NITRITE EFFECT ON THE PHOSPHORUS UPTAKE ACTIVITY OF PHOSPHATE ACCUMULATING ORGANISMS (PAO) IN PILOT-SCALE SBR AND MBR REACTORS

Gürkan Sin[1], Kwinten Niville[2], Giulia Bachis[3], Tao Jiang[4], Ingmar Nopens[5], Stijn Van Hulle[6] and Peter A. Vanrolleghem[7]

[1] Post-doctoral fellow, BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
(**gurkan@biomath.ugent.be**)
[2] Graduate student, Departement PIH, Hogeschool West-Vlaanderen, Graaf Karel De Goedelaan 5, 8500 Kortrijk, Belgium
[3] Graduate student, BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[4] PhD candidate, BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[5] Post-doctoral fellow, BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[6] Post-doctoral fellow, Department PIH, Hogeschool West-Vlaanderen, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium
[7] Professor, modelEAU, Département de génie civil, Pavillon Pouliot, Université Laval, Québec G1K 7P4, Canada

ABSTRACT
Batch tests, performed to investigate the effect of nitrite on the P-uptake activity of biomass grown in pilot-scale SBR and MBR systems, showed that the nitrite has an inhibitory but reversible effect on the aerobic P-uptake activities of the SBR and the MBR biomass. The inhibitory effect was found more pronounced on the SBR biomass, in which the aerobic P-uptake was inhibited by 65 % and 37 % at 10 mgNO_2-N/l for the SBR and the MBR respectively. For the anoxic P-uptake activities, both the SBR and the MBR biomass were found capable of using nitrite as electron acceptor as effectively as nitrate. Moreover, for the SBR biomass the anoxic P-uptake rate using nitrite was found even higher (60%) than the P-uptake rate with nitrate. From an operational point of view, nitrite inhibition can be circumvented by preventing nitrite buildup in BNR systems, or by enhancing the denitrifying PAOs to proceed over the nitrite route instead of the nitrate. From a modeling point of view, the current mechanistic models require appropriate extensions to describe these various effects of nitrite. Hence, an extension of the ASM2d model has been provided. Last, until the underlying mechanism of nitrite inhibition is resolved, experimental determination of the nitrite effect is advised.

KEYWORDS
ASM2d, MBR, Modeling, Nitrogen and phosphorus removal, nitrite inhibition, Polyphosphate Accumulating Organisms, Phosphate uptake, SBR

INTRODUCTION

Microbiological investigation of the nitrite effect with a variety of physiological types of bacteria (from aerobes to facultative anaerobes) has shown that nitrite interferes with energy generation (e.g. by inhibiting oxygen uptake, oxidative phosphorylation or proton-dependent active transport of substrate) and growth mechanisms (Yarbrough et al., 1980; Rake and Eagon, 1980). In the field of activated sludge, the inhibitory effect of nitrite (its effect is mainly pH dependent and better represented as function of the nitrous acid concentration , HNO_2) on the nitrifying biomass activities have also been long recognized and documented (Anthonisen et al., 1976; Van Hulle et al. (2004); Vadivelu et al., 2006)

Several studies with Phosphorus Accumulating Organisms (PAOs) have also confirmed that elevated concentrations of nitrite negatively affect phosphorus uptake activities of PAOs under both aerobic and anoxic conditions (Meinhold et al., 1999; Saito et al., 2004). Note that denitrifying PAOs were also shown to be able to utilise nitrite as an electron acceptor without any adverse affect of course within a certain concentration range.

That said, however, these studies have reported different types of effect (e.g. inhibitory versus toxic effect) and different threshold values of inhibition by nitrite. For example, Meinhold et al (1999) found that a nitrite level of 6-8 $mgNO_2$-N/l completely halted the anoxic P-uptake and severely inhibited aerobic P-uptake for sludge taken from a pilot BIODENIPHO® plant. Saito et al (2004) reported that 2 $mgNO_2$-N/l already inhibited the aerobic P-uptake severely while more than 6 $mgNO_2$-N/l completely stopped it. Moreover, they also found that nitrite at 12 $mgNO_2$-N/l level reduced the anoxic p-uptake rate down to 65%. On the other hand, Anh et al (2001) and Hu et al (2003) could not find any severe effect on the anoxic P-uptake rate at elevated nitrite concentrations, even up to 40 and 35 $mgNO_2$-N/l respectively. The varying degree of adaptation of biomass to nitrite has been offered as explanation for these wide ranging threshold values of nitrite inhibition. Saito et al (2004), however, argued that chemical precipitation may occur if pH is not controlled during batch tests, which may corroborate some of the reported results.

Since the underlying biochemical mechanism of the nitrite effect is still far from being clear, in this study, we investigate and evaluate the effect of nitrite on aerobic and anoxic phosphate uptake by PAOs enriched in nutrient removing pilot-scale SBR and MBR systems. The knowledge of the nitrite effect in the system was necessary for two particular reasons: (i) to complement our understanding of the systems' performance and (ii) to help making an appropriate choice of model structure for properly describing possible nitrite inhibition of aerobic P-uptake as well as for adequately describing the mechanism of anoxic P-uptake with nitrite and nitrate by denitrifying PAOs (see e.g. Sin et al., 2006a). To this end, we have performed pH and temperature controlled batch experiments using sludges sampled from SBR and MBR systems respectively, where we measured and evaluated phosphorus uptake and anoxic respiration rates. In the following, the SBR and MBR systems and the experimental procedure are described, which is followed by a presentation of the results obtained. Next, the results are critically discussed in view of modeling EBPR systems and the paper ends with a future outlook and conclusions.

MATERIAL AND METHODS

The SBR and MBR systems

The sludges used for batch tests were sampled from the SBR and MBR reactors. These systems were fed with synthetic wastewater mimicking domestic sewage with total COD, total nitrogen, nitrate, total phosphate and suspended solids equal to 504±55 mgCOD/l, 64±7.5 mgN/l, 3.4±1.2 mgNO₃-N/l, 11±1.3 mgP/l and 3.3±0.1 mg SS/l respectively (Boeije *et al*., 1999).

All the devices controlling and monitoring the SBR and MBR set-ups are connected to a LabView data acquisition and control (DAC) system (National Instruments, USA). The DAC system consists of a computer, interface cards, meters, transmitters, sensors, valves, pumps and solid-state relays. The status and the time series of the signals from the sensors are also displayed on the computer and stored in a data file to be analyzed later on.

The SBR set-up

The sequencing batch reactor (SBR) has been operated for both nitrogen and phosphorus removal following an anaerobic-aerobic-anoxic-aerobic reaction sequence. The reactor has a total volume of 80 L but only 68 L was used during the operation. The volumetric exchange rate (VER) was set to 0.5, which corresponds to 34 L of filling volume in each cycle and a hydraulic retention time (HRT) of 12 hrs. The sludge wastage rate was set to 0.5 L per cycle to ensure a 17 days solids retention time (SRT), but the effective SRT was lower due to solids washout in the effluent (see results). The duration of one SBR cycle was 6 h which consisted of 60 min anaerobic of which the first 20 minutes was filling, 150 min aerobic, 60 min anoxic, 30 min aerobic and 60 min of settling/draw phases. During the aerobic phases, the oxygen is controlled around 2 mgO₂/l using a PID controller implemented in LabView®, which controls the airflow by manipulating a mass flow controller (Brooks®, Brooks Instrument, USA). While a detailed description of the set-up can be found in Insel *et al*. (2006), the configuration is shown in Figure 1.

**Figure 1 – Schematic diagram of the SBR system (Insel *et al*., 2006)**
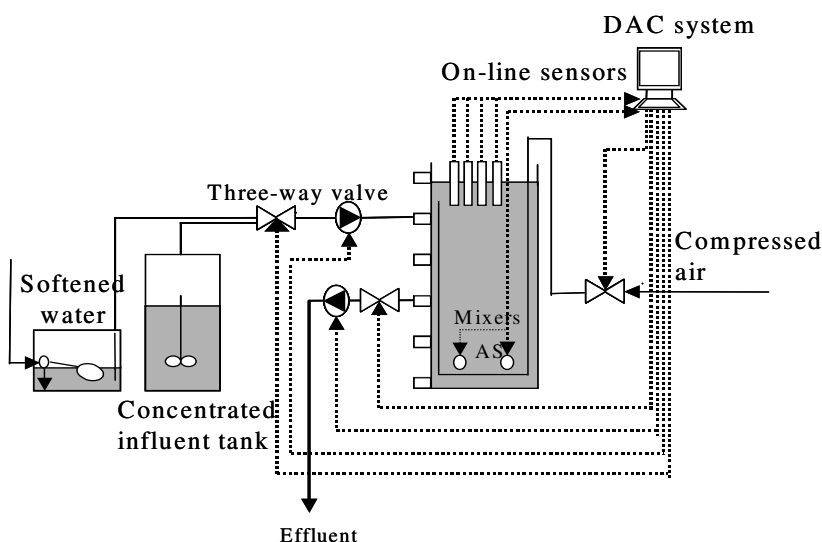
The MBR set-up

The membrane bioreactor (MBR) was operated continuously (unlike the SBR) following a UCT type configuration to achieve biological nitrogen and phosphorus removal. The MBR consists of an anaerobic compartment (8 L), an aerobic/anoxic compartment (17 L) and a membrane loop (3 L). The influent was continuously fed at 0.075 l/min rate to the anaerobic compartment (see Figure 2). The sludge was recycled (at 0.6 l/min rate) internally from the anoxic/aerobic compartment during the anoxic phase (i.e. it was intermittently recycled) back to the anaerobic compartment. The anoxic/aerobic compartment was intermittently aerated following a cycle of 40 minutes of which 17 min was aerobic and 23 min was anoxic. Every 6 hours 400 ml of sludge volume was wasted from the aerobic/anoxic compartment during the aerobic phase providing an SRT of 17 days.

The solid-liquid separation was achieved using a side stream membrane system. To this end, a tubular membrane module (X-flow UF membrane, Norrit, The Netherlands) with a 0.17 m^2 surface area was used. A sludge flow rate of 0.375 l/min was pumped into the membrane module and the permeate flow rate was fixed to 0.09 L/min which corresponds to a constant flux of 31.8 l/m^2-h. A more detailed description of the side stream MBR can be found in Jiang (2007).

The aeration is controlled by using a simple on-off controller at a set-point around 2 mg O_2/L with 0.5 mg O_2/L band. Further information about the configuration and operation of the SBR and MBR systems can be found in Insel *et al* (2006) and Jiang (2007) respectively.

Figure 2 – Schematic illustration of the MBR set-up (backwashing unit is not shown, adapted from Jiang, 2006)
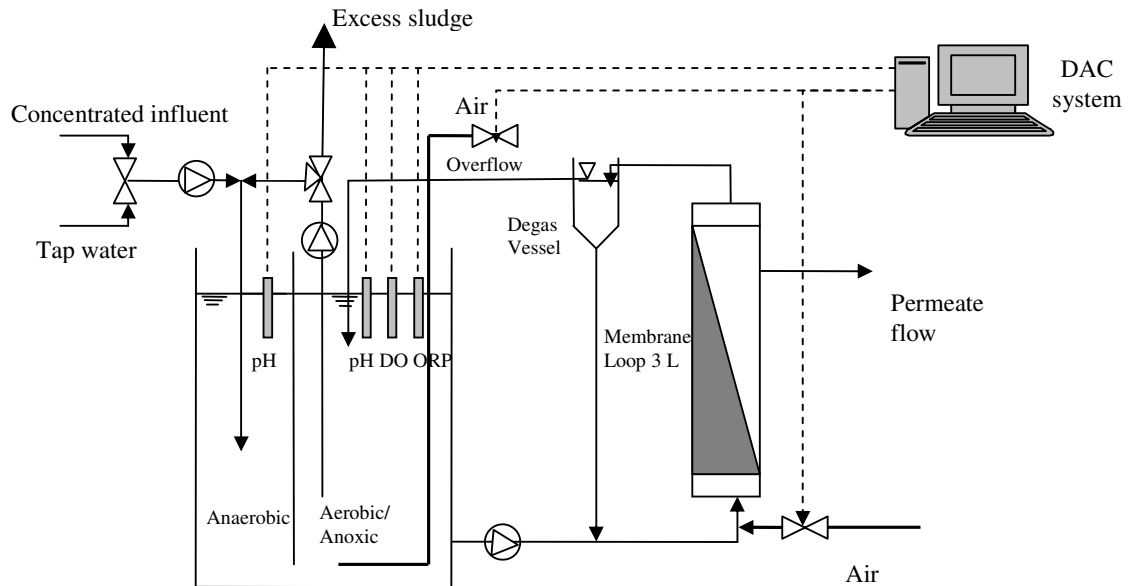
The phosphorus release and the phosphorus uptake batch tests

The phosphorus release and uptake tests were performed in a set-up consisting of 2 L glass reactor with double jacket enabling circulation of cooling liquid around the reactor for temperature control (See Figure 3). A Lauda Ecoline E303 was used as cooling system to maintain the temperature at $15 \pm 0.1\ ^{\circ}$C. Data acquisition, pH control and data processing are implemented in LabView software (National Instruments, NIDAQ 6.9 with AT-MIO-16XE-50 DAQ card and LabView 6.i). The dissolved oxygen is measured by an Inpro 6100 /120 /T /N (Mettler Toledo) oxygen electrode, which is connected to Knick Process 73 transmitter. The pH is measured in the aeration vessel with a HA405-DXK-S8/120 pH electrode (Mettler Toledo), which is connected to a Knick Stratos 2401 pH transmitter. Both electrodes were calibrated regularly (once every week). The data acquisition interval was set to 3 seconds and the pH was controlled within a narrow band 7.5 ± 0.03.The reactor is provided with a mixer and a tubular diffuser for supplying air or nitrogen gases as needed.

Figure 3 – Configuration of the experimental set-up used for batch tests.
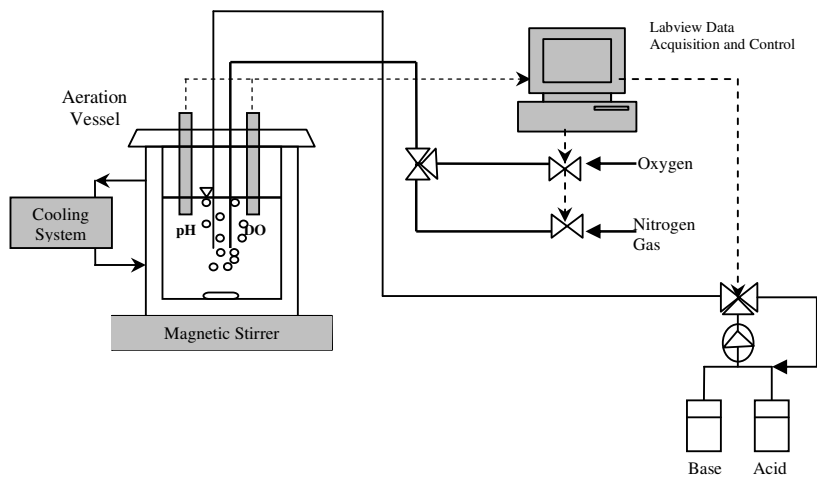
Experimental procedure for the phosphorus release and uptake tests

One litter of fresh sludge samples taken daily from the SBR and the MBR systems were used for the experiments. The sludge sample from the SBR was taken at the end of the second aerobic phase (see above), while the sludge sample from the MBR was taken from the aerobic/anoxic compartment during the aerobic sub-phase of the intermittent aeration. The pH and the temperature were controlled at 7.5 and 15 ^{0}C respectively during both tests. For the phosphorus release, the following procedure was followed:
1. Anaerobic phase: anaerobic conditions were reached and maintained for 75 minutes, by continuously nitrogen gas to the reactor.
2. Addition of acetate: small amounts of acetate (e.g. 50 -75 mgCOD-acetate/l) from a concentrated stock solution (10 gCOD/l) were added to the reactor. In case there was nitrate present in the sludge liquor, additional acetate was added to ensure complete denitrification of nitrate. To this end, a stoichiometry of 6 mgCOD/l was used to ensure a complete denitrification of 1 mgNO$_3$-N/l. For example, if there is 5 mgNO$_3$-

N/l present in the mixed liquor of the sludge sample, an additional of 30 mgCOD-acetate/l was added to the reactor on top of the 50 mgCOD/l.

3. Sampling: Activated sludge samples of 5 ml were directly taken from the reactor using a syringe. The first sample was taken before the addition of the substrate, in order to measure the initial ortho-phosphate and nitrate concentration (see item 2).

4. Filtration: Millipore filters (0.45 μm) were immediately used to filter the samples from the SBR sludge, which were immediately refrigerated at 4°C for later analysis. To separate sludge from liquid in the MBR sludge samples, the sludge sample was centrifuged at 3000 rpm (1200 g) for 4 min using a refrigerated centrifuge (Sorvall RC-5B, Du Pont Instruments), since it wasn't possible to use Millipore filters due to high solids concentration.

The phosphorus uptake tests (either aerobic or anoxic) were started right after the anaerobic phase. For the aerobic P-uptake test, a known amount of nitrite from a stock solution with 1 g N/L was added to the reactor. Then the nitrogen gas was switched off and the aeration was switched on to provide oxygen in excess (around 9 mgO$_2$/l) continuously throughout the test. For the anoxic P-uptake test, the test was started by appropriate addition of nitrite or nitrate under continuous supply of the nitrogen gas.

For the measurements of COD, NH$_4$-N, NO$_2$-N, NO$_3$-N and PO$_4$-P, commercial kits from HACH LANGE (Germany) were used. SVI, MLSS and MLVSS were measured according to standard methods (APHA, 1998).

RESULTS

MONITORING THE SBR AND MBR SYSTEMS

In this section, off-line (daily and flow-proportionally sampled) measurements of the SBR and MBR systems are presented to provide an adequate insight into the behavior of these systems during the batch experimental period. This is useful to help better interpret the batch results presented in the next section.

The SBR system

The average COD, nitrogen and phosphate removal in the SBR were around 81%, 61% and 72% respectively during the experimental period. The sludge concentration time series together with sludge settling quality as monitored by SVI are shown in Figure 4. One observes that the sludge concentration fluctuates in the reactor. Further, one also notices that starting from the beginning of June, the sludge concentration started to decrease until levelling off around 2 g/l. This decrease can be attributed to the increase of the SVI.

The deterioration of the settling quality subsequently leads to more washout of the sludge in the effluent. This also means that the SRT of the system is also decreased because of the higher solids washout (it was calculated to decrease from 17 days to ca 9 days). What induced this deterioration in the settling quality is usually a challenging task to understand. It could be due to parameters related to the process configuration/operation and or the microbiological activity, e.g. the substrate feeding pattern, F/M, nitrite at the end of aerobic phases, proliferation of filamentous organisms and the like (Sin *et al*., 2006b; Martins *et al*., 2004). A detailed discussion is however beyond the scope of this contribution.

Figure 4 - Time series of MLSS, MLVSS and SVI profiles in the SBR system
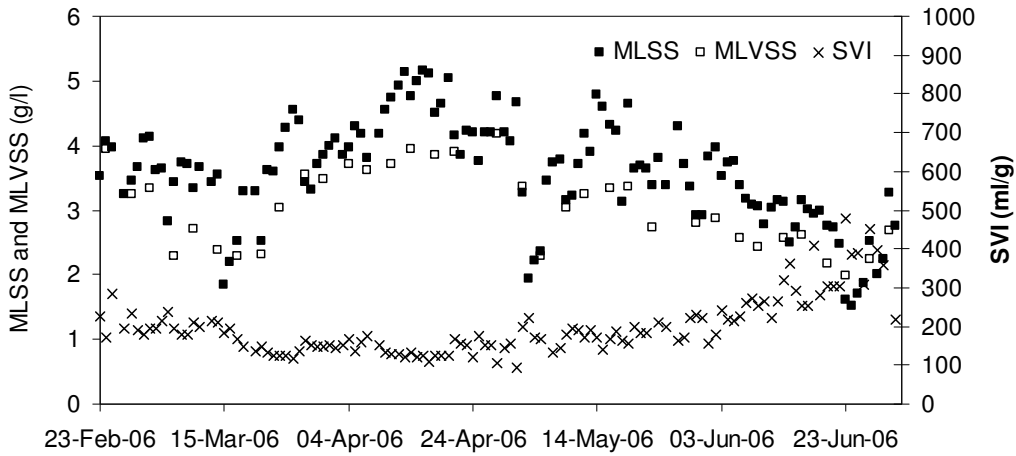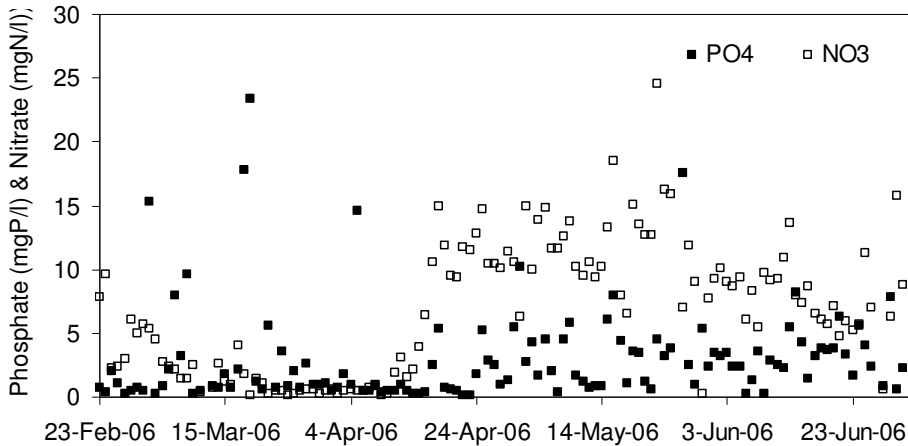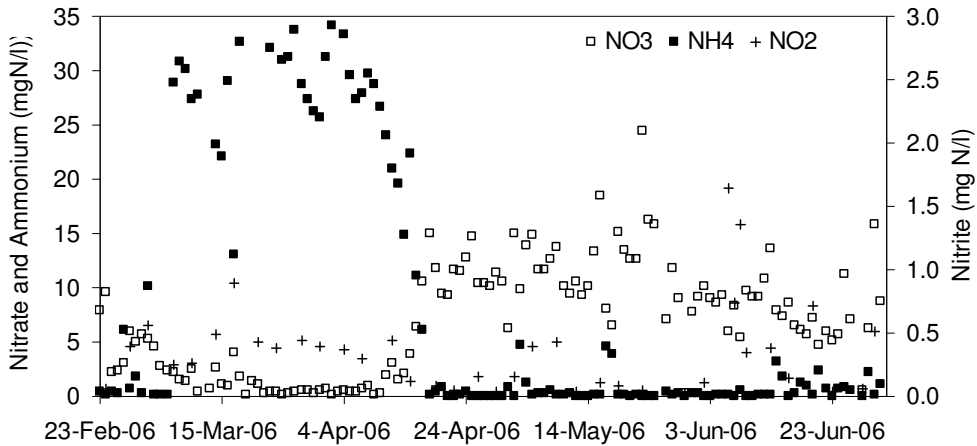

Figure 5 – The effluent quality of the MBR during the experimentation period: nitrogen (top) phosphate and nitrate (bottom).

Concerning the nitrogen removal, it is possible to detect a lack in nitrification from the beginning of March until mid-April (see Figure 5 (top)). High values in the effluent ammonia concentration correspond to the absence of nitrate production, i.e. no nitrification. After this period the ammonia concentration is observed to gradually drop back to zero and at the same time the nitrate starts to increase in the effluent, as expected from the stoichiometry of the nitrification. From April onwards, one notices complete nitrification (i.e. the effluent ammonium and nitrite concentration are zero), with some sporadic events where effluent nitrite was observed to be around 1.5 $mgNO_2$-N/l, indicating incomplete nitrification.

The nitrate and phosphate profiles are plotted together in Figure 5 bottom to check the well-known competition between ordinary denitrifying heterotrophic organisms (DHOs) and PAOs towards VFA (in this case acetate) uptake (Manning and Irvin, 1985; Wilderer *et al.*, 2001). The presence of nitrate during the anaerobic phase is commonly observed to slow down significantly the P-release rate of PAOs, since the presence of nitrate triggers denitrification by denitrifying ordinary heterotrophic organisms using VFA as the carbon source. Only after the nitrate has been completely removed from the system, the PAOs can take up acetate and release phosphate at their maximum rate. Therefore the extent of the P-release of PAOs will be determined by the extent of acetate available in the anaerobic phase which is again influenced by the amount of denitrification taking place using nitrate as electron acceptor.

From Figure 5 (bottom), one can observe that the effluent nitrate concentration began to rise starting from the middle of April (see the explanation for the rise of nitrate in the effluent above). Parallel to the observed increase in the effluent nitrate, the effluent phosphate concentrations started to increase as well. Moreover, one can also observe fluctuations in the effluent phosphate concentration between about 2 and 7 mg P/l. This indicates that the bio-P removal in the system became unstable as a result of the increased nitrate level in the anaerobic phases of the SBR.

The MBR system

The COD and nitrogen removal in the MBR are found to be around 98% and 76% respectively during the experimental period, both higher than in the SBR system. Whereas the average phosphate removal was about 43% for the MBR, worse than for the SBR system. Further, although the COD removal was quite stable due to the almost ideal separation of the solids from the effluent (results not shown), the nitrogen and phosphate removal were not (see below).

The MLSS profile of the MBR is provided in Figure 6. The SVI profile is absent from the figure since in the MBR system the solid-liquid separation is achieved by membrane filtration and therefore monitoring the quality of sludge settling is not required. Clearly detectable is an increase of around 50% in the MLSS in the final part of the figure, starting from the beginning of May, in which the average MLSS was around 6 gSS/l, and lasting until the end of June, in which the MLSS was around 9 gSS/l. This increase in the MLSS was induced by a change of the influent feeding system. This change resulted in an increase in the influent load (flow rate remained fixed, but the concentration of the wastewater was higher). This in turn meant a higher amount of sludge production and therefore an increase in the MLSS concentration since the sludge wastage rate remained constant during these periods (see the line in Figure 6).

Figure 6 – The MLSS and MLVSS profiles in the MBR. The line indicates the linear increase in the sludge concentration parallel to an increase in the influent wastewater concentration (see the text).
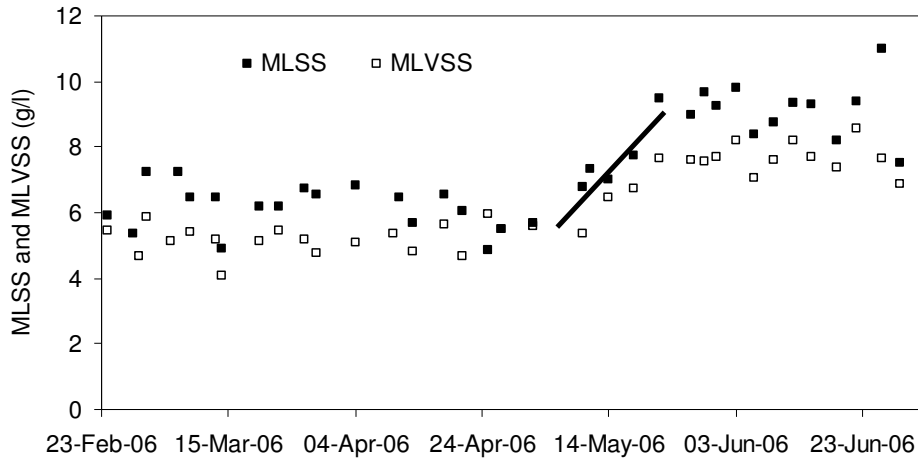

Figure 7 The effluent quality of the MBR: nitrogen (top) phosphate (bottom).
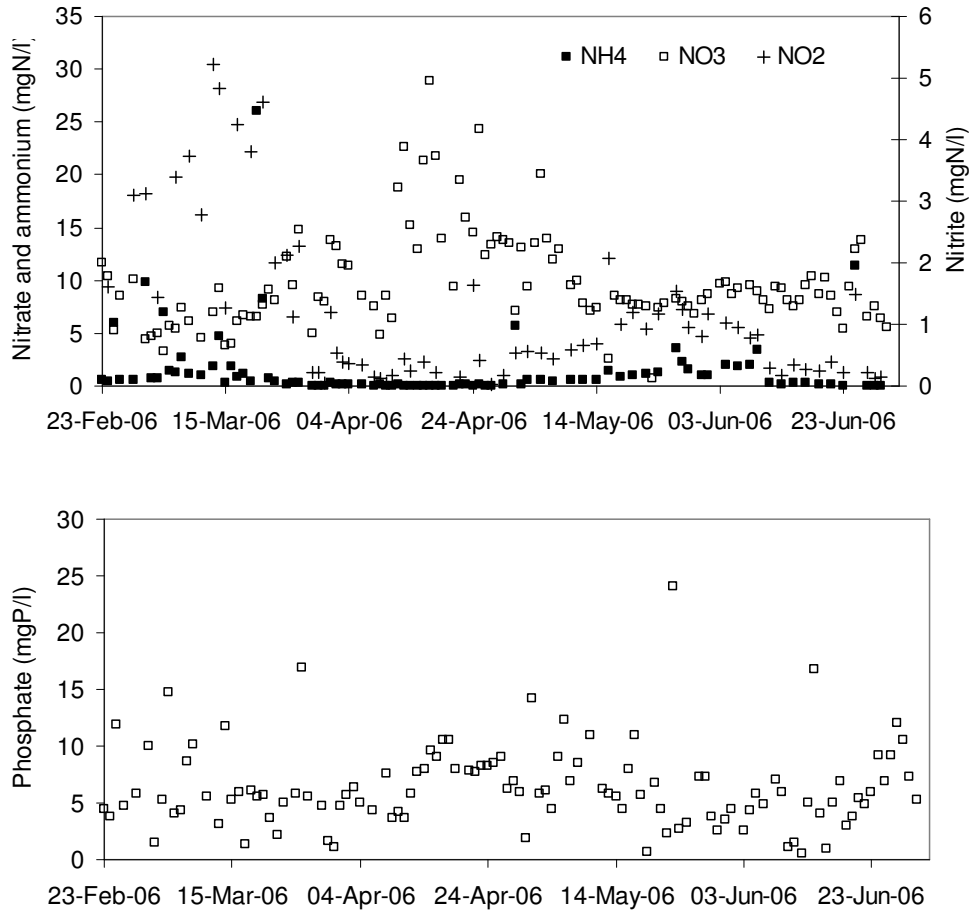
Figure 7 (top) illustrates the effluent ammonia, nitrate and nitrite time series. Incomplete nitrification can be deduced from the higher nitrite values (up to 5.23 mg/l) appearing in the effluent from March until April. In general the effluent nitrite was observed to fluctuate periodically from a low level (around 0 mgN/l), indicating complete nitrification, to levels higher than 1mgN/l indicating incomplete nitrification. Higher values of nitrate in the effluent can be observed in the middle of April, confirming a complete nitrification process, as also indicated by the ammonia and nitrite effluent values, which are close to zero in the same period.

In Figure 7 (bottom), one can observe that the effluent phosphate was fluctuating around 5 mgP/l from February onwards till the mid of April. Afterwards, the phosphate concentration increased almost to the influent level indicating a complete loss of P-removal from the system. The P-removal was observed to recover starting from the mid of May on. However, the P-uptake was not sufficient to completely remove the influent phosphate, most likely due to competition with the ordinary DHOs for the limited VFAs in the influent as explained above.

It is noted that during the period when the influent load (concentration wise) were increased (see above discussion concerning the MLSS increase in Figure 6), one observes that effluent nitrate concentration were slightly decreased and became stable (see Figure7top). This is contrary to what one would expect that an increase in the influent load would cause an increase in the effluent of the system. What causes the effluent nitrate concentration to decrease is probably related to an overall improvement of the denitrification capacity of the system. On the other hand, the effluent phosphate was observed to be slight higher during this period (Figure 7down). These observations confirm again that activated sludge systems respond in a non-linear fashion to a change in the input characteristics.

NITRITE EFFECT ON THE P-UPTAKE RATE OF THE SBR AND MBR SLUDGE

In this section, the results obtained from the batch tests performed with the SBR and the MBR sludges are presented. First, the aerobic P-uptake results are given and then the anoxic P-uptake results with nitrite and nitrate as electron acceptor.

**Nitrite effect on the aerobic phosphate uptake rate (PUR)**

The aerobic P-uptake results under different initial nitrite concentrations are presented in Figure 8 (top) and Figure 8 (bottom) for the SBR and MBR sludges respectively. In total three tests are shown per sludge sample. These tests correspond to 0, 5 and 10 $mgNO_2$-N/l additions at the beginning of the aerobic P-uptake phase (as indicated by the arrow in Figure 8). The resulting P-uptake profiles from these additions are marked by different symbols corresponding to each initial NO_2-N concentration (see the legend of Figure 8). The decreasing nitrite concentration profiles due to the activity of nitrite oxidizing bacteria (NOB) during these aerobic tests were also plotted indicating the exposure concentration and duration of PAO to nitrite throughout the aerobic P-uptake phase.

The P-release and P-uptake profiles obtained in this study (e.g. see Figure 8) indicate a typical *polyphosphate-accumulating metabolism* (PAM). During the anaerobic phase and in the presence of external VFA such as acetate, phosphate release occurs to generate the energy necessary to take up the external VFA and store internally in the form of PHA. During this phase internal glycogen is also expected to be used for balancing reducing equivalents (Mino

et al., 1987; Smolders *et al*., 1995; Schuler and Jenkins, 2003). In the following aerobic or anoxic phase, the internally stored PHA is used both as carbon and energy source (oxidative phosphorylation using electron acceptor as oxygen or nitrate) for P-uptake, but also for restoring the glycogen pool and the biomass growth (Smolders *et al*., 1995).

As shown in Figure 8, in the first 75 minutes of the batch test, which is anaerobic, the phosphate release took place. The phosphorus release rate was observed to be high in the beginning (e.g. between 0 – 40 min) thanks to the sufficient amount of acetate present externally and to the internal polyphosphate and glycogen pools present in the PAO organisms. The phosphate release later on slowed down until coming to a halt probably due to the depletion of the external carbon source. This P-release trend was observed in all of the 6 tests shown in Figure 8 for the SBR and the MBR sludge.

Figure 8 The aerobic P-uptake versus nitrite: for SBR sludge (top) and MBR sludge (bottom). The arrow indicates the end of the P-release and the start of the P-uptake
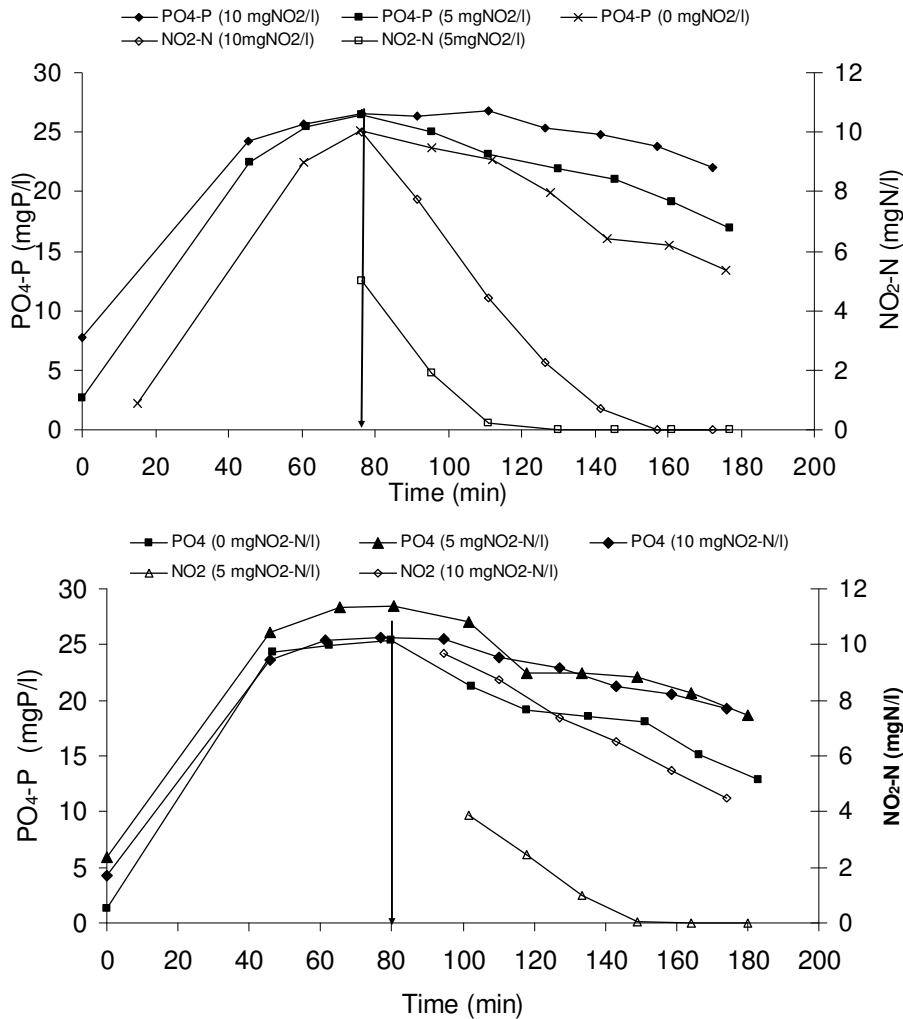
After the P-release phase ended, the aerobic P-uptake is started as soon as the aeration is switched on as indicated by the arrow. One observes that the P-uptake rate is significantly slower than the P-release rate (also for the reference tests, i.e. with zero nitrite addition). For the tests with the SBR sludge, one can clearly observe that the aerobic P-uptake was

affected by the presence of nitrite (see Figure 8 (top)). For example, when the initial nitrite concentration was 5 mgNO$_2$-N/l, the aerobic P-uptake rate was reduced by around 30% compared to the reference P-uptake rate without nitrite addition. Further, the aerobic P-uptake was found to completely stop at 10 mgNO$_2$-N/l and it was resumed in the same batch test only when the nitrite level had been reduced down to about 4 mgNO$_2$-N/l in the reactor by the nitrite oxidizing bacteria. From this point onward, the aerobic P-uptake proceeded at 50% of the reference aerobic P-uptake rate (the calculation of degree of inhibition presented below). This implies that the inhibitory effect of nitrite is reversible since with the decreasing nitrite concentration in the medium the P-uptake activity were restored back albeit slowly. The latter, i.e. the slow P-uptake recovery, indicates that PAOs didn't recover fully from the inhibitory effect of nitrite.

In the case of MBR sludge, the nitrite was observed to affect the aerobic P-uptake as well albeit at a smaller magnitude. For example, the aerobic P-uptake didn't completely cease at 10 mgNO$_2$-N/l but was reduced by 63% of the reference P-uptake rate of the MBR (Figure 8 (bottom)).A more detailed comparison of the aerobic P-uptake rate of the MBR and the SBR sludge is given below.

Nitrite effect on the anoxic phosphate uptake rate (PUR)

Typical results from the anoxic P-uptake tests are shown in Figure 9 for the SBR and the MBR biomass. Similar to the tests presented above, the first 75 minutes of the tests was anaerobic P-release phase, while the following phase was for the anoxic P-uptake either consisted of the nitrite (top of the figure) or nitrate (bottom of the figure) as indicated by the arrows.

Figure 9 Anoxic P-uptake with NO$_2$ (top) and NO$_3$ (bottom) as electron acceptor: SBR biomass (left) and MBR biomass (right). The arrows indicate the start of the P-uptake phase.
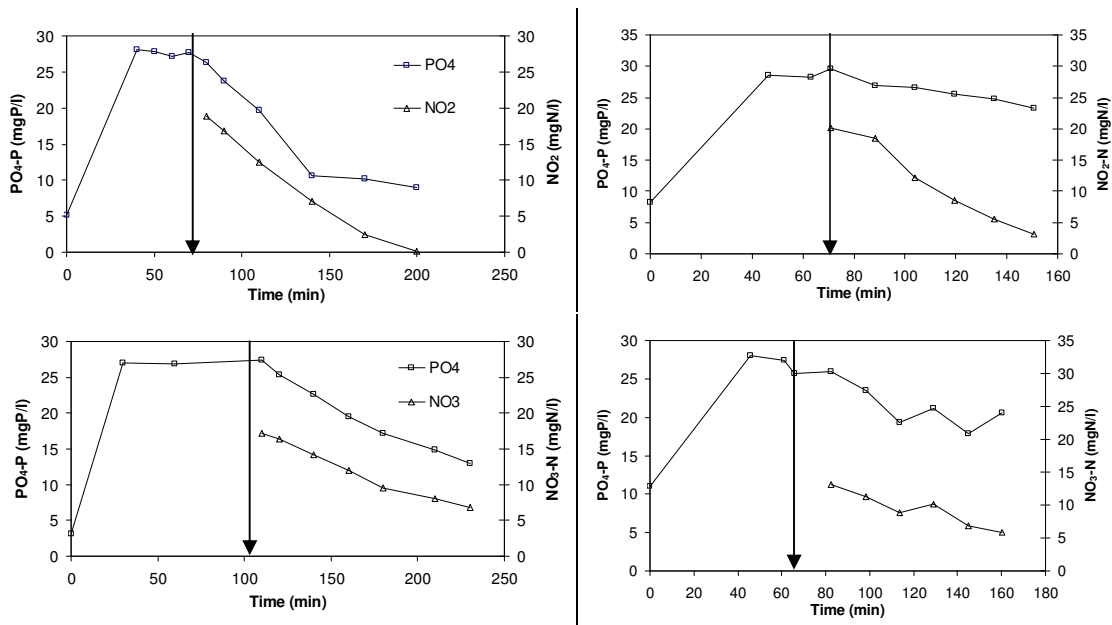
One observes that biomasses from both systems were able to use nitrite and nitrate as electron acceptor and perform the typical *polyphosphate-accumulating metabolism* (PAM) (Schuler

and Jenkins, 2003). Furthermore, within the studied range and up to 25 mgNO$_2$-N/l, no inhibitory effect of nitrite could be observed on the anoxic P-uptake activity of either SBR or MBR sludges. Since these results were obtained under pH controlled tests, it confirms that elevated nitrite concentrations may not pose significant inhibitory effects on the anoxic phosphate uptake. This is in contrast to the results of Meinhold *et al.* (1999) and expectation of Saito *et al.* (2004) but is in line with the results of Anh *et al.* (2001) and Hu *et al.* (2003) who found as well that at high nitrite levels PAOs are still able to show anoxic P-uptake activity.

Visual comparison of the anoxic P-uptake profiles for the SBR biomass shows that the P-uptake with nitrite appears to proceed faster than the P-uptake with nitrate as electron acceptor. For the MBR biomass, on the contrary, no significant difference can be observed between the anoxic P-uptake rates with nitrite and nitrate. A detailed comparison of the P-uptake rates with these different electron acceptors are given below in the discussion.

DISCUSSION

The calculation of the P-uptake rate

To quantify the extent of nitrite inhibition of the P-uptake rate, one can calculate and compare the P-uptake rate obtained from exposing the biomass to different levels of nitrite. However, one needs to exercise caution when calculating the P-uptake rate as the P-uptake process is an inherently non-linear one since it depends on several other processes taking place in the cell metabolism, e.g. the degradation rate of the internal organic storage polymers (X$_{PHA}$) as well as the saturation of the buildup of the polyphosphate pool (X$_{PP}$). For example, ASM2d model formulates the aerobic P-uptake process (the anoxic P-uptake rate is essentially the same but with nitrate as electron acceptor instead of oxygen) as follows:

$$PUR_{O2} = q_{PP} \cdot \frac{S_{O2}}{S_{O2} + K_{O2}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PS}}$$
$$\cdot \frac{X_{PHA} / X_{PAO}}{X_{PHA} / X_{PAO} + K_{PHA}} \cdot \frac{K_{MAX} - X_{PP} / X_{PAO}}{K_{IPP} + K_{MAX} - X_{PHA} / X_{PAO}} \cdot X_{PAO}$$

Eq.1

where, PUR$_{O2}$ is the aerobic P-uptake rate, q$_{PP}$ stands for the rate constant of P-storage; K$_{O2}$ and K$_{PS}$ are the saturation coefficients for oxygen and for ortho-phosphate storage respectively; S$_{O2}$ and S$_{PO4}$ are the oxygen and ortho-phosphate concentration in the bulk respectively; X$_{PHA}$ and X$_{PP}$, X$_{PAO}$ are the intracellular organic storage products, intracellular polyphosphate storage and concentration of PAOs respectively; K$_{PHA}$, is the saturation coefficient for PHA; K$_{MAX}$ and K$_{IPP}$ are the maximum ratio of X$_{PP}$/X$_{PAO}$ (the maximum ratio for polyphosphate storage in the cell) and inhibition coefficient for X$_{PP}$ storage (Henze *et al.*, 2000). Important to note is that while other models have formulated different mechanisms to describe P-uptake process, the non-linear nature of the process remains similar (Smolders *et al.*, 1995; Meijer *et al.*, 2001). This non-linear process can be assumed to proceed linearly under following assumptions:
    i.   Sufficiently high level of X$_{PHA}$, e.g. X$_{PHA}$/X$_{PAO}$ is higher than 0.01.
    ii.   X$_{PP}$ is much lower than the K$_{MAX,}$ e.g. X$_{PP}$/X$_{PAO}$ is lower than 0.34.
    iii.   Sufficiently high level of ortho-phosphate (S$_{PO4}$) and electron acceptor (e.g. S$_{O2}$)

Under these assumptions, the saturation and inhibition terms of Eq.1 will be rendered to the neighborhood of 1, which means that the PUR will be driven close to its maximum under the given conditions. Under such conditions, the PUR can be experimentally calculated as the slope of the observed P-uptake.

Figure 10 – Illustration of the calculation of the P-uptake rate from the P-uptake profiles obtained from the batch tests. The arrow indicates the beginning of the P-uptake.
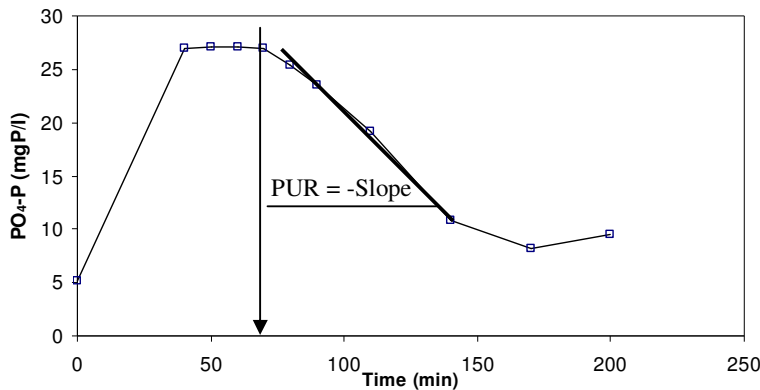
Concerning the batch experiments performed in this study, we assumed that the first hour of the P-uptake experiment is likely to meet the above mentioned assumptions and therefore to proceed independent of limitations/inhibitions. Hence, we approximated the PUR from the slope of the measured PO_4-P profile in this first hour of the P-uptake experiment. This is illustrated in Figure 10 for one P-uptake profile obtained from one of the anoxic batch tests in Figure 10.Although this approach is an approximation in its nature, it is still expected to provide a means to quantify the effect of nitrite on the P-uptake.

Nitrite inhibition of the aerobic P-uptake

The degree of nitrite inhibition was calculated on the basis of the observed aerobic P-uptake rates obtained under the different nitrite concentrations of Figure 8. The results are given in Table 1 both for the SBR and the MBR biomass.

The PUR(%) is calculated as $\left(\dfrac{PUR_{NO2}}{PUR_0} \times 100 \right)$, which is the percent ratio of the observed P-uptake rate at a certain nitrite level, PUR_{NO2}, to the reference P-uptake rate, PUR_0, obtained under zero nitrite concentration . The degree of inhibition is then calculated as $\left(\dfrac{PUR_0 - PUR_{NO2}}{PUR_{NO2}} \right)$. The degree of inhibition may take a value between 0 and 1, corresponding to no and severe inhibition respectively.

Table 1 indicates that the degree of nitrite inhibition of the PAOs from the SBR biomass is considerably higher than that of the PAOs in the MBR biomass. This shows that the PAOs from the SBR were more vulnerable to nitrite exposure. The underlying reason is not that clear. Looking at the long-term effluent monitoring of both systems, one notices that the nitrite concentration sporadically reaches up to 1.5 and 2.5 $mgNO_2$-N/l in the effluent of both

systems (Figure 5 and Figure 7). This means that both systems were sporadically exposed to nitrite. Hence one expects that *polyphosphate accumulating organisms* present in both systems would be adapted to nitrite to a similar degree, which apparently wasn't the case. One may expect that different organisms or perhaps, using the argument of Schuler and Jenkins (2003), different *polyphosphate-accumulating metabolism* (PAM) could be responsible for the observed difference in the response of the SBR and the MBR systems to nitrite exposure. Quantitative molecular techniques such as FISH-MAR are of course needed to support these speculative arguments (Seviour *et al.*, 2003), which is beyond the scope of current contribution.

Table 1- Degree of nitrite inhibition of the aerobic P-uptake

Initial NO_2-N	PUR (%)		Degree of inhibition	
$mgNO_2$-N/l	SBR	MBR	SBR	MBR
0	100	100	0	0
5	71	86	0.29	0.14
10	35	63	0.65	0.37

Electron acceptor preference of PAOs

A summary of the phosphate uptake rates obtained under three different redox conditions, namely with presence of oxygen, nitrate and nitrite, are shown for the SBR biomass in Table 2. A comparison of the phosphate uptake rates with these different electron acceptors show that the PUR obtained with nitrite was 60% faster than the PUR obtained with nitrate. It also follows that the nitrite uptake rate, as referenced to the electron content of nitrate, i.e. $3/5*N_{O2}UR$, was around 60% higher than the nitrate uptake rate (NUR_{NO3}). This suggests that the anoxic PUR was limited by the nitrate uptake rate. In short, for this particular biomass the anoxic P-uptake was faster with nitrite than with nitrate.

Table 2- P-uptake rates under different electron acceptor for the SBR biomass

	O_2	NO_2-N	NO_3-N	η_{PUR_NO2}[1]	η_{PUR_NO3}[1]
[2]Specific PUR (mgP/gSS-h)	3.9±0.4	3.1±0.4	2.0±0.4	0.80	0.52
[2]Specific NUR (mgN/gSS-h)	-	2.2±0.1	1.4±0.1		

[1]Anoxic reduction factor for aerobic PUR under anoxic conditions, i.e. for NO_2 and NO_3 respectively.
[2] Each rate is determined as the average of 4 batch tests.

When compared to the aerobic PUR, the reduction factor for PUR with nitrate and nitrite was found 0.5 and 0.8 respectively. The fact that different P-uptake rates under different electron acceptor conditions were observed suggests that the P-uptake rate is different under different e-acceptor conditions or that different fractions of PAOs are active under different electron acceptor conditions.

A classic question related to such results is whether one type of facultative PAO is able to perform *polyphosphate-accumulating metabolism* with oxygen, nitrate and nitrite (one organism, all functions) or rather that different types of PAOs specialize in performing different types of phosphorus uptake metabolism (different organisms, different functions). To address this question, we have performed dedicated tests with mixtures of nitrite and nitrate as electron acceptors.

The results, shown in Figure 11 provide several insights. First, they show that both electron acceptors were simultaneously utilized while the phosphate was being taken up and stored by the PAOs. Second, the nitrite uptake rate was faster than the nitrate uptake rate which explains the fact that no nitrite build-up was observed during nitrate-denitrification. Moreover, the observed P-uptake rate was found around 3.5 mgP/gSS-h in the presence of both nitrite and nitrate. This rate is higher than the average anoxic P-uptake rate with nitrate alone (see Table 2). This implies that the PUR corresponding to the presence of nitrite and nitrate may be due to a cumulative activity of PAOs performing nitrite and nitrate metabolisms. This in turn suggests that there might be different PAO types performing different functions in this particular SBR sludge.

Figure 11- Anoxic phosphate uptake with mixture of nitrate and nitrite as electron acceptor: 12mgNO$_3$-N/l+5 mgNO$_2$-N/l (Top) 12mgNO$_3$-N/l+2.5 mgNO$_2$-N/l (down)
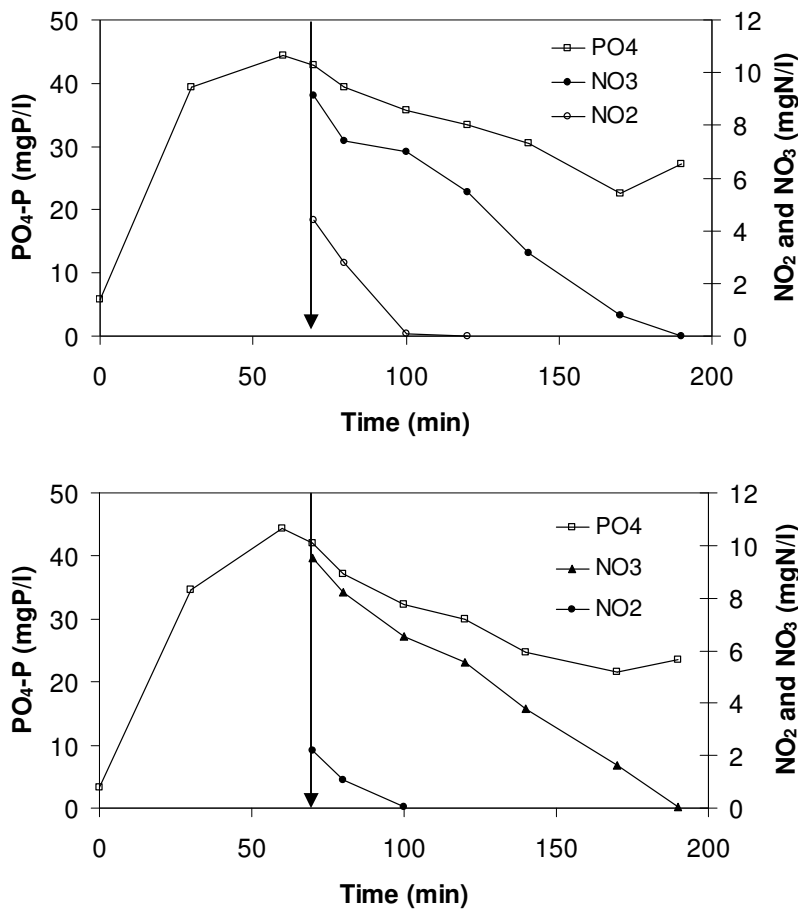
Mechanism and threshold value of nitrite inhibition?

A summary of the results obtained so far in several studies about the effect of nitrite on the phosphate-accumulating organisms' activity (both aerobic and anoxic) is given in Table 3. It becomes clear from this table that the reported inhibitory effect of nitrite for the aerobic and anoxic P-uptake activity of PAO has a wide range of threshold values. One of the main differences in these reported values is the sludge used to perform the batch tests, ranging from lab-scale reactors, fed with synthetic wastewaters, to pilot and full-scale systems with

domestic wastewater. This wide ranging effect of nitrite on the PAO activity implies that the nitrite effect is influenced by the history of the biomass used in these tests.

The "history includes" the particular environmental conditions the biomass has been exposed to prior to analysis in the batch tests. Indeed, there are many interacting factors including the operational strategy, type of wastewater, type of reactor, dynamic operational disturbances as well as competition/interaction with other microbial populations that may influence the response of PAO to the nitrite exposure. Indeed, these combined interactions are likely affect the physiology of the PAO enriched in a particular system.

The underlying mechanism of the nitrite inhibition is currently still unclear. Research at physiological and metabolic level of the PAOs combined with a detailed process analysis of the history of the system is expected to help uncover the mechanism of nitrite inhibition and explain the variation in the reported empirical results of nitrite inhibition of PAOs. Such investigations will also help establish a sound model structure for the nitrite inhibition (see below).

Implications to operation of alternating EBPR systems

In biological systems performing simultaneous nitrogen and phosphorus removal, such as the case of the SBR and MBR systems of this study, it is well-known that phosphate removal can become unstable and lose its efficiency because of the direct competition for substrate between PAOs and normal denitrifying heterotrophs, when nitrate reaches the anaerobic compartment (Manning and Irving, 1985; Wilderer *et al*., 2001). However, the instability of the system may also be caused by the accumulation of nitrite in the aerobic phase, which may result from a low oxygen concentration in the aerobic phases, low aerobic SRT and/or low temperatures that strongly affect the activity of nitrite oxidizers (Wiessman, 1994).

Results from this study as well as previous studies tend to converge to a common agreement that nitrite has an inhibitory effect particularly on the aerobic P-uptake activity of PAOs (see Table 3). The results also suggest that the inhibition is reversible. In other words, after removing nitrite from the mixed liquor, the aerobic p-uptake could resume albeit at a slower rate compared to the previously observed maximum PUR. This suggests that complete nitrification and denitrification will prevent any possible side effect of nitrite on the aerobic P-uptake activity of the EBPR systems.

In case nitrite is consistently building up in the system, one may consider to operate the system in favor of enhancing anoxic P-uptake activity to aerobic P-uptake activity. This suggestion is based on the experimental results of Hu *et al*. (2003) and Anh *et al* (2001) and this study (see Table 3) which consistently showed that PAOs are capable of using nitrite as electron acceptor at a rate equal to at least 60% percent (if not more) of the aerobic P-uptake rate. Our study also showed that some particular DPAOs may prefer nitrite over nitrate (e.g. because of selective enrichment of nitrite reducing PAOs in a particular system configuration).

In short, instead of confronting the nitrite problem with conventional preventive process engineering means, we argue to take up the opportunity presented itself in this problem. Extrapolating further from these empirical observations that some particular DPAOs may prefer to use nitrite at least as effectively as nitrate if not more, one may also speculate that denitrifying phosphorus accumulating systems may also be operated/configured to use *only*

nitrite as electron acceptor instead of using nitrate which is the common configuration/operation of denitrifying EBPR systems in current practice (see e.g. Kuba *et al.*, 1996). Such a configuration can be coupled to nitrogen removal via the nitrite route (van Benthum *et al.*, 1998; van Dongen *et al.*, 2001; Anthileo *et al*, 2003) thereby resulting in a potentially novel nitrogen and phosphorus removing system configuration.

Table 3- Overview of the response of PAO to nitrite under different electron acceptor conditions.

	Operation strategy	Type of reactor/wastewater	Inhibition level of nitrite	
			aerobic P-uptake	anoxic P-uptake
Meinhold *et al.*	Anoxic/aerobic	Pilot scale BPR plant fed with real wastewater	10-15 mg NO_2-N/l	5-8 mg NO_2-N/l
Ahn *et al.*	Anaerobic/aerobic	Lab-scale SBR fed with real wastewater	Not tested	No inhibition (up to 40 mg N/l)
Lee *et al.*	Anaerobic/ aerobic/anoxic /aerobic	Lab-scale SBR fed with synthetic wastewater, BNPR plant	Not tested	No inhibition (up to 10 mg N/l)
Hu *et al.*	Anaerobic/ anoxic	Lab-scale SBR fed with synthetic wastewater, BPR plant	Not tested	No inhibition (up to 35 mg N/l)
Saito *et al.*	Anaerobic/ anoxic/aerobic	Lab-scale SBR fed with real wastewater, BPR	2 mg NO_2-N/l partial; 6 mgNO_2-N/l complete inhibition	12 mg NO_2-N/l
Yoshida *et al.*	Anaerobic/aerobic and Anaerobic/anoxic	Lab-scale SBR fed with synthetic wastewater, BPR	1 mg NO_2-N/l for A/O; 3 mgNO_2-N/l for A2O sludge	Not tested
Soeijima *et al.*	Anaerobic/ aerobic/ anoxic/ SBR	Lab-scale SBR fed with synthetic wastewater, BNPR plant	Inhibition reported but not quantified	Not tested
This study	Anaerobic/ aerobic/ anoxic/ aerobic SBR and Anaerobic/ anoxic/ aerobic MBR	Pilot scale SBR and MBR fed with synthetic wastewater: BNPR plant	5 mg NO_2-N/l SBR sludge;10 mg NO_2-N/l MBR sludge	No inhibition up to 20 mgNO_2-N/l

The advantage of such a process configuration, a nitrite reducing DPAO system, is that more nitrite can be removed on mass basis per unit mass of PHA oxidized by DPAOs during the anoxic P-uptake phase. In this way, theoretically more nitrogen removal can be achieved using anoxic DPAO activity with nitrite, theoretically 3/5 times more than what can be achieved with a DPAO system using nitrate. In such a configuration, however, one needs to – again- account for the possible proliferation of denitrifying glycogen accumulating organisms (GAOs), which may prevent the system to achieve nitrogen and phosphorus removal over nitrite. Such an interference of GAO was shown in Zeng *et al.* (2003) for a particular lab-scale reactor configuration. From a process configuration and operation point of view, one also needs to overcome the challenges of maintaining a stable partial nitrification process (i.e. only ammonia conversion to nitrite), which is only possible under a certain reactor configuration, e.g. high temperature, high pH, bicarbonate limitation, oxygen limitation and the like.

Moreover granular activated sludge technology focusing on performing simultaneous nitrification, denitrification and phosphorus removal, which currently considers only oxygen and nitrate as electron acceptor for PAOs, may also benefit from including nitrite in the system optimization (see e.g. de Kreuk *et al.*, 2005). These theoretically possible configurations naturally require experimental evaluation, but in principle such an approach should offer an efficient solution to the inhibitory effect of nitrite on aerobic P-uptake.

Modeling the nitrite effect

As mentioned earlier, current deterministic models describing the behavior of EBPR systems ignores two important influences of nitrite on the activity of PAOs: (i) the inhibitory effect of nitrite on the aerobic P-uptake, and (ii) nitrite as a plausible electron acceptor for denitrifying PAO activity. These assumptions are likely to hold for many EBPR systems where there is no nitrite build up. However for systems with nitrite build-up such models are expected to fail and will require extension. For such an extension, the above mentioned fundamental research will be invaluable as it will allow developing a sound mechanistic structure for the nitrite inhibition mechanism. Lacking that, we propose the following two extensions based on the empirical observations obtained thus far (see Table 3).

Modeling nitrite inhibition of aerobic P-uptake:

A convenient and simple way of modeling the reversible inhibitory effect of nitrite would be to use a similar function as the one describing the inhibitory effect of oxygen on the denitrifying activity of heterotrophic organisms, i.e. using a switch function, in the form of $K_{INO2}/(S_{NO2} + K_{INO2})$, where K_{INO2} stands for inhibition concentration of nitrite. Therefore, the rate equation of P-uptake in ASM2d may become as follows:

$$PUR_{O2} = q_{PP} \cdot \frac{S_{O2}}{S_{O2} + K_{O2}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PS}} \cdot \frac{K_{INO2}}{S_{NO2} + K_{INO2}}$$
$$\cdot \frac{X_{PHA} / X_{PAO}}{X_{PHA} / X_{PAO} + K_{PHA}} \cdot \frac{K_{MAX} - X_{PP} / X_{PAO}}{K_{IPP} + K_{MAX} - X_{PHA} / X_{PAO}} \cdot X_{PAO}$$

Eq.2

As mentioned earlier, the K_{INO2} appears to vary from system to system and therefore needs experimental determination or calibration from inhibition tests. As our data is not sufficient for reliable parameter estimation (it is a non-linear process), we don't present an estimate for K_{INO2}. However one may state that the lower bound for this estimate seems to be around 5 $mgNO_2$/l and 10 $mgNO_2$-N/l for the SBR and the MBR biomass respectively. Important to note is that the nitrite inhibition coefficient is likely to be pH dependent as the previous nitrite inhibition studies on other bacteria have confirmed that free nitrous acid (HNO_2) is the true inhibitory compound.

Modeling anoxic activity of denitrifying PAOs

There are two possible pathways for modeling denitrification, one assumes a sequential reduction of nitrate to nitrite and then to nitrogen gas, i.e. $NO_3 \rightarrow NO_2 \rightarrow 1/2N_2$. Another plausible pathway is parallel and independent reduction of nitrate and nitrite all the way down to nitrogen gas, i.e. $NO_3 \rightarrow 1/2N_2$ and $NO_2 \rightarrow 1/2N_2$ (Sin *et al.*, 2006a).

Our experimental results showed that nitrite reduction by PAOs was faster than the nitrate reduction. This phenomenon can be described by both pathways, which mean one can not rule out one pathway over the other based on these empirical results. However, from a modeling perspective, opting for the sequential reduction of nitrate allows to describe more than one particular behavior of a system, e.g. both nitrite build-up and faster nitrite reduction behaviors can be modeled by appropriate manipulation of the rate parameters of the first and the second steps (see Eq3 and Eq4). Hence, we suggest the adoption of sequential reduction of nitrate as the model structure for the denitrifying PAOs, until evidence is provided another structure is needed. The rate equations for nitrate and nitrite reduction processes of ASM2d then becomes as follows:

$$PUR_{NO3} = q_{PP} \cdot \eta_{NO3} \frac{K_{O2}}{S_{O2} + K_{O2}} \frac{S_{PO4}}{S_{PO4} + K_{PS}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3}}$$
$$\cdot \frac{X_{PHA}/X_{PAO}}{X_{PHA}/X_{PAO} + K_{PHA}} \cdot \frac{K_{MAX} - X_{PP}/X_{PAO}}{K_{IPP} + K_{MAX} - X_{PHA}/X_{PAO}} \cdot X_{PAO}$$

Eq. 3

$$PUR_{NO2} = q_{PP} \cdot \eta_{NO2} \frac{K_{O2}}{S_{O2} + K_{O2}} \frac{S_{PO4}}{S_{PO4} + K_{PS}} \cdot \frac{S_{NO2}}{S_{NO2} + K_{NO2}}$$
$$\cdot \frac{X_{PHA}/X_{PAO}}{X_{PHA}/X_{PAO} + K_{PHA}} \cdot \frac{K_{MAX} - X_{PP}/X_{PAO}}{K_{IPP} + K_{MAX} - X_{PHA}/X_{PAO}} \cdot X_{PAO}$$

Eq. 4

In these equations, the competition between electron acceptors is not included, but in case this would be needed, they can be easily described following the concept of substrate competition already used elsewhere in ASM2d.

It is clear that with the introduction of nitrite, the model structure of ASM2d gets even more complex, i.e. the number of parameters increases significantly. In a way, it becomes more expensive to apply such model, since it requires knowledge/information about these additional parameters. However, we believe that such an extension is essential if not a must for proper description of simultaneous nitrogen and phosphorus removing systems characterized by significant nitrite build-up.

CONCLUSIONS

In this study, we investigated experimentally using batch phosphate uptake tests the nitrite effect on the aerobic and anoxic P-uptake activities of Polyphosphate Accumulating Organisms (PAOs) grown in an SBR and an MBR system fed with the same synthetic wastewater. We found that the nitrite has an inhibitory but reversible effect on the aerobic P-uptake activities of the SBR and the MBR biomass. The inhibitory effect was more pronounced on the SBR biomass than the MBR biomass, where the respective degree of inhibition for each biomass was 0.65 and 0.37 at 10 mgNO$_2$-N/l. This discrepancy may be due to different degree of adaptation of PAOs to the presence of nitrite enriched in these particular SBR and the MBR systems.

For the anoxic P-uptake activities, both the SBR and the MBR biomass were found capable of using nitrite as effectively as nitrate as electron acceptor. Moreover, for the SBR biomass the anoxic P-uptake rate using nitrite was found higher (60%) than the P-uptake rate with nitrite. These results seem to imply that in these particular systems different PAO organisms

specialized in different functions may have been developed instead of one type of PAO organism performing all of the functions, i.e. the assumption of most of mechanistic activated sludge models, e.g. ASM2d.

Overall, the results of this study as well as previous studies over nitrite effect of PAO activity, requires an appropriate extension of the current mechanistic models to properly describe biological nitrogen and phosphorus removing systems exposed nitrite buildup. Such a possible extension to ASM2d model has also been suggested. Until the underlying biochemical pathway of nitrite inhibition is resolved, experimental determination of the reduction factors for P-uptake with nitrite and nitrate are advised.

ACKNOWLEDGEMENT

REFERENCES

Ahn J., Daidou T., Tsuneda S., and Hirata A. (2001) Metabolic behavior of denitrifying phosphate-accumulating organisms under nitrate and nitrite electron acceptor conditions. *J Biosc. Bioeng.,* **92** (5), 442-446.

Anthonisen, A. C., R. C. Loehr, T. B. S. Prakasam, and E. G. Srinath, 1976, Inhibition of Nitrification by Ammonia and Nitrous-Acid. *J. Water Pollut. Control. Fed*, **48**(5), 835-852.

Antileo C., Roeckel M. and Wiesmann U. (2003) High nitrite buildup during nitrification in a rotating disk reactor. *Water Environ. Res.* **75**:151-162.

APHA (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association, Washington DC.

Boeije G., Corstanje R., Rottiers A. and Schowanek D. (1999). Adaptation of the CAS test system and synthetic sewage for biological nutrient removal. Part I: Development of a new synthetic sewage. *Chemosphere*, 38(4), 699-709.

de Kreuk,M., Heijnen,J.J., and van Loosdrecht,M.C.M. (2005) Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. *Biotech. Bioeng.,* **90** (6), 761-769.

Hu J.Y., Ong S.L., Ng W.J., Lu F., and Fan X.J. (2003) A new method for characterizing denitrifying phosphorus removal bacteria by using three different types of electron acceptors. *Water Res.,* **37** (14), 3463-3471.

Insel, G., Sin, G., Lee,D.S., Nopens, I., and Vanrolleghem,P.A. (2006) A calibration methodology and model-based systems analysis for SBRs removing nutrients under limited aeration conditions. *J Chem. Tech. and Biotech.,* **81** (4), 679-687.

Jiang, T. (2007) Characterization and modeling of soluble microbial products in membrane bioreactors. PhD thesis. Ghent University.

Kuba,T., VanLoosdrecht,M.C.M., and Heijnen,J.J. (1996) Phosphorus and nitrogen removal with minimal cod requirement by integration of denitrifying dephosphatation and nitrification in a two-sludge system. *Water Res.,* **30** (7), 1702-1710.

Lee, D. S., C. O. Jeon, and J. M. Park, (2001) Biological nitrogen removal with enhanced phosphate uptake in a sequencing batch reactor using single sludge system. *Water Res.*, **35**(16), 3968-3976.

Martins, A. M. P., K. Pagilla, J. J. Heijnen, and M. C. M. van Loosdrecht, 2004, Filamentous bulking sludge - a critical review. *Water Res.,* 38, 793-817.

Manning J.F. and Irvin R.L. (1985) The biological removal of phosphorous in a sequencing batch reactor. *J. Water Pollut. Control. Fed.* **57**: 87-94.

Meijer, S.C.F., van Loosdrecht, M.C.M., and Heijnen, J.J. (2001) Metabolic modelling of full-scale biological nitrogen and phosphorus removing WWTP's. *Water Res.,* **35** (11), 2711-2723.

Meinhold J., Arnold E., and Isaacs S. (1999) Effect of nitrite on anoxic phosphate uptake in biological phosphorus removal activated sludge. *Water Res.,* **33** (8), 1871-1883.

Rake, J. B., and R. G. Eagon, (1980) Inhibition, but not uncoupling, of respiratory energy coupling of 3 bacterial species by nitrite. *J Bacteriol.,* **144**(3), 975-982.

Saito T., Brdjanovic D., and van Loosdrecht M.C.M. (2004) Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Research* **38** (17), 3760-3768.

Schuler, A.J. and Jenkins, D. (2003) Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, part I: Experimental results and comparison with metabolic models. *Water Environ. Res.,* **75** (6), 485-498.

Seviour,R.J., Mino,T., and Onuki,M. (2003) The microbiology of biological phosphorus removal in activated sludge systems. *Fems Microbiol. Rev.,* **27** (1), 99-127.

Sin G. and Vanrolleghem P.A. (2006a) Evolution of an ASM2d-like model structure due to operational changes of an SBR process. *Wat. Sci. Technol.*, **53(12)**:237–245.

Sin, G., K. Villez, and P. A. Vanrolleghem, (2006b) Application of a model-based optimisation methodology for nutrient removing SBRs leads to falsification of the model. *Wat. Sci. Technol.*, **53**(4-5), 95-103.

Soejima,K., Oki,K., Terada,A., Tsuneda,S., and Hirata,A. (2006) Effects of acetate and nitrite addition on fraction of denitrifying phosphate-accumulating organisms and nutrient removal efficiency in anaerobic/aerobic/anoxic process. *Bioprocess Biosyst.Eng.,* **29** (5-6), 305-313.

Vadivelu, V. M., J. Keller, and Z. G. Yuan, (2006) Effect of free ammonia and free nitrous acid concentration on the anabolic and catabolic processes of an enriched Nitrosomonas culture. *Biotech. Bioeng.,* **95**(5), 830-839.

Van Benthum,W.A.J., Derissen,B.P., van Loosdrecht,M.C.M., and Heijnen,J.J. (1998) Nitrogen removal using nitrifying biofilm growth and denitrifying suspended growth in a biofilm airlift suspension reactor coupled with a chemostat. *Water Res.,* **32** (7), 2009-2018.

van Dongen,U., Jetten,M.S.M., and van Loosdrecht,M.C.M. (2001) The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Wat. Sci. Technol.*, **44** (1), 153-160.

Van Hulle, S.W.H., Volcke, E.I.P, López Teruel, J., Donckels, B., van Loosdrecht, M.C.M. & Vanrolleghem, P.A. (2004). Influence of temperature and pH on the kinetics of the SHARON nitritation process. In: *Proceedings 4th IWA World Water Congress and Exhibition*. Marrakech, Marocco, September, 19-24, 2004.

Weismann U. (1994) *Biological nitrogen removal from wastewater.* In: Fiechter A., editor. Advances in biochemical engineering/biotechnology, Vol 5. Berlin: springer-verlag Berling-Heidelberg.

Wilderer P., Irvine R.L. and Goronszy M.C. (2001) *Sequencing batch reactor technology*. IWA Scientific and Technical Report No. 10, IWA Publishing, London.

Yarbrough, J. M., Rake J. B., and Eagon R. G., (1980) Bacterial inhibitory effects of nitrite - inhibition of active-transport, but not of group translocation, and of intracellular enzymes. *Appl. Environ. Microbiol.,* 39(4), 831-834.

Zeng,R.J., Lemaire,R., Yuan,Z., and Keller,J. (2003) Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. *Biotech. Bioeng.,* **84** (2), 170-178.