Effect of nitrite, limited reactive settler and plant design configuration on the predicted performance of a simultaneous C/N/P removal WWTP

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ABSTRACT

This paper describes a modelling study where five new benchmark plant design configurations for biological nutrient removal (A\textsuperscript{2}/O, UCT, JHB, MUCT and BDP-5 stage) are simulated and evaluated under different model assumptions. The ASM2d including electron dependent decay rates is used as the reference model (A1). The second case (A2) adds nitrite as a new state variable, describing nitrification and denitrification as two-step processes. The third set of models (A3 and A4) considers different reactive settlers types (diffusion-limited/ non limited). This study analyses the importance of these new model extensions to correctly describe the nitrification behaviour and the carbon source competition between ordinary heterotrophic organisms (OHO) and polyphosphate accumulating organisms (PAO) under certain operation conditions. The economic and environmental aspects when meeting the P discharge limits by adding an external carbon source are also studied.

KEYWORDS

Benchmarking; EBPR; reactive settler; two step nitrification; two step denitrification.

1. INTRODUCTION

Nowadays, water shortage is forcing governments to impose stricter effluent discharge limits for nutrients to wastewater treatment plants (WWTPs). Consequently, upgrading current WWTPs with biological nutrient removal (BNR) including nitrification/denitrification and Enhanced Biological Phosphorus Removal (EBPR) should be a short-term aim. EBPR, the most sustainable technology for phosphorus (P) removal, is based on the enrichment of activated sludge with polyphosphate.
accumulating organisms (PAO), usually under sequential anaerobic and aerobic or anoxic conditions so that the electron donor (organic matter) and the electron acceptor (usually oxygen but also nitrate or nitrite) are physically separated (Metcalf and Eddy, 2003).

The most widespread mathematical models to describe EBPR in a WWTP are the Activated Sludge Model No. 2d (ASM2d) (Henze et al., 2000) as well as the extended Activated Sludge Model No. 3 (ASM3) incorporating EBPR process (ASM3-BioP) (Rieger et al., 2001). The formulation of these models includes some simplifications to reduce the model complexity and thus, they may not be valid for all scenarios (Sin and Vanrolleghem, 2006). For example the ASM2d default model structure does not differentiate amongst the anaerobic, anoxic and aerobic decay rates while experimental results show the contrary (Nowak et al., 1995; Siegrist et al., 1999). Gernaey and Jørgensen (2004) and Flores-Alsina et al. (2012) therefore formulated an updated ASM2d model with electron acceptor dependent decay rates. In addition, nitrite is not considered as a state variable in the ASM2d despite the fact that it is a key intermediate to describe accurately the anoxic organic matter consumption and the nitrification process for low dissolved oxygen (DO) concentrations. Regarding EBPR, the recent advances on anoxic P removal have increasingly set the focus on the denitrifying PAO (DPAO) fraction (Ahn et al., 2001; Tayà et al., 2011). He et al. (2007) reported that different types of DPAO have different denitrification capabilities, where nitrite is an important electron acceptor under some operational conditions. Hence, including nitrite in the ASM models is also essential for achieving a proper description of EBPR in a WWTP. Most of the studies that included nitrite as state variable considered two-step nitrification and denitrification processes. Although the
two-step nitrification assumption is commonly accepted, two-step denitrification modelling is not well established and different approaches have been proposed (Wett and Rauch, 2003; Sin and Vanrolleghem, 2006; Guerrero et al., 2011). Sin et al. (2008) analysed some of these models and proposed some guidelines for a consistent description of activated sludge systems including nitrite and considering two-step nitrification and denitrification.

The biological reactions occurring in the secondary settler are another factor to take into consideration when modelling BNR. Although the settling process is usually considered non-reactive (e.g. Takács et al., 1991), several studies (Siegrist et al., 1995) reported that biological reactions also occur, and in particular denitrification processes, despite of the mass transfer limitations present in the settler (concentration gradients and preferential pathways). Gernaey et al. (2005) and Flores-Alsina et al. (2012) presented a reactive settler model that considered each layer of the settler as a continuously stirred tank reactor (CSTR). Unfortunately this approach seems to overestimate the reactive capacity of the settler, since mass transport limitations were not considered. Clearly, more research should be conducted on this topic to correctly simulate a reactive settler.

In a real WWTP, EBPR has to coexist with biological nitrogen (N) removal based on the aerobic nitrification and anoxic denitrification processes. Coupling N removal and EBPR is not just as simple as adding an extra anaerobic zone after the influent inlet to favour PAO growth, since there can be some detrimental interactions between both processes. Most of the reported WWTP configurations for simultaneous N and P removal have an aerobic zone before the secondary settler which may result in the presence of some nitrate or nitrite (named NOX hereafter) in the external recycle (QEXT).
The NO\textsubscript{X} would then enter the anaerobic zone via the external recycle, leading to EBPR failure as reported for many full-scale WWTPs (Henze \textit{et al.}, 2008). The most commonly accepted hypothesis to describe this failure is that the NO\textsubscript{X} presence triggers the competition for the electron donor (i.e. organic matter) between ordinary heterotrophic organisms (OHO) and PAO. Guerrero \textit{et al.} (2011) experimentally observed that the nature of the carbon source rules such competition. Thereby, it was proved that OHO were able to outcompete PAO when the electron donor was a complex carbon source, while PAO won the competition when treating a wastewater with high volatile fatty acids content.

Among all the possible WWTP configurations, the A\textsuperscript{2}/O (anaerobic-anoxic-aerobic) configuration has been widely applied for municipal WWTP despite the obvious disadvantage that complete denitrification is not possible and some NO\textsubscript{X} will always enter the anaerobic phase via Q\textsubscript{EXT} (Henze \textit{et al.}, 2008). Thereby, alternative configurations have been designed to prevent such deleterious effect on EBPR by reducing the NO\textsubscript{X} in the inlet to the anaerobic phase (Figure 1). The Bardenpho 5-stage (BDP-5 stage) system (Barnard, 1976) improves N removal by adding an extra anoxic-aerobic zone and thus, limits the NO\textsubscript{X} load in the external recycle. Rabinowitz and Marais (1980) designed the UCT (University of Cape Town) system aiming at preventing the Q\textsubscript{EXT} from entering the anaerobic reactor directly. In this configuration, Q\textsubscript{EXT} is discharged to the anoxic reactor together with the internal recycle (Q\textsubscript{INT}) to denitrify the NO\textsubscript{X}. A new recycle is then required from the anoxic reactor to the anaerobic reactor to maintain the desired biomass concentration, which is named anaerobic recirculation (Q\textsubscript{ANAE}) in this study. However, it has been reported for this configuration (Henze \textit{et al.}, 2008) that avoiding NO\textsubscript{X} presence in Q\textsubscript{ANAE} is critical to
achieve a high EBPR activity, but this control is not always possible under full scale operation. A modification of the UCT (Modified UCT, MUCT) was proposed to avoid this problem and increase its efficiency. In the MUCT configuration, the $Q_{\text{EXT}}$ is directed to an anoxic reactor that does not receive the $Q_{\text{INT}}$ flow (Figure 1), easing the total NO$_X$ depletion in the $Q_{\text{ANAE}}$. On the other hand, most of the denitrification takes place in the second anoxic tank, which also receives the $Q_{\text{INT}}$ recycle flow. Finally, Osborn and Nicholls (1978) proposed another alternative to overcome the negative effect of NO$_X$ on EBPR, the Johannesburg process (JHB). Here, an anoxic reactor is located in the $Q_{\text{EXT}}$ line so that the NO$_X$ in the $Q_{\text{EXT}}$ is predenitrified. The electron donor for this process could be either part of the influent (influent bypass, IB) or an external carbon source addition.

Selecting the best of these configurations is not a straightforward issue because many variables affect the overall WWTP performance (i.e. influent characteristics, operational conditions or availability of an external carbon source) and thus, a defined framework is required to compare all the possible scenarios under unbiased conditions. For example, the Benchmark Simulation Model (BSM) has been widely used as a standardised simulation protocol to compare control strategies in a WWTP (Copp et al., 2002; Jeppsson et al., 2007; Nopens et al., 2010; Gernaey et al., 2013).

Along this line of thinking, the objective of this paper is to evaluate i) the effect of different model assumptions, and ii) the impact of different WWTP configurations on the performance of EBPR coupled to biological N removal. In order to address the first point, the inclusion of nitrite as state variable and biochemical reactions in the settler (with and without considering mass transfer limitations) were analysed and compared under long-term operation (364 days). On top of that, the previous model assumptions
were also applied to the five most common EBPR plant configurations found in full-scale WWTPs. Note that this is the first study where benchmark simulations have been conducted using these new plant configurations. Effluent quality, operational costs and discharge levels were used to evaluate the performance of the different plant configurations.

2. MATERIALS AND METHODS

2.1. Wastewater treatment plant configurations under study

Five different benchmark WWTP configurations for simultaneous carbon (C), N and P removal were considered in this study: 1) A²/O, 2) BDP-5stage, 3) UCT, 4) MUCT and 5) JHB (Figure 1). The most significant parameters of each configuration are summarised in Table 1. The volumes of the anaerobic/anoxic/aerobic zones were considered constant for all the plant configurations and the different configurations were implemented by changing the location of specific reactors and by adding the required recycle streams. As these new recycle streams (QANAE and IB) had not been reported in any previous benchmark study, their flow rates were set according to common textbook knowledge (Metcalf and Eddy, 2003; Henze et al., 2008). The volumes of the anoxic and aerobic reactors were determined according to the current plant-wide benchmark for C and N removal outlined in Nopens et al. (2010). The two additional anaerobic reactors included for EBPR were assumed to have a volume of 1250 m³ each.

2.2. Mathematical models

In the first step of the study, four different approaches to describe BNR and the settling process were evaluated (Table 2). The biological kinetic model used to describe BNR in this study was the ASM2d (Henze et al., 2000), similarly to other benchmark
studies on proposing new model extensions (Gernaey and Jørgensen, 2004; Flores-Alsina et al., 2012). For the first approach (A1), the ASM2d was extended with electron acceptor dependent decay rates as described by Gernaey and Jørgensen (2004). The secondary settler behaviour was modelled using the 10-layer (non-reactive) settler model of Takács et al. (1991). In the second approach (A2), A1 was modified including nitrite as a new state variable, considering nitrification and denitrification as two-step processes (see Supplementary Information S2 for the complete stoichiometric and kinetic description of the model). Once nitrite is considered, two alternative electron acceptors (nitrate and nitrite) are present for denitrification. Hence, a mixed substrate approach was used similar to the ASM2d mixed substrate implementation for acetate \((S_A)\) versus fermentable COD \((S_F)\) in biological carbon removal processes (i.e. including a \(\text{SNO}_2/(\text{SNO}_2+\text{SNO}_3)\) reduction term in the nitrite degradation rate and a \(\text{SNO}_3/(\text{SNO}_2+\text{SNO}_3)\) term in the nitrate degradation rate) (Sin and Vanrolleghem, 2006). The third approach (A3) aimed to introduce the reactive settler concept to consider biotransformations of both soluble and particulate compounds during the settling process. The full set of equations used in A2 was therefore considered in the settler, where each layer was simulated as a CSTR (Gernaey et al., 2005). However, it is known that this approach results in an overestimation of the reactive capacity of the settler since mass transfer problems or limitations (i.e. concentration gradients or preferential pathways) are not considered. For that reason, a fourth approach (A4) was proposed to describe such settler limitations by adding a reduction factor to the kinetics in the settler. The value of such reduction factor was 0.25, which was determined in order to obtain a denitrifying capacity similar to the one which was experimentally observed in real settlers (See Supplementary Information S3.).
All the simulations were conducted in accordance to benchmarking principles (Jeppsson et al., 2007): 300 days simulation to reach steady state using predefined constant influent data, then 609 days of long term dynamic influent. Only the last 364 days were used for evaluation and comparison purposes. The influent profile was generated following the principles outlined in Gernaey et al. (2011). All the plant configurations / mathematical models were simulated with identical influent flow rate (with an average value of 20648 m$^3$·d$^{-1}$) and pollutant loads in terms of COD (12250 kg·d$^{-1}$), N (932 kg·d$^{-1}$) and P (255 kg·d$^{-1}$), which are the default loads created by the influent generator (Gernaey et al., 2011). A daily/yearly temperature variation profile was also considered. The long term (LT) influent included daily, weekly and seasonal changes both in flow rate and pollutant loads. Finally, the influent generator described in Gernaey et al., (2011) was also used to simulate occasional events such as the dilution effect after a rainy period or the first flush of the particulates after a storm. The constant influent represents the average values of the 364-day dynamic input data used for comparison purposes.

2.3. Description of plant performance criteria

2.3.1. Operational cost index (OCI):

The OCI (Equation1) was calculated according to the BSM1 guidelines (Alex et al., 2008). Aeration energy (AE), mixing energy (ME), pumping energy (PE), sludge production (SP) and the external carbon source addition (EC), described below, were considered. AE and PE were calculated considering the new approaches described in the BSM2 since aeration was found to play a major role in the OCI and thus, AE has a significant impact on the evaluation process (Nopens et al., 2010).

$$OCI = AE + ME + PE + 5 \cdot SP + 3 \cdot EC$$  \hspace{1cm} \text{Equation 1}
2.3.2. Influent and effluent quality indexes (IQI and EQI)

\[
\text{IQI or EQI (kgPU·d}^{-1}\text{)}= \frac{1}{1000·t_{\text{total}}} \int_{t_{\text{start}}}^{t_{\text{end}}} \left[ PU_{\text{TSS}}(t) + PU_{\text{COD}}(t) + PU_{\text{BOD}}(t) + PU_{\text{TKN}}(t) + PU_{\text{NOx}}(t) + PU_{\text{TP}}(t) \cdot Q(t) \right] \cdot dt
\]

Equation 2

IQI and EQI (Equation 2) were evaluated similar to Copp (2002) where \( t_{\text{total}} \) is the total evaluation time and \( Q_j \) the influent or effluent flow rate. \( PU_X \) (pollutant units of component X) represents the product between weights \( \beta_X \) and the concentration of the considered pollutant at time (t). The weights \( \beta_X \) suggested by Gernaey and Jørgensen (2004) were used for IQI and EQI evaluation. However, the fact that the ammonium is more harmful for the environment than nitrate or nitrite (Carmango and Alonso, 2006) was also considered and thus, the weights for total Kjeldahl nitrogen (TKN) and for \( \text{NOx} \) were changed from 20 to 30 and from 20 to 10 respectively to take this effect into account (Nopens et al., 2010). Finally, the weight for total phosphorus (TP) was also increased from 20 to 50 in order to favour those plant configurations or operational conditions that resulted in higher bio-P removal.

3. RESULTS AND DISCUSSION

3.1. Nitrogen removal and EBPR performance under different model assumptions

The results obtained in the LT simulation of the A\(^2\)/O configuration are summarised in figure 2 for the four different model assumptions (Table 2). Note that the results for the rest of the plant configurations are included in the Supplementary Information S4. Similar results were obtained for the different plant configurations allowing to highlight the differences in the process performance when the plants are simulated for these four different sets of model assumptions.
3.1.1. Nitrite as state variable

According to figure 2, the predicted effluent total nitrogen (TN) and TP increased after the inclusion of nitrite as new state variable (i.e. comparing A2 simulations with A1). This increase was essentially due to the denitrification kinetics used when the model was extended with nitrite as an intermediary by using the mixed substrate approach (see section 2.2). This assumption indeed results in a lower denitrification capacity when nitrite and nitrate coexist in similar concentrations. For example, when simulating the A^2/O configuration using the A2 approach, nitrite and nitrate concentrations in the ANOX2 were 1.87 mg N-NO_2^-·L^{-1} and 2.59 mg N-NO_3^-·L^{-1}. Thus, the denitrification rate from nitrate to nitrite was reduced by around 42% and the denitrification from nitrite to nitrogen by around 58%, in comparison with the default ASM2d with single step denitrification where mixed substrate terms are not considered in the denitrification rates (i.e. a SNO_2^−/(SNO_2^−+SNO_3^−) and SNO_3^−/(SNO_2^−+SNO_3^−) are not included there). Despite of this behaviour, the mixed substrate approach was chosen for the inclusion of nitrite in ASM2d because is more conservative than considering only substrate limitations (i.e. nitrate limitations in denitratation and nitrite limitations in denitrification). The latter approach may lead to simultaneous nitrite and nitrate reduction and, consequently, to a denitrification rate which is higher than the aerobic respiration, which is incorrect from a bioenergetics point of view (Sin et al., 2008). Hence, two-step denitrification rates will be dependent on both the concentration of nitrite and nitrate under anoxic conditions. Not surprisingly, some studies that considered this mixed substrate approach when modelling experimental data observed that, depending on nitrate or nitrite
concentrations, sometimes denitrification was faster than denitrification and *vice versa* (Sin and Vanrolleghem, 2006; Wett and Rauch, 2003).

Regarding the EBPR process, the lower TN removal capacity after nitrite inclusion obviously resulted in an increase of the NO_{X} concentration in both the effluent and the Q_{EXT}. As could be expected, the increase of NO_{X} in the Q_{EXT} led to a decrease in EBPR activity because part of the carbon source in the anaerobic reactor was used to denitrify instead of for EBPR purposes (Kuba *et al.*, 1994; Cho and Molof, 2004). Similar results (higher N and P effluent concentrations for A2 with respect to A1) were also observed when the other plant configurations were simulated (see Supplementary Information S4. for further details).

### 3.1.2. Importance of considering nitrite to simulate certain operation conditions

A scenario analysis (SCA1) has been conducted to emphasise the importance of nitrite inclusion in the ASM2d. The A2/O configuration was simulated under different dissolved oxygen concentrations (the results for the other plant configurations can be found in the Supplementary Information S6.). In the first case scenario (SCA1-A) the default operation mode (table 1) was maintained while in the second case (SCA1-B) the air supply in AER1 and AER2 was decreased (from k_{L}a = 120 d^{-1} to k_{L}a = 80 d^{-1}).

Figure 3 presents the main results of SCA1 for the A2/O configuration using a constant influent wastewater. As can be observed for SCA1-A, after considering two step nitrification in the ASM2d (grey bars, A2), the effluent nitrate concentration increased again due to the reduction in the denitrification rates when considering the mixed substrates approach to simulate two-step denitrification (see above). When the air supply was reduced (SCA1-B) and nitrite was not considered (black bars, A1), the main nitrification product was nitrate. Contrary, when nitrite was considered in the model
(A2), the ammonium concentration in the effluent decreased when the air supply was reduced and nitrite accumulation was observed. These differences are explained due to the oxygen saturation coefficient used when autotrophic biomass is considered i) as a single group (K_{O2, AUT} = 0.5 g·O_2 m^{-3}) or ii) when it is divided into ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) (K_{O2, AOB} = 0.4 and K_{O2, NOB} = 1.0) (Wett and Rauch, 2003). The lower value for AOB explains a higher nitrification rate when A2 was used as the model, and as such resulted in a decrease of the ammonium concentration compared to the standard ASM2d (A1). The fact that both AOB and NOB have two different oxygen affinities also explains the fact that a nitrite accumulation was observed in A2. The dissolved oxygen concentration in the aerobic reactors was indeed always below 0.5 mg·L^{-1} in SCA1-B, so nitritation was favoured instead of the nitratation process (i.e. NOB were almost washed out from the system). Although it cannot be directly distinguished with the depicted results, the competition of PAO and OHO for the carbon source was also affected by the inclusion of nitrite. It is well-known that denitritation requires less COD per unit of nitrogen removed (around 40%) than denitratation (Seyfried et al., 2001). Therefore, when nitrite is the main nitrification product, less COD is required in the anaerobic phase to denitrify the nitrite inlet and then, more COD is available for the EBPR process (i.e. the PAO population in AER3 for SCA1-B increased from 943 mg COD·L^{-1} using A1 to 1180 mg COD·L^{-1} using A2). Similar results were also observed when this scenario was simulated for the other plant configurations (see Supplementary Information S6. for further details).

These results demonstrate the importance of including nitrite in the ASM2d to achieve a better description of all the processes where nitrogen species take part and to avoid the simulation of potentially non-realistic behaviour at certain operational
conditions (i.e. nitrification failures under low oxygen conditions). Moreover, this nitrite inclusion opens new possibilities in terms of developing operational strategies that can result in high energy savings by decreasing the aeration requirements and the decrease of COD demand for denitrification.

3.1.3. Biological reactions in the secondary settler

When a reactive settler was simulated (A3), part of the NO$_X$ was denitrified in the bottom of the secondary clarifier leading to a decrease of the NO$_X$ present in the Q$_{\text{EXT}}$ and in the effluent (Figure 2). P-removal was obviously improved (A2 versus A3 in figure 2) because less COD was consumed for denitrification in the anaerobic reactor, and thus became available for EBPR. Hence, the tremendous impact of considering the reactive settler approach when modelling EBPR processes was proved. Although modelling a reactive settler as a series of 10 CSTR is a good approximation to what really happens in full-scale settlers, the reactive capacity will be overestimated (i.e. mass transfer/diffusion limitations are not considered) leading to an unrealistic predicted EBPR activity due to the low nitrate concentrations obtained for the inlet of the anaerobic zone (Flores-Alsina et al., 2012). Limiting the reaction rates in the reactive settler should result in more realistic results. For this reason a last test was run (A4), essentially multiplying the biological reaction rates in the settler with a reduction factor. Different values for this factor were considered (see Supplementary Information S3.) in order to mimic the denitrification capacity observed in full-scale settlers. When the reduction factor was 0.25, the denitrifying capacity in the settler was around 17% of the TN denitrified in the system. This value is similar to the capacity reported for a real WWTP (Siegrist et al., 1995) and it was therefore kept during all the rest of the simulation study. When the approaches A3 and A4 are compared (non-limited and
diffusion limited settler, respectively), less optimistic denitrification rates in the bottom of the clarifier were obtained for scenario A4. As a result, there was a higher P concentration in the effluent since the amount of NO\textsubscript{X} entering into the anaerobic phase via Q\textsubscript{EXT} was higher. This reduction in the denitrification process efficiency was also evident in the fact that the TN concentration (mainly NO\textsubscript{X}) also increased in the effluent (Figure 2). On the contrary, a slight improvement of P-removal was still observed for A4 compared to A2 (non-reactive settler). Based on these results, it was concluded that it is important to consider the denitrifying capacity of the settler to properly describe EBPR in BNR. A similar behaviour was observed for the other studied WWTP configurations (see Supplementary Information S4. for further details).

3.1.4. Importance of considering reactive settler under certain operation conditions

To gain more insights about the importance of considering a reactive settler, a new scenario analysis is presented to study its effect on the overall BNR processes. Figure 4 presents the 10-layer settler model profiles for the A\textsuperscript{2}/O configuration (the results for the other plant configurations can be found in the Supplementary Information S7.). As can be observed, no big differences on the removal efficiency of the plant were obtained when comparing the reactive and non reactive settler approach for the default A\textsuperscript{2}/O operation (SCA2-A). Only the absence of oxygen in the bottom layers of the settler produced a small decrease of nitrate linked to an increase of nitrite. As can also be observed in figure 4, such behaviour became even clearer when no reactive limitations were considered in the settler (A3). The fast oxygen consumption in the lower layers of the settler highly favoured denitrification processes but the low COD available resulted in incomplete nitrate denitrification and thus, nitrite accumulation. However, the fact of considering each layer as a CSTR resulted in an overestimation of
the intensity of the processes occurring in the settler. When A3 was considered, the
denitrification capacity of the settler was around 43% of the TN denitrified in the
system, which is disproportionate when is compared to the 15% reported in the
literature for full-scale settlers (Siegrist et al., 1995).

A greater effect of the reactive settler (A3 and A4) can be observed in other
scenarios with a higher loaded WWTP. For that reason, a new constant influent was
simulated where the influent flow rate of the plant was increased by 25 % (SCA2-B)
compared to the default value (20648 m³·d⁻¹). Despite the decrease of the hydraulic
retention time in the settler (from 3.5h to 3.1h), the increase of the load resulted in an
increase of the biomass concentration in the system (the purge flow-rate was not
increased) and thus, higher reactivity in the settler was observed (figure 4). The limited
reactive settler (grey dots, A4) denitrified most of the nitrate in the lower layers after
oxygen depletion, thus providing an extra anoxic volume. In addition, it is important to
note that nitrite inclusion in the model also allowed describing nitrite occurrence in the
lower layers of the settler due to an incomplete denitrification process. Despite this
nitrite increase, less NOₓ entered in the anaerobic phase and thus, more COD was
available for the PAO, improving EBPR process compared to non-reactive settler
results (black dots, A2). Comparing SCA2-A to B, the flow rate increase resulted in a
lower oxygen concentration in AER3 and thus in a lower oxygen flux entering in the
settler (figure 4). This lower oxygen inlet increased the anoxic conditions in the settler
and then some denitrification activity was observed in the upper layers compared to
SCA1-A, where total oxygen depletion and denitrification only occurred in the lower
layers of the settler. Once again, not limiting the reactive capacity in the settler (A3)
resulted in an overestimation of the processes occurring in the settler (e.g. total NO$_X$ depletion or high ammonium production due to biomass decay in the lower layers).

These results demonstrated the importance of considering a reactive settler approach with systems with high biomass content and a high degree of anoxic conditions in the settler (SCA2-B); on the contrary, the traditional assumption of non-reactive settler (Takács et al., 1991) seems to be enough for describing the settling process in systems with a relatively low biomass content. Note as well that the reactive settler considered here includes a correction factor to easily simulate diffusion limitations in reaction rates (A4), resulting in more realistic results. This approach could be further extended by considering the effect of other physical parameters (e.g. an increase of settler inflow) on the diffusion limitations (i.e. the reduction factor estimate could be made flow rate dependent). Similar trends were also observed for the other plant configurations (see Supplementary Information S7.).

3.2. EBPR behaviour under different plant configurations

Taking the conventional A$^2$/O as a reference, this section compares alternative configurations (BDP-5stage, JHB, UCT and MUCT, see figure 1) that have been proposed to minimise the detrimental effect on EBPR of NO$_X$ entering the anaerobic phase. Based on the previous results, the inclusion of nitrite in AMS2d and the assumption of a diffusion-limited reactive settler (approach A4) were proved to be necessary to obtain a more realistic description of the BNR processes and thus, this approach was used for these simulations. As was mentioned above, this is the first study where benchmark simulations have been conducted using these plant configurations. Figure 5 shows the main results obtained for the long term plant operation (364 days).
As can be observed in figure 5, the effluent TN concentrations were below the N discharge level for all the configurations, providing the A\textsuperscript{2}/O plant the lower TN level (12.13 mg N·L\textsuperscript{-1}). On the other hand the TP effluent concentrations were all above the P discharge limit (1.5 mg P·L\textsuperscript{-1}). In this case, MUCT and JHB yielded the lower effluent P concentrations (3.69 and 4.61 mg P·L\textsuperscript{-1}, respectively) at the expense of a higher effluent TN (15.24 and 14.40 mg N·L\textsuperscript{-1}), as is also pointed out in Henze et al. (2008) and in Van Haandel and Van der Lubbe (2007). This is mainly because these configurations minimise the arrival of nitrate to the anaerobic section (and are thus favouring P release by PAO). For the MUCT and JHB plants, the purpose of the ANOX1 compartment is to denitrify the NO\textsubscript{X} from Q\textsubscript{EXT} before entering the anaerobic tank, while the ANOX2 compartment was only used to denitrify NO\textsubscript{X} from Q\textsubscript{INT}. However, on the basis of the simulations, it can be concluded that the denitrifying capacity was not fully exploited since ANOX1 was oversized considering the low NO\textsubscript{X} load originating from Q\textsubscript{EXT}, whereas ANOX2 was overloaded to denitrify the NO\textsubscript{X} fed by the Q\textsubscript{INT}.

In the A\textsuperscript{2}/O configuration on the contrary, a lower effluent NO\textsubscript{X} concentration was observed because both ANOX1 and ANOX2 were used to denitrify the NO\textsubscript{X} from the Q\textsubscript{INT} instead of only ANOX2 as occurred in the JHB and MUCT configurations. For example the NO\textsubscript{X} concentration at the end of the JHB-ANOX2 was 6.68 mg N·L\textsuperscript{-1} while it was 4.46 mg N·L\textsuperscript{-1} for A\textsuperscript{2}/O-ANOX2. Thus, it can be concluded that MUCT and JHB plants give the PAO a competitive advantage compared to denitrifying bacteria, since more of the influent carbon source was channelled in to the EBPR processes.
The UCT plant showed the highest effluent P concentration (Figure 5) contrary to what was expected taking into account that it is one of the most often reported configurations used to prevent NO\textsubscript{X} presence in the anaerobic reactor (Rabinowitz and Marais, 1980; Henze et al., 2008). UCT plant simulation results revealed that total NO\textsubscript{X} depletion was not achieved at the end of the anoxic phase (5.08 mg N·L\textsuperscript{-1}) favouring denitrification instead of P release in the anaerobic reactors. This fact is in agreement with statements made in some engineering manuals (Henze et al., 2008; Metcalf and Eddy, 2003) that pointed out that total anoxic NO\textsubscript{X} denitrification is critical to achieve high biological P removal in the UCT plant. This issue is tackled by the MUCT, which separates the Q\textsubscript{EXT} and Q\textsubscript{INT} inlet points at the expense of decreasing even more the TN removal capacity.

Finally, the BDP 5-stage resulted in a high effluent P (Figure 5). This could be explained due to the location of ANOX2 in this configuration, which was placed after the AER2 and before AER3 (Figure 1). Thus, the Q\textsubscript{INT} only fed the ANOX1, resulting in less denitrifying capacity mainly for two reasons: i) a reduction of the anoxic volume to denitrify NO\textsubscript{X} brought by the Q\textsubscript{INT} (similar to what occurred for JHB and MUCT); and, ii) the low COD available for denitrification that entered into ANOX2 after the aerobic phase (e.g. NO\textsubscript{X} concentration only decreased from 12.77 to 10.29 mg N·L\textsuperscript{-1} in such a reactor). As reported by Van Haandel and Van der Lubbe (2007), the BDP-5 stage configuration can perform well with high P-removal as long as sufficient denitrification is ensured in the second anoxic reactor. Otherwise the system is not capable to prevent nitrate to enter in the anaerobic reactor. Barnard (1976) and Osborn and Nicholls (1978) reported some examples of this problem in a BDP-5 stage pilot.
plant. To solve this problem, external carbon dosage could be introduced in ANOX2 to ensure sufficiently high COD levels to allow such denitrification.

Figure 6 (black bars) presents the summary of the simulation results for the different plant configurations in terms of benchmarking criteria. The configuration with the best EQI was MUCT (9108 kg PU·d\(^{-1}\)) and that with the lowest OCI was BDP-5 stage (15986 kg PU·d\(^{-1}\)). As can be observed, the configurations with the best removal capacity presented also the highest operational cost (i.e. a decrease in the EQI leads to an increased OCI) and vice versa. These differences were directly related to the sludge production and its processing cost (Figure 6 C). The higher BNR efficiencies in the JHB and MUCT plants (15% and 20% less than the EQI for \(A_2^2/O\)) also resulted in a higher solids production (172 kg·d\(^{-1}\) more for JHB and 271 kg·d\(^{-1}\) more for MUCT, compared to the amount of SP for \(A_2^2/O\), the reference configuration) and thus, higher costs associated to solid processing. These results demonstrate clearly that effluent quality and the operating costs need to be traded off against each other. Such an observation has been made in several studies (Jeppsson et al., 2007; Alex et al., 2008; Guerrero et al., 2012).

3.2.1. Effect of carbon addition for the different WWTP configurations

Regarding P removal, the simulation results show that none of the plant configurations met the legal effluent P discharge limit of 1.5 mg P·L\(^{-1}\) (Figure 5). This is mainly because of the low COD content in the wastewater, and the competition between PAO and OHO for the electron donor in the anaerobic reactor. A conventional solution in real systems is the addition of an external carbon source in the ANAE1 tank in order to provide a supplementary amount of readily biodegradable organic matter for EBPR and denitrifying processes (Olsson et al., 2005). In the last scenario analysis
(SCA3), the necessary quantity of external carbon source (simulated as $S_A$) was calculated to obtain an average effluent TP concentration of $1.50 \pm 0.03 \text{ mg·L}^{-1}$ (see Supplementary Information S8.). Results showed that the MUCT configuration required the lowest amount of external carbon source ($612 \text{ kg·d}^{-1}$) whereas the BDP-5 stage required the highest amount ($2000 \text{ kg·d}^{-1}$) in order to reduce the effluent TP concentration to the effluent limit for P. These results were in agreement with the fact that MUCT favoured the EBPR process and thus achieved the highest P removal efficiency whereas BDP-5 stage favoured OHO denitrification and achieved the worst P removal efficiency (Figure 5). The external carbon addition reduced the effluent pollutant content resulting in similar EQI results for all the plant configurations (Figure 6A). When no carbon source was added, effluent TP played a major role in the EQI calculation favouring the MUCT and JHB configurations (black bars). However, when carbon source was added, the effluent phosphorus concentration was drastically reduced and TN caused the main differences in the EQI values (white bars). Thus, in this case JHB and MUCT achieved the highest EQI values (Figure 6A) due to a higher effluent TN concentration ($11.74$ and $12.64 \text{ mg N·L}^{-1}$, respectively). When it comes to OCI criteria, using an external carbon source reduced the effluent pollutant loads but at the expense of an increasing OCI (Figure 6B). The fact of adding external carbon source implies a cost (Equation 1) and thus, the configurations that required higher carbon addition obviously also resulted in higher OCI values. The external carbon addition also resulted in an increase of the SP (Figure 6C), which also contributed to increased OCI values due to the considerable sludge processing cost. The BDP-5 stage plant resulted in the highest OCI value not only because more external carbon source addition was required to meet the P limit, but also because it resulted in a higher SP.
If EQI and OCI are considered simultaneously, A²/O can be considered the most balanced plant configuration. This configuration did not require any excessive carbon source addition (920 kg·d⁻¹) to meet P discharge limits and it presented lower OCI values than BDP-5-stage or UCT plants (Figure 6B white bars). In addition, the EQI value was one of the lowest obtained (6070 kg PU·d⁻¹) due to the low effluent TN obtained for the A²/O plant, in contrast to the MUCT or JHB plants. The latter result gains more importance taking into account that in the last years the TN discharge limit has become stricter, for example 10 mg N·L⁻¹ according to the Council Directive 91/271/EEC. If this directive was applied, A²/O would be considered the best plant configuration because the TN values for MUCT and JHB (11.74 and 12.64 mg N·L⁻¹, respectively) would be above the discharge limit.

3.3. Practical implications of the study

The list of major (practical) implications extracted from this study can be summarised as follows:

- The inclusion of nitrite allows a better description of N removal in systems with low aeration because it could avoid the prediction of potential nitrification failure. Instead of that, partial nitrification to nitrite is described in those cases. Moreover, inclusion of nitrite allows a better accounting of the organic matter needed to denitrify (i.e. denitritation requires less COD than denitratation), which enables a better description and understanding of the competition between PAO and OHO for the carbon source, especially in systems with carbon shortage.

- The reactive settler approach with a diffusion-limitation factor (0.25) allowed a more realistic description of the settling process in terms of biological process rates that can be achieved in settlers. If the assumption of a reactive settler model is not considered,
the real denitrifying capacity of the system is not reflected and a wrong EBPR failure could be predicted (anaerobic NO\textsubscript{X} in the inlet is overestimated). On the contrary, not limiting the reactive settler due to diffusion limitation could result in unrealistically high denitrification rates. In addition, the consideration of reactive settler gains importance in systems with high biomass content because of the higher reactivity of the settler. On the contrary, in systems with low biomass content, only physical processes could be used to simulate settling phenomena.

- The NO\textsubscript{X} presence under anaerobic conditions played an important role on EBPR performance. When NO\textsubscript{X} is present under anaerobic conditions OHO outcompete PAO for the carbon source. Therefore, those configurations that reduced the NO\textsubscript{X} in the inlet to the anaerobic reactor resulted in the highest TP removal (MUCT and JHB) while in the rest, OHO denitrification was favoured instead of EBPR.

- Finally, the results presented establish a comparative basis that can be used in future research studies. It is important to note that the operational limits of each configuration are not presented in this study. Hence, future work could be conducted on the volume optimisation for each reactor or the implementation of different control strategies since they have been reported as promising alternatives to improve WWTP operation (Nopens et al., 2010; Gernaey et al., 2013).

4. CONCLUSIONS

The improvement provided by the nitrite inclusion in the ASM2d model was clearly demonstrated, avoiding the prediction of N removal failure in systems with low aeration. Diffusion-limited reactive settler model also allowed a more realistic description of the settling process and thus, the settler reactivity was not overestimated.
Regarding the effect of the plant configurations on biological C/N/P removal, the highest biological P removal was obtained for JHB and MUCT (65% and 55%, respectively). UCT and BDP-5-stage configurations resulted in the lowest TP removal because high amounts of NO\(_X\) entered the anaerobic zone, favouring OHO denitrification instead of EBPR.

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Table 1 Characteristics for the plant configurations

| Parameters                      | Values                           |
|---------------------------------|----------------------------------|
| Reactor volumes                 |                                  |
| Anaerobic, ANAE 1 and 2         | 1250 m$^3$                      |
| Anoxic, ANOX 1 and 2            | 1500 m$^3$                      |
| Aerobic, AER 1, 2 and 3         | 3000 m$^3$                      |
| $k_L$ for AER 1, 2 and 3        | 120 d$^{-1}$, 120 d$^{-1}$ and 60 d$^{-1}$ |
| Influent (Average flow-rate)    | 20648 m$^3$·d$^{-1}$            |
| Internal recycle, $Q_{INT}$     | 61944 m$^3$·d$^{-1}$ (300% Influent) |
| External recycle, $Q_{EXT}$     | 20648 m$^3$·d$^{-1}$ (100% Influent) |
| Anaerobic recycle, $Q_{ANAE}$*  | 41296 m$^3$·d$^{-1}$ (200% Influent) |
| Influent bypass, IB **          | 6814 m$^3$·d$^{-1}$ (33% Influent) |
| Waste sludge, $Q_w$             | 385 m$^3$·d$^{-1}$              |

* UCT and MUCT  **JHB
Table 2 Summary of the modelling approaches studied in this work.

| Approach | ASM2d | ASM2d + Nitrite Inclusion | Reactive Settler | Limited reactive settler |
|----------|-------|---------------------------|------------------|--------------------------|
| A1       | X     |                           |                  |                          |
| A2       |       | X                         |                  |                          |
| A3       |       | X                         | X                |                          |
| A4       |       | X                         |                  | X                        |
Figure 1 Plant configurations for simultaneous C/N/P removal: A²/O, BDP-5stage, UCT, MUCT and JHB. Inf: Influent, Eff: Effluent, Q_w: Waste sludge or purge and IB: Influent bypass.
Figure 2 Average effluent concentrations obtained for the four model assumptions studied with the A^2/O configuration, compared to the discharge levels.
Figure 3 Effluent concentrations obtained for SCA1-A ($k_{L,a}$ AER 1 and 2 = 120 d$^{-1}$) and SCA1-B ($k_{L,a}$ AER 1 and 2 = 80 d$^{-1}$) in the A$^2$/O plant configuration when the nitrification / denitrification are described as single (approach A1, black) or two step processes (approach A2, grey). $S_{NH4}$ corresponds to ammonium nitrogen, $S_{NO3}$ to nitrate nitrogen, $S_{NO2}$ to nitrite nitrogen and $S_{PO4}$ to orthophosphate phosphorus.
Figure 4 Effect of the 25% influent flow rate increase on $S_{O_2}$, $S_{RBS}$ ($S_A+S_P$), $S_{NH4}$, $S_{NO3}$, $S_{NO2}$ and $S_{PO4}$ for the A$^2$/O configuration. The non-reactive secondary settler (A2) is compared with a reactive settler (A3) or a diffusion-limited reactive settler (A4). Black circles correspond to A2, open circles correspond to A3 and grey circles to A4. $S_{O2}$ corresponds to dissolved oxygen, $S_{RBS}$ to readily biodegradable organic substrates, $S_{NH4}$ to ammonium nitrogen, $S_{NO3}$ to nitrate nitrogen, $S_{NO2}$ to nitrite nitrogen and $S_{PO4}$ to orthophosphate phosphorus.
Figure 5: Average effluent concentrations obtained for the different plant configurations under long-term conditions.
Figure 6 Simulations results for the five plant configurations without carbon source addition (black) and when adding an external carbon source to achieve 1.5 mg·L⁻¹ P-PO₄³⁻ in the effluent (white).