A nitrate biosensor based methodology for monitoring anoxic activated sludge activity

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Abstract An improved methodology based on a nitrate biosensor is developed and applied successfully for in-depth monitoring and study of anoxic activated sludge activities. The major advantages of the methodology are its simplicity, reliability and high data quality. The resulting data allowed for the first time to monitor anoxic respiration rate of activated sludge (nitrate uptake rate (NUR)) at a high time resolution making it clearly comparable with high frequency oxygen uptake rate (OUR) measurements obtained under aerobic conditions. Further, the anoxic respiration data resulting from a pulse addition of carbon source to endogenously respiring anoxic activated sludge shows a clear start-up phenomenon and storage tail that is usually also observed in high-frequency OUR measurements. Finally, the improved methodology can be expected to serve as an anoxic respirometer for activated sludge treatment plants where denitrification process occurs in single-step. Further, it can be used for a variety of purposes e.g. for toxicity and activity monitoring, process control and parameter estimation of the activated sludge process, similar to the aerobic respirometers.

Keywords Anoxic activated sludge; biosensor; monitoring; nitrate uptake rate; titrimetry

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Introduction

Techniques and methods currently available to study the anoxic respiration rate of the biomass defined as nitrate uptake rate (NUR) are rather limited and under-developed compared to the tools available for studying the aerobic processes. One example is respirometry, the measurement of the oxygen uptake rate (OUR) of the biomass (Spanjers *et al*., 1998). It has been shown to be a powerful tool to study in-depth the aerobic processes for various purposes: monitoring toxicity (Kong *et al*., 1996), characterisation of wastewater, estimation of kinetics and stoichiometric parameters (Vanrolleghem *et al*., 1999) and control (Spanjers *et al*., 1998).

The major limitation to obtain an equally popular tool for monitoring anoxic respiration has been mainly technical (Lynggaard-Jensen, 1999). In other words, a reliable, simple and on-line sensor for the measurement of nitrate at least as powerful as oxygen electrodes used in respirometry was not available. The anoxic respiration rate has been usually measured using automatic analysers based on colorimetric methods (Kristensen *et al*., 1992; Naidoo *et al*., 1998) or ion chromatography (McClintock *et al*., 1988) with 10–35 minutes of measurement interval. However, this is not sufficient to adequately monitor process dynamics that is often occurring at the minute scale (Vanrolleghem *et al*., 2004). Recently, several other methods were developed for on-line and reliable *in situ* measurement of nitrate based on various principles (Lynggaard-Jensen, 1999): potentiometric, e.g. ion selective electrode (ISE) (Rieger *et al*., 2002), photometric, e.g. UV-based sensors (Langergraber *et al*., 2003) and biosensors (Larsen *et al*., 2000). Each technique has limitations as well as advantages. The bottleneck of UV-based nitrate measurements is the likely interference of organic compounds present in the complex matrix of wastewater to the nitrate absorbance at 205 nm (Lynggaard-Jensen, 1999). A previously developed anoxic activated sludge

monitoring methodology was based on an ion-selective nitrate electrode (Sin *et al*., 2003). Long-term experience/working with this type of ion-selective electrode showed that the slope of the electrode drifts usually in a rather random and unpredictable way and even during the course of a relatively short (less than 1 hour) batch experiment (Petersen *et al*., 2002; Malisse, 2002). Based on this long-term experience, it appeared that this type of nitrate sensor was not sufficiently advanced to live up to the high requirements for a robust nitrate sensor.

The main motivation of this study is to develop a more robust, simple and reliable anoxic methodology for the monitoring of activated sludge based on the previously developed anoxic methodology (Sin *et al*., 2003). Particularly, the aim is that the methodology can be used as a reliable anoxic respirometer that provides high frequency (on-line) and high quality data to be used for a variety of purposes (mentioned above). To this aim, the ion-selective nitrate electrode of the previous methodology (Sin *et al*., 2003) is replaced with a novel nitrate measurement technique, a nitrate biosensor, demonstrated to provide reliable, online and fast measurements (Larsen *et al*., 2000). The paper is structured as follows: first, the step-response of the biosensor, which is a significant property of a sensor in view of high-frequency measurements, is analysed. This is followed by a rigorous analysis of the slope of the biosensor in relation to its robustness/stability. Further, the improved methodology will be applied to different activated sludge samples for the monitoring of anoxic activity. Finally, the results of the anoxic respirometer will be compared to the result of an aerobic respirometer for the same activated sludge.

Materials and methods

The anoxic respirometer developed in this study is shown in Figure 1. The set-up consists of a 1 L reactor with thermal jackets for temperature control by a cooling system (Lauda Ecoline E303).

Data acquisition, pH control and data processing are implemented by Labview software (National Instruments, NIDAQ 6.9 with AT-MIO-16XE-50 DAQ card and Labview 6.i). The nitrate is measured by a biosensor shown in Figure 1 (right) (Unisense, Denmark) and connected to a picoammeter (Unisense, Denmark) where the raw signals (nA) of the nitrate biosensor are amplified (mV) and sent to the DAQ card. The principle of the biosensor

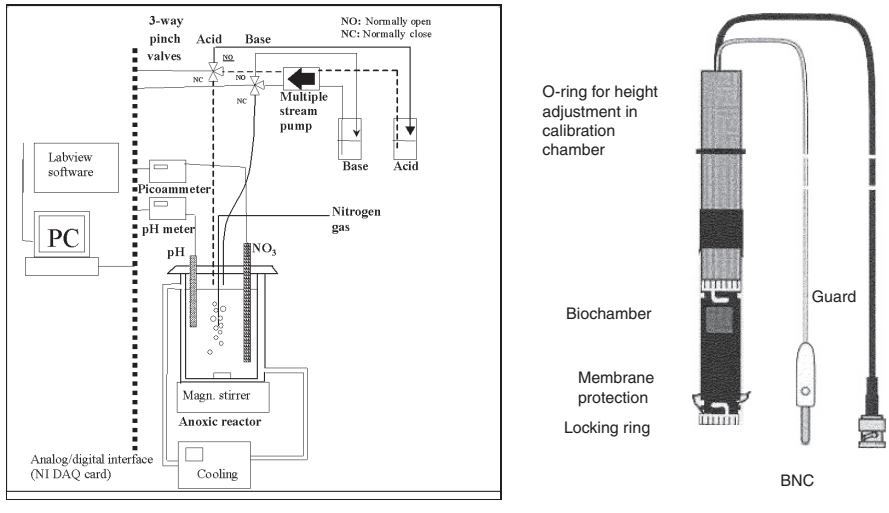

Figure 1 Illustration of the anoxic respirometer (left); Nitrate biosensor (right)

is simply that nitrate in the medium diffuses through an ion selective membrane into the biochamber. The nitrate is then reduced by specialised bacteria to nitrogen dioxide (N_2O), which is then measured by a Clark-type N_2O transducer (Larsen *et al*., 2000). The pH is measured in the aeration vessel with a HA405-DXK-S8/120 type pH electrode (Mettler Toledo), which is connected to a Knick Stratos 2401 pH transmitter. The data acquisition frequency of the sensor is set to 3 seconds. High frequency noise known to ride on top of the weak analog signals of electrodes was filtered using a low pass Savitzky-Golay filter (Sin *et al*., 2003) with central moving window size 11 and polynomial order 1. The pH is controlled within a pH set-band ± 0.03 as described in detail in Gernaey *et al*. (2002).

Activated sludge monitoring methodology

For the experimental work activated sludge was sampled from either a pilot scale 80-l SBR reactor (Sin *et al*., 2003) or Ossemeersen WWTP receiving mainly domestic wastewater (Aquafin, Belgium). During the experiments small substrate pulses of acetate (10 g COD/l), nitrate (1 g NO_3-N/l) and ammonium (1 g NH_4-N/l) stock solutions were dosed to the activated sludge. All experiments were performed at 20 ± 0.1°C. The following procedure was applied during the anoxic experiments: (1) Transfer 900 ml fresh sludge sample into the anoxic reactor. (2) Aerate the sludge sample overnight. (3) Stop aeration and provide N_2 gas to ensure anoxic conditions. (4) Wait for 20–30 min to determine the anoxic endogenous baseline. (5) Add 5 mg NH_4-N to ensure no ammonia limitation for growth. (6) Add nitrate (NO_3-N) stepwisely for in-line calibration. (7) Add acetate as carbon source according to certain C/N ratios. (8) Repeat steps 5 and 6 to obtain anoxic experiments under different C/N ratios. (9) Take regular samples for off-line nitrate/nitrite measurements.

Results and discussion

Step response of the nitrate biosensor and reconstruction of true nitrate concentration

Every sensor has a transient dynamic response to a step change in the physical quantity under measurement, e.g. nitrate (see e.g. for DO sensors in Lee and Tsao (1979)). It is, therefore, important to identify and account for the dynamic response of the biosensor to prevent measurement errors. This is especially significant in studies dealing with the determination of the kinetics of the biological processes (Vanrolleghem *et al*., 2004). To this aim, the step-response of the nitrate biosensor was analysed and shown in Figure 2 (left) for two different nitrate pulse additions (3.45 and 6.9 mgNO_3-N/l). Similar to the oxygen electrodes (Lee and Tsao, 1979), the response of the biosensor can be modelled by a first order model (see Eq. (1)

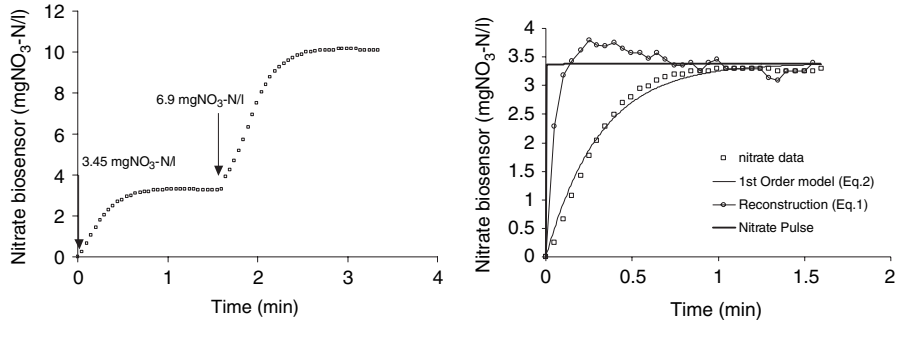

Figure 2 Step-response of the nitrate biosensor to 2 consecutive additions of known nitrate pulses (left), first order model prediction (Eq. (2)) fitted to the first step response and reconstruction of the real nitrate concentration in the medium (using Eq. (1)) (right)

and Figure 2 (right)):

$$E_{real}(t) = E(t) + \tau \cdot \frac{dE(t)}{dt} \quad (1)$$

$$E(t) = A \cdot (1 - e^{-t/\tau}) \quad (2)$$

where $E_{real}(t)$ is the ideal output of the nitrate biosensor (mgNO_3-N/l), $E(t)$ is the output of the nitrate biosensor (mgNO_3-N/l), τ is the first order time constant of the nitrate biosensor (min) and t is time (min). The time constant of the biosensor,τ, can be estimated using Eq. (2), where A is a constant referring to the ideal output of the biosensor after nitrate pulse addition (mgNO_3-N/l). With Eq. (2) the model fit to the biosensor output is shown for the first nitrate pulse in Figure 2 (right). Using non-linear regression, the time constant was determined to be 0.31 ± 0.01 (min) (where A was also simultaneously estimated and found to be 3.39 ± 0.04 mgNO_3-N/l). The time constant of the nitrate biosensor is in the same order of magnitude as that of oxygen electrodes (Lee and Tsao, 1979; Vanrolleghem *et al.*, 2004).

The reconstruction of the true nitrate measurements using Eq. (1) is also shown in Figure 2 (right). From a practical application point of view, the results are acceptable and show that it is possible to approximate the actual nitrate concentrations in the medium.

Slope of the nitrate biosensor: in-line calibration

In contrast to ion-selective electrodes that show log-linear behaviour, the biosensor provides mV signals linearly proportional to the nitrate concentration in the medium (Larsen *et al.*, 2000) and has no intercept, i.e. absence of nitrate gives a 0 mV signal. Nitrate is therefore obtained simply by multiplying the biosensor signals with its slope. The accurate estimation of the slope is therefore essential to obtain accurate nitrate measurements. Equally significant is the stability/drift of the slope for short-term (during an experiment) and long-term applications. With this in mind, the drift/stability of the biosensor's slope was studied in two different activated sludge samples: a pilot-scale SBR and the Ossemeersen WWTP.

The slope of the biosensor in the activated sludge from pilot-scale SBR was found to be higher than the slope in the sample from the Ossemeersen WWTP (almost twice, see Table 1). The reason of this change in the characteristics of the biosensor in different samples could be due to the differences in composition of the samples e.g. salinity (Manual, Unisense). However, it is beyond the scope of this study to go into detail on the causes. In short, the biosensor should be calibrated in the medium in which it is being used.

The slope of the biosensor was observed to drift in time albeit at a different rate depending on the activated sludge (see Figure 3 for activated sludge from SBR). In comparison, the slope and the offset of the ion-selective nitrate electrode used in the previous methodology (Sin *et al.*, 2003) was also monitored for long-term in the same pilot-scale SBR (Malisse, 2002).

It was clearly observed that the drift in the slope of the ion-selective electrode is rather random and chaotic with no specific pattern and ranging between 45 and 75 mV/log(NO_3-N/l) on consecutive days (results not shown). Moreover, the offset of the ion-selective electrode was rather high and changing randomly between 230–280 mV (results not shown). In contrast, the drift in the slope of the biosensor is rather stable/predictable in the sense that it is

Table 1 The slope of the biosensor in experiments with different activated sludge samples

Activated Sludge Source	Slope mV/(mgN/l)	Drift (%/day)
Pilot-scale SBR	0.022	−32% over 12 days (−2.68%/day)
Ossemeersen WWTP	0.013	−14% over 3 days (−4.65%/day)

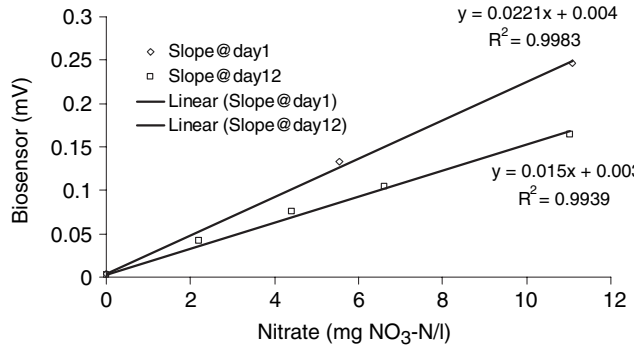

Figure 3 Drift of the biosensor slope in SBR sludge

decreasing linearly in time. Moreover, the offset of the biosensor was observed to be rather stable and almost zero (between 0.003–0.004 mV) in different activated sludge samples. A regular calibration therefore seems appropriate to deal with the drift for the biosensor.

It is important to note that in combined nitrate-titrimetric experiments, the ionic strength of the medium may change slightly due to, for instance, continuous addition of acid (see Figure 4). This was also observed to affect the slope of the biosensor (results not shown) but could also be compensated for appropriately with the in-line calibration that is available in each pulse addition of nitrate.

Application of the improved methodology

The improved anoxic methodology was applied to the pilot-scale SBR to study the anoxic response of activated sludge to pulse additions of carbon source (see Figure 4). From now on, the presented nitrate measurements are all corrected for the response time of the biosensor and for the slope drift. The nitrate uptake rate (NUR) indicating the denitrification activity of the biomass is calculated as ($NUR = dNO_3/dt$) which is similar to the calculation of OUR in LSS type respirometers (Spanjers *et al*., 1998)). The derivative of the nitrate concentration (dNO_3/dt) is calculated by taking the slope of the nitrate profile using a central moving window approach with 3 data points.

The response of the endogenously respiring biomass to the first pulse addition of substrate (see Figure 4) shows that the maximum nitrate uptake rate (NUR_{max}) is reached following a

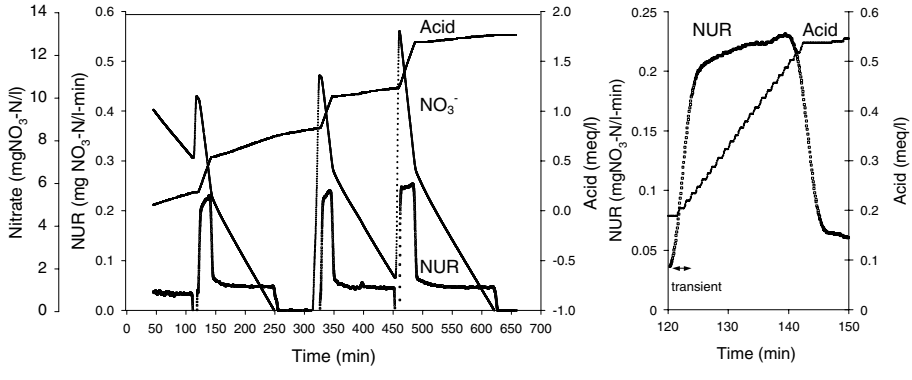

Figure 4 Anoxic monitoring of pilot-scale SBR sludge with three consecutive pulse additions: (10 mgNO_3-N + 30 mgCOD), (11 mg NO3-N + 30 mgCOD) and (12.5 mgN + 40 mgCOD). NO_3, NUR and titrimetric data (left); Transient response in NUR zoomed from the first pulse addition (right)

fast transient response taking 2–3 minutes to complete. This transient phenomenon is often observed in OUR from similar batch experiments (Vanrolleghem *et al*., 2004). Further, after the external carbon source is removed completely from the medium (this is indicated by a strong bending point in the nitrate and acid addition profiles (see Figure 4 (left)), NUR decreases drastically to a level clearly higher than the previous endogenous level (see Figure 4 (left)). This elevated endogenous level implies that the nitrate reduction continues at a rate higher than the endogenous rate probably using intracellular storage polymers produced during the feast phase (Van Loosdrecht and Heijnen, 2002). The sharp decrease (around 80%) in the denitrification rate of the biomass when the external carbon source is depleted is noteworthy. It clearly indicates the bottleneck of denitrification i.e. the availability of a readily biodegradable carbon source. Further on, the endogenous level drops to zero when all nitrate is removed from the reactor (around $t = 250$ min, Figure 4 (left)). Moreover, it is worthwhile to mention that the anoxic response of biomass to the pulse addition of acetate is repeated in a similar way in the following two pulse additions, with one exception: in the third pulse addition NUR reaches a maximum plateau implying that the previous two acetate pulse additions induced a wake-up effect on the biomass as discussed in detail in Vanrolleghem *et al*. (1998) for OUR.

Similar results were obtained and discussed with the previous version of the anoxic methodology (Sin *et al*., 2003). However, the significance of the modification presented in this paper is its high-quality nitrate measurements, which enable much higher-quality NUR calculation thereby improving the quality of the anoxic activated sludge monitoring. In addition, the nitrate biosensor provides linear signals and can also measure at the low concentration range as opposed to the log-linear signals of the ion-selective electrode in the previous methodology that were practically limited to concentrations above 2 mgNO_3-N/l. As a result, the biosensor enables to study/estimate nitrate affinity constants of biomass, which was not possible with the ion-selective electrode used in the previous methodology. Moreover, it is easy to calibrate the biosensor and convert its signals into the nitrate data. Finally, although the drift in the slope of the biosensor exists, it is predictable (see above) which allows it to be compensated for appropriately by in-line calibration in contrast to the chaotic drift in the ion-selective electrode.

For the interpretation of the titrimetric data, the conceptual model developed and described in detail elsewhere (Petersen *et al*., 2002; Sin *et al*., 2003) is used. The conceptual model in general takes into account various pH effects of biological and physico-chemical processes occurring during anoxic batch experiments. In the endogenous period before the first pulse addition, a linear acid addition rate (i.e. proton consumption rate) is observed (see Figure 4) mainly to compensate for the protons removed due to CO_2 stripping by the nitrogen gas. Upon pulse addition of acetate, the acid addition rate is immediately increased (see Figure 4 (left)). This is mainly required to compensate for the immediate uptake of the acetate, which is accompanied by removal of protons from the medium (by stoichiometry, each mmol of acetate removed consumes approximately 1 mmol of proton from the medium, see Petersen *et al*. (2002) and Sin *et al*. (2003)). During the feast period, i.e. in the presence of the external carbon source, the acid addition rate is maximum and linear in time; however, the acid addition rate decreases drastically to a lower rate similar to the previous level i.e. during the famine period. The titrimetric data is also observed to produce reproducible results in three consecutive pulse additions of acetate. For the famine period, however, the titrimetric data show a slow-down in the rate due to the reduction in the CO_2 stripping process (rate).

The nitrate and titrimetric data are observed to display different dynamics about the denitrification process. The acid addition is immediately started after pulse addition of substrate but a transient is clearly shown in the NUR data (see Figure 4 (right)). The same

phenomenon was also observed in combined OUR-titrimetric measurements (Gernaey *et al*., 2002; Vanrolleghem *et al*., 2004). The difference in the observed dynamics is hypothesised to be resulting from the substrate metabolism of the cell where substrate uptake is an earlier process in the metabolism than the nitrate uptake. The experimental results obtained here further support the aforementioned hypothesis of Vanrolleghem *et al*. (2004) as it is obtained here under anoxic conditions.

Comparison of NUR and OUR under high resolution

In Figure 5, the anoxic respiration rate (NUR) is compared with the aerobic respiration rate (OUR) of the sludge from the Ossemeersen WWTP. The aim is to compare the performance of the anoxic respirometer developed in this study with a well-established aerobic respirometer (Sin *et al*., 2003).

The NUR was obtained using the methodology described above with C/N equal to 1 while the OUR was obtained with a pulse addition of 46.88 mgCOD/l acetate following the methodology and the respirometer described in Sin *et al*. (2003). Biomass concentrations in both experiments were significantly different, 6,800 versus 2,700 mgVSS/l for aerobic and anoxic tests respectively. The data frequency and quality of NUR is the same as the OUR. These results firmly suggest that the anoxic respirometer developed in this study is an equally powerful tool as the aerobic respirometer.

The anoxic set-up is ready to meet the high requirements for reliable, robust and accurate determination of the anoxic respiration rate of biomass. It is observed that the pattern of acetate degradation is similar under aerobic and anoxic conditions and under both feast and famine periods albeit, as expected, the rate and the stoichiometry of the processes are different. No stoichiometric and rate comparisons are made here since it is part of an ongoing study.

Limitation of the improved methodology

The biosensor measures the sum of nitrate and nitrite concentration in the medium with one to one molar weight (Larsen *et al*., 2000). It is therefore very important to quantify the nitrite in the medium to ensure correct NUR calculations. In fact, the ISE used in the previous methodology (Sin *et al*., 2003) was also sensitive to nitrite concentrations. In this study, the nitrite build-up was regularly monitored using off-line nitrite measurements and was observed to be at maximum around 0.22 mgNO_2-N/l indicating that nitrite build-up was negligible in this study. Further, since the biosensor is also sensitive to possible N_2O build-up during denitrification (Larsen *et al*., 2000), the anoxic respirometer based on the biosensor

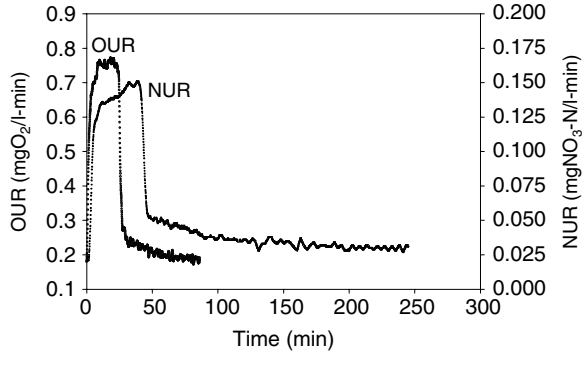

Figure 5 Comparison of OUR (46.88 mgCOD/l) and NUR (38.89 mgCOD/l) for the Ossemeersen sludge

becomes *essentially* more suitable to study the single-step denitrification process with negligible intermediate accumulation. On the other hand, the anoxic methodology can easily be extended to study denitrification intermediates as new sensors/tools become available to measure nitrite and N_2O separately (e.g. McMurray *et al*., 2004).

Conclusions

An improved methodology has been developed and successfully applied for the monitoring of anoxic activated sludge activity. The major improvement of the methodology is its simplicity, robustness and high quality data resulting from the use of a novel nitrate biosensor. The output of the biosensor is quite fast with a first order time constant equal to 0.31 min. Moreover, the drift of the biosensor slope was observed but it can easily be corrected for by in-line calibration. The resulting high frequency data allowed for the first time to monitor anoxic respiration rate of activated sludge, NUR, at a higher resolution (every 3 s) and down to lower nitrate concentrations allowing to even estimate nitrate affinity constants. Similar to the OUR, the NUR resulting from a pulse addition of carbon source to endogenously respiring anoxic activated sludge exhibits a clear start-up phenomenon and storage tail. Finally, the methodology is expected to serve as an anoxic respirometer to study particularly single-step denitrification process. It can be used for various purposes, e.g. process control, wastewater characterisation, estimation of kinetics and stoichiometry of denitrification process in view of calibration of activated sludge models.

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References

Gernaey, K., Petersen, B., Nopens, I., Comeau, Y. and Vanrolleghem, P.A. (2002). Modelling aerobic carbon source degradation processes using titrimetric data and combined respirometric-titrimetric data: II. Structural and practical identifiability. *Biotech. Bioeng*, **79**, 754–769.

Kristensen, H.G., Elberg Jørgensen, P. and Henze, M. (1992). Characterisation of functional microorganism groups and substrate in activated sludge and wastewater by AUR, NUR and OUR. *Wat. Sci. Tech.*, **25**(6), 43–57.

Kong, Z., Vanrolleghem, P.A., Willems, P. and Verstraete, W. (1996). Simultaneous determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer. *Water Res.*, **30**, 825–836.

Langergraber, G., Fleischmann, N. and Hofstaedter, F. (2003). A multivariate calibration procedure for UV/VIS spectrometric quantification of organic matter and nitrate in wastewater. *Wat. Sci. Tech.*, **47**(2), 63–71.

Larsen, L.H., Damgaard, L.R., Kjaer, T., Stenstrom, T., Lynggaard-Jensen, A. and Revsbech, N.P. (2000). Fast responding biosensor for on-line determination of nitrate/nitrite in activated sludge. *Water Res.*, **34**, 2463–2468.

Lee, Y.H. and Tsao, G.T. (1979). Dissolved oxygen electrodes. *Adv. Biochem. Eng.*, **13**, 35–86.

Lynggaard-Jensen, A. (1999). Trends in monitoring of wastewater systems. *Talanta*, **50**, 707–716.

Malisse, K. (2002). *Monitoring van de denitrificatie in actief slib aan de hand van nitraat- en titrimetrische gegevens*. Engineers Thesis, Faculty of Agricultural and Applied Biological Sciences, Ghent University, 130 p [*in Dutch*].

McClintock, S.A., Sherrard, J.H., Novak, J.T. and Randall, C.W. (1988). Nitrate versus oxygen respiration in the activated sludge process. *J. Water Pollut. Control Fed.*, **60**, 342–350.

McMurray, S.H., Meyer, R.L., Zeng, R.J., Yuan, Z. and Keller, J. (2004). Integration of titrimetric measurement, off-gas analysis and NO_x^- biosensors to investigate the complexity of denitrification processes. *Wat. Sci. Tech.* 135–141 (following article).

Naidoo, V., Urbain, V. and Buckley, C.A. (1998). Characterisation of wastewater and activated sludge from European municipal wastewater treatment plants using the NUR test. *Wat. Sci. Tech.*, **38**(1), 303–310.

Petersen, B., Gernaey, K. and Vanrolleghem, P.A. (2002). Anoxic activated sludge monitoring with combined nitrate and titrimetric measurements. *Wat. Sci. Tech.*, **45**(4–5), 181–190.

Rieger, L., Siegrist, H., Winkler, S., Saracevic, E., Votava, R. and Nadler, J. (2002). In-situ measurement of ammonium and nitrate in the activated sludge process. *Wat. Sci. Tech.*, **45**(4–5), 93–100.

Spanjers, H., Vanrolleghem, P.A., Olsson, G. and Dold, P.L. (1998). *Respirometry in Control of the Activated Sludge Process: Principles*. IAWQ Scientific and Technical Report No. 7. London, UK.

Sin, G., Malisse, K. and Vanrolleghem, P.A. (2003). An integrated sensor for the monitoring of aerobic and anoxic activated sludge activities in biological nitrogen removal plants. *Wat. Sci. Tech.*, **47**(2), 141–148.

van Loosdrecht, M.C.M. and Heijnen, J.J. (2002). Modelling of activated sludge processes with structured biomass. *Wat. Sci. Tech.*, **45**(6), 13–23.

Vanrolleghem, P.A., Gernaey, K., Coen, F., Petersen, B., De Clercq, B. and Ottoy, J.P. (1998). Limitations of short-term experiments designed for identification of activated sludge biodegradation models by fast dynamic phenomena. In: *Proceedings 7th IFAC Conference on Computer Applications in Biotechnology CAB7*, 31 May–4 June 1998, Osaka, Japan, pp. 567–572.

Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P. and Takács, I. (1999). Estimating (combinations of) Activated Sludge Model No. 1 parameters and components by respirometry. *Wat. Sci. Tech.*, **39**(1), 195–215.

Vanrolleghem, P.A., Sin, G. and Gernaey, K. (2004). Transient response of aerobic and anoxic activated sludge activities to sudden concentration changes. *Biotechnol. Bioeng.*, **86**, 277–290.