Limited simultaneous nitrification-denitrification (SND) in aerobic granular sludge systems treating municipal wastewater: Mechanisms and practical implications

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ABSTRACT

Simultaneous nitrification-denitrification (SND) is, in theory, a key advantage of aerobic granular sludge systems over conventional activated sludge systems. But practical experience and literature suggests that SND and thus total nitrogen removal are limited during treatment of municipal wastewater using AGS systems. This study thus aims at quantifying the extent and understanding the mechanisms of SND during treatment of municipal wastewater with aerobic granular sludge (AGS) systems. Experiments (long-term and batch-tests) as well as mathematical modelling were performed. Our experimental results demonstrate that SND is significantly limited during treatment of low-strength municipal wastewater with AGS systems (14–39%), while almost full SND is observed when treating synthetic influent containing only diffusible substrate (90%). Our simulations demonstrate that the main mechanisms behind limited SND are (1) the dynamics of anoxic zone formation inside the granule, (2) the diffusibility and availability of electron-donors in those zones and (3) the aeration mode. The development of anoxic zones is driven by the utilisation of oxygen in the upper layers of the granule leading to transport limitations of oxygen inside the granule; this effect is closely linked to granule size and wastewater composition. Development of anoxic zones during the aerobic phase is limited for small granules at constant aeration at bulk dissolved oxygen (DO) concentration of 2 mgO2 L−1, and anoxic zones only develop during a brief period of the aerated phase for large granules. Modelling results further indicate that a large fraction of electron-donors are actually utilised in aerobic rather than anoxic redox zones in the bulk or at the granule surface. Thus, full SND cannot be achieved with AGS treating low strength municipal wastewater if a constant DO is maintained during the aeration phase. Optimised aeration strategies are therefore required. 2-step and alternating aeration are tested successfully using mathematical modelling and increase TN removal to 40–79%, without compromising nitrification, and by shifting electron-donor utilisation towards anoxic redox conditions.

1. Introduction

van Loosdrecht and Brdjanovic, 2014 In AGS systems, mass transfer is limited by diffusion, which leads to concentration gradients of electron-donors/final acceptors within the granules. During the aerated phase, an oxygen gradient develops within the granules whereby the outer layers are aerobic and the inner core is anoxic or anaerobic (De Kreuk et al., 2007a). Those different redox conditions within the granules allow nitrifying, denitrifying and facultative anaerobic organisms to coexist (Winkler et al., 2013). As a result, it is usually well accepted that simultaneous nitrification and denitrification (SND) is a key feature of AGS and that SND is the main nitrogen removal pathway in AGS systems (De Kreuk et al., 2005; Adav et al., 2008; Pronk et al., 2015).

In theory, complete total nitrogen (TN) removal via SND could be achieved in one single reactor and within a single aerobic phase of the SBR cycle. However, when analysing data from literature, it is less evident that high TN removal via SND occurs in AGS systems.
treating municipal wastewater (MWW) (Fig. 1). SND efficiencies reported for lab-scale AGS systems fed with synthetic influent (mostly volatile fatty acids, VFA) are highly variable. Values ranging from 10 to 100% are reported for different dissolved oxygen (DO) concentrations in the bulk liquid (Kocaturk and Erguder, 2016; De Kreuk et al., 2005; Lochmatter et al., 2013). But for those systems, high SND efficiencies of more than 75% are typically observed for DO values below 4 mgO₂ L⁻¹ (Fig. 1). In AGS systems fed with MWW, SND efficiencies smaller than 50% on average are reported (Fig. 1) (Pronk et al., 2015; De Kreuk and Van Loosdrecht, 2006; Świątczak and Cydzik-Kwiatkowska, 2018; Lashkarizadeh et al., 2015; Liu et al., 2010; Ni et al., 2009; Wagner et al., 2015). High variability of SND and/or low efficiencies lead to high TN and NO₃ effluent concentrations in the case of AGS-SBR operation without explicit anoxic phases. Low SND and TN removal of AGS is problematic in areas with stringent treatment requirements. It is therefore crucial to understand the extent and mechanisms of SND in order to further optimise TN removal of AGS systems.

SND requires (1) simultaneous occurrence of aerobic (for nitrification) and anoxic (for denitrification) redox conditions, and (2) electron-donor availability in anoxic redox conditions (for denitrification). Fig. 1 illustrates both effects. Lower DO concentrations generally tend to increase SND performances in both activated sludge and AGS systems (Pochana and Keller, 1999; Zeng et al., 2003; Third et al., 2003; He et al., 2017, 2019). In activated sludge flocs, anoxic micro-zones form due to high oxygen utilisation rates at the surface of the flocs (Li and Bishop, 2004), or by maintaining a bulk dissolved oxygen (DO) concentrations below the oxygen half-saturation constant (KO₂) of denitrifying organisms (Daigger et al., 2007). Operation of activated sludge system at a DO set-point below KO₂ thus results in SND without strict anoxic conditions. In comparison to activated sludge, larger SND efficiencies are observed for AGS systems across all DO concentrations (Fig. 1, green and orange dots). Higher SND in AGS systems results from the formation of anoxic zones inside the granules, which is driven by the limited diffusion of oxygen and simultaneous diffusion/production of NO₃. Another important observation is the distinct SND efficiency of AGS fed by synthetic WW – mostly composed of readily available VFA – vs. real MWW (Fig. 1). Indeed, the type of electron-donor and its availability partially determines SND efficiency (Pochana and Keller, 1999). Therefore, the distinct effects of the electron-donor availability, type and anoxic zone formation on SND in AGS systems need to be clarified.

AGS systems treating low strength MWW are typically characterized by slower start-up, more heterogeneous granule sizes, lower granule fractions, and an increased floc fraction in comparison to VFA-only WW fed AGS (Layer et al., 2019). The size of granules vary from d = 0.5 mm (or smaller) after few months (Ni et al., 2009; Liu et al., 2010; Wagner et al., 2015; Layer et al., 2019) up to d > 2 mm after few years of operation (Prónk et al., 2015). The granule size, together with the penetration depth of O₂ in theory determines the extent of anoxic zone formation inside the deeper layers of the granule (Li et al., 2008). If the granule diameter impacts the penetration of O₂, it is then key to evaluate the mechanisms of SND for both small (several hundred μm) and

Fig. 1. SND efficiencies for different DO bulk concentrations reported in literature for activated sludge systems and aerobic granular sludge fed with synthetic acetate/propionate based influent (AGS synthetic) or MWW (AGS municipal WW) (Mosquera-Corral et al., 2005; Kishida et al., 2006; Lochmatter et al., 2013; Kocaturk and Erguder, 2016; Isanta et al., 2012; He et al., 2018; He et al., 2019; He et al., 2017; Wang et al., 2015; Semerci and Haselc, 2016; Rollemberg et al., 2019; Prónk et al., 2015; Wang et al., 2009; De Kreuk and Van Loosdrecht, 2006; Liu et al., 2011; Świątczak and Cydzik-Kwiatkowska, 2018; Derlon et al., 2016; Lashkarizadeh et al., 2015; Liu et al., 2010; Ni et al., 2009; Wagner et al., 2015; Pochana and Keller, 1999; Third et al., 2003; Zeng et al., 2003; Wang et al., 2015; Lo et al., 2010; Marin et al., 2019). Data on SND were collected directly from literature (whenever given), or calculated from batch-test data or in-cycle concentration profiles of N-species (Supplementary Information S1).
large (several mm) granules, representative of young and mature granules, respectively. The extent of anoxic zones in smaller granules might thus limit SND compared to larger granules. Another determinant of the extent of SND is the availability of (diffusible) electron-donors in the anoxic zones. Municipal WW contains a large fraction of electron-donors in the non-diffusible particulate form ($X_p$), typically representing 50% of the total chemical oxygen demand (COD) (Metcalf and Eddy, 2014). If most of the electron-donors contained in MWW are not diffusible, it is then hypothesized that denitrification might also be limited during treatment of MWW. Understanding the distinct effects of the WW compositions and granule sizes on anoxic zone formation and electron-donor availability, and in turn on the SND and TN removal in AGS systems is therefore crucial.

The objectives of this study were therefore (1) to experimentally assess that SND and thus TN-removal is limited during treatment of low-strength MWW in comparison to 100%-VFA synthetic WW and (2) to then identify which mechanisms limit the extent of SND, e.g., the dynamic of anoxic zone formation, the availability of different electron-donors inside the granules, and (3) to identify how to improve SND and TN removal by optimising the aeration strategies in AGS systems (2-step aeration, alternating aeration). Both experiments and mathematical modelling were conducted. SND and TN removal were quantified during long-term and batch experiments for different AGS systems fed with different WWV to better understand the influence of DO, influent WW and sludge composition on the SND efficiency. An AGS model was then used to identify the effect of (1) electron-donor availability and contribution to SND and (2) anoxic zone formation inside the granules. The AGS model was then used to evaluate different aeration strategies in order to maximize TN removal.

2. Materials and methods

2.1. Experimental approach and reactor configuration

AGS were cultivated in 13 L column SBRs fed with 100%-VFA synthetic (R1), complex synthetic (R2), primary effluent (R3) and raw WW (R4), respectively (Layer et al., 2019). Influent composition in terms of electron-donor was either very simple (only soluble and highly diffusible organic acids) (R1), or increasingly complex in terms of electron-donor composition (R2, R3 and R4) (Table 1). All systems were operated at constant volume and SBR cycles were as follows: anaerobic plug-flow feeding (1.5 h), aerobic phase (4 h), settling (variable time), and excess sludge removal after settling. The total cycle length was 5.6 h. The DO concentration during the aerobic phase was controlled at a set-point of 2 mg O$_2$ L$^{-1}$. The reactor model used in this study is an SBR. The SBR sequence set in the model was similar to the experimental one. All process steps were modelled in fully-mixed conditions. Effluent total suspended solids (TSS) were set to 20 mg L$^{-1}$, in the range of the values measured experimentally (Layer et al., 2019). A solid retention time (SRT) of 20 d was maintained, in accordance to other AGS studies (De Kreuk et al., 2007b; Ni and Yu, 2010; Layer et al., 2019). SRT ($d$) was calculated based on Equ. (1).

$$\text{SRT}_{\text{target}} = \frac{\text{TSS}_{\text{f}} \cdot V_{f}}{\text{TSS}_{\text{eff}} \cdot Q_{\text{eff}} + \text{TSS}_{\text{bulk}} \cdot Q_{\text{ex}}}$$

(1)

TSS$_f$ is the TSS concentration in the reactor (gTSS L$^{-1}$), $V_f$ is the reactor volume (L), TSS$_{eff}$ is the TSS concentration in the effluent (gTSS L$^{-1}$), $Q_{eff}$ is the effluent flow rate (L d$^{-1}$), TSS$_{bulk}$ is the TSS concentration in the bulk compartment (gTSS L$^{-1}$) and $Q_{ex}$ is the excess sludge flow rate (L d$^{-1}$). $Q_{ex}$ was automatically calculated based on SRT$_{target} = 20$ d. Excess sludge is only withdrawn from the bulk compartment. Supplementary Information S2 provides detailed information on the reactor model.

Specific model adaptations: Granules of diameters of 0.5 mm (young MWW granules) and 2.0 mm (mature VFA granules) were considered, in accordance with our experimental results collected.
over 1 year of operation (Table 1, Layer et al., 2019). Granule size of $d = 2.0 \text{ mm}$ for MWW (mature granules) was also selected in accordance with literature (Pronk et al., 2015). The individual layer thickness of the granule were set to $25 \text{ mm}$ for all layers ($d = 0.5 \text{ mm}$) or $25 \text{ mm}$ for the 4 outer layers and $150 \text{ mm}$ for the residual 6 inner granule layers ($d = 2.0 \text{ mm}$). Decreasing the thickness of the 4 outer layers of the large granules increases the resolution in those layers, which is required to best predict the concentration gradients and redox conditions within the granules. For data plotting and interpretation, the distinction between aerobic, anoxic and anaerobic redox conditions is based on the half-saturation constants of growth on $O_2$ and $NO_3$ of $OHO$ of the biokinetic model (Table 2).

### 2.2.2. Modelling scenarios

Different modelling scenario were performed to (1) validate the overall performance of the model by comparing them to the batch test experiments (batch test scenarios), (2) understand the mechanisms of SND in AGS systems (mechanism scenarios), and (3) evaluate different optimised aeration strategies to maximize TN

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**Table 1**

| Reactor | 100%-VFA synthetic WW | complex synthetic WW | primary effluent WW | raw WW |
|---------|-----------------------|----------------------|---------------------|--------|
| R1      | Synthetic influent (50% acetate, 50% propionate) | Synthetic influent (33% VFA, 33% fermentable substrate, 33% particulate substrate) | Low-strength municipal WW after primary sedimentation | Raw low-strength municipal WW |
| R2      | Total COD [mgCOD L$^{-1}$] 582 | Soluble COD [mgCOD L$^{-1}$] 582 | 331 | 469 |
| R3      | Soluble COD/particulate COD-ratio [ ] | 9.9 | 1.3 | 1.1 |
| R4      | TN [mgN L$^{-1}$] 43 | NH$_4$-N [mgN L$^{-1}$] 40 | 33 | 41 |
|         | Total COD/TN-ratio [mgCOD mgN$^{-1}$] 13.5 | 20 | 24 | 29 |
|         | TP [mgP L$^{-1}$] 5.4 | PO$_4$-P [mgP L$^{-1}$] 5.0 | 3.3 | 4.4 |
|         | Sludge loading rate [kg totalCOD kgVSS$^{-1}$ d$^{-1}$] 0.26 | 0.21 | 0.26 | 0.26 |
|         | Sludge volume index after 30 min [SVI$_{10}$] [mL gTSS$^{-1}$] 43 | 51 | 84 | 65 |
|         | Granule fraction $d > 0.25 \text{ mm}$ 93% | 63% | 61% | 74% |
|         | Typical granule diameter after 1–3 year$^c$ 0.25–0.63 mm | 0.25–0.63 mm | 0.25–0.63 mm |

$^a$ Detailed WW characterization can be found in Layer et al. (2019).
$^b$ Long-term average, not considering the start-up phase.
$^c$ Based on biomass size-fractions (Layer et al., 2019).

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Fig. 2. Conceptual model of the biofilm and biokinetic model used in this study. The compartments are composed of bulk and biofilm (granule) layers 1 to 10. The biofilm models mass balances based on the mass-transfer mechanisms (1) diffusion of soluble and colloidal compounds (S and C), (2) displacement of Xi, (3) attachment of Xi and (4) internal transfer of Xi. The biokinetic model is active in all compartments.
Table 2
Conditions of DO and NOx-N that were defined as aerobic, anoxic and anaerobic conditions during modeling.

| Redox condition | DO [mgO2 L⁻¹] | NOx-N [mg NOx-N L⁻¹] |
|-----------------|---------------|----------------------|
| Aerobic         | ≥0.05         | –                    |
| Anoxic          | <0.05         | ≥0.03                |
| Anaerobic       | <0.05         | <0.03                |

removal (optimisation scenarios) (Table 3).

The influent composition of the MWWW cases was based on the “standard” fractionation provided by SUMO. The influent composition of the VFA cases were comprised of VFA as sole source of organic substrate, NH₄⁺ as sole N source and PO₄³⁻ as sole P source. See Supplementary Information Table S14 for detailed influent WW fractionation of MWWW and VFA influent. All scenarios were comprised of a TSS of 5.9–6.6 gTSS L⁻¹, and flocs represented 1–2% (VFA cases) to 7–9% of TSS (MWWW cases), respectively. An overview of the relevant simulation parameters is given in Table 3 for the different scenario.

The “batch test” modelling-scenarios were performed to assess the ability of the model to correctly predict the experimental observations. The “mechanism” scenarios were performed to better understand the mechanisms influencing SND for different influent WW (MWWW and VFA) and granule diameters (young and mature granules, d = 0.5 and 2.0 mm). Influent concentrations of 500 mg COD L⁻¹, 41 mg N L⁻¹ and 5.1 mg P L⁻¹ were selected as the basis of MWWW. The VFA WW consisted of the biodegradable fractions of COD, TN and TP of MWWW and comprised 403 mg COD L⁻¹, 40 mg N L⁻¹ and 5.0 mg P L⁻¹, respectively. For the different conditions, simulations were first run for 150 d at DO = 2.0 mg O₂ L⁻¹. DO was then changed, over two SBR cycles only, to different values of 0.5–6.0 mg O₂ L⁻¹. Data of the second cycle were then extracted. Matlab (Version R2018a, MathWorks, USA) was used for further data analysis, and SigmaPlot (Version 12.0, Systat, USA) was used for visualization of the data.

Finally, two different aeration strategies were tested to evaluate how to optimise the SND and TN removal during treatment of municipal WW with AGS (optimisation scenarios): alternating aeration (scenario #1) and 2-step aeration (scenario #2) (Table 3). For the “alternating aeration” scenario, the DO was switched on (DO = 2.0 mg O₂ L⁻¹) and off (DO = 0.0 mg O₂ L⁻¹) every 15 min during the aerobic SBR phase. For the “2-step aeration” scenario, the DO set-point was at 2.0 mg O₂ L⁻¹ for 15 min, followed by 0.5 mg O₂ L⁻¹ for the residual 270 min of the aerobic SBR phase.

2.3. Calculations

The specific rate of ammonium removal (mgN gVSS⁻¹ h⁻¹) was calculated using Eq. (2).

Equation (2) includes only the rate of NOx produced simultaneously during nitrification.

The specific rate of NOx-N accumulation (mgN gVSS⁻¹ h⁻¹) is calculated using Eq. (3).

The SND efficiency (%) was calculated by dividing the amount of NOx denitriﬁed by the amount of NH₄⁺ removed, according Eqs. (4)–(7). These calculations neglect the contribution of N-assimilation to NH₄⁺ or NOx removal.

The aerobic, anoxic and anaerobic electron-donor utilisation rates (“substrate utilisation rates”) were extracted separately for each electron-donor (S₅, VFA, GLY and PHA) directly from SUMO for each compartment (bulk and all granule layers). Supplementary Information Table S15 provides a detailed list of electron-donors utilisation by OHO, PAO and GAO in different redox conditions.

2.4. Analytical methods

Samples of influent and effluent during long-term experiments were analysed for total nitrogen (TN) using photochemical tests (Hach Lange, Germany, ICK 238 and 338). Cations (NH₄⁺-N) and anions (NO₂⁻–N, NO₃⁻–N) were analysed using ﬂow injection analysis (Foss, FLAstar flow injection 5000 analyzer, Denmark) and anion chromatography (Methrom 881 compact IC, Switzerland), respectively.

Table 3
Overview of simulation scenarios and their corresponding granule diameters, biomass composition, aeration strategy and influent composition.

Table 3

| WW Type | Batch test scenarios | Mechanism scenarios | Optimisation scenarios |
|---------|----------------------|---------------------|----------------------|
|         | MWWW | VFA | MWWW | VFA | MWWW | MWWW |
| Granule diameter [mm]⁴ | 0.5 | 2.0 | 0.5/2.0 | 2.0 | 0.5/2.0 | 0.5/2.0 |
| Aeration strategy | Constant DO | Constant DO | Constant DO | Constant DO | Alternating | 2-step |
| COD [mg L⁻¹] | 600 (as VFA) | 403 (as VFA) | 500⁴ | 500⁴ | 500⁴ | 500⁴ |
| TN [mg L⁻¹] | 42 (as NH₄⁺-N) | 40 (as NH₄⁺-N) | 41⁴ | 41⁴ | 41⁴ | 41⁴ |
| TP [mg L⁻¹] | 6 (as PO₄³⁻) | 5.0 (as PO₄³⁻) | 5.1⁴ | 5.1⁴ | 5.1⁴ | 5.1⁴ |
| Objective | validate the overall performance of the model by comparing them to the batch test experiments | better understand the mechanisms of SND in AGS systems | evaluate different optimised aeration strategies to maximize TN removal |

⁴ as experimentally observed.

⁵ with SUMO® standard WW fractionation, Supplementary Information Table S14.
3. Results

3.1. How is SND influenced by WW composition during long-term operation? (experimental results)

The nitrogen influent and effluent concentrations were monitored over the course of 300–400 days for the 4 reactors (Fig. 3). TN influent concentrations were similar for all reactors, ranging from 30 to 50 mg N L\(^{-1}\) on average. Also, low ammonium effluent concentrations (<2 mg NH\(_4\)-N L\(^{-1}\)) were measured, indicating that full nitrification occurred in all reactors. In addition, NO\(_2\)-N effluent concentrations were negligible in all reactors. The effluent NO\(_3\)- concentrations and thus the SND efficiencies were however significantly influenced by the influent composition. Average effluent NO\(_3\)- concentrations below 4 mg NO\(_3\)-N L\(^{-1}\) were measured for AGS system fed by 100%-VFA synthetic WW only, while values of 5–15 mg NO\(_3\)-N L\(^{-1}\) were measured in the effluents of the complex synthetic, primary effluent and raw WW AGS systems, respectively.

3.2. How does DO concentration affect SND performance? (experimental and modelling results)

Batch tests and model simulations were performed at different bulk DO concentrations to confirm the effect of the influent composition in terms of simple vs. complex electron-donor composition on SND (Fig. 4). Results from batch-tests confirmed observations made over long-term operation of the 4 reactors, i.e., that SND is strongly influenced by the influent composition in terms of organic substrate. Low SND, characterized by high NO\(_3\), accumulation rates, was observed for AGS systems treating complex WW (complex synthetic + real municipal WW). On the contrary, high SND was observed for AGS fed with 100%-VFA synthetic WW, independent from the DO concentration maintained in bulk (Fig. 4).

NH\(_4\) removal rates increase with an increasing bulk DO concentrations for all tested WW conditions. Maximum NH\(_4\) removal rates of 2 mg NH\(_4\)-N gVSS\(^{-1}\) h\(^{-1}\) were measured for bulk DO concentrations larger than 2 mg O\(_2\) L\(^{-1}\). The NO\(_3\) accumulation rates almost match the NH\(_4\) removal rates for AGS systems fed with complex WW (complex synthetic, primary effluent and raw WW, Fig. 3), indicative of the absence of SND in these systems. High SND only occurred in the AGS systems treating 100%-VFA synthetic WW, as indicated by the (very) low NO\(_3\) accumulation rates, i.e., high denitrification rate.

Modelling results (batch test scenarios) correctly matched experimental observations of NH\(_4\) removal- and NO\(_3\) accumulation rates – and thus SND performance - with changing DO (Fig. 4, plain line). Low NO\(_3\) accumulation rates (high SND) were predicted by the model for AGS fed with VFA WW, while high NO\(_3\) accumulation rates (low SND) were predicted for AGS fed by MWW. NH\(_4\) removal rates increased for increasing DO bulk concentrations, and overall higher NH\(_4\) removal rates were observed for young AGS (d = 0.5 mm) fed with MWW, in comparison to AGS fed with VFA WW (d = 2.0 mm).

3.3. Dynamics of redox zone formation (modelling results)

The formation of the different redox zones was predicted during the aerobic phase for different DO concentration in the bulk (mechanism scenarios) (Fig. 5). The simulations indicate that (1) the formation of anoxic zones inside the granules depends on influent composition, granule size and bulk DO concentration and that (2) the formation of these anoxic zones is particularly dynamic, and thus significantly changes during the aerobic phase.

The composition of the influent WW governs the formation of the redox zones. Penetration depth of oxygen significantly reduces towards the deeper layers of the granule in case of VFA influent, compared to the MWW case with the same granule diameter (d = 2.0 mm) at the same DO concentration. In addition, the granule diameter governs how fast and deep oxygen penetrates the granule. Oxygen penetration is limited to 50–100 µm from the surface for AGS fed with VFA and DO = 2 mgO\(_2\) L\(^{-1}\) during the entire aerobic phase, while oxygen penetrates the entire granule immediately after aeration starts (d = 0.5 mm) or 2 h of aeration (d = 2.0 mm) for AGS fed by MWW. The third influencing factor affecting anoxic zone formation and dynamic is the DO concentration in the bulk. Increasing DO concentrations generally result in faster and deeper penetration of oxygen towards the deeper granule layers, independent of influent WW composition or granule size.

3.4. Which electron donors are actually used for denitrification? (modelling results)

The denitrification rates were predicted for each electron-donor and in each layer of the granule during constant aeration at DO = 2.0 mgO\(_2\) L\(^{-1}\) (Fig. 6A). The concentration of each electron-donor available at the end of the anaerobic phase in each granule layer is also provided (Fig. 6B). The model predictions suggest that the higher the availability of electron-donor at the end of the anaerobic phase, the higher the denitrification during SND. The availability and utilisation of electron-donor via denitrification is highly influenced by the influent WW composition and granule diameter.

![Fig. 3.](image_url) Long-term effluent NH\(_4\)-N and NO\(_3\)-N and influent TN concentrations for 100%-VFA synthetic, complex synthetic, primary effluent and raw WW fed AGS systems.
The denitrification rates predicted for AGS fed with VFA WW are 5–6 fold larger than those predicted for MWW influent with young granules (d = 0.5 mm) and 2–4 fold over the MWW influent with mature granules (d = 2.0 mm). For AGS systems fed with VFA WW, the main electron-donor for denitrification is glycogen (GLY). A minor fraction of NOx is removed via assimilation or readily biodegradable SB. For small granules (d = 0.5 mm) fed with MWW, denitrification does not occur via GLY but rather through assimilation. Assimilation is the dominant pathway for TN removal for small granules fed with MWW. For large granules (d = 2.0 mm), the majority of denitrification is achieved utilising GLY as electron-donor, and to a smaller extent PHA and SB. Concentrations of internally stored electron-donors PHA and GLY after anaerobic conditions are a good proxy for their contribution to denitrification. Electron-donors are however only utilised in denitrification, if anoxic conditions also occur in those granule layers (cf. Figure 5, Figure 6AB).

3.5. Is there potential for electron-donor shift towards anoxic utilisation pathways? (modelling results)

Aerobic, anoxic and anaerobic utilisation pathways of the different electron donors (readily biodegradable (SB), VFA, glycogen (GLY) and PHA) were analysed during the aerobic phase with constant aeration at DO = 2.0 mgO2 L\(^{-1}\) (Fig. 7). A main observation is that a major fraction of the electron-donors is utilised aerobically. Also, almost no electron-donors are utilised in anoxic conditions in AGS fed with MWW, while there is some anoxic utilisation in AGS fed with VFA.

![Fig. 4. Change in the rates of NH4-\(\text{N}\) removal and NOx-N accumulation for AGS systems fed with different types of WW: 100%-VFA synthetic, complex synthetic, primary effluent and raw WW fed AGS systems during batch tests (rate NH4-\(\text{N}\), NOx-N exp, indicated by dots), and model simulations of VFA and MWW (batch test scenarios, rate NH4-\(\text{N}\), NOx-N sim, indicated by lines).](image)

![Fig. 5. Effect of influent composition and granule diameter on dynamics of redox zone formation for different DO concentrations during the aerated phase in the bulk: 0.5, 1, 2, 3 and 6 mgO2 L\(^{-1}\) for VFA (granule diameter 2.0 mm) and MWW (granule diameters of 0.5 and 2.0 mm). Anaerobic zones are indicated in red (Anae), anoxic zones in yellow (Anox), aerobic zones in green (Aero). Granule thickness from core (0 \(\mu\)m) to the surface (250 or 1000 \(\mu\)m). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image)
Only VFA fed AGS gathered the prerequisites needed for significant anoxic utilisation of GLY in multiple granule layers, while readily biodegradable SB is mostly utilised aerobically. No PHA utilisation neither aerobic nor anoxic is observed in VFA fed AGS, which is probably linked to competition with GLY. In MWW fed AGS on the other hand, aerobic growth is the main utilisation pathway for SB, VFA, GLY and PHA. Anoxic electron-donor utilisation is almost absent in MWW conditions for young granules \((d = 0.5 \text{ mm})\) and only occurs to a minor extent in the inner granule layers via anoxic utilisation of GLY for mature granules \((d = 2.0 \text{ mm})\). The model predictions indicate a large potential for shifting the utilisation of SB, PHA and GLY from aerobic to anoxic conditions and thus to increase SND and overall TN removal performances.

3.6. Can SND and TN removal be improved by optimising the aeration strategy? (modelling results)

If most of SB, PHA and GLY is utilised via aerobic utilisation pathways, a main question is to what extent can the aeration strategy be optimised to increase SND and ultimately TN removal? The performance of alternating aeration and 2-step aeration were thus compared to the constant DO base case scenarios MWW \(d = 0.5\) and \(2.0\) mm in terms of nitrification and denitrification performance (Table 4, Fig. 8 and Fig. 9). Applying an optimised aeration help to significantly increase denitrification efficiencies, while full nitrification was maintained for both young \((d = 0.5 \text{ mm})\) and mature granules \((d = 2.0 \text{ mm})\).

Denitrification efficiencies were increased by a factor of 2–4 to 40–61\% for young \((d = 0.5 \text{ mm})\) and by a factor of 1.5–2 to 65–79\% for mature \((d = 2.0 \text{ mm})\) granules, compared to 14–39\% for AGS composed of young and mature granules operated at constant DO aeration, respectively. Despite the high increase in the SND efficiencies, optimising the aeration strategy does not allow achieving full denitrification. Optimised aeration helps to better control the formation of anoxic zones within the granule, and in turn the anoxic electron-donor utilisation (Fig. 8A, Fig. 9). Granule size still influences the extent of anoxic zone formation: larger anoxic zones develop for a longer period for mature granules \((d = 2.0 \text{ mm})\) than for young granules \((d = 0.5 \text{ mm})\). In the case of young granules \((d = 0.5 \text{ mm})\), the main contributor to denitrification shifted from assimilation in the constant DO base case (due to absence of anoxic redox zones) to SB and PHA in the optimisation scenarios (Fig. 9). For mature granules \((d = 2.0 \text{ mm})\), the main electron-donor for

![Fig. 6. A) Contribution of the electron-donors SB, VFA, Assimilation of NO\textsubscript{x}, PHA and GLY to the denitrification rate for VFA influent, granule diameter 2.0 mm (VFA \(d = 2.0 \text{ mm}\)) and MWW influent, granule diameter 0.5 mm and 2.0 mm (MWW \(d = 0.5 \text{ mm}\) and \(d = 2.0 \text{ mm}\)) during the aerobic phase at constant aeration at 2 mgO\textsubscript{2} L\textsuperscript{-1} displayed over the depth of the granule. B) Concentration of substrate (electron-donors) SB, VFA, PHA and GLY at the end of the anaerobic phase normalized to the reactor volume.](Image)
denitrification shifted from glycogen (GLY) in constant DO aeration to Sb in the optimisation scenarios, and the contribution of assimilation to SND decreased to almost zero.

4. Discussion

4.1. SND and TN removal is limited in AGS systems treating municipal WW

It is usually well recognised that high TN removal via SND is a key attribute of AGS systems (Chen et al., 2011; De Kreuk et al., 2005; He et al., 2017). However, our long-term experiments indicated that limited SND is observed for AGS systems treating municipal WW, while full TN removal via SND is representative of systems fed with VFA only. Those results are supported by both experiments and mathematical modelling (Figs. 3, 4 and 6). Experimentally, only AGS fed with VFA achieved high SND and NO3-N effluent concentrations below 4 mg NO3-N L−1. Effluent NO3-N larger than 10 mg NO3-N L−1 were on the other hand measured for AGS treating municipal WW in our experiments. Other studies indicate a similar trend. Limited SND was previously reported for AGS treating municipal WW (Derlon et al., 2016; Wagner et al., 2015) and effluent NO3-N of 2–6 mg NO3-N L−1 and TN effluent concentrations of 5–15 mg N L−1 were measured for a Nereda® full-scale plant (Pronk et al., 2015). But in the study of Pronk et al. (2015), the majority of denitrification actually occurs once the DO
Fig. 8. Redox-conditions and concentrations of NH$_4^+$, NO$_x$ and DO during aerobic conditions of the simulated SBR cycle for MWV influent and young granules (d = 0.5 mm) (A,B) and mature granules (d = 2.0 mm) (C,D), for the three aeration strategies tested: constant DO, 2-step and alternating aeration.
and GLY to the denitrification for MWW in too low, nitri-
cation (Hibiya et al., 2004). On the contrary, if oxygen availability is
granules limits the formation of anoxic zones and thus denitri-
fication. A too fast and deep penetration of oxygen inside the
granule and (2) simultaneous lack of electron-donor available
in anoxic zones of the granule. The presence of OHO at the
surface can hamper the biological phosphorus removal (Majed et al., 2012).
Growth of OHO. OHO in turn outcompete GAO and PAO. PAO/GAO
favour granule formation, overall process stability and are essential
to the formation of granules, as this promotes aerobic
utilisation in denitrification. The absence of
utilisation in denitrification for municipal WW conditions. Ideally, most of the
influent SB is stored as intracellular polymers (GLY and PHA) under
anaerobic conditions, in order to favour granule formation. But
anaerobic utilisation of SB can become the major electron-donor source during SND in such sys-
tems, depending on the presence of GAO/PAO. The absence of
denitrification on PHA in the VFA influent AGS case can be
explained by the high COD/P ratio in the influent, which promotes
the growth of GAO over PAO (Majed and Gu, 2019).

4.2. What mechanisms limit SND in AGS systems treating municipal
WW?

Low SND efficiency in AGS systems treating municipal WW is
mainly governed by (1) the formation of limited anoxic zones inside the
granule and (2) simultaneous lack of electron-donor available for
denitrification. Concurrently, nitrification is not limiting SND.

Penetration of oxygen is one of the key factors influencing
denitrification inside granules or biofilms in general (Nielsen et al.,
1990). Our results demonstrate that, as a result of the SBR mode, the
formation of anoxic conditions within the granules is a very dy-
namic process. A too fast and deep penetration of oxygen inside the
granule limits the formation of anoxic zones and thus denitrifi-
cation (Hibiya et al., 2004). On the contrary, if oxygen availability is
too low, nitrification is then limited. When DO is controlled at a
constant value of 2 mg L⁻¹, the entire granule volume is anaerobic
during the first minutes of aeration, due to the simultaneous
absence of oxygen (quickly consumed due to high microbial activ-
ties) and of NO₃ (nitrification has not started yet). After few
minutes, NO₃ are then produced by nitrification and diffuse
through the granules, resulting in the formation of anoxic

conditions towards the granule core. Oxygen gradually penetrates
deep inside the granules as substrates get converted (reduced
oxygen uptake rate at the surface). This deeper penetration of ox-
ygen in turn reduces the anoxic zone. Increasing bulk DO thus re-
ультs in (1) a faster appearance of anoxic zones inside the granules
at the beginning of the aerobic phase, but also (2) a faster dis-
appearance of those anoxic zones at the end of the aerobic phase. Our
modelling and batch experiment results confirm that SND can be
greatly influenced by the DO applied in the bulk phase of the
reactor (Mosquera-Corral et al., 2005; He et al., 2019; Third et al.,
2003). Granule size is another important determining factor of
anoxic zone formation (Chen et al., 2011). Hereby, larger granules
sustain anoxic zones for longer time. Granule size is important
since it governs the volume and persistence of anoxic zones inside the
granule, and larger diameters prevent from too fast and deep oxygen penetration through diffusion (Li et al., 2008). A main challenge is in finding a fine balance in terms of aeration, in order to achieve full nitrification and to establish anoxic zones in the core of the granules.

In addition to the dynamics of anoxic zone formation, the
diffusion of electron-donors also strongly limit denitrification in
AGS systems (Derlon et al., 2016). Only diffusible carbon sources, like VFA or SB, can reach the deeper layers of the granule, in which
anoxic conditions are more likely to occur. VFA can be utilised directly or be stored as GLY or PHA during the prior anaerobic phase by GAO and PAO, respectively. The availability of VFA in the deep layers of the granule during the anaerobic SBR phase is crucial for subsequent denitrification during the aerobic SBR phase. In the case of municipal WW the lack of VFA (<5–10% of total COD) in the influent WW thus strongly limits SND. Readily biodegradable
substrate SB therefore becomes the most important electron-donor
for denitrification in municipal WW conditions. Ideally, most of the
influent SB is stored as intracellular polymers (GLY and PHA) under
anaerobic conditions, in order to favour granule formation. But
anaerobic utilisation of SB can occur when part of SB leaks into the
aerobic phase, or is produced by hydrolysis of XS. The presence of
readily biodegradable SB in the aerobic SBR phase can benefit the
growth of OHO. SB can be used by OHO for denitrification if it is available in anoxic zones of the granule. The presence of OHO at the
granule surface layers might also promote the establishment of strong oxygen gradients within the granule, which ultimately helps
forming anoxic conditions inside the granule. However, a higher availabil-
ity of SB during the aerobic SBR phase can also be detri-
mental to the formation of granules, as this promotes aerobic
growth of OHO. OHO in turn outcompete GAO and PAO. PAO/GAO
favour granule formation, overall process stability and are essential
for nutrient removal in AGS systems (De Kreuk and Van Loosdrecht,
2004). Another main outcome of our study is about the role of GAO.
GAO are usually considered detrimental in EBPR systems, as they
can hamper the biological phosphorus removal (Majed et al., 2012).
However, their presence can be beneficial for TN removal, as sug-
gested by our simulations and literature (Weissbrodt et al., 2013).

In the case of AGS fed with VFA WW, the overall electron-donor utilisation in denitrification is strongly shifted towards internally
stored GLY by GAO. Thus, storage compounds such as GLY or PHA become the major electron-donor source during SND in such sys-
tems, depending on the presence of GAO/PAO. The absence of
denitrification on PHA in the VFA influent AGS case can be
explained by the high COD/P ratio in the influent, which promotes
the growth of GAO over PAO (Majed and Gu, 2019).

Our results thus demonstrate that different mechanisms govern
SND, depending on the influent WW composition and granule
diameter. But in the case of municipal WW, our results indicate that
the main electron-donor utilisation pathways of SB, VFA, GLY and PHA are almost exclusively aerobic. If most of the electron-donors

![Fig. 9. Contribution of the electron-donors SB, VFA, Assimilation of NOx (Assim), PHA and GLY to the denitrification rate during aerobic conditions of the simulated SBR cycle for MWW influent, young granules (d = 0.5 mm) and mature granules (d = 2.0 mm) for the three aeration strategies tested: constant DO, 2-step and alternating aeration.](image-url)
are used aerobically during the aerobic phase operated at constant DO, optimised aeration strategies might help to direct electron-donors towards anoxic utilisation.

4.3. How to optimise for TN removal in AGS systems?

Our results indicate that large granule diameter is one of the parameters that promotes anoxic zone formation and thus the capability of the system to achieve high SND efficiency. However, in practice, controlling the granule diameter is very challenging as granule size is influenced by the granules age and the organic loading (Layer et al., 2019). Large granule diameters have been reported for full-scale AGS systems, but rather after few years of operation and during treatment of WW with a high readily biodegradable S$_{5}$ content (Prönk et al., 2015). For this reason, improving SND via engineering of the granule size does not represent a relevant approach for optimising TN removal. Inoculation with large granules could impair start-up and denitrification performance, since SND could occur from the start of the system. Nevertheless, the final extent of SND remains strongly hampered by the low availability of diffusible electron-donors in municipal WW. Therefore, other approaches must be considered to improve the TN removal. Pre- or post-denitrification are typical options to increase TN removal in conventional activated sludge SBR systems or in AGS systems (Prönk et al., 2015). However, both come with drawbacks. Pre-denitrification does not prevent from high TN effluent concentrations and post-denitrification is associated with very low rates, and hence SBR cycle duration must be increased.

Our results demonstrate that optimising the aeration strategy represents a simple and efficient approach to improve the TN removal of AGS systems. An increase from 14-37% (constant DO aeration) to 65–79% (2-step and alternating aeration) of the denitrification efficiency could be achieved. The increase in the denitrification efficiency results from an improved utilisation of the electron-donors under anoxic conditions. In previous studies, optimised aeration strategies for AGS systems have successfully increased TN removal too, like e.g. mathematical modelling of 2-step aeration (Sun et al., 2019), adaptable DO setpoint operation (Isanta et al., 2013), or lab-scale experiments on alternating aeration (Lochmatter et al., 2013). On full-scale installations, a 2-step aeration strategy is applied to overcome limited TN removal (Prönk et al., 2015; Supplementary Information S1). A recent patent on the aeration strategy of Nereda® reactors also confirms that specific measures must be taken to increase SND in AGS systems (Derlon et al., 2016; Layer et al., 2019), i.e., to control the aeration rate based on a targeted NO$_3$ concentration (Van Dijk et al., 2018). However, those studies lack fundamental understanding on anoxic zone formation or electron-donor utilisation affecting TN removal.

Our simulation results indicate that anoxic zone formation is much better controlled and prolonged when a 2-step and or an alternating aeration is applied. Especially, we observed that the electron-donor utilisation can be shifted, from mostly aerobic to anoxic. Significantly more S$_{5}$ was thus utilised via anoxic pathways, and the contribution of assimilation to TN removal was almost zero, in comparison to constant DO aeration. In the case of large granules, VFA could be produced via fermentation and utilised inside the granule, additionally contributing to denitrification. Both effects result in a much higher denitrification efficiency in comparison to constant DO aeration. Therefore, high fractions and concentrations of diffusible electron-donors are not only favourable to improve start-up time and settling performance of AGS systems, but also SND performance (Layer et al., 2019). Limitations in TN removal due to unfavourable influent WW conditions can be overcome by optimised aeration strategies. The microbial pathways of electron-donor utilisation can directly be influenced and engineered by application of different aeration strategies.

However, drawbacks of the proposed aeration strategies to increase TN removal also exist. During 2-step and alternating aeration, a balance must be found between the high DO period (to achieve full nitrification) and low DO period (to maximize denitrification). Transient DO conditions — present in both optimised aeration strategies - were reported to trigger growth of filamentous bacteria, or breakage of granules (Martins et al., 2004; Sturm et al., 2004), or increased NO$_2$ formation during nitrification (Alleman, 1985). Accumulation of NO$_2$ ultimately increases the risk of N-losses via N$_2$O production during nitrification/denitrification (Law et al., 2012). N$_2$O has the potential to be the main greenhouse gas emission during WW treatment (Gruber et al., 2019). Therefore, N$_2$O production during nitrification and denitrification should be avoided.

5. Conclusions

- Limited SND and TN removal is observed in AGS systems treating low-strength municipal wastewaters, thus resulting in high NO$_3$ and TN effluent concentrations. Denitrification, not nitrification, is limiting SND in AGS systems.
- SND is limited by the very dynamic formation of anoxic zones and the availability of electron-donors within the granules. Anoxic zones only develop during a short period in a small volume of the granule. Larger granules and lower bulk DO concentrations prolong anoxic zones inside the granules and hence increase SND.
- The mechanisms and extent of anoxic substrate conversion at constant DO operation is governed by the composition of the influent WW (municipal or VFA-only WW). Internal storage compounds PHA and glycogen accounted for 40% (AGS municipal WW) or > 90% (AGS VFA-only WW) of electron-donors used in denitrification at constant DO operation. In municipal WW fed AGS systems, S$_{5}$ is an important electron-donor in denitrification, too.
- Aeration strategies must be optimised to increase SND and TN removal during treatment of low-strength municipal WW using AGS systems. Alternating and 2-step aeration strategies help to increase TN removal from 13% to more than 65% during treatment of municipal low-strength WW.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Adav, S.S., Lee, D.-J., Show, K.-Y., Tay, J.-H., 2008. Aerobic granular sludge: recent advances. Biotechnol. Adv. 26, 411–423.
Ali, M., Wang, Z., Salam, K.W., Hari, A.R., Prönk, M., Van Loosdrecht, M.C.M., Saikaly, P.E., 2019. Importance of species sorting and immigration on the bacterial assembly of different-sized aggregates in a full-scale Aerobic granular sludge plant. Environ. Sci. Technol. 53, 8291–8301.
Allemann, J.E., 1985. Elevated nitrite occurrence in biological wastewater treatment systems. Water Sci. Technol. 17, 409–419.

Chen, F.-Y., Liu, Y.-Q., Ning, P., Pang, H., 2011. Operational strategies for nitrogen removal in granular sequencing batch reactor. J. Hazard Mater. 187, 342–348.

Daigger, G., Adams, C., Steller, H., 2007. Diffusion of oxygen through activated sludge flocs: experimental measurement, modeling, and implications for simultaneous nitrification and denitrification. Water Environ. Res. 79, 375–386.

De Kreuk, M.K., Heijnen, J.J., Van Loosdrecht, M.C., 2005. Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. Biotechnol. Bioeng. 90, 761–769.

De Kreuk, M.K., Kishida, N., Van Loosdrecht, M.C.M., 2007a. Aerobic granular sludge—state of the art. Water Sci. Technol. 55, 75–81.

De Kreuk, M.K., Picicoreanu, C., Hosseini, M., Xavier, J.B., Van Loosdrecht, M.C.M., 2007b. Kinetic model of a granular sludge SBR: influences on nutrient removal. Water Sci. Technol. 56, 35–44.

De Kreuk, M.K., Van Loosdrecht, M.C., 2004. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci. Technol. 49, 9–22.

De Kreuk, M.K., Van Loosdrecht, M.C.M., 2006. formation of aerobic granules with domestic sewage. J. Env. Eng. 132 (6).

Deroian, N., Wagner, J., Da Costa, R.H.R., Morgenroth, E., 2016. Formation of aerobic granules for the treatment of real low-and-strength municipal wastewater using a sequencing batch reactor operated at constant volume. Water Res. 105, 341–350.

Dynamita, 2019. Dynamita Process Modelling [Online]. [Accessed]. http://www.dynamita.com.

Figdore, B.A., Stensel, H.D., Winkler, M.-K.H., 2018. Comparison of different aerobic granular sludge types for activated sludge nitrification bioaugmentation potential. Bioreusor. Technol. 251, 189–196.

Gruber, D., Villegas, K., Wunderlin, P., Siegert, H., Vogt, L., Joss, A., 2019. N2O emission in full-scale wastewater treatment: proposing a refined monitoring strategy. Sci. Total Environ. 134157.

He, Q.L., Chen, L., Zhang, S.J., Chen, R.F., Wang, H.Y., 2019. Hydrodynamic shear force shaped the microbial community and function in the aerobic granular sequencing batch reactors for low carbon to nitrogen (C/N) municipal wastewater treatment. Bioresour. Technol. 278, 195–204.

Hirsh, N., H., 2016. Fate of carbon, nitrogen and phosphorus removal in a post-anoxic system treating low strength wastewater. Int. Biodeterior. Bioegr. 108, 166–174.

Sturm, B., Irvine, R., Wilderer, P., 2004. The effect of intermittent feeding on aerobic granular structure. Water Sci. Technol. 49, 19–25.

Sun, F., Lu, Y., Wu, J., 2019. Comparison of operational strategies for nitrogen removal in aerobic granular sludge sequencing batch reactor (AGS-SBR): a model-based simulation. Environ. Eng. Chem. 7.

Świątczak, P., Cydzik-Kwiatkowska, A., 2018. Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant. Environ. Sci. Pollut. Control Ser. 25, 1655–1669.

Third, K.A., Burnett, N., Cord-Ruwisch, R., 2003. Simultaneous nitrification and denitrification using stored substrate (phb) as the electron donor in an SBR. Biotechnol. Bioeng. 83, 706–720.

Van Dijck, E.J.H., Van Schagen, K.M., Oosterhoff, A.T., 2018. Controlled Simultaneous Nitrification and Denitrification in Wastewater Treatment. Pct/Ep2018/063599, Varga, E., Hauduc, H., Barnard, J., Dunlap, P., Jimenez, J., Menniti, A., Schauer, P., Varga, E., Hauduc, H., Barnard, J., Dunlap, P., Jimenez, J., Menniti, A., Schauer, P., 2018. Recent advances in bio-P modelling — a new approach verified by full-scale observations. Water Sci. Technol. 78, 2119–2130.

Wagner, J., Guinérais, P., Akaboci, T.R.V., Costa, R.H.R., 2015. Aerobic granular sludge technology and nitrogen removal for domestic wastewater treatment. Water Sci. Technol. 71, 1040–1046.

Wang, S.-G., Gai, L.-H., Zhao, L.-J., Fan, M.-H., Gong, W.-X., Gao, B.-Y., Ma, Y., 2009. Aerobic granules for low-strength wastewater treatment: formation, structure, and microbial community. J. Chem. Biotechnol. Biotechnol. 84, 1015–1020.

Wang, X., Wang, S., Xue, T., Li, B., Dai, X., Peng, Y., 2015. Treating low carbon/nitrogen (C/N) wastewater in simultaneous nitrification-denitrification and phosphorus removal (SNDRP) systems by strengthening anaerobic intracellular carbon storage. Water Res. 77, 191–200.

Wassner, O., Reichert, P., 1996. Mathematical modeling of mixed-culture biotechnological processes. Biotechnol. Bioeng. 48, 172–184.

Weissbrodt, D.G., Neu, T.R., Kuhlicke, U., Rappaz, Y., HOLLIGER, C., 2013. Assessment of bacterial and structural dynamics in aerobic granular biofilms. Front. Microbiol. 4, 175–175.

Winkler, M.K.H., Bassin, J.P., Kleerebezem, R., Sorokin, D.Y., Van Loosdrecht, M.C.M., 2012. Unravelling the reasons for disproportion in the ratio of AOB and NOB in activated sludge. Water Res. 46, 5010–5017.

Majed, N., Chernenko, T., Diem, M., Gu, A.Z., 2012. Identification of functionally relevant populations in enhanced biological phosphorus removal processes based on intracellular phosphorus profiles and insights into the metabolic diversity and heterogeneity. Environ. Sci. Technol. 46, 5010–5017.

Majed, N., Gu, A.Z. 2019. Impact of Influent Carbon to Phosphorus Ratio on Performance and Phenotypic Dynamics in Enhanced Biological Phosphorus Removal (Ebrp) Systems. Water Environ. Res. 91, 375–385.

Marin, J.C.A., Caravelli, A.H., Zartiky, N.E., 2019. In: Jacob-Lopes, Eduardo, Queiroz Zepka, Leila (Eds.), Performance of anoxic-oxic sequencing batch reactor for nitrification and aerobic denitrification. Biotechnology and Bioengineering, IntechOpen. https://doi.org/10.4137/interopen.S84775X.

Martins, A.M.P., Pagilla, K., Heijnen, J.J., Van Loosdrecht, M.C.M., 2004. Filamentous bulking sludge—a critical review. Water Res. 38, 793–817.

Mehta, C., 2004. Micro-pro...