Titrimetric monitoring of a completely autotrophic nitrogen removal process

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Abstract Fully autotrophic nitrogen removal processes, such as the combined SHARON-Anammox process, help to improve the sustainability of wastewater treatment. Successful operation of such a completely autotrophic system is, among others, based on the strict control of the SHARON reactor in order to produce an Anammox-suited influent with a 1:1 ammonium:nitrite ratio. The high quality and high frequency measurements provided by a titrimetric set-up measuring the total ammonium (TAN) and total nitrite (TNO_2) concentrations facilitate this control considerably. In this study, the use of a titrimetric set-up for monitoring the combined SHARON-Anammox process is investigated. The technique that interprets on-line collected titration curves was applied to a lab-scale system. Comparison with classic colorimetric results gave statistically indistinguishable results for TAN and TNO_2 concentrations in the SHARON reactor. In the Anammox reactor, only TAN could be determined by the investigated method due to the very low TNO_2 concentrations. Phosphate, a potential inhibitor of the Anammox process, is available as an additional measurement in the effluent of the SHARON reactor. Three measurements are thus combined in one single instrument. The proposed measuring technique holds different advantages over the other TAN and TNO_2 measurement techniques such as on-site availability, easy automation, the absence of the need for high dilutions and cost reduction.

Keywords Anammox; control; nitrification; sensors; SHARON; titration

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Introduction

Applying the SHARON-Anammox process (van Dongen *et al.*, 2001) can significantly reduce the pressure on nitrogen removal in the main wastewater treatment plant (WWTP) by separately treating the sludge digester effluent (typically $1\,gN\,L^{-1}$) before this stream is recycled to the entrance of the WWTP. This highly loaded nitrogen stream is first partially oxidised in the SHARON reactor which works at a sludge retention time (SRT) of 1 to 1.5 days and a temperature between 30 and 40 °C. As such, ammonium oxidisers are maintained in the reactor, while nitrite oxidisers are washed out and further nitrification of TNO_2 to nitrate is prevented. The SHARON reactor can produce an almost 1:1 total ammonium to total nitrite (TAN:TNO_2) ratio depending on the total ammonium to total inorganic carbon (TAN:TIC) ratio in the influent of the SHARON reactor (Van Hulle *et al.*, 2003). The effluent of the SHARON reactor is then sent to the Anammox reactor where the remainder of the TAN is oxidised anoxically with TNO_2 as electron acceptor (Jetten *et al.*, 1999). With the combined SHARON-Anammox process, low nitrogen effluent concentrations can be obtained, while aeration costs are significantly reduced, no additional carbon source is needed and sludge production is very low (Jetten *et al.*, 1997). Although the application of this completely autrotophic system is very promising, difficulties in operation are to be expected in view of the inhibition of the Anammox organisms by oxygen, phosphate and TNO_2 (Strous *et al.*, 1999). Careful control of the preceding SHARON reactor is therefore essential for successful operation. A key factor

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in this control is the availability of high quality and high frequency measurements of TAN and TNO_2 as these are the principal components of the combined system. Up to now, the methods used for the off-line measurement of these components (e.g. spectrophotometry and ion chromatography) are time-consuming and difficult to automate, especially in view of the concentration ranges typical for the SHARON reactor ($1\,gN\,L^{-1}$). Indeed, for this, large dilutions are required. In this contribution, therefore, the use of a titrimetric device for the monitoring of the combined SHARON-Anammox process is studied.

Materials and methods

SHARON reactor

The lab-scale SHARON used in this study is a 2 L continuously stirred tank reactor (CSTR) without biomass retention. The influent is pumped to the reactor at a rate of 1.3 L per day. The pump flow rate of this influent pump determines both the hydraulic residence time (HRT) and the sludge residence time, since both residence times are equal and defined as the ratio of the volume to the flow rate. The reactor is fed with synthetic influent. The influent TAN concentration is 1,000 mg TAN-N L^{-1} and the TAN:TIC ratio is 1:1 as decribed by Van Hulle *et al.* (2003). After 26 days of operation the amount of KH_2PO_4 was 20 times reduced (from 228 mgPO_4^{3-}-P L^{-1} to 11 mgPO_4^{3-}-P L^{-1}) to avoid toxic conditions for the Anammox organisms. The reactor is aerated through a pumice stone using air from a compressor (1 bar). The normal operational temperature is 35 °C, as is usual in practice for the SHARON process. The dissolved oxygen (DO) and pH in the reactor are logged with Labview® software. The pH was allowed to vary freely in a broad pH control range (pH 6–8), although the average pH during the experimental period was 6.9. The oxygen was always above 5 mgO_2 L^{-1}. Start-up and operation of the SHARON reactor are described in detail by Van Hulle *et al.* (2003).

Anammox reactor

The Anammox reactor is a 2 L sequencing batch reactor (SBR). The reactor is a Biostat B2 (BBI, Germany) and is equipped with sensors for pH, DO, redox and temperature. Operation and monitoring of the reactor is controlled via the BRAUN MFCS® software, as described by Wyffels (2004). The pH and the temperature were controlled at 7.5 and 35 °C respectively when necessary, N_2 gas was flushed through the reactor to ensure anaerobiosis. The reactor was fed with the effluent of the SHARON reactor that was diluted to obtain a TAN and TNO_2 concentration of 400 mgN L^{-1} each. If necessary, the TNO_2 or TAN influent concentration was adjusted with $NaNO_2$ or $(NH_4)_2SO_4$. The biomass washed out from the SHARON reactor was not separated from the Anammox influent. The reactor was operated at an almost infinite SRT and a HRT of 4 days, although several times during operation the influent and effluent pumps were stopped in case TNO_2 concentrations became too high as this would cause serious inhibition of the Anammox biomass.

Chemical analyses and standard solutions

Concentrations of TAN, TNO_2 and NO_3^- were analysed on a daily basis using colorimetric methods (Dr Lange GmbH, Germany). Concentrations of TAN and TNO_2 were also analysed with a titrimetric set-up (Metrohm Titrino 716, Metrohm, Switzerland) and the software discussed by Zaher and Vanrolleghem (2004), of which a small summary is given in the following paragraph. Before titration, the samples were acidified to pH 2 by the addition of a 37% HCl solution. The titrant (NaOH) was prepared by adding an

appropriate amount of NaOH pellets to distilled water. Standard solutions were prepared by appropriate dilution from a 4 $gN\,L^{-1}$ $NaNO_2$ and $(NH_4)_2SO_4$ stock solution.

Interpreting the titration curve with the BCS software

Titrimetric determination of buffers such as the monoprotic components TAN and TNO_2 (pKa values at 25 °C, respectively 9.2 and 3.4, Stumm and Morgan, 1996) and the triprotic component phosphate (with pKa values at 25 °C, of 2.1, 7.2 and 11.9, Stumm and Morgan, 1996) is accomplished with the BCS software that performs a model-based interpretation of experimentally determined buffer capacity curves (Zaher and Vanrolleghem, 2004). From the measured buffer capacity curve, estimates of the different buffering components are computed using model selection and parameter estimation techniques. The methods used for model selection and parameter estimation are described elsewhere (Van De Steene *et al.*, 2002).

The buffer capacity curve is calculated from the titration curve, which is obtained by measuring the pH in function of a stepwise addition of base. From this measured titration curve (typically around 30 to 50 points), the buffer capacity at each pH point is calculated as the derivative of the amount of base needed ($meq\,l^{-1}\,pH^{-1}$). This buffer capacity curve consists of the sum of the buffer capacities of individual buffering components in the solution (Van Vooren *et al.*, 2001), as demonstrated in Figure 1.

In Figure 1, a typical example of a sample taken from a partial nitrifying reactor is depicted. The buffer capacity curve as measured by the titrimetric device is shown as well as the contribution to the buffer capacity curve by TAN, phosphate and TNO_2, with a respective concentration of 10 mM (140 $mgTAN\text{-}N\,L^{-1}$), 5 mM (217 $mgPO_4^{3-}\text{-}P\ L^{-1}$) and 7 mM (98 $mgTAN\text{-}N\,L^{-1}$). These components are very critical for autotrophic nitrogen removal operation. Also, the contribution to the buffer capacity of the water buffer is given in the figure. The area of each peak is related to the concentration of the component, while the position is depending on the pKa value(s) of the component. From the figure, it can be seen that possible interference of the water buffer with the TNO_2 buffer might impede the determination of the TNO_2 concentration, especially if the pKa value of TNO_2 shifts to a lower value because of the effect of salt content, for instance.

The model based interpretation is performed as follows. The automatic model building of the BCS software starts with calibrating an initial model that is defined and initialised on the basis of the available information of expected buffer components in a certain system. The residuals (differences between model and data) are analysed and used to suggest a candidate model extension, i.e. adding an additional buffer with unknown pKa and concentration. Then, another fitting cycle is performed with the extended model, the residuals are analysed, a new extension is defined, and so on, until one of the stop criteria is

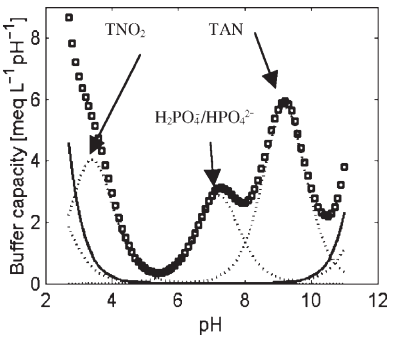

Figure 1 Contribution of individual components (…) (in casu TNO_2, phosphate and TAN) and the water buffer (–) to the buffer capacity curve (□).

reached. The stop criteria are based on a set of model selection techniques, which determine the optimum buffer capacity model and pKa values and concentrations of the significant buffers. Also, based on the fitting results, standard deviations are estimated for each of the pKa and concentration values.

Automation, with a measurement every 30 min, of this method is straightforward and is already available through the Anasense® titrimetric analyser (De Neve *et al.*, 2004) currently applied to titrimetric monitoring of anaerobic digesters (measuring VFA, bicarbonate, TAN, phenol and lactate).

Results and discussion

Titration with standard solutions

Before using the titrimetric method with the actual system, it was tested with standard solutions. Solutions of TAN and TNO_2 ranging from 10 to 4,000 mgN L^{-1} were prepared from the stock solution and analysed with the titrimetric set-up in a 50 ml sample without applying any dilution. Titrant concentrations of 0.5, 0.2, 0.1 and 0.05 M were used in order to determine the optimal titrant concentration. Comparison between expected and measured concentrations with the different titrants revealed that only with the lowest concentration (0.05 M) was an acceptable agreement obtained. With higher titrant concentrations, the difference between expected and calculated concentration was increasing when low TAN and TNO_2 concentrations had to be measured. However, concentrations up to 10 mgN L^{-1} could be measured. Of course, the application of such a lowly concentrated titrant limits the use of the titrimetric set-up to samples with a total nitrogen concentration of approximately 1,000 mgN L^{-1} since the physical limitations of the titrimetric vessel do not allow higher concentrations, i.e. more titrant must be dosed than the volume of the test vessel. Essentially the method had a dynamic range of 10–1,000 mgN L^{-1}. Based on these preliminary measurements with stock solutions, it was decided to use a 0.05 M titrant for the determination of the TAN and TNO_2 concentrations in the SHARON and Anammox reactors. These measurements were compared to measurements of TAN and TNO_2 with colorimetric methods (Dr Lange GmbH, Germany) as presented below.

Monitoring the SHARON reactor

Samples from the SHARON reactor were taken daily and analysed once with the colorimetric method and twice with the titrimetric method. For practical reasons, one of the two samples analysed with the titrimetric set-up was diluted twice and a 50 ml sample was used. For the other sample, only a 35 ml volume was used. Three times, around day 55, day 95 and day 110 (see Figure 2), a disturbance occurred in the reactor causing the TAN concentration to increase and the TNO_2 concentration to decrease. With both

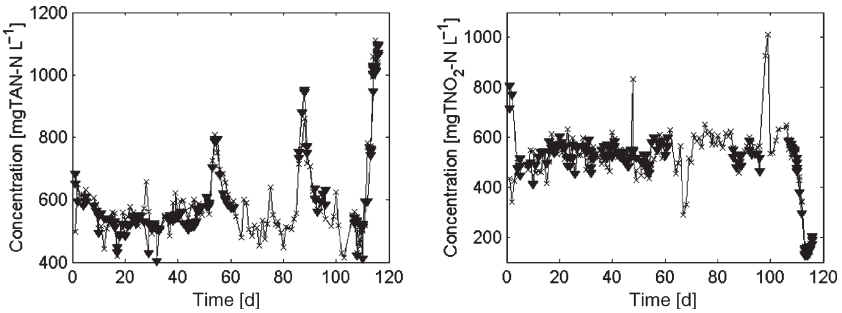

Figure 2 Top: Comparison between colorimetric (x) and titrimetric measurements (▼) for TAN concentrations (left) and TNO_2 (right) in the SHARON reactor

methods this disturbance could be detected. The rest of the studied period the reactor operated stably. In Figure 2, the comparison between colorimetric and titrimetric measurements is made for TAN and TNO_2 concentrations in the SHARON reactor. For TAN, the concentrations determined with both methods are very similar. The TNO_2 concentrations determined with both methods also follow the same trend, but here the colorimetric results show somewhat more scattering. This high variability may be due to the high dilution (100 times) that needs to be applied for determining the TNO_2 concentrations with the colorimetric method. As such, small errors during sample handling are amplified in the final result.

To statistically support the hypothesis that the titrimetric set-up offers an alternative to colorimetric methods for measuring the TAN and TNO_2 concentrations in a SHARON reactor, 17 samples were analysed twice with each method. (Un)fortunately during the sampling period, the reactor performance was not stable and TAN and TNO_2 concentrations fluctuated between 150 and 1000 $mgN\,L^{-1}$. The t-values calculated for a paired t-test (Weiss, 2002) were 1.027 and 1.065 ($n = 17$) for TAN and TNO_2, respectively. As the tabulated t value for the 95% confidence interval is 2.12 ($t_{16,0.975}$), the hypothesis that both methods are the same can not be rejected with 95% confidence. In Figure 3 the TAN and TNO_2 concentrations measured with the colorimetric and titrimetric methods are compared in a q-q plot. In this q-q plot, the points have as abscissa the sample concentration obtained with the colorimetric method and as ordinate the sample concentration obtained with the titrimetric method. In case both methods gave the same result, then all points should lie on the bisector.

As such, the excellent agreement is obvious. The average pKa values of TAN and TNO_2 determined with the BCS software for these 17 samples was 9.42 ± 0.05 and 2.85 ± 0.06, respectively. This indicates that a shift in pKa values occurred compared to the values mentioned by Stumm and Morgan (1996), possibly caused by temperature and salinity effects. The BCS software, however, easily deals with these shifts as it allows some freedom around the default values.

In addition to cost-effective TAN and TNO_2 measurements, the titrimetric measurement also offers the determination of phosphate, a component that was present in the influent of the SHARON reactor. However this only works on condition that the concentration is sufficiently high.

From day 1 until day 26 the concentration of phosphate in the influent was 228 $mgPO_4^{3-}$-$P\,L^{-1}$, after day 26 the phosphate concentration was reduced 20 times in view of the possible toxic effects of phosphate on the Anammox biomass (van de Graaf *et al.*, 1996). On average, the phosphate concentration determined before day 26 was

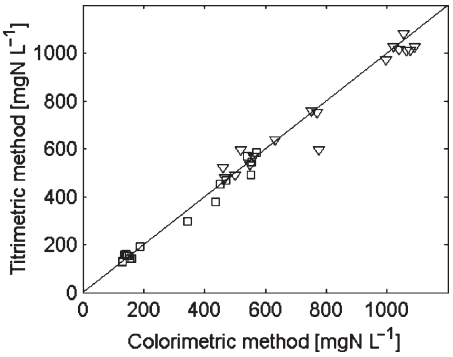

Figure 3 The q-q plot of TAN (▽) and TNO_2 (□) for comparing concentration measured in the 17 samples with the colorimetric and titrimetric method

244 ± 35 mgPO_4^{3-}-P L^{-1}. This concentration is somewhat higher than the one present in the influent, possibly because of the concentrating effect of the evaporation that occurs in the SHARON reactor (Van Hulle *et al.*, 2003). After day 26, phosphate could no longer be detected because the concentration was too low compared to the TNO_2 and the TAN concentrations. Typical buffer capacity curves of the period before and after the phosphate reduction are depicted in Figure 4.

Monitoring the Anammox reactor

Samples from the Anammox reactor were also taken on a daily basis and analysed once with the colorimetric method and twice with the titrimetric method. A 50 ml sample without dilution was applied for the titrimetric method. Unfortunately, the Anammox reactor did not operate stably at the time of the study. Possible reasons for this are phosphate and TNO_2 inhibition. Also, the presence of oxygen can inhibit the Anammox process, but the reactor was regularly purged with nitrogen gas and no oxygen was detected in the reactor. Figure 5 compares between colorimetric and titrimetric measurements for TAN concentrations in the Anammox reactor. It can be seen that both analytical methods give approximately the same result, although the TAN concentration fluctuates considerably. Because of the unstable operation it was not possible to perform a further elaborated comparison, as was the case with the SHARON reactor.

Unfortunately, for the TNO_2 concentration, no close agreement between both methods was obtained. TNO_2 concentrations determined with the titrimetric device were overestimated up to two times compared to the colorimetric method. Possibly, interferences with other components present (e.g. phosphate or bicarbonate) may have led to the

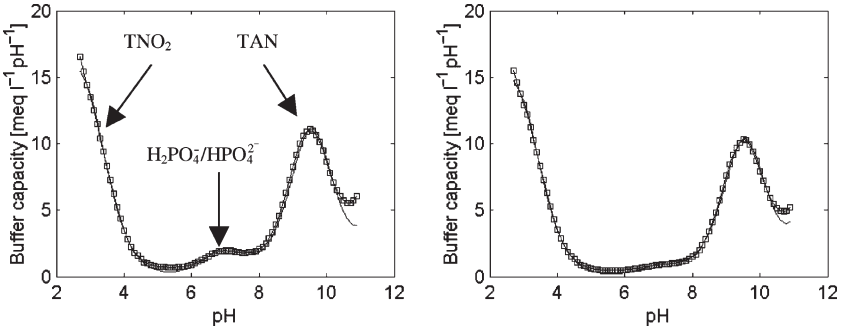

Figure 4 Typical buffer capacity curves for the period with 228 mgPO_4^{3-}-P L^{-1} phosphate in the influent (left) and the period with 11 mgPO_4^{3-}-P L^{-1} phosphate in the influent (right)

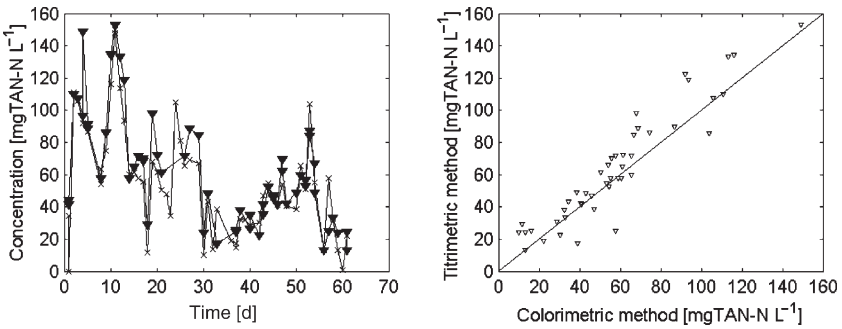

Figure 5 Comparison between colorimetric and titrimetric measurements for TAN concentrations in the SHARON reactor (left) and corresponding q-q plot (right)

unidentifiability of low TNO_2 concentrations in the Anammox sample. This is disappointing as exactly the TNO_2 concentration in the Anammox reactor should be monitored closely in view of the inhibition of Anammox at elevated TNO_2 concentrations. In view of control systems design, the preferred strategy would therefore be to install the on-line titrimetric sensor in between the SHARON and Anammox reactor. Control actions would then be applied in feedback mode to the SHARON process to achieve an Anammox suited effluent (Volcke *et al.*, 2003). An excess of TAN compared to TNO_2 will safeguard the Anammox reactor for TNO_2 inhibition.

Conclusions

In this study, the possibility of using a titrimetric set-up for the determination of TAN and TNO_2 when monitoring the combined SHARON-Anammox process was investigated. First, the optimal titrant concentration was determined to be 0.05 M based on measurements of standard solutions within the typical concentration range. Then, measurements with both methods were compared for samples taken from a lab-scale SHARON reactor. For both nitrogen components the different methods gave similar results, although the TNO_2 concentrations determined with the colorimetric device varied considerably. A statistical test showed that both methods could not be distinguished from each other with 95% confidence. For the Anammox reactor, only the TAN concentrations obtained with both methods agreed, while the TNO_2 concentrations were overestimated with the titrimetric set-up due to the low concentration of TNO_2 in the effluent and possible interferences by other components. Basically, the titrimetric set-up gives the possibility to replace part of the analyses work by a cheaper and easy to automate the method that requires no dilution and hence is less sensitive to errors. Next to TAN and TNO_2, phosphate could also be detected as an additional measurement in the SHARON reactor.

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