



## NITRIFICATION MONITORING IN ACTIVATED SLUDGE BY OXYGEN UPTAKE RATE (OUR) MEASUREMENTS

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(First received August 1994; accepted in revised form October 1995)

**Abstract**—A simple measuring system was developed that yields information about the presence of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  nitrogen in mixed liquor samples. In addition, it allows monitoring of the rate of both  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation simultaneously with the carbon substrate oxidation using OUR measurements. The method is based on the subsequent addition of  $\text{NaClO}_3$  and allylthiourea, selective inhibitors of *Nitrobacter* and *Nitrosomonas* respectively, to the mixed liquor sample in a closed batch respirometer. The presented method is valuable for detailed monitoring of the nitrification process in a reactor: it is simple, inexpensive, robust and generally applicable for nitrifying reactor systems. The measurement of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation rates enables the operator to detect the presence of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  species. This allows action to be taken to improve the performance of the system. The method is validated on a SBR. It is indicated that optimal SBR phase scheduling can be based on such OUR measurements. Copyright © 1996 Published by Elsevier Science Ltd.

**Key words**—biological nutrient removal, nitrification, activity measurements, allylthiourea, sodium chlorate, sequencing batch reactor, control, inhibition, oxygen uptake rate

### INTRODUCTION

Removal of nitrogen and phosphorus has become an essential part of activated sludge processes as a result of the concern for eutrophication of the effluent receiving waters. Anoxic, anaerobic and aerobic conditions are essential to accomplish biological nutrient removal.

For nitrogen removal, a sequence of nitrification and denitrification is necessary. Nitrification is performed by two populations: the *Nitrosomonas* group oxidizes  $\text{NH}_4^+$ -N to  $\text{NO}_2^-$ -N; the *Nitrobacter* group subsequently converts  $\text{NO}_2^-$ -N to  $\text{NO}_3^-$ -N.

It is difficult to monitor and adjust nitrification in a plant in operation. The decrease of the substrate ( $\text{NH}_4^+$ -N) and increase of the end-product ( $\text{NO}_3^-$ -N) gives information but does not reflect the actual activity of the nitrifiers. There would be more control possibilities when changes in activity of the nitrifiers could be observed. Influent with strongly varying concentrations of COD and nutrients can cause two types of non-optimal behaviour of the treatment plant. Overloading of the activated sludge will endanger complete nitrification; the presence of  $\text{NH}_4^+$ -N in the effluent will be the consequence. Underloading of the activated sludge will give rise to a wastage of aeration energy.

It could be useful to have a biological method capable to detect the presence of  $\text{NO}_2^-$ -N in mixed liquor samples. Nitrite is a toxic nitrification intermediate whose formation has already been studied intensively (Balmelle *et al.*, 1992; Yang and Alleman, 1992; Abeling and Seyfried, 1993; Baten *et al.*, 1993).

The nitrification part of the biological nutrient removal process is generally recognized as the most vulnerable activated sludge process, depending on many factors which are often interconnected. To monitor the activity of the nitrifying populations present in activated sludge, Nowak and Svardal (1993) estimated the kinetic parameters of nitrifying biomass under the inhibiting conditions obtained by the presence of particular wastewaters. This was carried out by measuring the OUR of the nitrifying biomass at different  $\text{NH}_4^+$ -N concentrations. Allylthiourea (ATU), a selective inhibitor of the *Nitrosomonas* group, was used to inhibit nitrification. Vanrollegheem and Verstraete (1993) characterized mixed heterotrophic and nitrifying activated sludge populations using respirometry. They could distinguish the OUR for nitrification and carbon oxidation by choosing the appropriate composition of the calibration substrate used in their on-line RODTOX respirographic biosensor.

Selective inhibitors of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation have been described to determine the nitrifying activity of a sludge. In this paper, the nitrifying activity is defined as the amount of  $\text{NH}_4^+$  or  $\text{NO}_2^-$

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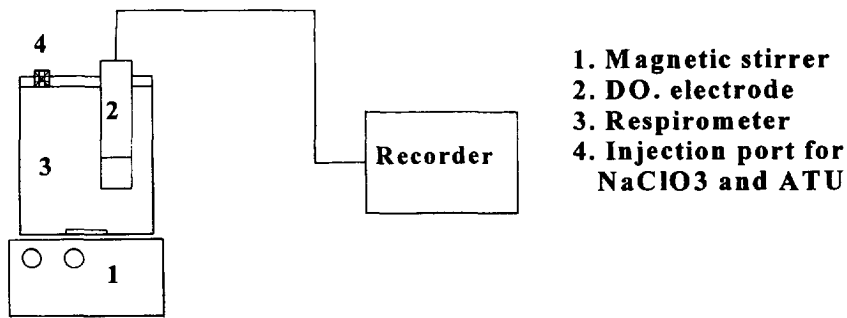


Fig. 1. Fed batch respirometer used for recording oxygen uptake rate profiles.

oxidized per liter sludge per hour and is expressed as the corresponding oxygen consumption rate in mg O<sub>2</sub>/l·h. The inhibitors commonly used are NaClO<sub>3</sub> and ATU (Völsch *et al.*, 1990). NaClO<sub>3</sub> is an inhibitor of NO<sub>2</sub><sup>-</sup> oxidation by *Nitrobacter* at a concentration of 20 mM (Belsler and Mays, 1980; Hynes and Knowles, 1983; Völsch *et al.*, 1990). NaClO<sub>3</sub> exerts no immediate negative effects on NH<sub>4</sub><sup>+</sup>-N oxidation by *Nitrosomonas* bacteria. Its inhibitory effect on *Nitrosomonas* becomes detectable only after approx. 30 min, due to the slow conversion of NaClO<sub>3</sub> to NaClO<sub>2</sub>, which inhibits both *Nitrobacter* and *Nitrosomonas* (Hynes and Knowles, 1983). ATU is a selective inhibitor of NH<sub>4</sub><sup>+</sup> oxidation by *Nitrosomonas* at a concentration of 5 mg/l (Wood *et al.*, 1981; Völsch *et al.*, 1990). Völsch *et al.* (1990) proposed a method for the determination of NH<sub>4</sub><sup>+</sup> or NO<sub>2</sub><sup>-</sup> degradation rates. They used the NO<sub>2</sub><sup>-</sup>-N production rate of a mixed liquor sample in the presence of NaClO<sub>3</sub> (*Nitrobacter* inhibited) as a measurement for the NH<sub>4</sub><sup>+</sup>-N degradation rate. The NO<sub>2</sub><sup>-</sup>-N degradation rate in the presence of ATU (*Nitrosomonas* inhibited) was used as a measure for the *Nitrobacter*

activity. The completion of one test took about 30 min.

The purpose of this work is to develop a rapid, simple and robust method for the determination of NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N oxidation rates by measuring the OUR of mixed liquor samples before and after addition of selective nitrification inhibitors in a specific experimental setup. It was verified if the measured NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N oxidation rates corresponded with the NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N concentrations in the mixed liquor. Validation of the method was done on a SBR.

#### PRINCIPLE OF THE METHOD

A conventional fed batch respirometer (Fig. 1) with a small, completely closed reactor vessel (volume = 100 ml) was used for the measurements. The respirometer contained an oxygen electrode connected to a recorder. Mixed liquor samples were taken from the SBR, saturated with oxygen by shaking the sample and transferred to the vessel. This vessel was carefully closed, with no air bubbles

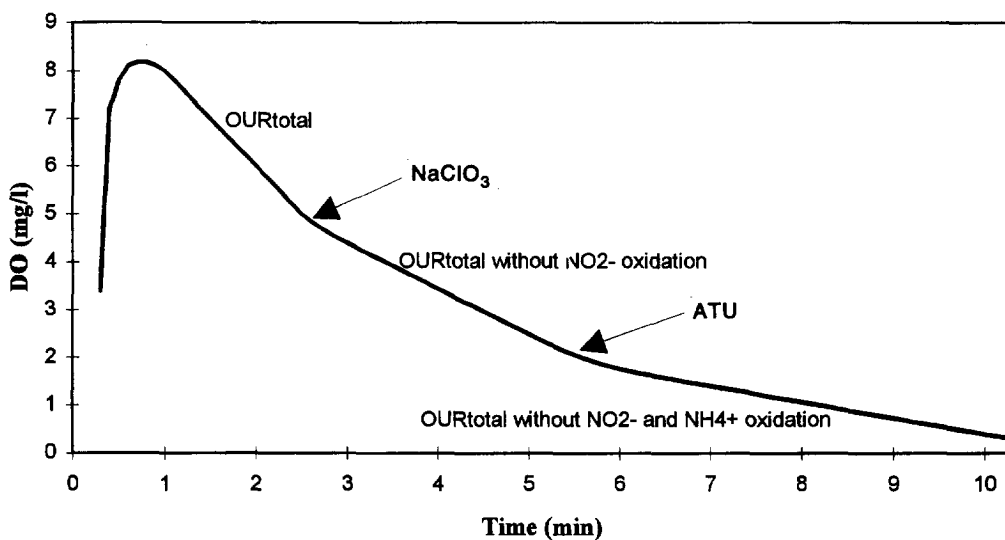


Fig. 2. Schematic representation of a typical oxygen uptake profile recorded with the developed nitrification activity determination method.

remaining in it. Samples were mixed during measurements by means of a magnetic stirrer at 150 r.p.m. Temperature and pH in the respirometer were the same as in the SBR.

The activity measurements result in oxygen uptake profiles as illustrated in Fig. 2. The operating procedure is as follows. First, the total OUR is determined. After the DO concentration has decreased with about 3 mg/l,  $\text{NaClO}_3$  is added to the mixed liquor sample (final concentration is 20 mM  $\text{NaClO}_3$  or 2.13 g/l) and the OUR is determined. The difference between the total OUR and the OUR measured in the presence of  $\text{NaClO}_3$  is considered as the oxygen uptake due to the  $\text{NO}_2^-$ -N oxidation (Fig. 3). Finally, after the DO has decreased with another 2 mg/l, ATU is added to the mixed liquor sample (final concentration is 5 mg/l) and the remaining OUR is measured. The difference between the OUR with  $\text{NaClO}_3$  and both inhibitors,  $\text{NaClO}_3$  and ATU, represents the oxygen uptake due to  $\text{NH}_4^+$ -N oxidation. The OUR measured in presence of both inhibitors reflects the oxygen consumption of the heterotrophs. The activity of the heterotrophic organisms consists of two components: oxygen consumption for substrate oxidation and endogenous respiration. It is not possible with this method to distinguish the heterotrophic substrate oxidation rate from the endogenous respiration rate. The selective inhibitors are freshly prepared stock solutions of ATU (125 mg/l) and  $\text{NaClO}_3$  (0.5 M). Each time, the OUR is obtained by calculating the slope of the linear parts of the recorded DO profiles as illustrated in Fig. 2. The linearity of the curves was always checked. A linear curve indicates 0 order kinetics.

The repeatability of the method was verified in a separate experiment. A vessel containing 5 l of activated sludge was aerated overnight, until all COD and  $\text{NH}_4^+$ -N were converted (endogenous respiration). The  $\text{NH}_4^+$ -N oxidation rate was determined 5

times immediately after adding 1 mg  $\text{NH}_4^+$ -N to 5 different 100 ml sludge samples taken from the 5 l of sludge. An average activity of  $18.5 \pm 1.6$  mg  $\text{O}_2/\text{l}\cdot\text{h}$  has been calculated (initial  $\text{NH}_4^+$ -N concentration in mixed liquor = 10 mg/l). Hence, the repeatability was good. Subsequently, the  $\text{NO}_2^-$ -N oxidation rate was determined 5 times after adding  $\text{NO}_2^-$ -N to 100 ml sludge samples (initial concentration = 10 mg  $\text{NO}_2^-$ -N/l mixed liquor). An average  $\text{NO}_2^-$ -N oxidation rate of  $2.7 \pm 0.45$  mg  $\text{O}_2/\text{l}\cdot\text{h}$  was obtained. The repeatability of the  $\text{NO}_2^-$ -N oxidation rate determination is less, probably because the activity of *Nitrobacter* bacteria was rather low.

#### MATERIALS AND METHODS

To evaluate the new method, experiments were performed on a 430 l pilot scale sequencing batch reactor (SBR) for nutrient removal. The reactor receives sewage from the city of Gent. The sewage mainly consists of domestic wastewater but at regular intervals also contains inputs (up to 20% of the COD load) of textile industry wastewater. The pilot plant was operated in a mode ensuring both organic carbon and nutrient (N and P) removal (Demuyne *et al.*, 1994). Aeration (C1), stirring devices (R1), dosing pumps (DPA and DPB) and valves were controlled by a PC with input/output card (Fig. 4).

Presetting of the raw wastewater was realised by means of a parallel plate separator (PPS). The presettled influent is then stored in a flow-through buffer tank, where it is gently stirred to avoid further settling of suspended matter. The hydraulic retention time in the buffer tank was about 6 min, due to the high flow rate of presettled wastewater coming from the parallel plate separator (50 l/min). At the beginning of a cycle, 200 l influent from the buffertank is added to the 230 l of mixed liquor remaining after decantation. The SBR cycles were performed according to the time schedule given in Table 1 to achieve nitrogen, phosphorus and BOD removal. Every day, 4 cycles of 6 h were performed.

During aerobic phase I (Table 1), mixed liquor samples were taken at regular time intervals from the SBR. No measurements were performed during the other periods of

**Total oxygen uptake rate**

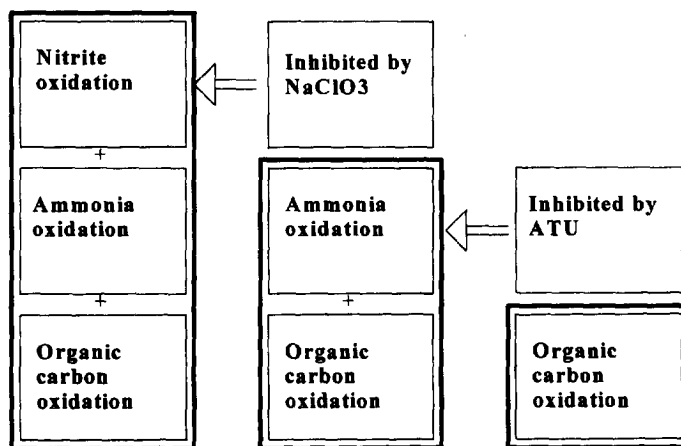


Fig. 3. Schematic representation of the action of  $\text{NaClO}_3$  and ATU on the respiratory activity of the activated sludge.

## SBR pilot plant

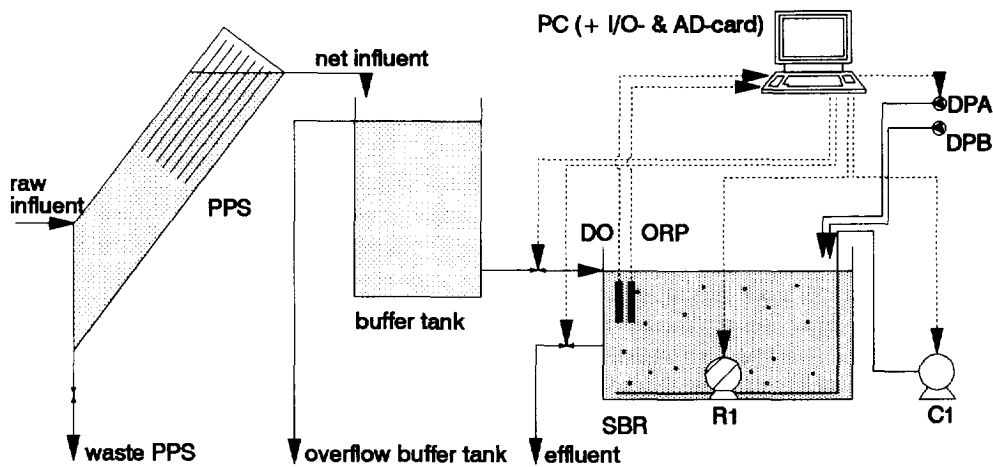


Fig. 4. Schematic overview of the SBR pilot-plant. PPS = parallel plate separator ( $V = 160$  l); buffer tank ( $V = 300$  l); SBR = sequencing batch reactor ( $V = 430$  l); DO = DO-electrode; ORP = oxidation-reduction potential electrode; DPA, DPB = dosing pumps and valves; R1 = stirring device; C1 = aeration pump ( $Q_{\max} = 22$  m<sup>3</sup>/h).

the SBR cycle. To avoid errors due to sampling, samples were always taken at the same well mixed place in the SBR. Each mixed liquor sample was divided into 3 parts (Fig. 5). A first part was used for the measurement of  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N oxidation rates, as described above. A second part of the sample was filtered and then immediately used for the determination of  $\text{NO}_2^-$ -N. Nitrite was determined colorimetrically according to the method of Montgomery and

Dymock (1961). Ammonium and  $\text{NO}_2^-$ -N oxidation rates and  $\text{NO}_2^-$ -N concentration were measured immediately after sampling from the SBR. Finally, a third part of the mixed liquor sample was stored at 4°C after filtration and acidification with a few drops of concentrated HCl. The acidified samples were afterwards used in the lab for COD,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N determination. COD and nitrogen compounds concentrations were analysed according to

Table 1. SBR reaction sequence for nutrient removal (Demuyne *et al.*, 1994)

Phase	Purpose	Length (min)
Anaerobic + filling	P release	60
Aerobic 1	P uptake, nitrification, COD removal	150
Anoxic	Denitrification	60
Aerobic 2	Excess COD removal, N <sub>2</sub> stripping	30
Settling	Sedimentation	45
Decanting	Decantation	15
	Total cycle time	360

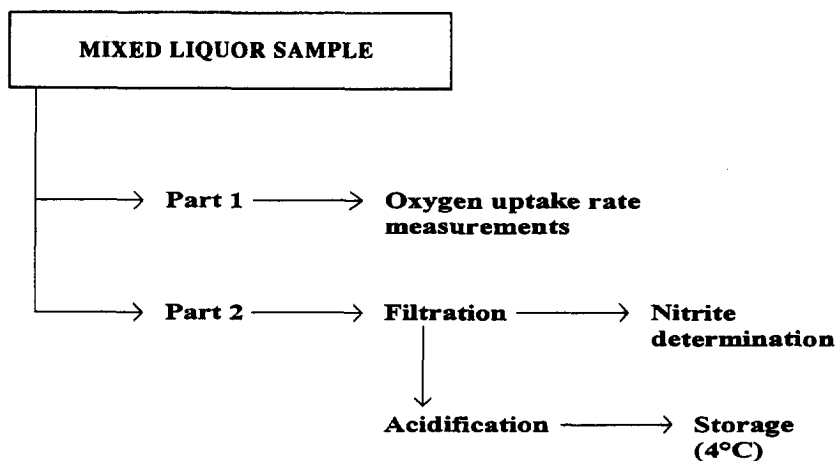


Fig. 5. Sampling procedure for the SBR. Each time, a mixed liquor sample was divided into 3 parts. The first part was used for OUR measurements, the second part for  $\text{NO}_2^-$ -N determination and the third part for lab analyses.

Standard Methods (APHA, 1985). Short term BOD was determined with the RODTOX activated sludge biosensor (Vanrolleghem *et al.*, 1994).

Three test runs were performed with the new method. A situation of normal loading was compared to situations with underloaded and overloaded sludge.

### RESULTS

An example of the variable BOD concentration in the influent of the SBR pilot plant is shown in Fig. 6(A). This figure, representing BOD concentrations measured on the presettled wastewater in the buffer tank during a one week period, clearly shows how the influent BOD concentration fluctuates from hour to hour, resulting in strongly varying loading conditions for the treatment plant. Figure 6(B) illustrates the evolution of  $\text{NH}_4^+\text{-N}$  and TKN concentrations in the influent during a representative period of approximately one month. With sufficient plant flexibility, it can be expected that process control could be useful to cope with the variable influent composition.

The experiments to validate the method were performed under three totally different nitrogen loading conditions (Table 2). Different loading conditions were caused by the variable influent composition, as illustrated before (Fig. 6).

Figure 7 represents the evolution of the COD and the nitrogen species [Fig. 7(A)] and different oxidation rates [Fig. 7(B)] for aerobic phase I (see

Table 2. Approximate nitrogen loading conditions used in the 3 experiments performed to evaluate the nitrification activity measurement device

Loading conditions	mg Kj-N/l·cycle	mg Kj-N/l·d
(1) Normal loading	15	60
(2) Underloaded sludge	5	20
(3) Overloaded sludge	25	100

Table 1) of a SBR cycle. The activated sludge had a normal loading and the aeration time was sufficient to obtain complete nitrification. After 60 min, COD removal was completed and the residual COD level remained constant. This is illustrated by the finding that the heterotrophic OUR stabilized at a low level corresponding with endogenous respiration [Fig. 7(B)]. After 80 min,  $\text{NH}_4^+\text{-N}$  was almost completely eliminated from the mixed liquor and the  $\text{NH}_4^+\text{-N}$  oxidation rate became zero. After 100 min, nitrification was completed: all  $\text{NO}_2^-\text{-N}$  had been converted to  $\text{NO}_3^-\text{-N}$ . At that moment the  $\text{NO}_3^-\text{-N}$  concentration reached a maximum. With 50 min remaining in the aerobic phase I, this shows that the SBR could handle a higher nitrogen loading under the given circumstances. In other words, aeration could already be stopped after 80 min and the denitrification phase could be initiated.

A limited  $\text{NO}_2^-$  build-up was observed. When  $\text{NO}_2^-$  was at its highest level (1.55 mg  $\text{NO}_2^-$ -N/l), the  $\text{NO}_2^-$ -N oxidation rate reached a maximum (24.5 mg  $\text{O}_2$ /l·h) [Fig. 7(B)]. Subsequently the activity of  $\text{NO}_2^-$  oxidizing bacteria decreased with decreasing

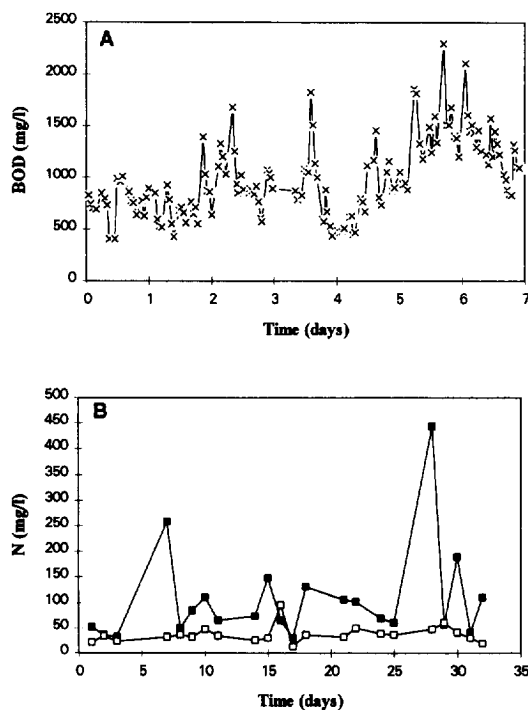


Fig. 6. Variable influent composition of the wastewater entering the wastewater treatment plant. (A) Typical BOD variation in the influent of the SBR pilot plant during a one-week period. (B) Daily variation of influent TKN (■) and  $\text{NH}_4^+\text{-N}$  (□).

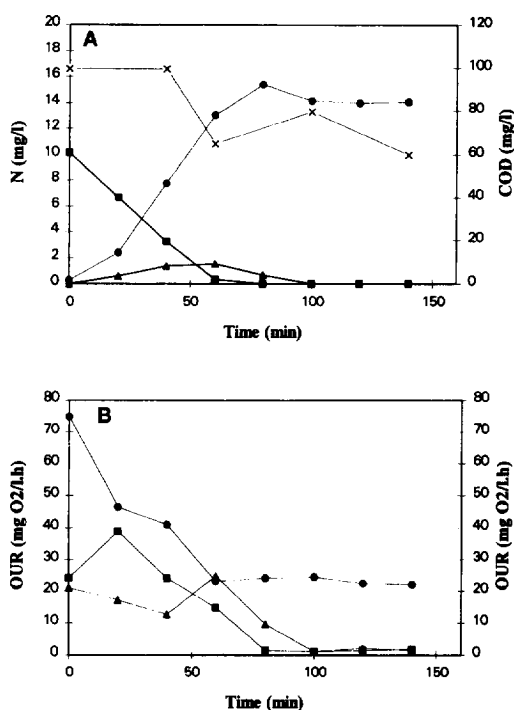


Fig. 7. Typical aerobic I phase in a SBR cycle with normal nitrogen loading. (A) COD (x),  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and  $\text{NO}_3^-\text{-N}$  (●) concentration. (B)  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and heterotrophic substrate oxidation rate (●).

$\text{NO}_2^-$ -N concentrations to become nil when the substrate for *Nitrobacter* bacteria was exhausted. An almost identical relationship was observed between the  $\text{NH}_4^+$ -N concentration and the  $\text{NH}_4^+$ -N oxidation rate. Only at the very beginning of the aerobic phase, the  $\text{NH}_4^+$ -N oxidation rate was low with regard to the  $\text{NH}_4^+$ -N concentration in the reactor.

A similar relationship between changes of the nitrogen species and  $\text{NH}_4^+$  and  $\text{NO}_2^-$ -N oxidation rates was found for underloaded sludge. Underloaded sludge needed about 60 min to complete both  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N oxidation (Fig. 8). Under this loading condition, the maximum  $\text{NO}_2^-$ -N concentration (0.70 mg  $\text{NO}_2^-$ -N/l) and the maximum  $\text{NO}_2^-$ -N oxidation rate (21 mg  $\text{O}_2$ /l·h) coincided after 20 min of aeration. After 1 h, neither the COD nor the heterotrophic OUR were subject to further changes. The oxidation rate measurements showed that for this cycle substrate oxidation processes were fully completed after approximately 60 min of aeration.

A different picture was observed in case of sludge overloading. The preset time of 150 min for nitrification was found too short: only the  $\text{NH}_4^+$ -N oxidation was nearly finished (Fig. 9). After an aeration period of 150 min, there was still 1.0 mg  $\text{NH}_4^+$ -N/l present in the mixed liquor. As the  $\text{NH}_4^+$ -N concentration decreased, the  $\text{NH}_4^+$ -N oxidation rate first increased and then, after 120 min, when  $\text{NH}_4^+$ -N started to become rate limiting, the  $\text{NH}_4^+$ -N oxidation

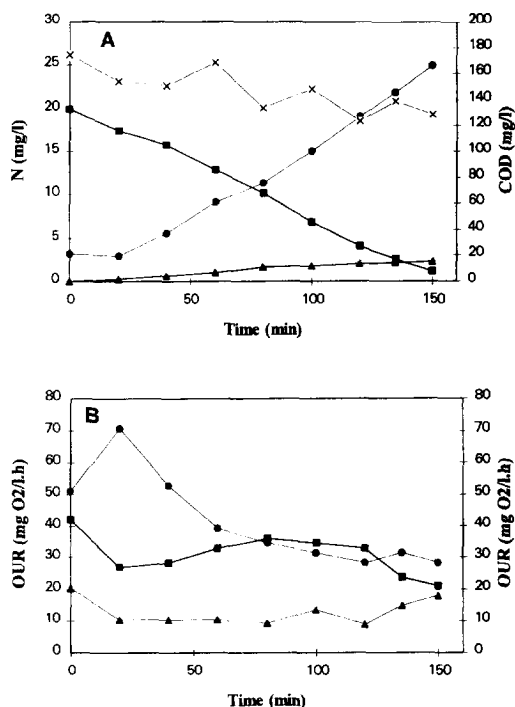


Fig. 9. Aeration step in a SBR cycle with overloaded sludge. (A) COD (x),  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and  $\text{NO}_3^-$ -N (●) concentration. (B)  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and heterotrophic substrate oxidation rate (●).

rate decreased. Yet, it did not drop to very low levels, suggesting the ongoing metabolic activity of the *Nitrosomonas* population. This corresponds with the low amount of  $\text{NH}_4^+$ -N that was still remaining in the reactor liquid. The fact that none of the 3 oxidation rates (for  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N and heterotrophic substrate oxidation respectively) reached a low level at the end of the cycle reflected the incompleteness of the oxidation processes. For this cycle, oxidation rate measurements clearly showed that 150 min aeration was insufficient to complete  $\text{NH}_4^+$ -N and COD oxidation. Also, based on the  $\text{NO}_2^-$ -N oxidation rate, it could be concluded that the mixed liquor still contained  $\text{NO}_2^-$ -N at the end of the aerobic cycle.

Ammonium oxidation rates determined under 3 different loading conditions were plotted against  $\text{NH}_4^+$ -N concentrations. The oxidation rates at the beginning of the aerobic phase (time = 0) were not used for these plots, because these values are endowed with large standard errors. Aerobic conditions are not always completely fulfilled at that moment (short transition phase between anaerobic and aerobic phases), which could result in low oxidation rates due to the fact that oxygen is a limiting substrate for the nitrifying bacteria.

Figure 10 shows that a relationship was found between  $\text{NH}_4^+$ -N oxidation rate and  $\text{NH}_4^+$ -N concentration. According to this figure, the  $\text{NH}_4^+$ -N oxidation rate is inhibited by its own substrate. The

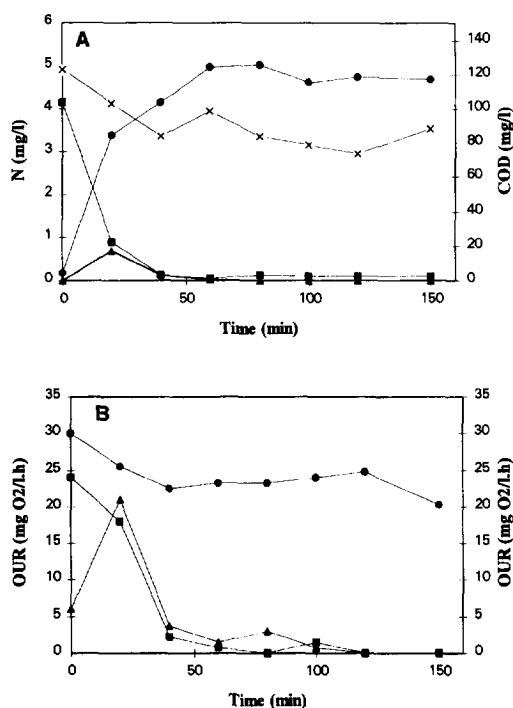


Fig. 8. Aeration step in a SBR cycle with underloaded sludge. (A) COD (x),  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and  $\text{NO}_3^-$ -N (●) concentration. (B)  $\text{NH}_4^+$  (■),  $\text{NO}_2^-$  (▲) and heterotrophic substrate oxidation rate (●).

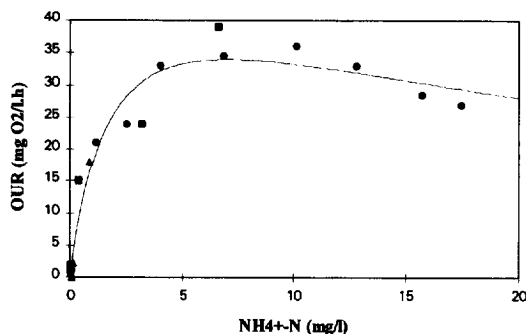


Fig. 10.  $\text{NH}_4^+$  oxidation rate plotted as a function of  $\text{NH}_4^+$ -N concentration. Experimental datapoints (■ = data for normal loaded sludge; ▲ = data for underloaded sludge; ● = data for overloaded sludge) are compared with the curve (full line) representing the Haldane competitive inhibition kinetics model with  $r_{\max} = 52.24 \text{ mg O}_2/\text{l}\cdot\text{h}$ ,  $K_M = 1.9 \text{ mg NH}_4^+\text{-N/l}$  and  $K_I = 26.27 \text{ mg NH}_4^+\text{-N/l}$ .

experimental datapoints were fitted by the Haldane competitive substrate inhibition kinetics model (Han and Levenspiel, 1988). This model can be described as

$$r = r_{\max} \frac{C_s}{K_M + C_s + \frac{C_s^2}{K_I}}$$

The parameters for the curve that fitted the datapoints were:  $K_M = 1.9 \text{ mg NH}_4^+\text{-N/l}$ ,  $r_{\max} = 52.24 \text{ mg O}_2/\text{l}\cdot\text{h}$  and  $K_I = 26.27 \text{ mg NH}_4^+\text{-N/l}$ . No trend could be deduced for the  $\text{NO}_2^-$ -N oxidation rates plotted as a function of  $\text{NO}_2^-$ -N concentration.

#### DISCUSSION

Ammonium and nitrite oxidation rates could be measured with a batch respirometer using selective inhibitors to nitrifying sludge samples. The method is fast (one measuring cycle takes approx. 10 min) and its repeatability is good, which makes it a powerful instrument to monitor nitrification in activated sludge tanks. Respirometry without use of inhibitors measures the oxygen consumption for C oxidation and nitrification as one signal. ATU was already used to distinguish between nitrification and C oxidation (Stensel *et al.*, 1976; Kroiss *et al.*, 1992; Nowak and Svardal, 1993). With this paper, it is shown that dosing both  $\text{NaClO}_3$  and ATU gives extra value to respirometric methods. When operated this way, respirometry can give a detailed view of 3 different oxidation processes: C oxidation,  $\text{NH}_4^+$  oxidation and  $\text{NO}_2^-$  oxidation. The use of two selective inhibitors allows to determine  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N oxidation rates with one single experiment. Völsch *et al.* (1990) also used  $\text{NaClO}_3$  and ATU as selective nitrification inhibitors, but they needed 2 experiments to get the same information as in the proposed method. One experiment took about 30 min while the method proposed in this paper takes about 10 min.

Vanrolleghem and Verstraete (1993) did not use inhibitors because the sludge in the respirometer used for their experiments is used for several consecutive substrate injections, when operated in the on-line mode. However, a method was proposed to still distinguish between OUR for nitrification and carbon oxidation. It is based on an appropriate design of the composition of the calibration substrate used in the on-line RODTOX respirometric biosensor. Our method maybe yields less information but is inexpensive and can be carried out more easily than the procedure proposed by Vanrolleghem and Verstraete (1993). Moreover, the proposed method evaluates the current state of the sludge and the nitrogen species in the monitored system, while the method of Vanrolleghem and Verstraete (1993) evaluates the heterotrophic and nitrifying activity of the sludge in the RODTOX. Nowak and Svardal (1993) aerated sludge samples until  $\text{NO}_2^-$ -N was completely oxidized, before they used ATU as a nitrification inhibitor to measure the total autotrophic nitrification activity. Hence, no differentiation could be made.

During the measurements with the proposed method, a limited  $\text{NO}_2^-$  build-up takes place after  $\text{NaClO}_3$  addition, because *Nitrobacter* bacteria are at that moment inhibited. For a measured  $\text{NH}_4^+$ -N oxidation rate of  $20 \text{ mg O}_2/\text{l}\cdot\text{h}$ , this will result in a  $\text{NO}_2^-$  build-up of about  $0.6 \text{ mg NO}_2^-\text{-N/l}$  assuming that  $\text{NaClO}_3$  addition took place 6 min before the end of the measurement. Such  $\text{NO}_2^-$ -N levels do not inhibit nitrification according to Anthonisen *et al.* (1976).

To optimize operating conditions, OUR measurements for the determination of  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N oxidation rates are proposed. When for instance being applied on a nitrifying activated sludge system, the method should allow to detect the presence of  $\text{NO}_2^-$ -N in the mixed liquor. These data could be used to optimize the activated sludge process in such a way that  $\text{NO}_2^-$ -N is converted completely to  $\text{NO}_3^-$ -N. The proposed method is in fact generally applicable for nitrifying systems but was validated on a SBR. The results show that it is possible to detect the end of the  $\text{NH}_4^+$ -N oxidation during the aerobic phase of a SBR cycle by performing OUR measurements. When the  $\text{NH}_4^+$ -N oxidation rate drops to a very low level,  $\text{NH}_4^+$ -N has been completely removed. The same goes with regard to the  $\text{NO}_2^-$ -N oxidation rate and the  $\text{NO}_2^-$ -N concentration. For the specific case of a SBR, aeration could then be stopped and denitrification allowed to start. The use of these OUR measurements can result in aeration energy cost savings and a more constant effluent quality. In the case of an overloaded sludge for example, the aeration period could be prolonged to complete  $\text{NH}_4^+$ -N removal.

A remarkable property of the method is that not only the possibility exists to distinguish between the  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N oxidation rate, but also the fact that the OUR data give information about COD

oxidation. This enables the operator of a SBR plant to stop aeration when all  $\text{NH}_4^+\text{-N}$  is converted to  $\text{NO}_2^-\text{-N}$  (aerobic phase I). Indeed, neither COD removal nor  $\text{NO}_2^-\text{-N}$  oxidation have to be completed at this moment. Denitrification works also with  $\text{NO}_2^-$  as electron acceptor (Abeling and Seyfried, 1993; Baten *et al.*, 1993). The excess COD that can still be present in the mixed liquor is useful as carbon source during the denitrification process, which results in the addition of less external carbon source for denitrification. The amount of COD present in the wastewater after completing  $\text{NH}_4^+\text{-N}$  oxidation can be estimated with the organic carbon respiration: a low activity of the heterotrophs means that the readily biodegradable COD has been converted and vice versa.

In view of the typical daily variations of the influent composition (e.g. Fig. 6) occurring in many wastewater treatment plants, the controlled phase scheduling of a SBR could allow to cope with the varying load. At higher loading, longer cycles could be established, while short cycles could be used in underloaded situations. It is evident that some storage capacity of the wastewater is needed to allow for this type of control.

Haldane competitive substrate inhibition kinetics could be extracted for  $\text{NH}_4^+\text{-N}$  oxidation rate as a function of  $\text{NH}_4^+\text{-N}$  concentration. Substrate inhibition of *Nitrosomonas* activity by  $\text{NH}_4^+\text{-N}$  can explain why in the case of an overloaded sludge (Fig. 9) the  $\text{NH}_4^+\text{-N}$  oxidation rate became higher with decreasing  $\text{NH}_4^+\text{-N}$  concentration. Substrate inhibition kinetics could not be observed for  $\text{NO}_2^-\text{-N}$  oxidation rates plotted as a function of  $\text{NO}_2^-\text{-N}$  concentration. This could be due to the fact that  $\text{NO}_2^-$  never reached concentrations higher than 2.5 mg  $\text{NO}_2^-\text{-N/l}$ , which is still in the range of  $K_s$  for *Nitrobacter* reported by Barnes and Bliss (1983) ( $K_s = 0.06\text{--}8.4$  mg  $\text{NO}_2^-\text{-N/l}$ ). Haldane  $K_M$  values can be compared with  $K_s$  values originating from normal Monod-type models. The value for  $K_M$  (1.9 mg  $\text{NH}_4^+\text{-N/l}$ ) is in the range of  $K_s$  values found by Barnes and Bliss (1983) for the *Nitrosomonas* group of bacteria ( $K_s = 0.06\text{--}5.6$  mg  $\text{NH}_4^+\text{-N/l}$ ). Abeling and Seyfried (1993) also found modified Haldane kinetics, which are very similar to normal Haldane kinetics, for the conversion of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  by *Nitrosomonas*. The similarity between the results obtained with the new nitrification activity determination method and the results of other researchers corroborates the applicability of the method in practice.

#### CONCLUSIONS

By means of simple measurements of the OUR combined with the addition of two selective nitrification inhibitors, insight can be obtained into  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  oxidation rates of the mixed liquor in an activated sludge system.

Changes of the nitrogen species and COD concentrations coincided well with the changes of the  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and COD oxidation rates. This approach shows potential for control and optimization of nitrification and aeration in biological nutrient removal processes.

*Acknowledgement*—This research has been funded by a scholarship from the Flemish Institute for the Improvement of Scientific-Technological Research in the Industry (IWT).

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