For the DO sensor, time constants and precision were most affected by the measurement direction and DO concentration level, with faster responses for decreasing concentrations and better precision at low concentrations. DO and pH sensor dynamics were found to fit a modeled 2nd order and 1st order response, respectively.

The nitrite estimates determined with the UKF for ideal sensors were excellent. The maximum error in the estimation was of 1.7 $mgNO_2^--N/L$. With sensor dynamics, the nitrite increase could still be estimated, but the UKF performance was severely affected. Nitrite concentrations were then underestimated by up to 100 $mgNO_2^--N$ /L, linked to an overestimation of the NOB activity. The uncertainty of these estimates also increased drastically, with the standard deviation of the estimate reaching up to 38 $mgNO_2^--N/L$.

It may be possible in the future to use this soft sensor as part of a warning system, if not a control system, but a number of further evaluations are first necessary. Furthermore, this study has been undertaken with a simulated reality. Model performance will have to be taken into account in addition to soft sensor performance before it can be put to use.

7. References

Alex, J., Rieger, L., Winkler, S., & Siegrist, H. (2003) Progress in sensor technology – progress in process control? Part II: results from a simulation benchmark study, *Water Science and Technology,* 47 (2), 113-120.

Beaupré, M. (2010) *Characterization of on-line sensors for water quality monitoring and process control* (Unpublished master's thesis). Université Laval, Québec, Canada.

Bourgeois, W., Burgess, J. E., & Stuetz, R. M. (2001) Review – On-line monitoring of wastewater quality: a review, *Journal of Chemical Technology and Biotechnology*, 76, 337-348.

Endress + Hauser (2008) *Technical Information Orbisint CPS11/CPS11D pH electrodes, analog or with digital Memosens technology.* Retrieved from http://www.endress.com/en/Tailor-made-field-instrumentation/liquid-analysis-product-overview/pH-digital-sensor-cps11d

Endress + Hauser (2012) *Operating Instructions Oxymax COS61D Dissolved oxygen sensor With Memosens protocol.* Retrieved from http://www.endress.com/en/Tailor-made-field-instrumentation/liquid-analysis-product-overview/oxygen-optical-sensor-cos61d

Etter, B., Udert, K.M., & Gounden, T. (Eds.). (2015). *VUNA Final Report*. Dubendorf, Switzerland: Eawag.

Etter, B., Hug, A., Udert, K.M. (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.

Fumasoli, A., Morgenroth, E., & Udert, K. M. (2015) Modeling the low pH limit of Nitrosomonas-type bacteria in high-strength nitrogen wastewaters, Manuscript submitted for publication.

Garbani, L. (2014) *Urine nitrification system model documentation*, Unpublished manuscript. Internal Eawag work.

GFA, Gesellschaft zur Förderung der Abwassertechnik (1996) *ATV M 20E Measurement of Oxygen Transfer in Activated Sludge Aeration Tanks with clean Water and in Mixed Liquor.* Bonn, Germany : J. F. CARTHAUS GmbH & Co.

Gonzalez, G. D. (1999) Soft Sensors for Processing Plants**.** In Proceedings of the Second International Conference on Intelligent Processing and Manufacturing of Materials (IPMM '99). Honolulu, Hawaii, 10-15 July.

Hess, A. (2015). Feasibility of UV-Vis spectrophotometry for nitrite estimation in urine nitrification systems (Unpublished master's thesis). ETH, Zurich, Switzerland.

ISO (1994) *ISO 5725-1: Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.* Geneva, Switzerland: International Organization for Standardization.

ISO (2003). ISO 15839: Water quality – On-line sensors/analysing equipment for water – Specifications and performances tests. Geneva, Switzerland: International Organization for Standardization.

Julier, S.J., & Uhlmann, J.K. (2004) Unscented filtering and nonlinear estimation. *Procedings of the IEEE*, 92(3), 401-422.

Kadlec, P., Gabrys, B., & Strandt, S. (2009) Review – Data-driven Soft Sensors in the process industry, *Computers and Chemical Engineering*, 33, 795-814.

Kämpf, G. (2015) Calibration and validation of the urine nitrification model (Unpublished master's thesis). ETH, Zurich, Switzerland.

Masic, A., & Villez, K. (2014) Model-based observers for monitoring of biological nitrification of urine in decentralized treatment. In *Proceedings of the 2nd IWA Specialized International Conference EcoTechnologies for Wastewater Treatment (ecoSTP2014)*. Verona, Italy, 23-27 June.

Metcalf & Eddy (2004) *Wastewater Engineering – Treatment and Reuse Fourth Edition*, New York, USA: McGraw-Hill.

Philichi, T. L., & Stenstrom, M. K. (1989) Effects of dissolved oxygen probe lag on oxygen transfer parameter estimation, *Water Pollution Control Federation,* 61 (1), 83-86.

Rieger, L., Alex, J., Winkler, S., Boehler, M., Thomann, M., & Siegrist, H. (2003) Progress in sensor technology – progress in process control? Part I: Sensor property investigation and classification, *Water Science and Technology*, 47 (2), 103-112.

Rieger, L., Thomann, M., Gujer, W.,& Siegrist, H. (2005) Quantifying the uncertainty of on-line sensors at WWTPs during field operation, *Water Research*, 39, 5162-5174.

Rieger, L., Takacs, I., Villez, K., Siegrist, H., Lessard, P., Vanrolleghem, P. A., Comeau, Y. (2010) Data reconciliation for wastewater treatment plant simulation studies – Planning for high-quality data and typical sources of errors, *Water Environment Research,* 82 (5), 426-433.

Spanjers, H., & Olsson, G. (1992) Modelling of the dissolved oxygen probe response in the improvement of the performance of a continuous respiration meter, *Water Research*, 26 (7), 945-954.

Thomann, M., Rieger, L., Frommhold, S., Siegrist, H., & Gujer, W. (2002) An efficient monitoring concept with control charts for on-line sensors, *Water Science and Tehcnology*, 46 (4), 107-116.

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

Wan, E. A., & van der Merwe, R. (2001). Chapter 7 The Unscented Kalman Filter. In Haykin, S. (Ed.), *Kalman Filtering and Neural Networks* (pp. 221-280). New York, USA: John Wiley & Sons, Inc..

WHO (2011) *Nitrate and Nitrite in Drinking-water - Background document for development of WHO Guidelines for Drinking-water Quality.* Geneva: Author.

A.1 Selected states for the reduced model, and initial conditions after running the model at steady state with an inflow and outflow of 24 L/d.

Reduced model	Full model	States	Reduced Model	Units
	1	V	120	L
	2	T	25	°C
1	3	XAOB	0.1521	$gCOD \cdot L^{-1}$
2	4	XNOB	0.0789	$gCOD \cdot L^{-1}$
3	5	O2	1.95E-04	$mol \cdot L^{-1}$
4	6	NH3	8.18E-05	$mol \cdot L^{-1}$
5	7	NH4	0.0667	$mol \cdot L^{-1}$
6	8	HNO2	5.47E-07	$mol \cdot L^{-1}$
7	9	NO2	8.05E-04	$mol \cdot L^{-1}$
8	10	NO3	0.0583	$mol \cdot L^{-1}$
9	11	proton	4.69E-07	$mol \cdot L^{-1}$
	12	K	0.0227	$mol \cdot L^{-1}$
	13	Na	0.0416	$mol \cdot L^{-1}$
	14	Ca	2.50E-04	$mol \cdot L^{-1}$
	15	Mg	0.0015	$mol \cdot L^{-1}$
10	16	OH	3.71E-08	$mol \cdot L^{-1}$
	17	Cl	0.0516	$mol \cdot L^{-1}$
11	18	CO3	2.43E-07	$mol \cdot L^{-1}$
12	19	HCO3	8.04E-04	$mol \cdot L^{-1}$
13	20	CO2	4.69E-04	$mol \cdot L^{-1}$
	21	PO4	3.71E-09	$mol \cdot L^{-1}$
	22	HPO4	7.90E-04	$mol \cdot L^{-1}$
	23	H2PO4	1.93E-03	$mol \cdot L^{-1}$
	24	H3PO4	7.35E-08	$mol \cdot L^{-1}$
	25	NH4H2PO4	9.29E-05	$mol \cdot L^{-1}$
	26	NH4HPO4	3.50E-04	$mol \cdot L^{-1}$
	27	KH2PO4	5.03E-05	$mol \cdot L^{-1}$
	28	K2HPO4	1.04E-06	$mol \cdot L^{-1}$
	29	KHPO4	4.70E-05	$mol \cdot L^{-1}$
	30	NaH2PO4	9.22E-05	$mol \cdot L^{-1}$
	31	Na2HPO4	2.31E-06	$mol \cdot L^{-1}$
	32	NaHPO4	1.27E-04	$mol \cdot L^{-1}$
	33	HSO4	3.64E-08	$mol \cdot L^{-1}$
	34	SO4	2.41E-03	$mol \cdot L^{-1}$
	35	NH4SO4	5.67E-04	$mol \cdot L^{-1}$
	36	KSO4	1.28E-04	$mol \cdot L^{-1}$
	37	NaSO4	1.82E-04	$mol \cdot L^{-1}$
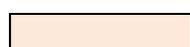
Varying states in the reduced

A.2 Original model description: Kinetics and Process matrix parameters

Kinetics

process	process rates	variable	unit	unit
AOB				
aerobic growth	mue_AOB*S_NH3/(K_NH3_AOB+S_NH3)*I_HNO2_AOB/(S_HNO2+I_HNO2_AOB)*(1-K_pH*(pHmin-pH))*X_AOB	r_NH_Growth	gCOD/m³/d	gCOD·L⁻¹·d⁻¹
NOB				
aerobic growth	mue_NOB*S_HNO2/(K_HNO2_NOB+S_HNO2+S_HNO2^2/I_HNO2_NOB)*X_NOB	r_NO_Growth	gCOD/m³/d	gCOD·L⁻¹·d⁻¹
Acid-base equilibrium				
HCO3 -> CO3 + H	k_eq_CO3*(S_HCO3*fA1-S_CO3*fA2*S_proton*fA1*10^pK_CO3)	eq_CO3	gC/m³/d	mol·L⁻¹·d⁻¹
H2CO3 -> HCO3 + H	k_eq_HCO3*(S_CO2-S_HCO3*fA1*S_proton*fA1*10^pK_HCO3)	eq_HCO3	gC/m³/d	mol·L⁻¹·d⁻¹
NH4 -> NH3 + H	k_eq_NH3*(S_NH4*fA1-S_NH3*S_proton*fA1*10^pK_NH3)	eq_NH3	gN/m³/d	mol·L⁻¹·d⁻¹
HNO2 -> NO2 + H	k_eq_NO2*(S_HNO2-S_NO2*fA1*S_proton*fA1*10^pK_NO2)	eq_NO2	gN/m³/d	mol·L⁻¹·d⁻¹
H3PO4 -> H2PO4- + H+	k_eq_H2PO4*(S_H3PO4-S_H2PO4*fA1*S_proton*fA1*10^pK_H2PO4)	eq_H2PO4		mol·L⁻¹·d⁻¹
H2PO4- -> HPO42- + H+	k_eq_HPO4*(S_H2PO4*fA1-S_HPO4*fA2*S_proton*fA1*10^pK_HPO4)	eq_HPO4	gP/m³/d	mol·L⁻¹·d⁻¹
HPO42- -> PO43- + H+	k_eq_PO4*(S_HPO4*fA2-S_PO4*fA3*S_proton*fA1*10^pK_PO4)	eq_PO4	gP/m³/d	mol·L⁻¹·d⁻¹
HSO4- -> SO42- + H+	k_eq_SO4*(S_HSO4*fA1-S_SO4*fA2*S_proton*fA1*10^pK_SO4)	eq_PO4	gP/m³/d	mol·L⁻¹·d⁻¹
OH equilibrium rate constant	k_eq_OH*(1-S_OH*fA1*10^(pK_OH-pH))	eq_OH		mol·L⁻¹·d⁻¹
Gas exchange				
CO₂(aq) → CO₂(g)	(H_CO2*S_CO2-S_CO2_air)*Q_gas/Volume*(1-exp(-KLa_CO2*Volume/H_CO2/Q_gas))	r_CO2_strip	gC/m³/d	mol·L⁻¹·d⁻¹
NH₃(aq) → NH₃(g)	(H_NH3*S_NH3-S_NH3_air)*Q_gas/Volume	r_NH3_strip	gN/m³/d	mol·L⁻¹·d⁻¹
HNO₂(aq) → HNO₂ (g)	(H_HNO2*S_HNO2-S_HNO2_air)*Q_gas/Volume	r_HNO2_strip		mol·L⁻¹·d⁻¹
O2(g) → O2(aq)	KLa_O2*(S_O2_sat-S_O2)	r_aeration	gO2/m³/d	mol·L⁻¹·d⁻¹
Komplexes				
2 K+ + H2PO4- --> K2HPO4 + H+	k_eq_K2HPO4*(S_K^2*fA1^2*S_H2PO4*fA1-S_K2HPO4*S_proton*fA1*10^pK_K2HPO4)	eq_K2HPO4		mol·L⁻¹·d⁻¹
K+ + H2PO4- --> KH2PO4	k_eq_KH2PO4*(S_K*fA1*S_H2PO4*fA1-S_KH2PO4*10^pK_KH2PO4)	eq_KH2PO4		mol·L⁻¹·d⁻¹
K+ + H2PO4- --> KHPO4- + H+	k_eq_KHPO4*(S_K*fA1*S_H2PO4*fA1-S_KHPO4*fA1*S_proton*fA1*10^pK_KHPO4)	eq_KHPO4		mol·L⁻¹·d⁻¹
K+ + SO42- --> KSO4-	k_eq_KSO4*(S_K*fA1*S_SO4*fA2-S_KSO4*fA1*10^pK_KSO4)	eq_KSO4		mol·L⁻¹·d⁻¹
2 Na+ + H2PO4- -> Na2HPO4 + H+	k_eq_Na2HPO4*(S_Na^2*fA1^2*S_H2PO4*fA1-S_Na2HPO4*S_proton*fA1*10^pK_Na2HPO4)	eq_Na2HPO4		mol·L⁻¹·d⁻¹
Na+ + H2PO4- -> NaH2PO4	k_eq_NaH2PO4*(S_Na*fA1*S_H2PO4*fA1-S_NaH2PO4*10^pK_NaH2PO4)	eq_NaH2PO4		mol·L⁻¹·d⁻¹
Na+ + H2PO4- -> NaHPO4- + H+	k_eq_NaHPO4*(S_Na*fA1*S_H2PO4*fA1-S_NaHPO4*fA1*S_proton*fA1*10^pK_NaHPO4)	eq_NaHPO4		mol·L⁻¹·d⁻¹
Na+ + SO42- -> NaSO4-	k_eq_NaSO4*(S_Na*fA1*S_SO4*fA2-S_NaSO4*fA1*10^pK_NaSO4)	eq_NaSO4		mol·L⁻¹·d⁻¹
NH4+ + H2PO4- -> NH4H2PO4	k_eq_NH4H2PO4*(S_NH4*fA1*S_H2PO4*fA1-S_NH4H2PO4*10^pK_NH4H2PO4)	eq_NH4H2PO4		mol·L⁻¹·d⁻¹
NH4+ + HPO42- -> NH4HPO4-	k_eq_NH4HPO4*(S_NH4*fA1*S_HPO4*fA2-S_NH4HPO4*fA1*10^pK_NH4HPO4)	eq_NH4HPO4		mol·L⁻¹·d⁻¹
NH4+ + SO42- -> NH4SO4-	k_eq_NH4SO4*(S_NH4*fA1*S_SO4*fA2-S_NH4SO4*fA1*10^pK_NH4SO4)	eq_NH4SO4		mol·L⁻¹·d⁻¹

Parameters of the process matrix

Class	parameter		expression	unit	description	source
biol	iN		1/160	molN/gCOD	Nitrogen fraction of biomass	assumed composition of biomass: $C_5H_7O_2N$
biol	iC		5/160	molC/gCOD	Carbon fraction of biomass	assumed composition of biomass: $C_5H_7O_2N$
biol	iH		7/160	molH/gCOD	Hydrogen fraction of biomass	assumed composition of biomass: $C_5H_7O_2N$
biol	iO		2/160	molO/gCOD	Oxygen fraction of biomass, takes into account other oxygen demands	assumed composition of biomass: $C_5H_7O_2N$
AOB						
biol	mueStarAOB		1.28*10^12/1.2545	d^{-1}	maximal growth rate	Jubany et al. (2009)
biol	bStarAOB		1.651*10^11	d^{-1}	decay rate	Jubany et al. (2009)
biol	temperatureConstantAOB		8183	K		Jubany et al. (2009)
biol	Y_AOB		2.52	gCOD/molN	Growth yield	Jubany et al. (2009), 0.18 gCOD/gN
biol	K_NH3_AOB		2.23*10^(-5)	mol·L-1	Substrate limitation constant	Corinne implementation
biol	I_HNO2_AOB		0.00019	mol·L-1	Inhibition cnostant	Corinne implementation
biol	K_pH		2.1	-	fitting parameter	experimentally determined
biol	pH_min_1		0.002	-	constant 1 to compute the above pH_min in ReactRateSlow	experimentally determined
biol	pH_min_2		0.00037	-	constant 1 to compute the above pH_min in ReactRateSlow	experimentally determined
biol	pH_min		(S_HNO2+0.0020088)/0.00037119	-	minimal pH	experimentally determined
NOB						
biol	mueStarNOB		6.69*10^7/1.2545	d^{-1}	maximal growth rate	Jubany et al. (2009)
biol	bStarNOB		8.626*10^6	d^{-1}	decay rate	Jubany et al. (2009)
biol	temperatureConstantNOB		5295	K		Jubany et al. (2009)
biol	Y_NOB		1.12	gCOD/molN	Growth yield	Jubany et al. (2009)
biol	K_HNO2_NOB		5.71425*10^(-7)	mol·L-1	Substrate limitation constant	Corinne implementation
biol	I_HNO2_NOB		3.214*10^(-5)	mol·L-1	Inhibition cnostant	Corinne implementation
Acid-base						
chem	pK_CO3	HCO3 -> CO3 + H	10.33	-	acidity constant of HCO3 -> CO3 + H	Thermo_mintey.dat, Visual minteq
chem	pK_HCO3	H2CO3 -> HCO3 + H	6.35	-	acidity constant of H2CO3 -> HCO3 + H	Thermo_mintey.dat, Visual minteq
chem	pK_NH3	NH4 -> NH3 + H+	9.24	-	acidity constant of NH4 -> NH3 + H	Thermo_mintey.dat, Visual minteq
chem	pK_NO2	HNO2 -> NO2 + H+	3.25	-	acidity constant HNO2 -> NO2 + H	Lide (2009)
chem	pK_H2PO4	H3PO4 -> H2PO4- + H+	2.15	-	acidity constant of H3PO4 -> H2PO4- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_HPO4	H2PO4- -> HPO42- + H+	7.2	-	acidity constant of H2PO4- -> HPO42- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_PO4	HPO42- -> PO43- + H+	12.38	-	acidity constant of HPO4 -> PO43- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_SO4	HSO4- -> SO42- + H+	1.99	-	acidity constant of HSO4- -> SO42- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_OH	H2O --> OH- + H+	14	-	OH equilibrium rate constant	Thermo_mintey.dat, Visual minteq
chem	k_eq_CO3		1000	d^{-1}	CO3 forward reaction rate constant	assumed
chem	k_eq_HCO3		1000	d^{-1}	HCO3 forward reaction rate constant	assumed
chem	k_eq_NH3		1000	d^{-1}	NH3 forward reaction rate constant	assumed
chem	k_eq_NO2		1000	d^{-1}	NO2 forward reaction rate constant	assumed
$H_3PO_4/H_2PO_4^-$ - equilibrium						
chem	k_eq_H2PO4		1000	d^{-1}	H2PO4 forward reaction rate constant	assumed
chem	k_eq_HPO4		1000	d^{-1}	HPO4 forward reaction rate constant	assumed
chem	k_eq_PO4		1000	d^{-1}	PO4 forward reaction rate constant	assumed
chem	k_eq_SO4		1000	d^{-1}	SO4 forward reaction rate constant	assumed
chem	k_eq_OH		1000	d^{-1}	OH forward reaction rate constant	assumed
Gas Exchange						
gas	H_CO2		1.2	g C(g)/ g C(aq)	Henry coefficient for CO_2	Stumm and Morgan (1996) p.214
gas	H_NH3		7.2E-04	g NH_3-N(g)/ g NH_3-N(aq)	Henry coefficient for NH_3	Stumm and Morgan (1996) p.214
gas	H_HNO2		8.3E-04	g HNO_2-N(g)/ g HNO_2-N(aq)	Henry coefficient for HNO_2	Stumm and Morgan (1996) p.214
gas	H_O2		32.4	g O2(g)/g O2 (aq)	Henry coefficient for O_2	Stumm and Morgan (1996) p.214
gas	k_KLa_CO2		36.7933	1/d^2		
gas	k_KLa_O2		10*38.73	1/d^2		
gas	k_KLa_HNO2		1.0E+06	1/d^2		
gas	k_KLa_NH3		1000000	1/d^2		
gas	c1		0.082057	l*atm/mol/K	Constant to compute S_CO2_air	from Stumm and Morgan (1996)
gas	absoluteZeroCelsius		273.15	°K, °C?	Absolute zero	Everywhere
gas	S_NH3_air		0	molN(g)/L	concentration of NH3 in the air	Assumption
gas	S_HNO2_air		0	molN(g)/L	concentration of NHNO2 in the air	Assumption
gas	S_O2_sat		8.25/32/1000	mol/L	saturation concentration for O2	Skript Siegrist (2009)
Komplexes						
chem	pK_K2HPO4		6.07	-	equilibrium constant of 2 K+ + H2PO4- -> K2HPO4 + H+	Thermo_mintey.dat, Visual minteq
chem	pK_KH2PO4		-0.3	-	equilibrium constant of K+ + H2PO4- -> KH2PO4	Thermo_mintey.dat, Visual minteq
chem	pK_KHPO4		6.3	-	equilibrium constant of K+ + H2PO4- -> KHPO4- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_KSO4		-0.85	-	equilibrium constant of K+ + SO42- -> KSO4-	Thermo_mintey.dat, Visual minteq
chem	pK_Na2HPO4		6.25	-	equilibrium constant of 2 Na+ + H2PO4- -> Na2HPO4 + H+	Thermo_mintey.dat, Visual minteq
chem	pK_NaH2PO4		-0.3	-	equilibrium constant of Na+ + H2PO4- -> NaH2PO4	Thermo_mintey.dat, Visual minteq
chem	pK_NaHPO4		6.13	-	equilibrium constant of Na+ + H2PO4- -> NaHPO4- + H+	Thermo_mintey.dat, Visual minteq
chem	pK_NaSO4		-0.74	-	equilibrium constant of Na+ + SO42- -> NaSO4-	Thermo_mintey.dat, Visual minteq
chem	pK_NH4H2PO4		-0.1	-	equilibrium constant of NH4+ + H2PO4- -> NH4H2PO4	Martell et al. (1997)
chem	pK_NH4HPO4		-1.3	-	equilibrium constant of NH4+ + HPO42- -> NH4HPO4-	Martell et al. (1997)
chem	pK_NH4SO4		-1.03	-	equilibrium constant of NH4+ + SO42- -> NH4SO4-	Thermo_mintey.dat, Visual minteq
chem	pK_NaCO3		-1.27			Corinne implementation
chem	pK_NaHCO3		0.25			Corinne implementation
chem	k_eq_K2HPO4		1000	d^{-1}	K2HPO4 equilbrium rate constant	assumed
chem	k_eq_KH2PO4		1000	d^{-1}	KH2PO4 equilibrium rate constant	assumed
chem	k_eq_KHPO4		1000	d^{-1}	KHPO4 equilibrium rate constant	assumed
chem	k_eq_KSO4		1000	d^{-1}	KSO4 equilibrium rate constant	assumed
chem	k_eq_Na2HPO4		1000	d^{-1}	Na2HPO4 equilbrium rate constant	assumed
chem	k_eq_NaH2PO4		1000	d^{-1}	NaH2PO4 equilibrium rate constant	assumed
chem	k_eq_NaHPO4		1000	d^{-1}	NaHPO4 equilibrium rate constant	assumed
chem	k_eq_NaSO4		1000	d^{-1}	NaSO4 equilibrium rate constant	assumed
chem	k_eq_NH4H2PO4		1000	d^{-1}	NH4H2PO4 equilibrium rate constant	assumed
chem	k_eq_NH4HPO4		1000	d^{-1}	NH4HPO4- equilibrium rate constant	assumed
chem	k_eq_NH4SO4		1000	d^{-1}	NH4SO4 equilibrium rate constant	assumed

A.3 ISO standard definitions and notes from ISO 5725-1:1994 (*) and ISO 15839:2003 (**)

Precision*	The closeness of agreement between independent test results obtained under stipulated conditions. *Note 1 to entry*: Precision depends only on the distribution of random errors and does not relate to the true value or the specified value. *Note 2 to entry*: The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation. *Note 3 to entry*: "Independent test results" means results obtained in a manner not influenced by any previous result on the same or similar test object. Quantitative measures of precision depend critically on the stipulated conditions. Repeatability and reproducibility conditions are particular sets of extreme conditions.
Trueness*	The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value. *Note 1 to entry*: The measure of trueness is usually expressed in terms of bias. *Note 2 to entry*: Trueness has been referred to as "accuracy of the mean". This usage is not recommended.
Response time**	Time interval between the instant when the on-line sensor/analysing equipment is subjected to an abrupt change in determinand value and the instant when the readings cross the limits of (and remain inside) a band defined by 90 % and 110 % of the difference between the initial and final value of the abrupt change. *Note 1 to entry*: In laboratory testing, the response time of the on-line sensor/analysing equipment is measured. In field testing, it is the whole measurement chain which is tested.

A.4 Experimental protocols

PROTOCOL 1: Field-specific medium sampling (Day before the experiment)
Estimated time needed: Sedimentation (2 hours), Filtration (1.5 h), Autoclaving (7.5 h)

Required products:
1) 2.5 L from the urine tank (Column 1)
2) Nanopure water

Required hardware:
Sampling
1) 2.5 L container for sampling
2) Another waste container

Sedimentation
3) 2 Imhoff cones of 1 L with valves
4) 2 beakers

Filtration
5) 0.7 μm filter paper (MN GF-1 ϕ90mm)
6) Glass Filter
7) Vacuum pump and air tube
8) Forceps
9) Intermediate container(s) for storing
10) Final 2.5 L glass container with a lid
11) Funnel
12) 1-2 beakers

Autoclaving
13) Autoclaver
14) 4 pressure resistant closed bottles (250 mL, DURAN glass)
15) Funnel
16) Oven hand glove
17) 2.5 L glass container with a lid

Steps:
1) Take a 2.5 L sample from the urine reactor
   a) Open the tap behind the reactor
   b) Throw away the first mLs
      -using the waste container
      -rinse this waste container after use
   c) Fill the 2.5 L container, add lid, and take to the lab

2) Sedimentation
   a) Empty the sample in the Imhoff cones
      i) Leave a couple hours to settle
      ii) Swing the Imhoff cones from side to side to help with the settling from time to time
   b) Remove the sedimented sludge with a beaker by opening the valve.
   c) Afterwards, retrieve the sample for filtering from the bottom with another beaker

3) 0.7 μm filtration

a) Put together the filter and plug in the vacuum pump
b) Place the 0.7 μm filter paper, humidify it so as to avoid air bubbles forming
c) Add the top part of the filter
   i) Well centered
   ii) Press on the edges once in place to make sure it is sealed
   iii) Add the metal part, well centered, and tighten the seal
d) Rinse the filter
   i) Wet the filter paper with a bit of nanopure water and only afterwards turn the pump on
   ii) Empty the filtered water
      - Stop the pump and remove the air tube
      - Slowly remove and place the top part of the filter on an empty beaker, making sure that this does not dip into any non-filtered solution
      - Empty out the collected liquid
   iii) Add a bit of the urine sample and turn pump on
   iv) Empty out the collected liquid as in step (3.d.ii)
e) Filter urine
   i) Add the urine supernatant
   ii) Turn the vacuum pump on for a **2-3 sec at a time only**,
   iii) Wait until the filtration rate slows down again before pumping again
f) Collecting the filtered solution
   i) Stop the pump and remove the air tube
   ii) Slowly remove and place the top part of the filter on a clean beaker, making sure that this is not submerged into any non-filtered solution
   iii) Pour the collected solution into the intermediate container until the filtration rate with the vacuum pump slows down too much,
   iv) Stop the pump and remove the air tube
   v) Empty the remaining liquid waiting to be filtered back into the Imhoff cones
   vi) Loosen the metal part and remove the top part of the filter
   vii) Check for the quality of the seal on the filter paper
      - **If in doubt**, the "filtered" sample in the intermediate container should go back into the Imhoff cone, and a new intermediate container should be used
      - Otherwise, the filtered sample is poured into the final 2.5 L container
   viii) Remove the filter paper with the forceps
g) Add a new filter paper and REPEAT steps (b) to (h) until the whole urine sample is filtered
h) Label the 2.5 L container with "NAME – Column 1 Urine Reactor sample Filtered 0.7 μm, DATE"

4) Autoclave the 1L solution
   a) With a funnel, fill the pressure resistant bottles no more than 250 mL of filtrated solution
   b) Autoclave the four bottles (30 min, 1.2 bar, 120°C)
      i) Check that the water level is just underneath the security valve
      ii) Place the 4 bottles on the train inside
      iii) (If needed, "on" is at the bottom right of the instrument)
      iv) Make sure the program 3 Erlenmeyer flask is activated
      v) Close the door by turning an extra 2 turns after the "close door" light turns on
      vi) Press start

c) Wait 1.5 hours (or till the Exksikator exhaust light is no longer on and temperature has cooled down)
   i) Take the bottles out with the oven hand glove
   ii) Wait about 1 hour for the autoclaver and bottles to cool down.
   iii) Empty the autoclaved bottles into the 2.5 L container and close the lid (to avoid evapotranspiration)
d) Label the 2.5 L container with "NAME – Column 1 Urine Reactor sample Filtered 0.7 µm and Autoclaved, DATE sample taken".
e) REPEAT Step 4 until the whole solution has been autoclaved.

PROTOCOL 2: Preparation of the oxygen-saturated media (day before)

Required products:
1) 1 L of field-specific medium (Protocol 1)
2) 500 mL nanopure water
3) 3 tubes of pressured humidified air

Required hardware:
1) 3 tall 1 L beakers
2) 3 gas diffusers
3) Tubes and air valve connecting to the humidified pressured air used for the reactor
4) Parafilm
5) Air flow regulator clips

Steps:
1) Remove any water accumulated in the tube
   a) Remove the diffusers from the tube
   b) Put the tubes in a waste beaker
   c) Open the air flow valve and wait for any water to flow out
   d) Close the air flow valve
2) Place the diffusers in the beakers labelled "2", "4", and "6"
   a) Connect the diffusers to the air tube
   b) Form a circle with the diffuser (end to end)
   c) Slowly lower it down inside the beaker, making sure that it holds tightly on the edges
3) Fill the beakers with about 500 mL ("2" and "6" with field-specific medium; "4" with nanopure)
4) Cover with parafilm to avoid spatter
5) Allow the air flow in by turning the air valve, until large bubbles are reached
6) Adjust the regulator clips so that this is similar in all beakers
7) Leave overnight
8) (continue with Protocol 5 the next day)

PROTOCOL 3: Cobalt dosage with a security factor of 15

Required products:
1) Cobalt (II) chlorid-Hexahydrat [Catalyst] **C10**

Required hardware:
1) 3 small containers
2) mg-precise scale
3) (Parafilm)

Steps:

1) Roughly dose three times 15 mg of Cobalt(II) chlorid-Hexahydrat
   a) Place a small container on the scale, close the glass, and press tare
   b) Label the containers with CoCl.H2O and the concentration, name, date
2) Close the containers or cover them with parafilm
3) Bring these to the urine reactor room

PROTOCOL 4: Sodium sulfite preparation with a security factor of 5

Required products:
1) Sodium sulfite **N19**

Required hardware:
1) 3 small containers or lids
2) mg-precise scale
3) Parafilm

Steps:

1) Roughly dose three times 200 mg sodium sulfite
   a) Place the same containers as above on a small lid on the scale, close the glass, and press tare
   b) Label the containers with name, date, Na_2SO_3 , and the concentration added.
2) Close/cover the containers with parafilm
3) Bring these to the urine reactor room

PROTOCOL 5: Step change experiment – Dissolved Oxygen

Required products:
1) 2 beakers of aerated field-specific medium (Protocol 2)
2) 1 L of Field medium with Cobalt (II) addition (Protocol 3)
3) 500 mL of nanopure with Cobalt (II) addition (Protocol 3)
4) Nanopure water
5) 3 Sodium sulfite preparations (Protocol 4)

Required hardware:
1) Timer (1 sec resolution)
2) Camera

3) Bucket
4) 7 beakers
5) 2 L Container to recover the field-specific medium

6) 2 Magnetic stirrers
7) Magnetic stick to recover the stir bars
8) Minimum of 2 stir bars
9) Metallic arm to hold the sensor upright

10) SD card

Steps:
The night before
1) All solutions should already be at room temperature (bring from Lab the night before)
2) **Protocol 2** for beakers 2, 4, and 6

Two hours before
3) Stop the aeration in beakers 2,4,6
4) Remove and rinse the diffusers from beakers 2,4,6

Initial steps (which can be done during those 2 hours)
5) Set the measurement interval for the DO sensor in column 1 at 1 sec
    a) On the measurement transformer: Menü→ Setup→ Allgemeine Einstellungen→ Logbücher→ Datenlogbücher→ Datenlogbuch 4 DO 1
    b) Change the Abtastzeit to „00:00:01"
6) Note in the lab book for urine reactors: "NAME – Column 1 DO sensor dynamic experiments"
7) Take the DO sensor slowly out of the reactor in Column 1
    a) Place a bucket underneath to prevent dripping
    b) Turn the 1st handle and to untighten the sensor and pull the sensor out until you see the screws
    c) Pull the 2nd handle up till it touches the reactor to close the valve
    d) Completely turn and remove the 1st handle, making sure that the valve is well closed
    e) Take off the rings around the sensor
    f) Empty the bucket but keep the carriers that might have fallen out

g) Allow at least 20 min for the sensor to acclimatize to this air temperature

8) While waiting, **Protocols (3 and) 4** for beakers 1, 3, and 5

9) Set up the metallic arm so that the sensor will be able to be changed easily from aerated to non-aerated

10) Note the Temperature of the room with the thermometer next to the computer

11) Note the Atmospheric pressure on the CoDeSys from the distiller

12) Find the expected saturation with the excel file
Q:\Abteilungsprojekte\eng\EngData\ProcEng\VUNA\nitri\Sonden-log.xlsx

13) Timer
a) Start the timer at the same time as the Messumformer
b) Take a picture of the Timer and Messumformer time together
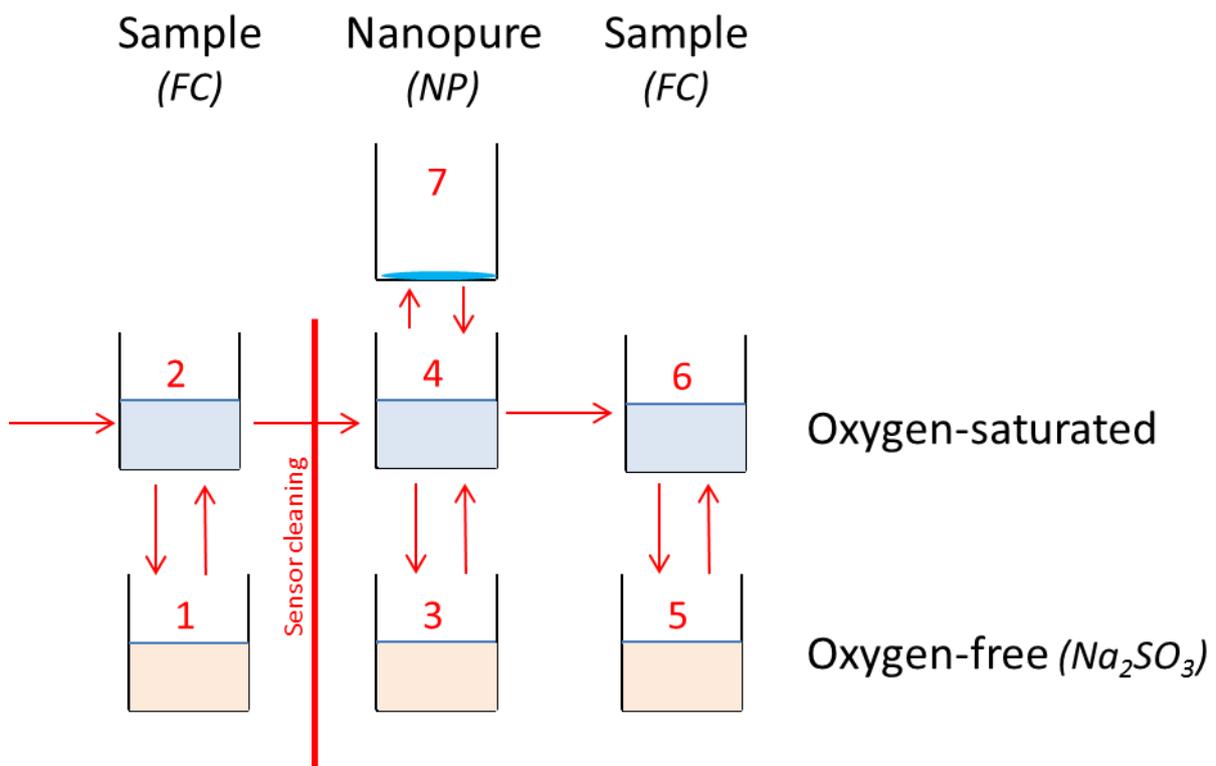
Measurement Procedure (Overview in the diagram above)

At every step/beaker:
➔ Note the exact time on the timer that the sensor was put IN and OUT of the beakers, as well as the final value reached
➔ Wait 15 min at each change of beaker (or till the value on the Messtransformer is stable for 3 min)

14) First experiment set (field conditions without cleaning the sensor): beakers 1 and 2
a) Place a magnetic stir bar into beakers 2 and 1, and place these on the magnetic stirrers
b) Turn the stirrer on to the marked frequency for beaker 2
c) Place the DO sensor (without cleaning) into beaker 2 so that the cap is submerged

x

d) About 1min before changing the sensor, add one of the chemical preparations (**Protocol 3** if cobalt is not already present in the beaker) (**Protocol 4**) in beaker 1
  - Rinse the container in the solution if necessary
  - Cover with parafilm
  - Stir a couple seconds
  - Place the sensor directly in beaker 1
e) Then place the sensor back in beaker 2

15) Cleaning of the sensor membrane
  a) With nanopure and a hand glove
  b) With nanopure and a tissue
  c) With nanopure and a hand glove

16) 2nd experiment set (nanopure cleaned sensor): beakers 3 and 4
  a) Place a stir bar in beakers 3 and 4
  b) Start mixing for beaker 4 and place the DO sensor in beaker 4 so that the cap is submerged
  c) About 1min before changing the sensor, add one of chemical preparations (**Protocol 3** if cobalt is not already present in the beaker) (**Protocol 4**) in beaker 3
    - Dip the container in the solution if necessary
    - Cover with parafilm
    - Stir a couple seconds
    - Place the sensor directly in beaker 3
  d) Place the sensor back in beaker 4
  e) Place the DO sensor straight up in beaker 7 above a of nanopure water for air humidity (sensor membrane not touching water)
  f) Place the sensor back in beaker 4

17) While waiting, perform **step 28** for the FC samples from beakers 1 and 2

18) Evaluate the data quality: note if 0.1 or 0.2 difference between the expected value calculated in **step 12** and the measured value (calibration limit).

19) 3rd experiment set (field conditions): beakers 5 and 6
  a) Place the beakers 5 and 6 on the magnetic stirrers and turn these on to the marked frequency
  b) Place the DO sensor into beaker 6
  c) About 1min before changing the sensor, add one of chemical preparations (**Protocol 3** if cobalt is not already present in the beaker) (**Protocol 4**) in beaker 5
    - Dip the container in the solution if necessary
    - Cover with parafilm
    - Stir a couple seconds
    - Place the sensor directly in beaker 5
  d) Place the sensor back in beaker 6

20) Clean up and rinse all beakers with nanopure

21) *If Calibration (1 point) is still necessary after cleaning*
  a) *Clean the sensor again*

b) *Place the DO sensor straight up in beaker 7 above nanopure water for air humidity (sensor membrane not touching water)*
c) *leave for 10 min, and*
d) *On the measurement transformer, Cal→ CH 2:1 (or CH 3:2) Sauerstoff*
e) *Start by calibrating the temperature according to the thermometer on the conductivity meter*
f) *Then calibrate the oxygen concentration (→ Steigung → Luft 100% →ok…)*
g) *Write the new slope (Steigung) and atmospheric pressure (Bezugsdruck) in the excel file: Q:\Abteilungsprojekte\eng\EngData\ProcEng\VUNA\nitri\Sonden-log.xlsx (F1 is column 1, even if it receives urine from tank H2)*

End of measurements
22) Place DO sensor back in the urine reactor, as well as any carriers that might have fallen out
23) Recover all the FC solutions in the 2 L container
24) Rinse the beakers and stir bars with nanopure
25) Take a picture of the Timer and Messumformer time together
26) At least 30 min after putting the sensor back in the reactor:
    Set the measurement interval for the DO sensor in column 1 back at 1 min
    a) On the measurement transformer: Menü→ Setup→ Allgemeine Einstellungen→ Logbücher→ Datenlogbücher→ Datenlogbuch 4 DO 1
    b) Change the Abtastzeit to „00:01:00"
27) Reading out the data
    a) Follow the steps for "Backup PLS" on the monitor
    b) Leave SD card overnight
    c) Put it into the computer
    d) Save in "Backup PLS" file on the desktop or under Q:\Abteilungsprojekte\eng\EngData\ProcEng\Alexandra\Doktorat\Labresults\FC\Datensicherung\Messumformer

28) Repeat ***Protocol 1: Field-specific medium sampling*** from Step 3 with the FC solutions (filter and autoclave again)

29) Wait 1 week for biomass growth before repeating ***Protocol 2,3 and 5***

PROTOCOL 6: Extensive cleaning of the pH sensors

Required products:
1) Nanopure
2) Ethanol
3) Hydrochloric acid 32%

Required hardware:

1) Nanopure wash bottle
2) Safety glasses
3) Two 100 mL flasks
4) mg-precise scale
5) Two pipettes
6) 4 small plastic bottles
7) 1 rinsing beaker
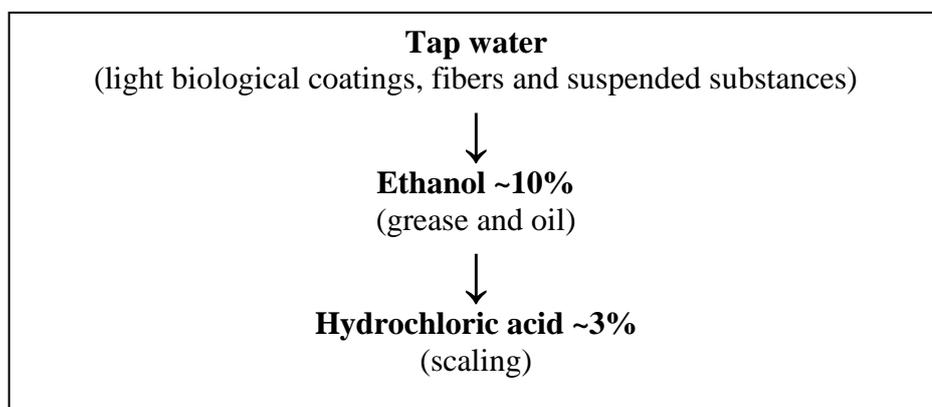
```
┌──────────────────────────────────────────────────────────────┐
│                          Tap water                             │
│   (light biological coatings, fibers and suspended substances) │
│                                                                │
│                             ↓                                  │
│                       Ethanol ~10%                             │
│                       (grease and oil)                         │
│                                                                │
│                             ↓                                  │
│                   Hydrochloric acid ~3%                        │
│                          (scaling)                             │
│                                                                │
└──────────────────────────────────────────────────────────────┘
```

DO NOT leave the sensors in the air as they should not dry up.
DO NOT use nanopure or distilled water.

Tap water (not nanopure!) under pressure
    1) Clean the sensors extensively with water
    2) Let them soak

Preparation
→Ethanol ~10%
    3) Put on gloves and safety glasses
    4) Place the flask on a scale and press tare
    5) Pour about 12.6 g of ethanol in the graduated container (10 mL)
    6) Fill with nanopure until the 100 mL limit
    7) Pour this into small plastic bottles and label "NAME, Ethanol 10%, DATE"

→ Hydrochloric acid ~3%
    8) Put on gloves and safety glasses
    9) Pour about 9.4 g (9.4 mL) of the hydrochloric acid
    10) Fill with nanopure until the 100 mL limit
    11) Pour this into two small plastic bottles and label "NAME, HCl 3%, DATE"

Water under pressure

    12) Clean the sensors again extensively with water

Ethanol ~10%

    13) Soak the sensors about 10 min in the ethanol 10% bottles

    14) Rinse with water

    15) Dispose of this ethanol solution in the sink.

Hydrochloric acid ~3%

    16) Soak the sensors no longer than 1-2 min

    17) Rinse with water

    18) Place the HCl cleaning solution in a sealed container to bring to the other building.

    19) Dispose safely (in the acid bucket available)

PROTOCOL 7: Preparing the HCl-adjusted urine reactor sample at pH 4 (no dilution)

Required products:
1) HCl 32% solution [10.17 M; density 1.159 g/mL]
2) 0.1 M HCl soluton [3.65 g_HCl/L]

3) Nanopure
4) Standard solution pH 4
5) 1 L urine filtered and autoclaved reactor sample (**Protocol 1**)

Required hardware:
1) Safety glasses
2) 2 Microdrop pipettes

3) Cleaned pH sensor
4) Magnetic stirrer and stir bar
5) Magnetic stir bar remover

Steps
1) Clean the sensor extensively with water
2) Weigh the bottle containing the solution to be adjusted with the stir bar inside
3) Place the pH sensor in a pH 4 buffer solution
4) Write down the pH and voltage values(achievement goal)
5) Place the container on the magnetic stirrer for mixing
6) Place the sensor in the solution
7) Wait 5 min or stabilization of the signal and note down the time, pH and voltage signal
8) HCl 32% adjustment (about 0.08 pH change per drop)
    a) Put on gloves and safety glasses
    b) Add the HCl 32% solution drop by drop
    c) At regular intervals (max 5 drops) write down the
       time, number of drops, pH and voltage signal
    d) Stop when the pH goes below 4.6 (Should be about 15-20 drops)
    e) Weigh the solution with the stir bar inside
    f) Store the rest of the HCl solution securely "Name, HCl 32%, 10.2 M, Date" with a
       corrosion warning sticker
9) HCl 0.1 M fine adjustment (about 0.002 pH change per drop)
    a) Put on gloves and safety glasses
    b) At regular intervals (max 40 drops) write down the
       time, number of drops, pH and voltage signal
    c) Stop when the pH and voltage signal correspond to that of the buffer solution (Should
       be about 300 drops)
    d) Weigh the solution with the stir bar inside
    e) Store the rest of the HCl solution securely "Name, HCl 0.1 M, Date" with a corrosion
       warning sticker

10) Place the pH sensor in a pH 4 buffer solution
11) Write down the pH value and voltage signal
12) Take out the magnetic stir bar and rinse
13) Label the sample with "NAME, pH4-adjusted urine sample, DATE OF PREPARATION"
14) Read out the data

PROTOCOL 8: Set the pH 7 for the Field condition sample (before every experiment)

Required products:
1) 2g of NaOH (pellets)
2) Nanopure
3) Standard solution pH 7
4) 1 L urine filtered and autoclaved reactor sample (**Protocol 1**)

Required hardware:
1) Safety glasses
2) Scale
3) 25 mL flask
4) 50 mL container for the stock solution
5) 2 microdrop pipettes
6) Cleaned pH sensor (**Protocol 6**)
7) Magnetic stirrer and stir bar
8) Magnetic stir bar remover

Steps
1) Preparation of the Stock Solution
   a) Put on gloves and safety glasses
   b) Weigh about 2g in NaOH pellets
   c) Place these in the 25 mL flask and add nanopure until the meniscus reaches the 25 mL mark
   d) Mix until completely dissolved
   e) Place in a sealed container
   f) Label "NAME, NaOH 2 M, DATE" with a corrosion warning sticker

2) Clean the sensor extensively with water
3) Weigh the bottle containing the solution to be adjusted
4) Place the pH sensor in a pH 4 buffer solution
5) Write down the pH and voltage value(achievement goal)
6) Place the container with a stir bar inside on the magnetic stirrer for mixing
7) Place the sensor in the solution
8) Note down the time, pH and voltage signal
9) HCl 32% adjustment (about 0.08 pH change per drop)
   a) Put on gloves and safety glasses
   b) Add the NaOH stock solution drop by drop
   c) At regular intervals (max 20 drops) write down the
      time, number of drops, pH and voltage signal
   d) Stop when the pH and voltage correspond to that of the buffer solution (Should be about 130 drops)

10) Place the pH sensor in a pH 4 buffer solution
11) Write down the pH value and voltage signal
12) Take out the magnetic stir bar and rinse

13) Weigh the bottle containing the final solution
14) Label the sample with "NAME, pH7-adjusted urine sample, DATE OF PREPARATION"
15) Read out the data

PROTOCOL 9: pH sensor experimental procedure
Estimated time needed:

Required products:
1) Standard solution pH 4
2) Standard solution pH 7
3) 1 L reactor urine with pH 4 (**Protocol 7**)
4) 1 L reactor urine with pH 7 (**Protocol 8**)
5) Tap water for rinsing

Required hardware:
1) 2 pH sensors in the reactor
2) Measurement transformer and SD card to read data
3) 6 beakers
4) Metallic arm for two sensors (so that they can be changed simultaneously)
5) 2 Magnetic stirrers
6) Magnetic stick to recover the stir bars
7) Minimum of 2 stir bars

Comments:
DO NOT leave the sensors in the air as they should not dry up.
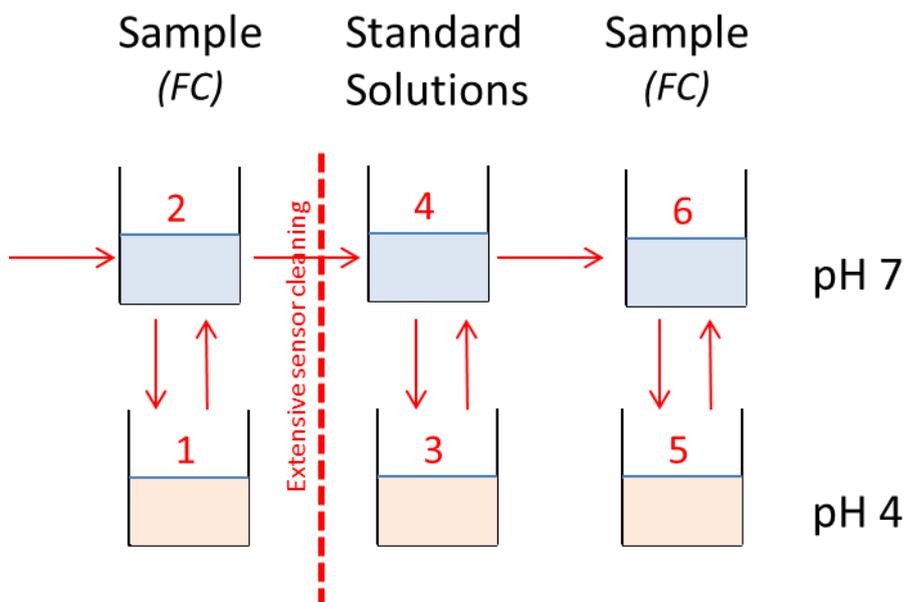*DO NOT use nanopure or distilled water.*
Make sure the step change is done as quickly as possible

Steps:
Day before
1) Set Check/adjust the pH of the urine samples (**Protocols 7 and 8**)

Measurement Procedure (Overview in the diagram above)

1st Experiment set (field conditions without cleaning the sensor): beakers 2 and 1
2) Set up beakers 1 and 2
   a) Pour about 500 mL of sample pH 4 and 7, respectively
   b) Place a magnetic stir bar into beakers 2 and 1 and place these on the magnetic stirrers
   c) Turn the stirrer on to the marked frequency for beaker 2
3) Take the pH sensors (without cleaning) out of the reactor
   a) Write the time on the measurement transformer (MT)
   b) Set up the arm to enable easy beaker changes
   c) Place both sensors in beaker 2 (pH 7) so that the cap is submerged
   d) After 5 min write down the Temperature, pH and voltage
4) Step change into 1 and then back into 2.
   a) Note the exact time on the timer that the sensor was put IN and OUT of the beakers
   b) Note the final pH and voltage values reached
   c) Wait 2 min after the value on the MT is stable before each change of beaker

Extensive cleaning
5) See **Protocol 6**
6) Keep the sensors in water when waiting or in between cleaning steps

2nd Experiment set (standard solutions): beakers 4 and 3
7) Set up beakers 3 and 4
8) Place the sensors in beakers 4 → 3 → 4
   a) Note the exact time on the timer that the sensor was put IN and OUT of the beakers
   b) Note the final pH and voltage values reached
   c) Wait 2 min after the value on the MT is stable before each change of beaker
9) Place the sensor in water while setting up the 3rd experiment

3rd Experiment set (field conditions with a cleaned sensor): beakers 6 and 5
10) Set up beakers 6 and 5
11) Place the sensors in beakers 6 → 5 → 6
   a) Note the exact time on the timer that the sensor was put IN and OUT of the beakers
   b) Note the final pH and voltage values reached
   c) Wait 2 min after the value on the MT is stable before each change of beaker

12) Filter and autoclave the pH 4 and pH 7 solutions separately.
13) Determine the slope and intercept value
   a) Go to the "pH-Kalibration" file on the laboratory computer.
   b) In Sheet 1, fill in row 3 and 4 with the voltage signal for each experimental set
   c) Write down the subsequent b and c values
14) *if the measured value > ± 0.2 pH from the expected value of the standard solutions.*
   Calibrate the sensors by following the instructions on the MT.
15) Read out the data from the Measurement transformer

A.5 Matlab codes for the determination of DO sensor characteristics

Highlighted parts correspond to other functions, as found below.

1) DO sensor characteristics

```
%% Prcess data manually beforehand
%delete title, leave only columns 1 and 6. Turn column 1 into number
%save as old version of Excel xls and not xlsx

%% Read and re-group the data
% clear all
close all
clear all
clc

%New data path if necessary
%addpath('\\eaw-homedirs\grimonel$\My Documents\Data\FC_O2\All data')

xls_1=xlsread('Exp11_results');
DO=xls_1(:,2);

date_num=(xls_1(:,1)+693960);

xls_2=xlsread('Exp11_times');
number=xls_2(:,1);
nb=num2str(number);  %turn it into string
DO_end=xls_2(:,4);
IN_num=(xls_2(:,2)+693960);
OUT_num=(xls_2(:,3)+693960);

%name the different beaker changes "initial beaker"->"final beaker"
step=zeros(length(number)-1,1);
for i=2:length(number);
    step(i-1)=number(i)-number(i-1);
end
    stepfind=find(or(step==1,step==-1)); %select step change OUT beaker

name=[];
for i=1:length(stepfind);
    a=stepfind(i);
    nam=strcat(nb(a),'->',nb(a+1));
    name=[name;nam];
end

%% Descriptive stats for all: table1= [beaker#; mean; std]
table1=[];
for i=1:length(number);
    out=OUT_num(i);
    a=find(date_num==out);
    range=DO(a-200:a-30); %when stable for 1min before !!!! will have to
change this?

    mean_DO=mean(range);
    std_DO=sqrt(var(range));
    beaker=number(i);
  table1=[table1; beaker mean_DO std_DO];
end
```

```matlab
%% correct the initial time when the sensor was put in
%"IN_final" the final optimized start times
%table 2 =[beaker_OUT; mean_DO; std_DO cuttoff_value; t_corr]
IN_final=[];
table2=[];
for i=1:length(stepfind); %number of step changes
    %precision
    out=OUT_num(stepfind(i));
    a=find(date_num==out); %a=location of t_OUT for the previous beaker
    range=DO(a-200:a-30); % POSSIBLE TO CHANGE range selected
        mean_DO=mean(range);
        std_DO=sqrt(var(range));

    sec_later=0;
    if DO(a)>6 %DO will go down
        cuttoff_value=std_DO*3;
        while [DO(a-1)-DO(a)]<cuttoff_value
            a=a+1 ;
            sec_later=sec_later+1;
        end
    else %DO going up
        cuttoff_value=std_DO*5;
        while [DO(a)-DO(a-1)]<cuttoff_value
            a=a+1 ;
            sec_later=sec_later+1;
        end
    end
     b=stepfind(i);
     beaker_OUT=number(b);

  IN_final=[IN_final;date_num(a)];
  table2=[table2; beaker_OUT mean_DO std_DO cuttoff_value sec_later];
end

%% CI determination _ 10% of the difference between initial and final
beaker
%CI_cutoff; RT_CI
CI=zeros(length(stepfind),1);
CI_cutoff=zeros(length(stepfind),1);
for i=1:length(stepfind)
    a=stepfind(i);
    CI(i)=0.1*(table1(a,2)-table1(a+1,2));
    CI_cutoff(i)=table1(a+1,2)+CI(i);
end

%start at IN_final ... and determine the nb of sec till cutoff value
RT_CI=zeros(length(stepfind),1);
abc=[];
for i=1:length(IN_final); %number of step changes
    in=IN_final(i);
    a=find(date_num==in); %a=location of t_OUT for the previous beaker
%while its above/below the cutoff value.
    sec_later=0;
    if DO(a)>6 % DO! going down (not DO!)
        while DO(a)> CI_cutoff(i)
            a=a+1 ;
            sec_later=sec_later+1;
        end
    else % --> DO going up
```

```matlab
        while DO(a)< CI_cutoff(i)
            a=a+1 ;
            sec_later=sec_later+1;
        end
    end
    RT_CI(i)=sec_later ;
end

%% objective function
%X=[y_o y_eq tau]
X_initial=[];
H=[];
for i=1:length(IN_final)
 %not always the same with table 1
a=find(date_num==IN_final(i));
m=stepfind(i);
y_m=DO(a:a+198);

y_zero=table1(m,2);
y_final=table1(m+1,2);
tau1=RT_CI(i);
tau2 = tau1;

X=[tau1 tau2];
X_initial=[X_initial; y_zero y_final tau1 tau2];

tt = (1:1:length(y_m))';

%2 parameters optimized (tau1,tau2)
[x,fval,exitflag]=fminsearch(@(X)obj_fun3_tau2(X,y_zero,y_final,y_m,tt),X);
H=[H;x fval exitflag];
tau1=H(i,1);
tau2=H(i,2);

%plot the data
yy_o=[y_zero y_zero];
[tt,y]=ode45(@(tt,y)modelpred4(tt,y,y_final,tau1,tau2),tt,yy_o);

figure
plot(tt,y_m,'.',tt,y(:,2),'r-')
end

%% plot all data measurements
figure
plot(date_num,DO,'-k')

%start time 1h before first solution (0.0417 = 1h)
%end time 1h after last solution taken out
x_start=IN_num(1)-0.0417;
x_end=OUT_num(end)+0.0417;
datetick('x','HH:MM')% Define date format that will be displayed 'dd.mm.yy'
HH:MM
xlim([x_start x_end])
xlabel('time [s]')
ylabel('DO [mg/l]')
grid on;
```

2) Objective function which is minimized for the fitting of the DO curve

```matlab
function J=obj_fun3_tau2(X,y_zero,y_final,y_m,tt)
tau1=X(1);
tau2=X(2);

tt = (1:1:length(y_m))';
yy_o=[y_zero y_zero];

[T,yy]=ode45(@(tt,y)modelpred4(tt,y,y_final,tau1,tau2),tt,yy_o);

y_m= y_m(:);
e_sq =(yy(:,2)-y_m).^2;
J=(sum(e_sq))/length(y_m);
end
```

3) Model which is fitted for the tau values

```matlab
function dy= modelpred4(tt,y,y_final,tau1_x,tau2_x)
dy=zeros(2,1);
dy(1)=(y_final-y(1))/tau1_x;
dy(2)=(y(1)-y(2))/tau2_x;
end
```

A.6 Matlab codes for the determination of pH sensor characteristics

Highlighted parts correspond to other functions, as found below.

1) pH sensor characteristics

```
%% Prcess data manually beforehand
%delete title, leave only columns 1 and 6. Turn column 1 into number
%save as old version of Excel xls and not xlsx

%% Read and re-group the data
% clear all
close all
clear all
clc

%New data path if necessary
%addpath('\\eaw-homedirs\grimonel$\My pHcuments\Data\FC_O2\All data')

xls_1=xlsread('Exp2_1_results');
pH=xls_1(:,2);

date_num=(xls_1(:,1)+693960);

xls_2=xlsread('Exp2_times');
number=xls_2(:,1);
nb=num2str(number);   %turn it into string
IN_num=(xls_2(:,2)+693960);
OUT_num=(xls_2(:,3)+693960);


%name the different beaker changes "initial beaker"->"final beaker"
step=zeros(length(number)-1,1);
for i=2:length(number);
    step(i-1)=number(i)-number(i-1);
end
    stepfind=find(or(step==1,step==-1)); %select step change OUT beaker

name=[];
for i=1:length(stepfind);
    a=stepfind(i);
    nam=strcat(nb(a),'->',nb(a+1));
    name=[name;nam];
end

table1=[];
%% Descriptive stats for all: table1= [beaker#; mean; std]
for i=1:length(number);
    out=OUT_num(i);
    a=find(date_num==out);
    if isempty(a)>0
        a=find(date_num==out-1.1574*10^-5);
    end
    range=pH(a-150:a-10); %Could change the 150 value.

    mean_pH=mean(range);
    std_pH=sqrt(var(range));
    beaker=number(i);
```

```matlab
    table1=[table1; beaker mean_pH std_pH];
end

%% correct the initial time when the sensor was put in
%"IN_final" the final optimized start times
%table 2 =[beaker_OUT; mean_pH; std_pH cuttoff_value; t_corr]
IN_final=[];
table2=[];
for i=1:length(stepfind); %number of step changes
    %precision
    out=OUT_num(stepfind(i));
    a=find(date_num==out); %a=location of t_OUT for the previous beaker
        if isempty(a)>0
        a=find(date_num==out-1.1574*10^-5);
                if isempty(a)>0
                    a=find(date_num==out-2*(1.1574*10^-5));
                end
        end
    range=pH(a-150:a-10); %can change the 150
        mean_pH=mean(range);
        std_pH=sqrt(var(range));

    sec_later=0;
    b=a;
    if pH(a)>6 %pH will go pHwn
        cuttoff_value=std_pH*3;
        while [pH(a-1)-pH(a)]<cuttoff_value
            a=a+1 ;
        end
    else %pH going up
        cuttoff_value=std_pH*5;
        while [pH(a)-pH(a-1)]<cuttoff_value
            a=a+1 ;
            sec_later=sec_later+1;
        end
      sec_later=(date_num(a)-date_num(b))/(1.1574*10^-5);
    end

     c=stepfind(i);
     beaker_OUT=number(c);

  IN_final=[IN_final;date_num(a)];
  table2=[table2; beaker_OUT mean_pH std_pH cuttoff_value sec_later];
end

%% CI determination _ 10% of the difference between initial and final
beaker
%CI_cutoff; RT_CI
CI=zeros(length(stepfind),1);
CI_cutoff=zeros(length(stepfind),1);
for i=1:length(stepfind)
    a=stepfind(i);
    CI(i)=0.1*(table1(a,2)-table1(a+1,2));
    CI_cutoff(i)=table1(a+1,2)+CI(i);
end

%start at IN_final ... and determine the nb of sec till cutoff value
RT_CI=zeros(length(stepfind),1);
for i=1:length(IN_final); %number of step changes
    in=IN_final(i);
```

```matlab
    a=find(date_num==in)-1; %a=location of t_OUT for the previous beaker -
1!!!! as it's so quick!
    b=a;
%while its above/below the cutoff value.
    sec_later=0;
    if pH(a)>5.7 % pH! going pHwn (not pH!)
        while pH(a)> CI_cutoff(i)
            a=a+1 ;
        end
    else % --> pH going up
        while pH(a)< CI_cutoff(i)
            a=a+1 ;
        end
    end

    RT_CI(i)=(date_num(a)-date_num(b))/(1.1574*10^-5);
end

%% objective function
%X=[y_o y_eq tau]
X_initial=[];
H=[];
for i=1:length(IN_final)
 %not always the same with table 1
a=find(date_num==IN_final(i));
m=stepfind(i);
y_m=pH(a:a+100);

y_zero=table1(m,2);
y_final=table1(m+1,2);
tau1=RT_CI(i)/5;

%Increase nb of evaluations
options = optimset('MaxFunEvals',1e20);

% X_initial=[X_initial; y_zero y_final tau1 tau2];
X=[tau1] ; %X_initial(i,:);
X_initial(i,:) = [y_zero y_final X];
tt = (1:1:length(y_m))';

y=zeros(199,1);
[x,fval,exitflag]=fminsearch(@(X)obj_fun6_1tau(X,y_zero,y_final,y_m,tt),X,o
ptions);
H=[H;x fval exitflag];
tau1=x;

%plot
yy_o=[y_zero];
[tt,yy]=ode45(@(tt,y)modelpred6_1tau(tt,y,y_final,tau1),tt,yy_o);
figure, hold on
    plot(y_m,'.')
    plot(yy,'r-')

end
%% plot
figure
plot(date_num,pH,'-k')

%start time 1h before first solution (0.0417 = 1h)
```

```
%end time 1h after last solution taken out
x_start=IN_num(1)-0.0417;
x_end=OUT_num(end)+0.0417;
datetick('x','HH:MM')% Define date format that will be displayed 'dd.mm.yy'
HH:MM
xlim([x_start x_end])
xlabel('time')
ylabel('pH')

grid on;
```

2) Objective function which is minimized for the fitting of the pH curve

```
function J=obj_fun6_1tau(X,y_zero,y_final,y_m,tt)
%X=[tau1]
tau1=X(1);


tt = (1:1:length(y_m))';
yy_o=[y_zero];
[T,yy]=ode45(@(tt,y)modelpred6_1tau(tt,y,y_final,tau1),tt,yy_o);
y_m= y_m(:);
e_sq =(yy(:,1)-y_m).^2;
J=(sum(e_sq))/length(y_m);
end
```

3) Model which is fitted for the tau values

```
function dy= modelpred6_1tau(tt,y,y_final,tau1)
dy=zeros(1,1);
dy(1)=(y_final-y(1))/tau1;
end
```

A.7 Matlab codes for sensor model

1) Model simulation with the sensor model

```matlab
clear all
close all
clc

global DEBUG;    % Debug mode (1=active): perform checks while execution
DEBUG = 0;       % i.e. check for negative states, negative flows, ...
                 % Slows down the execution (+ ca. 10%)

%% Add AdditionalFunctions to path
% so that the functions inside can be called.
thisPath = [pwd,'\AdditionalFunctions'];
addpath(thisPath);

%% Prepare data:
% Get all the parameters from Writeparameters function
parameters     = WriteParameters();
parameters.biol.mueStarAOB= parameters.biol.mueStarAOB/3;
parameters.biol.mueStarNOB= parameters.biol.mueStarNOB/3;
% Compute stoichiometric matrix for fast (chemical) reactions
parameters.SMF = WriteStoichiometricMatrixFast(parameters);
% Compute stoichiometric matrix for slow (biological) reactions
parameters.SMS = WriteStoichiometricMatrixSlow(parameters);

%% Scenario evaluations:
factor       =   [3]             ;
% factor      = [3:6]            ;
n_factor     =   length(factor)  ;

for i_factor = 1:n_factor
    %% Initialization
    %load SteadyStateFullModel
    load SteadyStateReducedModel
    xR  = data ;
    clear data

%only have NH3,NO2,OH,CO3,HCO3 chem reactions left.
    parameters.exp.customRRF(6:20) = 0 ;
    %parameters.exp.customRRF(3) = 0 ; %desactivate OH equilibrium reaction


    %find states which are variable:
    SMA =[parameters.SMS ; parameters.SMF(parameters.exp.customRRF==1,:)] ;
    dynstates =  find(any(SMA~=0,1)) ;
    constates = setdiff(1:size(SMA,2),dynstates) ;
    parameters.dynstates = dynstates;
    parameters.constates = constates;
    parameters.convalues = xR(constates) ;


    %find DO and pH initial values
    xR_DO = xR(GetStateNumber(parameters.names,'S_O2'));
    xR_proton = xR(GetStateNumber(parameters.names,'S_proton'));
```

```matlab
    [fA1,~,~]=ComputeFAFunctions(xR,parameters);
      xR_pH=-log10(xR_proton.*fA1);

      %add 3 additional initial states, DO 2nd order, pH 1st order
    xR   =        [xR(dynstates);xR_DO;xR_DO;xR_pH]        ;

% Initialize reactor state
    u    = zeros(3,1);                    % set control inputs
    u(1) = 24;                            % flow input to reactor:    24 L/d
    u(2) = 24;                            % flow output from reactor : 24 L/d
    u(3) = 24*1500;                 % gas flow rate: 1-1.5 m^3/h, 24*1300 L/d

    HRT    =   parameters.mech.reactorVolume/u(1) ;
    v    = zeros(4,1);                    % set disturbances
    t0   = 0;                             % beginning of simulation [day]
    tEnd = 1*HRT;                          % end of simulation: 10 day
    % note: influent composition is computed in InfluentComposition()

    %% Start Reactor Simulation
    tReactor = t0 ;
    uReactor = u(:)' ;
    yAll = xR(:)' ;
    model.parameters = parameters ;
    dt = 1/24 ;

    for period=1:2
        disp(['Period: ' num2str(period)])
        switch period
            case 1
                u(1)    =   24  ;
                tEnd    =   1   ;
            case 2
                u(1)    =   u(1)*factor(i_factor)  ;
                tEnd    =   2*HRT   ;
        end
        u(2) = u(1) ;

        tic
[tReactorPeriod,yReactorPeriod] = ode23s(@(t,x) GRE_ODErector_sensor_model4 ...
                                (t,x,u,v,model),t0:dt:tEnd,xR);
        uReactorPeriod =  repmat(u(:)',[length(tReactorPeriod) 1]) ;
        tReactor = [ tReactor ; tReactorPeriod(2:end,:)+tReactor(end) ];
        uReactor    =   [ uReactor ; uReactorPeriod(2:end,:)  ];
        yAll = [ yAll ; yReactorPeriod(2:end,:) ];
        toc

        xR = yReactorPeriod(end,:) ;
        xR = xR(:) ;
    end

    %% Reconstruct full states corrected
    %divide up
yReactor=yAll(:,1:13);
y_DO=yAll(:,14:15);
y_pH=yAll(:,16);

    %original model data
    nSamples = size(yReactor,1) ;
    yReactorDyn     =   yReactor ;
    yReactorCon     =   repmat(parameters.convalues',[nSamples 1])     ;
```

```matlab
    yReactor       = nan(nSamples,size(yReactorDyn,2)+size(yReactorCon,2))  ;
    yReactor(:,parameters.dynstates)  =  yReactorDyn;
    yReactor(:,parameters.constates)  =  yReactorCon;

    %with DO and pH sensor corrected data
    [fA1,~,~]=ComputeFAFunctions(yReactor,model.parameters);
        ySensor=yReactor;
len=length(yReactor(1,:));
ySensor(:,len+1)=y_DO(:,1);
ySensor(:,len+2)=y_DO(:,2);
ySensor(:,len+3)=y_pH;


    %% Save final state as new initial state.
    data = yReactor(end,:)';%#ok<NASGU>  % End state as column vector, used
in next row

    FileOut = [ 'NitAcc_NoCTRL_Scenario' num2str(i_factor) ];
    save(FileOut,'tReactor','uReactor','yReactor');

     FileOut = [ 'NitAcc_NoCTRL_Scenario_sensor' num2str(i_factor) ];
    save(FileOut,'tReactor','uReactor','ySensor');
```

2) Combined ODE function: reduced model and sensor model solved for simultaneously

```matlab
function [dxdt] = GRE_ODErector_sensor_model4(t,x,u,v,model)
%x : reactorState
%u : control inputs
%v : disturbances

 %% divide the matrix
yReactor=x(1:13);
y_DO=x(14:15);
y_pH=x(16); % y_pH=x(16);

 %% get measurements
[dyReactordt]=ODEreactor_reduced(t,yReactor,u,v,model);

 %% determine fA1 activity
% constant state values extended
    reactorStateCon =   (model.parameters.convalues);
% input = dynamic states only
   reactorStateDyn =   yReactor  ;
reactorState =
      nan(length(yReactor(:,1)+length(model.parameters.convalues),1);
reactorState(model.parameters.constates)  =  reactorStateCon;
reactorState(model.parameters.dynstates)  =  reactorStateDyn;

[fA1,~,~]=ComputeFAFunctions(reactorState,model.parameters);

 %% quick GSN for O2 and proton
dynstates=model.parameters.dynstates';
StateNames_Reduced=model.parameters.names(dynstates);
GSN_O2=GetStateNumber(StateNames_Reduced,'S_O2');
GSN_proton=GetStateNumber(StateNames_Reduced,'S_proton');
```

```matlab
 %% DO correction
y_final_DO=yReactor(GSN_O2);
[dy_DOdt]=GRE_dydt_O2_simple(t,y_DO,y_final_DO);

%% pH correction
% yy_o_pH=-log10(xR_sensor(GSN_proton)*xR_fA1); %xR_fA1 better as added as
input as it changes otherwise no?
% y_pH=-log10(y_proton*fA1);
y_final_pH=-log10(yReactor(GSN_proton).*fA1);
[dy_pHdt]=GRE_dydt_pH_f(t,y_pH,y_final_pH);
% dy_protondt=((10^-dy_pH))*ln(10)/fA1;

%% New states vector
dxdt=vertcat(dyReactordt,dy_DOdt,dy_pHdt);
end
```

3) DO sensor model

```matlab
function [dy] = GRE_dydt_O2_simple(tt,y,y_final)

dy=zeros(2,1);
tau1_x=22/3600/24;
tau2_x=22/3600/24;

    dy(1)=(y_final-y(1))/tau1_x;
    dy(2)=(y(1)-y(2))/tau2_x;
end
```

4) pH sensor model

```matlab
function dy= GRE_dydt_pH_f(tt,y,y_final)
dy=zeros(1,1);
tau1_x=3.5/24/3600; %tau was originally determined in sec --> days

    dy(1)=(y_final-y(1))/tau1_x;
end
```

5) UKF

```matlab
clear all
close all
clc

global DEBUG;    % Debug mode (1=active): perform checks while execution
DEBUG = 0;       % i.e. check for negative states, negative flows, ...
                 % Slows down the execution (+ ca. 10%)

%% Add AdditionalFunctions to path
% so that the functions inside can be called.
thisPath = [pwd,'\AdditionalFunctions'];
addpath(thisPath);
addpath(genpath('\\eaw-homedirs\grimonel$\My
Documents\3.2_Model+UKF+Sensor\UKF'))

 %% Prepare data:
% Get all the parameters from Writeparameters function
parameters     = WriteParameters();
parameters.biol.mueStarAOB= parameters.biol.mueStarAOB/3;
parameters.biol.mueStarNOB= parameters.biol.mueStarNOB/3;
% Compute stoichiometric matrix for fast (chemical) reactions
parameters.SMF = WriteStoichiometricMatrixFast(parameters);
% Compute stoichiometric matrix for slow (biological) reactions
parameters.SMS = WriteStoichiometricMatrixSlow(parameters);

%% Scenario evaluations:
factor      =   [3]              ;
n_factor    =   length(factor)  ;

for i_factor = 1:n_factor
     %% Initialization
    %load SteadyStateFullModel
    load SteadyStateReducedModel
    xR  = data ;        %initial conditions (37 states)
    clear data

%initial states for DO and pH
xR_DO = xR(GetStateNumber(parameters.names,'S_O2'));
xR_proton = xR(GetStateNumber(parameters.names,'S_proton'));
[fA1,~,~]=ComputeFAFunctions(xR,parameters);
xR_pH=-log10(xR_proton.*fA1);
xR(38:40)=[xR_DO;xR_DO;xR_pH];

    parameters.exp.customRRF(6:20) = 0 ;
    %parameters.exp.customRRF(3) = 0 ;
    % % find states which are variable:
    SMA = [parameters.SMS ; parameters.SMF(parameters.exp.customRRF==1,:)];
    dynstates =  find(any(SMA~=0,1)) ;
    constates = setdiff(1:size(SMA,2),dynstates) ;

%Add additional DO and pH sensor states to the dynastates
    dyn_sensor = [dynstates,38,39,40];

    parameters.dynstates = dynstates;
    parameters.constates = constates;
    parameters.convalues = xR(constates) ;
```

```matlab
%% LOAD DATA
FileIn = [ 'NitAcc_NoCTRL_Scenario_sensor' num2str(i_factor) ];
load(FileIn);

%% SETUP UKF
% -------------
nSamples = size(ySensor,1) ; % ySensor : yReactor +DO*2 states +pH

nState   =   length(dyn_sensor)  ;
dt       =   min(diff(tReactor))     ;

% Observer model
Bounds   =   [   zeros(nState,1)     inf(nState,1)   ] ; % Bounds on the
state variables


%    - Dimensions
mdl.NumOfStateVar        =   nState   ;
mdl.NumOfInputs          =   3        ;
mdl.NumOfInputNoiseVar   =   3        ;
mdl.NumOfOutputs         =   2        ;
mdl.NumOfOutputNoiseVar  =   2        ;

%    - Describe model
mdl.fformat =    'c'                              ;
mdl.f       =    'GRE_ODErector_sensor_model4'        ;
mdl.parameters= parameters                       ;

mdl.bounds  =    Bounds                           ;
mdl.dt      =    dt                               ;
mdl.Q       =    .01*eye(mdl.NumOfInputNoiseVar)      ;

%    - Measurement model
mdl.h       =    'GRE_h_DO_pH'                    ;
mdl.D       =    diag([.014/(32*1000) .004]) ; %std dev for DO and pH
mdl.R       =    1*eye(mdl.NumOfOutputNoiseVar)  ;

% -------------
% Tuning parameters

visual = false ;

%  - Chosen: following settings are inspired by [1].
version =    'Haykin2001'   ;
alpha   =    1              ;
beta    =    0              ;
kappa   =    2              ;

% -------------
% Further setup and initialization
xhat        =    ySensor(1,dyn_sensor)'                   ;
Phat        =    diag((xhat/10).^2)                       ;
[settings]  =    UKFsetup(mdl,kappa,version,alpha,beta)  ;

%% --------------------------
% SIMULATE MEASUREMENTS
```

```matlab
    % --------------------------
    yReactorRed       =     ySensor(:,dyn_sensor)            ;
    w                 =     randn(nSamples,mdl.NumOfOutputNoiseVar) ;
    U                 =     uReactor'                         ;
    Y                 =     GRE_h_DO_pH(mdl,yReactorRed',w')          ;

    %% --------------------------
    %   RUN UKF IN BATCH MODE
    %  --------------------------

%   U = U(:,1:25);
%   Y = Y(:,1:25);
    [Agg_xhat,Agg_Phat] =   UKFbatch(mdl,settings,xhat,Phat,U,Y,visual) ;

    FileOut = [ FileIn '_UKF'];

    save(FileOut,'Agg_xhat','Agg_Phat')

end
```

6) Adding uncertainty due to precision

```matlab
function [y] = GRE_h_DO_pH(sys,x,w)

i_O2    =   13+2      ;
i_pH     =  13+3 ;
y    =   [   x(i_O2,:)   ;   x(i_pH,:)    ]   ;
y    =       y + sys.D*w ;

end
```

A.8   Previously tested protocols and lessons learned

Experiment #	Protocol tests with nanopure water	Lessons learned
1	N_2 bubbling in 3 beakers Step changes: air ↔ N_2	• Do not take the diffusers out • Too much N_2 gas used when bubbled all night
2	N_2 bubbling in 3 beakers (keeping the diffusers in during the measurement) Step changes : air ↔ N_2 and air ↔ Co(II) + Na_2SO_3	• N2 bubbling : can reach 0 mgO2/l • 10 min intense bubbling before is enough
3	N_2 bubbling in 3 beakers, aeration in 2 beakers air ↔ N_2 (x1) and aerated water ↔ N_2 (x2) air ↔ Co(II) + Na_2SO_3 (left over)	• Importance of mixing • Response time increases with lower aeration • Mixing important when Na_2SO_3 limited
4	Repeated experiments air ↔ N_2 (x2) and aerated water ↔ N_2 (x5) with stronger aeration	• 10 min sometimes too short to calculate response time • Insufficient repeatability of the experiments • Saturated air measurement are different
5	Start the aeration with the sensor in Before and after calibration aerated water ↔ N_2 aerated water ↔ Co(II) + Na_2SO_3	• Minimum of 20 min aeration needed • Still no repeatability
6	air ↔ Co(II) + Na_2SO_3 (x3) Mixing of the Na_2SO_3 solution with a stir bar and magnetic stirrer	• Position and moving of the sensor in air affects the measurements • With continuous mixing, 140 Na_2SO_3 mg/500 mL lasts <1 h
7	Testing the performance of the 2 magnetic stirrers : air ↔ Na_2SO_3 (x4) Air ↔ Saturated water (x3)	• Similar response times obtained with both mixers • O2 concentration : Saturated water (rested 1h30 after aeration) > humidified air
8	aerated water ↔ Co(II) + Na_2SO_3 (x3 with different beakers)	• Rinsing the sensor necessary when leaving the Na_2SO_3 beaker (may affect the saturation concentration in the next beaker) • Decrease the Na_2SO_3 dosage to a security factor of 2+
9	As 8 but with lower Na_2SO_3 (now 100 mg/500mL)	• Still decreases in the 2nd saturated value
10 Urine	4 Urine beakers, 2 nanopure aerated water ↔ Co(II) + Na_2SO_3	• Na_2SO_3 and/or Co(II) dosage too low