Rapid automated detection of nitrification kinetics using respirometry

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Abstract
There is no doubt that respirometry is a useful measurement principle in the field of wastewater treatment. Although a large variety of methods and case studies have been published, respirometry has become neither a standard tool for control nor for assessment and optimisation of treatment plants. The drawback of the conventional method for determining nitrification kinetics is the long experimental time. This disadvantage can be avoided by “turning over” the experiment. Starting with low ammonia concentrations the steep slope of the Monod curve is measured first. The low concentration branch of the Monod curve is also the part where e.g. inhibition can be detected. Therefore the proposed procedure allows us to speed up the measurement of nitrification kinetics and to measure nitrification inhibition on-line.

Keywords
Inhibition; nitrification kinetics; on-line measurement; respirometry

Introduction
Respirometry is the measurement and interpretation of the respiration rate of activated sludge. The respiration rate is the amount of oxygen consumed by the microorganisms per unit time and unit volume. The respiration rate reflects the two most important biochemical processes in a wastewater treatment plant, biomass growth and substrate consumption. Although a large variety of methods and case studies have been published, respirometry has become neither a standard tool for control nor for assessment and optimisation of treatment plants.

A respiration rate value or a percentage inhibition deduced from respiration rate measurements cannot be interpreted without additional information about some measurement attributes. Indeed, it is a reactor in itself where different components are brought together to perform what may be called an “In-Sensor Experiment” and in which the experimental conditions generally have a very large influence on the measurement results (Spanjers et al., 1998). A precondition for rapid and accurate respiration rate measurements is a high quality of the oxygen measurement regarding the accuracy and the response time of the oxygen sensor.

Due to the fact that nitrification is the most critical process in activated sludge plants the rapid measurement of the influent ammonia concentration and the detection of a possible nitrification inhibition is of great importance for the operation of wastewater treatment plants (Spanjers and Vanrolleghem, 1995; Kong et al., 1996). Nitrification processes are characterized by a high oxygen consumption and it is therefore evident that respirometry has been adapted to monitor these processes.

Materials and methods

The respirometer
The utilisation of respirometry has not become standard perhaps due to the lack of appropriate instrumentation. On the one side there are respirometers but without predefined experiments. On the other side there are instruments with just one predefined experiment. Both models are obtained at a considerably high price.
An experimental respirometer set-up (Figure 1) was developed that complies with a variety of demands. It is completely automated, provides high flexibility for implementation of new experimental set-ups, measures with high accuracy and succeeds in speeding up the measurement process. Fast measurements are a precondition for the integration of respirometric data in control systems. The instrument also delivers evaluated rather than raw data, which is also important in monitoring systems.

The instrument consists of two respiration vessels for parallel experiments, one storage tank (endogenous vessel), two dosage pumps (µl–ml), various sensors, A/D and I/O cards and a standard PC and software (based on LabView®). The resolution of the oxygen measurement is 5–10 µg.l⁻¹ (depending on the sampling rate).

The respirometer provides simultaneous visualisation of respiration rates and other sensor data (such as pH and temperature). There is a possibility to implement a variety of respirometric experiments, including automatic aeration as well as dosage of substrates, inhibitors and other chemicals. Optimal choice of the oxygen sensor and data evaluation procedures has led to very rapid responses and a high level of accuracy.

The respirometer is controlled by a computer programme developed under the LabView® environment. The LabView® programme also provides facilities to control aeration and to operate the valves and pumps (they can be operated manually as well). Sequences of different experiments, depending on the information needed, can be defined and performed with the instrument.

The respirometer does not include temperature or pH stabilisation. The intention was to maintain the attractive simplicity of the respirometric principle, while using the instrument under conditions similar to those found in activated sludge tanks. However both parameters are measured and stored.

At the moment the instrument has the potential to measure actual respiration rates, short
term BOD (or oxygen consumption due to readily biodegradable substrate), nitrification capacity and kinetics, and nitrification inhibition (as alarm parameter).

**Accuracy of respiration rate measurements**

Usually the substrate concentrations in the aeration tank of wastewater treatment plants are low (actual respiration rates in a wastewater treatment plant are in the range of 5 to 20 mgO₂.l⁻¹.h⁻¹). Therefore a high accuracy for the oxygen measurement is a precondition for the measurement of the respiration rate itself and later on for measuring small changes of the respiration rate (Vanrolleghem and Spanjers, 1998; Marsili-Libelli and Tabani, 2002).

There is a relationship between the required measurement time and the respiration rate to be measured at a given resolution of the oxygen sensor due to the electrochemical principles of the sensor (Figure 2). The required measurement time for an oxygen sensor with a resolution of 15 µg.l⁻¹ to measure a respiration rate of 15 mgO₂.l⁻¹.h⁻¹ with an accuracy of ± 5% is 37 s. If a sensor with a resolution of 4.6 and 2 µg.l⁻¹ is available the measurement time can be reduced to 11 and 4.8 s respectively.

Later on the response time of the oxygen sensor is of great importance. The oxygen probe dynamics can be described using a first or second order transfer function (Spanjers and Olsson, 1992; Vanrolleghem and Spanjers, 1998). Figure 3 shows a calculated “measured” oxygen concentration and respiration rate for 2 theoretical oxygen sensors with a response time of \( t_{90} = 95 \) s and \( t_{90} = 205 \) s respectively. However, the “faster” sensor is also too slow for the use in a respirometer. There the required response time is about \( t_{90} = 10 \) s. The response time \( t_{90} \) of a sensor is defined as the time from the application of a step signal until the measured signal reaches 90% of its final value (Olsson and Newell, 1999). It can be clearly seen that for a given respiration rate of 1 mgO₂.l⁻¹.min⁻¹ = 60 mgO₂.l⁻¹.h⁻¹ the results for the two sensors differ significantly. Using a “slower” oxygen sensor no reliable respiration rates can be measured. The difference between the “faster” and “slower” oxygen sensor is smaller but also not negligible if smaller respiration rates have to be measured. The compensation of the response time is possible and an increase in accuracy can be achieved (Spanjers and Olsson, 1992).

The results show that a high quality of the oxygen measurement regarding the accuracy and the response time of the oxygen sensor is required to get reliable, rapid and accurate respiration rate measurements.

**Determination of nitrification kinetics**

First the standard method for the determination of nitrification kinetics (e.g. Vanrolleghem *et al.*, 1999) is described. The activated sludge is aerated for at least 12 hours to guarantee endogenous respiration and to remove ammonia from the activated sludge. After
endogenous respiration is reached sludge is pumped into the respirometer. Exactly dosed volumes of the ammonia standard (560 mgN/l; composed as given in DIN ISO 9509, 1995) are added to provoke substrate respiration and the consumption of oxygen is recorded.

A small amount of the ammonia standard is dosed at the beginning of the experiment to determine the stoichiometric factor for nitrification of the sludge used. The stoichiometric factor for nitrification gives the amount of oxygen consumed per g ammonia nitrified. It differs from the theoretical value of 4.57 $\text{gO}_2\cdot\text{gN}^{-1}$ due to the uptake of nitrogen in the biomass and can be calculated as given in Gujer et al. (2000) as

$$i_N = 4.57 - Y_A$$  \hspace{1cm} (1)

where $Y_A = \text{yield factor for autotrophic bacteria (gCOD,BM}\cdot\text{gN}^{-1})$. The stoichiometric factor has to be determined at every measurement due to changing sludge characteristics (Spanjers and Vanrolleghem, 1995). For the experiments 0.2 ml standard were used for the determination of the stoichiometric factor. Also with this low amount of standard a good reproducibility of the measurement could be achieved.

Knowing the measured stoichiometric factor of nitrification, the amount of ammonia standard added and the oxygen consumption one can calculate the actual ammonia concentration in the respirometer vessel. Calculated ammonia concentrations show a good correlation with measured concentrations using a standard lab method (Nowak, 1996). Fleischmann and Kreuzinger (2000) compared measured respiration rates with calculated respiration rates (from standard lab analysis of 2 h composite samples) at a municipal wastewater treatment plant and found a high correlation coefficient of 93%.

Figure 4 shows the standard experiment for a dosing of 9 ml ammonia standard. This results in an initial concentration of 3.9 $\text{mgNH}_4\cdot\text{N}^{-1}$. The endogenous respiration measured in this experiment was about 7 $\text{mgO}_2\cdot\text{L}^{-1}\cdot\text{h}^{-1}$. The main disadvantage of this type of respirometer is, that during the re-aeration periods no respiration rates can be measured. For these periods calculated respiration rates have to be used. However, these data can be reproduced by measurements in the parallel reactor vessel. The whole measurement takes about 35 minutes. The respiration rates and ammonia nitrogen concentrations are used to determine the coefficients $R_{\text{MAX}}$ and $K_S$ for the Monod-type equation

$$\frac{c}{c_S} = \frac{1}{1 + \frac{c}{K_S}}$$  \hspace{1cm} (2)

with $c_S = \text{substrate concentration (ammonia nitrogen in this case)}$. The maximum respiration rate was determined as $R_{\text{MAX}} = 31.0 \text{mgO}_2\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and the half saturation coefficient $K_S$ as 0.071 $\text{mgN}\cdot\text{L}^{-1}$. The confidence interval (95%) of $R_{\text{MAX}}$ is between 30.4 and 31.6 $\text{mgO}_2\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ (plus/minus about 2% of the actual number). For $K_S$ the confidence interval is between 0.056 and 0.085 $\text{mgN}\cdot\text{L}^{-1}$ (plus/minus about 20% of the actual number).
Under certain circumstances problems can occur with the identifiability of $R_{\text{MAX}}$ and $K_S$. It is well known that an error in one parameter can be compensated by the other (Olsson and Newell, 1999).

The arrow in Figure 4 (right) indicates that the measurements start with high substrate concentrations and therefore it takes a long time until the result is obtained. Additionally only a few data points for low ammonia concentrations can be measured due to the fact that the remaining substrate is consumed rather quickly at the end of the experiment. It is well known that it is difficult to get accurate results for determining the $K_S$ value (Vanrolleghem et al., 1995; Marsili-Libelli and Tabani, 2002).

**Rapid determination of nitrification kinetics**

The drawback of the standard method is the long experimental time for the determination of the coefficients for the Monod equation. This can be avoided by either using low substrate concentrations for the standard experiment as described above or by “turning over” the experiment.

**Low substrate concentrations.** It is quite clear that using only low ammonia nitrogen initial concentrations can speed up the experimental time. The accuracy of this method has to be determined.

“Turning over” the experiment. Starting the determination of the Monod curve with low substrate concentrations leads to “turning over” the experiment, shown in Figure 5. The steep slope of the Monod curve is measured first. Defined amounts of substrate are added to increase the concentration and further measurement points are obtained. Once an acceptable accuracy of the estimated parameters is reached the measurement can be interrupted. This procedure on the one hand reduces the measurement time and on the other hand should improve the accuracy for the measured $K_S$ value.

**Results and discussion**

**Determination of nitrification kinetics using low initial substrate concentrations**

Figure 5 summarises the results using different ammonia nitrogen initial concentrations. For the maximum respiration rate $R_{\text{MAX}}$ the residuals were within $\pm 15\%$ with ammonia nitrogen initial concentrations higher than 0.3 mg$_{\text{NH4-N}}$l$^{-1}$ whereas for $K_S$ the residuals of the estimated values were higher also for higher initial concentrations. Using low initial concentrations one can only get good results for the maximum respiration rate.

The reproducibility of the results was obtained by running several identical experiments one after the other. A confidence interval (95%) of 10% can be reached for $R_{\text{MAX}}$ whereas for $K_S$ the confidence interval varies strongly. Therefore for a good estimation of $K_S$ other methods are needed.

“Turning over” the experiment

Figure 6 shows the results for the proposed experiment. At the very beginning the stoichiometric factor is determined using a dosing of 0.2 ml ammonia standard. After endogenous respiration is reached again 0.6, 0.8, and 0.8 ml standard are dosed in an interval of 2 minutes. The respiration rates measured 1 minute after the dosing are taken into consideration for the determination of the nitrification kinetics. This lag-time after adding the standard until the actual respiration rate reached its limits shows the total reduction in measurement time that can be achieved. The obtained Monod-type function is shown in Figure 6 (right, with $R_{\text{MAX}} = 22.6$ mg$_{\text{O2}}$l$^{-1}$.h$^{-1}$, and $K_S = 0.067$ mg$_{\text{N}}$l$^{-1}$). Usually good results can be achieved when the measured values for three times dosing of the standard are included.
whole measurement including the determination of the stoichiometric coefficient takes about 15 minutes.

Vanrolleghem et al. (1995) showed for batch experiments that the results of the parameter estimation for a single Monod model can be improved significantly by adding additional substrate pulses at the end of the experiment. The addition of more additional pulses results in only marginal improvements. Gernaey et al. (2001) showed similar results for a two-step nitrification model.

Can the measurement time be shortened by leaving out the determination of the stoichiometric coefficient? To answer this question the sensitivity of the calculated parameters on the stoichiometric coefficient were checked. It turned out that using $\pm 10\%$ of the measured stoichiometric coefficient to calculate ammonia nitrogen the value of the estimated $R_{\text{MAX}}$ changed only by $\pm 2\%$ whereas the $K_S$ value varied by $\pm 10\%$.

Therefore if only $R_{\text{MAX}}$ is of interest the determination of the stoichiometric coefficient can be left out and the measurement time then reduces to less than 10 minutes. The described method can therefore also be used for on-line measurements of the nitrification kinetics.

**Conclusion**

Respirometry is a quick and easy method to measure ammonia concentrations and nitrification kinetics. To get rapid and accurate respiration rate measurements a high quality of the oxygen measurement regarding the accuracy and the response time of the oxygen sensor is a precondition. The proposed procedure will allow us to speed up the measurement of nitrification kinetics (up to a factor of 3) and therefore e.g. on-line inhibition measurements will be possible that can be further used for control purposes. An improvement regarding the accuracy of $K_S$ measurements can be achieved if the ammonia standard is dosed continuously and allowing the measured respiration rate to adjust a given ammonia concentration in the reactor.
The proposed procedure could also be a step forward for respirometry to leave its academic status and to become a tool more often used at wastewater treatment plants for on-line measurement nitrification kinetics. The main drawback – the long measurement time– can be avoided by the new method and makes it an interesting tool for plant operators.

References


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