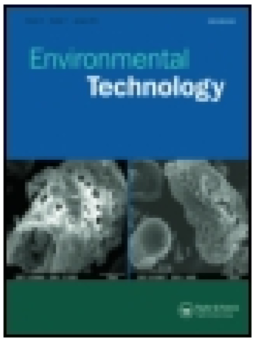

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Possible mechanism of efficient mainstream partial nitritation/anammox (PN/A) in hybrid bioreactors (IFAS)

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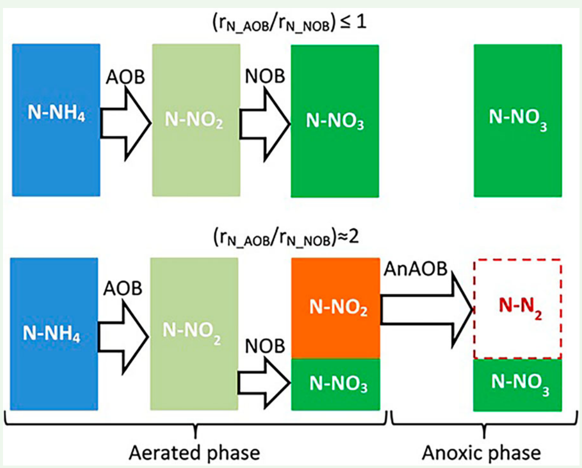
ABSTRACT

An explanation of possible mechanism of efficient PN/A in hybrid bioreactors was presented. The bottleneck process is nitritation. Surplus nitrite production by ammonium oxidizing bacteria (AOB) is required for assuring the activity of anammox bacteria and eliminating nitrite oxidizing bacteria (NOB). It will be possible if nitrogen removal rate by AOB (r_{N_AOB}) is higher than NOB (r_{N_NOB}). It was shown that in biofilm AnAOB bacteria should out-compete NOB, whereas nitrogen transformation rates by AOB are usually lower than NOB. However, the growth of r-AOB in activated sludge allows out-selecting NOB. Impact of ammonium-, nitrite–nitrogen and suspended biomass concentration in hybrid PN/A systems on nitrogen removal rates in the temperature ranges from 10°C to 25°C was presented and discussed. Because bulk liquid ammonium nitrogen concentration can be higher in SBR bioreactors (after certain period of time after aeration starts) or in the initial zones of plug-flow systems than in fully mixed systems, conditions for running efficient PN/A are more favourable in intermittently aerated 'IFAS-SBR' or 'IFAS-plug flow' bioreactors.



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Anammox; nitritation; autotrophic deammonification; mainstream wastewater; IFAS

Introduction

Efficient and ecologically sustainable nitrogen removal from mainstream wastewater is a necessary element for the application of the new concept of a wastewater treatment plant. It is to be an energy autarchic system for recovering of valuable resources – above all water but also nitrogen, phosphorous and hydrocarbons [1,2]. The technology in focus is the autotrophic deammonification based on two basic processes: partial nitritation (PN) and anammox (A).

Application of the PN/A process to mainstream wastewater treatment is a challenging goal. It is caused by the low concentration of ammonium–nitrogen and low temperature of wastewater inflowing to bioreactors of the main technological line of wastewater treatment plant [3–5]. As a result, decline of the activity of autotrophic organisms takes place in the PN/A systems, along with undesired nitrite oxidation by NOB (nitrite oxidizing bacteria), which negatively influences the efficiency and capacity of the process. Nonetheless

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strong scientific attention is given to achieving this purpose because of the possible benefits of the PN/A. During the last few years, some important studies were conducted, whose results can be recognized as the 'mile-stones' towards mainstream PN/A. Regmi et al. [6,7] and Al-Omari et al. [8] described the impact of an intermittent aeration with high DO concentration during short aerated phases on stable partial-nitritation (first stage of autotrophic deammonification) under mainstream wastewater conditions (see Figure 1(A,C). Successful operation of PN/A process in the conventional activated sludge bioreactors enriched with granular anammox biomass was explained as the effect of intermittent aeration strategy by Wett et al. [3]. Several studies described the impact of low ammonium concentration on the efficiency, capacity and kinetics of PN/A process [4,9–12]. Then activity of the main bacterial groups in the PN/A systems and process performance under low temperatures were the scope of the experimental studies [5,9,13–17]. Another breakthrough point was the evidence of the advantage of running mainstream PN/A in hybrid systems (IFAS – integrated fixed film activated sludge system), in which biomass grows in parallel as activated sludge and biofilm or granules [3–5,18]. An important part in finding the proper measures for NOB bacteria suppression are basic studies on ammonium oxidizing bacteria (AOB) and NOB growth kinetics under variable environmental conditions, especially linking experimental observations with theory of 'r-' and 'K-selection' of organisms [19,20]. Polishing of PN/A effluent by the removal of residual nitrates with the application of denitrification or partial dissimilatory nitrate reduction to ammonia (PDNR) is also intensely examined [21–27].

Reports of achieving highly efficient mainstream PN/A in the temperature ranges from about 15°C to 30°C have been presented recently. The progress was made mostly in the laboratory-scale systems and some pilot-scale experimental set-ups. Most of the described bioreactors were a kind of sequencing batch reactors (SBR), sequencing – integrated fixed film biofilm reactors (IFAS-SBR) or IFAS – plug flow systems. Positive results from fully mixed, continuous flow systems were presented as well [4,15,18,25,26,28–35]. An efficient and stable mainstream PN/A process was also accomplished by the dedicated treatment of biomass with free ammonia or free nitrous acid-rich wastewater, which allowed to suppress NOB's growth [36,37].

Although the studies of Regmi et al. [6,7] and Al-Omari et al. [8] explained the logic of application of an aeration strategy with a relatively high dissolved oxygen concentration (above 1 mgO_2/l) and intermittent aeration, in the

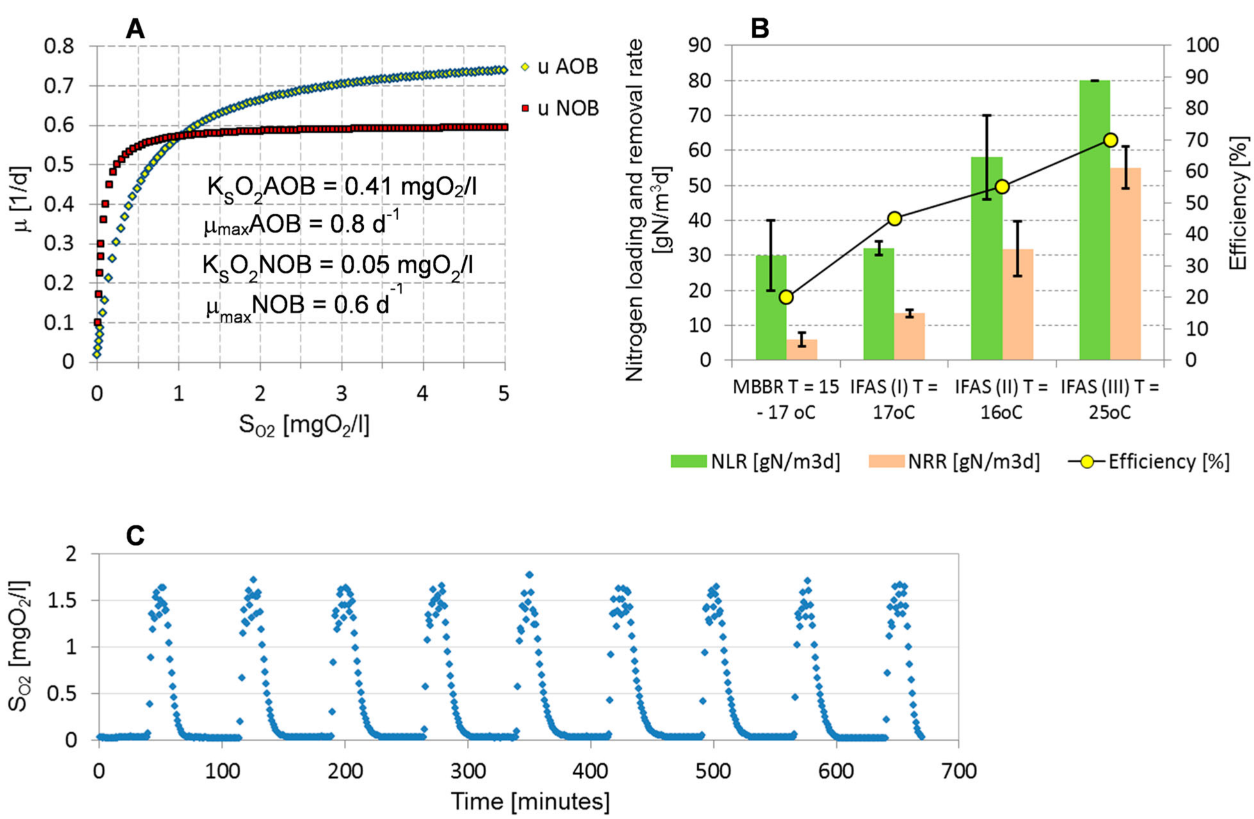

Figure 1. Intermittent aeration strategy as a method for NOB bacteria growth suppression (A – based on results of Malovanyy et al. [4,34]; B – based on results of Trojanowicz et al. [5], Plaza et al. [35], Malovanyy et al. [4]; C – based on the data of Trojanowicz et al. [5]).

available literature there is no explicit proof that a specific range of dissolved oxygen concentration (DO) and intermittent aeration leads to efficient mainstream PN/A process (Figure 1(B) 'MBBR') [4,5,31,38–40]. That strategy had a desired effect only when AOB bacteria were kept in the system, in the form of activated sludge, by recirculating a detached biofilm from the secondary clarifier to bioreactor or inoculation of the MBBR bioreactor with the activated sludge originated from a conventional SBR for municipal wastewater treatment (transition of the bioreactor operation from MBBR to IFAS mode) [4,5]. It allowed to achieve high efficiency and capacity of the process at 25°C, even under conditions of low substrate concentrations (Figure 1(B) 'IFAS III').

As it can be seen from the presented examples, the hybrid system, in which biomass grows in the form of biofilm and activated sludge, enables to suppress NOB and to increase the capacity and efficiency of PN/A process substantially. In this regard, questions arise concerning mechanisms of the described phenomena:

- How does it happen that NOB growing in activated sludge can be more easily eliminated from the system by applying intermittent aeration than from biofilm?
- What is the mechanism, which determines that nitrite flux generated during aerated phase by AOB is not simultaneously utilized by NOBs present in the biofilm and activated sludge of the hybrid system (IFAS)?
- Is actually intermittent aeration (IA) strategy, with setting of high DO concentration, the most important factor? The presented results indicate that IA is only one of more key elements for successful mainstream PN/A, so maybe the foundation for effective process running is a high activity of AOB, what was suggested by other authors [4,15,18,34]?

Answering to those questions might allow optimize the PN/A process and increase its efficiency to the almost stoichiometric levels.

The goal of this paper was to propose the explanation of the mechanism of the efficient PN/A process in IFAS bioreactor. Outcomes from theoretical analyses were discussed and compared with the results obtained by other research groups. The most promising PN/A systems outlook were recommended.

Materials and methods

It was assumed that efficient PN/A is strictly connected with high activity of AOB and AnAOB bacteria and at the same time low activity of NOB. The rate of substrate removal by those bacteria depends on their growth rates under given conditions and their abundance in the system. Therefore, searching for the mechanisms of the effective PN/A in IFAS bioreactor was based on the calculation and comparison of specific growth rates (μ) and nitrogen removal rates (r_N) values for AOB, NOB and AnAOB bacteria, under conditions of different values of factors influencing those parameters (substrate's concentration, temperature and intrinsic kinetic parameters of bacteria). The scope was to find the engineered system conditions and layout which promote nitrite–nitrogen production by AOB (high 'r_{N_AOB}' values for AOB and 'r_{N_AOB}' values for AOB higher than 'r_{N_NOB}' values for NOB) and utilization of this substrate by anammox bacteria (high 'r_{N_AnAOB}' values for AnAOB). The conducted analyses included:

- comparison of the substrate and inhibitor concentration gradients in a biofilm zone of MBBR and IFAS reactors,
- calculation of the specific nitrogen removal rates and specific growth rates of autotrophic biomass at variable temperature and substrate concentrations in IFAS system,
- estimation of the ratio between nitrogen removal rate of AnAOB and NOB (r_{N_AnAOB}/r_{N_NOB}) in biofilm, and between nitrogen removal rate of AOB and NOB (r_{N_AOB}/r_{N_NOB}) in biofilm and activated sludge.

Tracing the substrate and inhibitor concentration curves within cross-sectional area of biofilm

Calculations of distribution of the selected substrate and inhibitor concentrations in biofilm were done with Aquasim 2.1f computer program [41], with the application of Wanner and Reichert [42] biofilm model and the validated, mathematical model of PN/A process in biofilm reactors [11]. Simulations were conducted with the set of input parameters values presented in Table 1. Complete description of the applied model structure, its parameter values and validation results have been presented in the separate paper [11].

Analysis of the impact of substrate concentrations on specific nitrogen removal rate and specific growth rates of autotrophic biomass at variable temperature in IFAS system

The analysis was divided into two stages ('St-1' and 'St-2'). In the first phase, four scenarios ('Case A1-A2' and 'Case B1-B2') of the IFAS system's operation were considered, which differed with regard to the assumed concentrations of ammonium–nitrogen and total volatile

Table 1. Input values of selected parameters utilized for simulation.

Lp.	Parameter	Symbol [unit]	Value
1	pH	pH	6.9
2	Temperature	T [°C]	15.0
3	Influent flow rate	Q [m^3/d]	0.2592
4	Inorganic carbon (alkalinity)	ALK [molHCO$_3$/m^3]	5.0
5	Chemical oxygen demand	COD [gO$_2$/m^3]	106
6	Biodegradable fraction of COD	f_B [–]	0.4
7	Ammonium nitrogen concentration in the influent	N–NH$_4$ [gN/m^3]	42.5
8	Dissolved oxygen concentration	S_{O2} [gO$_2$/m^3]	1.5
9	Aeration: 20 min 'aerated phase' + 40 min 'non-aerated phase'		
10	Activated sludge concentration in IFAS	X_{AS} [gO$_{2B}$/m^3]	1036
11	Bioreactor volume	V_R [m^3]	0.2
12	Biofilm area	A [m^2]	37.5
13	Biofilm thickness	LF [μm]	1000

suspended solids in the bioreactor. In the second part, impact of the presence of 'r-' or 'K-strategists' AOB in the bioreactor on specific nitrogen removal rates and biomass specific growth rates, was additionally taken into account. Parameter values of the IFAS system, which were utilized for the analysis, are presented in Table 2.

Theoretical analysis of the effect of ammonium- and nitrite–nitrogen concentrations in the IFAS bioreactor on nitrogen removal rate of autotrophic biomass, in the temperature range from 10°C to 25°C, was conducted utilizing Equation (1) and the sets of parameters presented in Table 2:

$$r_N = X_i \cdot \frac{1}{Y_i} \cdot \mu_i, \tag{1}$$

where r_N – volumetric nitrogen 'N' utilization rate [gN/m^{3}d], X_i – concentration of biomass 'i' [gO$_{2B}$/m^3] ('gO$_{2B}$' – gram of biomass expressed in COD units), Y_i – yield coefficient of biomass 'i' [gO$_{2B}$/gN], μ_i – specific growth

Table 2. Parameter values utilized for the analysis of the effect of substrate's concentrations on specific nitrogen removal rate and specific growth rates of autotrophic biomass at variable temperatures in IFAS reactor.

Lp.	Parameter	Value		
Stage: 'St-1' and 'St-2'				
1	Bulk N–NH$_4$ [gN/m^3]	5.0 (*Case A1, A2, A3*) 1.0 (*Case B1, B2, B3*)		
2	Bulk N–NO$_2$ [gN/m^3]	0.1 (*Case A1, A2, A3 and B1, B2, B3*)		
3	VSS [gO$_{2B}$/m^3]	1000 (*Case A1, B1*) 4000 (*Case A2, B2*)		
4	pH	6.9		
5	T [°C]	10–25		
		Autotrophic bacteria		
		AOB	**NOB**	**AnAOB**
6	DO [gO$_2$/m^3]	1.5	1.5	0.0
7	f_i [%]	5.6 [30]	0.9 [30]	10.8 [30]
8	Y_i [gO$_{2B}$/gN]	0.2 [11]	0.041 [11]	0.15 [11]
9	$K_{S,O2}$ [gN/m^3]	0.41 [4]	0.05 [4]	n.a.
10	θ [1/K]	0.087 [49]	0.038 [49]	0.089 [this study]
Stage: 'St-1'				
11	$\mu_{max,i}$ [1/d]	0.8 [11]	0.6 [11]	0.05 [11]
12	$K_{S,FA}$ [gN/m3]	0.3 [11]	n.a.	2.72E-4 [11]
13	$K_{S,FNA}$ [gN/m^3]	n.a.	8.723E-4 [11]	1.29E-5 [11]
Stage: 'St-2'				
14	$\mu_{max,i}$ [1/d]	1.45 (r-AOB)/ 0.39 (K-AOB) [19]	0.67 (K-NOB) [6]	0.05
15	$K_{S,FA}$ [gN/m^3]	0.0203 (r-AOB)/ 0.002 (K-AOB) [19]	n.a.	2.72E-4 [20]
16	$K_{S,FNA}$ [gN/m^3]	n.a.	5.4E-5 (K-NOB) [6]	1.29E-5 [20]

rate of biomass 'i' [1/d] defined as Equation (2), 'i' – type of autotrophic biomass: ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), anammox bacteria (AnAOB).

Specific growth rate of AOB, NOB and AnAOB was calculated with the following formula and parameter's values presented in Table 2:

$$\mu_{\mathrm{i}} = \mu_{\mathrm{max,\,i}} \cdot \prod \frac{S_{\mathrm{j}}}{K_{\mathrm{Sj}} + S_{\mathrm{j}}} \cdot \mathrm{e}^{\theta(T-20)}, \qquad (2)$$

where $\mu_{max,i}$ – maximum growth rate of biomass 'i'[1/d], S_j – concentration of substrate 'j' [gN/m^3] [gO$_2$/m^3] (j = FA or FNA or DO), K_{Sj} – half saturation coefficient for substrate 'j' [gN/m^3] [gO$_2$/m^3], θ – temperature coefficient [1/K], T – temperature [^{o}C].

Substrates 'j' were: free ammonia (FA) and dissolved oxygen (DO), free nitrous acid (FNA) and dissolved oxygen (DO) and free ammonia (FA) and free nitrous acid (FNA) for AOB, NOB and AnAOB, respectively. Concentrations of FA and FNA were calculated based on the theory presented by Anthonisen et al. [43] with assumed ammonium- and nitrite–nitrogen concentrations, pH and temperature values in the bioreactor (Table 2). Constant DO values of 1.5 and 0.0 mgO$_2$/l were considered for the growth of aerobic nitrogen oxidizers and anammox bacteria, respectively.

Concentration of autotrophic biomass (X_i) was calculated by multiplying values of fraction 'f_i' of AOB, NOB or AnAOB bacteria in the total volatile suspended solids by assumed total volatile suspended solid concentration in IFAS bioreactor 'VSS' (defined as the concentration of biomass growing in the form of both biofilm and activated sludge). Values of biomass fractions 'f_i' in IFAS system were estimated on the basis of experimental studies conducted by Yang et al. [30]. They were 5.6%, 0.9% and 10.8% for AOB, NOB and AnAOB, respectively.

Concentration gradients of substrates in the biofilm were not considered. It was assumed also that inhibition by FA, FNA and DO does not exist in the bioreactor, due to their low concentrations (below their inhibition levels for all autotroph groups being analysed).

Calculation of the ratio between nitrogen removal rate of AnAOB and NOB (r_{N_AnAOB}/r_{N_NOB}) in biofilm, and between nitrogen removal rate of AOB and NOB (r_{N_AOB}/r_{N_NOB}) in biofilm and activated sludge

The ratio values were calculated by dividing values of nitrogen removal rate by AnAOB or AOB and NOB in biofilm or activated sludge '(r_{N_AnAOB}', 'r_{N_AOB}', 'r_{N_NOB}'), which are defined with Equations (1) and (2). Utilized parameters values are presented in Table 2. Concentrations of FA, FNA and DO in the cross-sectional area of biofilm and in the bulk liquid of IFAS bioreactor were the outcome of PN/A simulation in IFAS (as described earlier). Concentration of autotrophic biomass (X_i) in IFAS bioreactor was calculated by multiplying values of relative abundance 'a_i' of AOB, NOB or AnAOB bacteria in the biofilm and activated sludge by assumed total volatile suspended solid concentration in biofilm and activated sludge in IFAS bioreactor (VSS). Literature values of biomass relative abundance 'a_i' in IFAS system were used [30]. In biofilm they were assumed as: 0.008, 0.006 and 0.33 for AOB, NOB and AnAOB, respectively. In activated sludge they were: 0.077, 0.01 and 0.009 for AOB, NOB and AnAOB, respectively. Assumed VSS concentrations of activated sludge and biofilm in the bulk liquid were 2160 and 4870 mg/l, respectively.

Results and discussion

Implications of substrate and inhibitor concentration gradients within cross-sectional area of biofilm for PN/A process performance

In order to gain better insight into the possible mechanisms underlying the success of PN/A in hybrid bioreactors (IFAS), concentration curves of the main substrates in biofilm were traced. Presented data derived from simulations of MBBR and IFAS systems under conditions of mainstream wastewater inflow and at a temperature of 15°C (Table 1). This value of temperature was selected because of the knowledge that is possible to run a single-stage PN/A under such conditions [5,15,35].

Distribution of the main substrates and inhibitors in cross-sectional area of biofilm is shown in Figure 2. It can be seen that at the lower activity of microorganisms in biofilm (related with lower concentrations of substrates – below affinity constants of the main bacterial groups) and under high concentration of dissolved oxygen in the bulk-liquid, biofilm can be fully penetrated with oxygen (Figure 2(A)). It has a high importance, since AnAOB are strongly inhibited by oxygen and anammox process can be suppressed during aeration phase and for some period of time after aeration is ceased [44]. This justifies also a scheme of stopping aeration periodically in the system (intermittent aeration).

Another issue concerns setting the proper DO concentration in the bioreactor. Probably it should be related with the activity of biomass in the biofilm and nitrogen loading rate (NLR). The higher activity of aerobic nitrogen oxidizers (higher nitrogen removal rates) and generated nitrogen flux, the sharper drop of

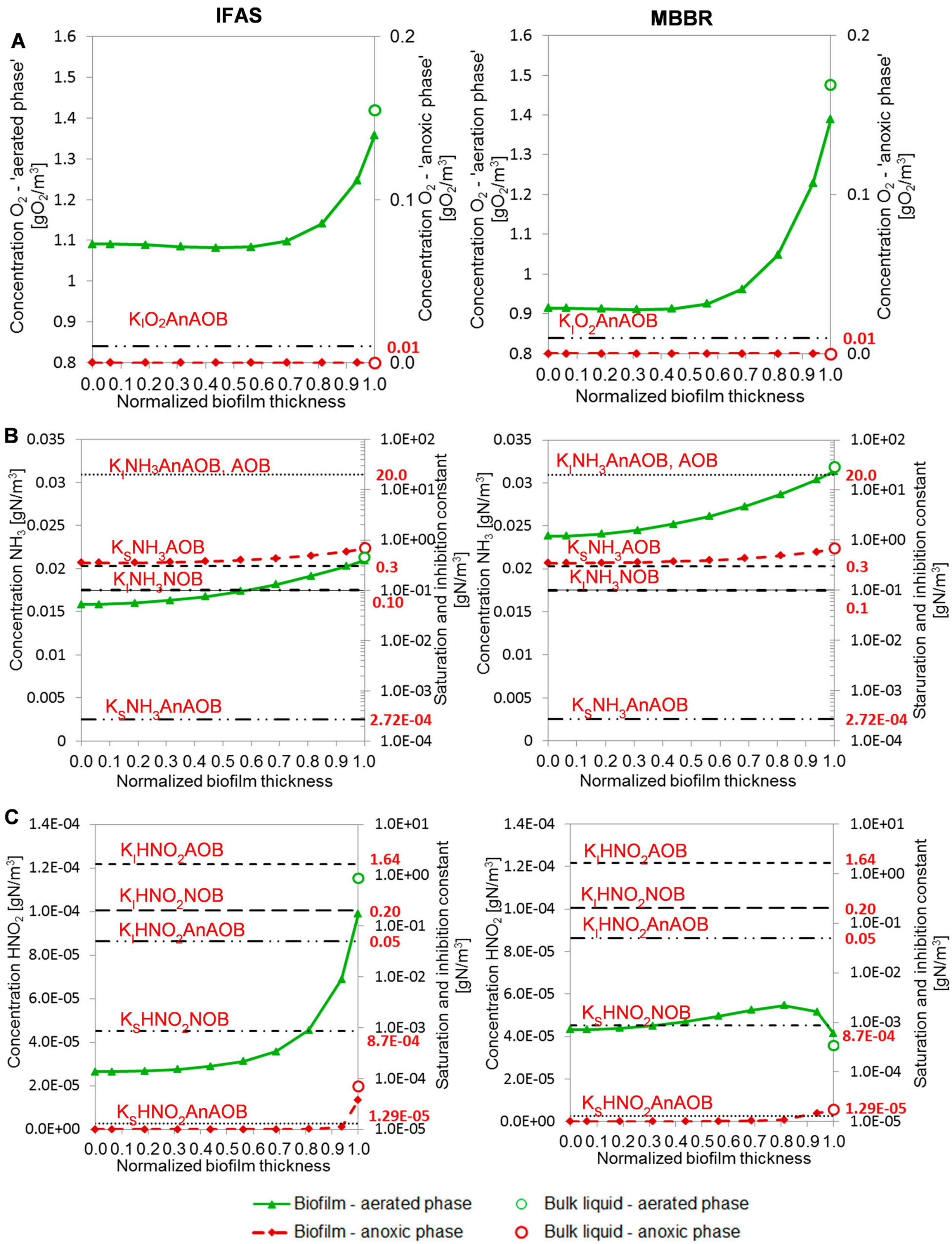

Figure 2. Concentration patterns of dissolved oxygen, free ammonia and free nitrous acid within the biofilm during aerated and non-aerated phases of MBBR and IFAS bioreactors at 15°C – theoretical predictions.

DO across biofilm should be expected [11]. Because of this, we should consider the strategy of decreasing DO set point along with lowering of NLR, so as we could create stable, oxygen impenetrable zone in biofilm, where AnAOB would not be suppressed.

It is also clear that inhibition of autotrophic bacteria by free ammonia and free nitrous acid is less likely under the examined conditions of the PN/A process (Figure 2(B,C)).

Important observation concerns increased concentration of free nitrous acid within the entire biofilm in IFAS bioreactor compared to MBBR (Figure 2(C)). Higher affinity of AnAOB than NOB to HNO_2 ($K_{S,FNA\_AnAOB} < K_{S,FNA\_NOB}$), with simultaneous greater amount of nitrite–nitrogen generated by AOB in activated sludge, and by this increase in its concentration in biofilm, could explain achieving competitive advantage of anammox in biofilm over NOB, when the process is run in a hybrid bioreactor. More $N–NO_2$ is available for AnAOB because the rate of $N–NH_4$ oxidation by AOB is higher in IFAS.

Impact of substrate concentrations on specific nitrogen removal rate of autotrophic biomass in different compartments of IFAS system

Expected gradients of the ratio between nitrogen removal rate by AnAOB and NOB in IFAS (r_{N_AnAOB}/r_{N_NOB}) within cross-sectional area of biofilm are presented in Figure 3(A). Two scenarios were taken into account, in which NOB bacteria are characterized by maximum growth rate (μ_{NOB}) of 0.6 and 0.67 d^{-1} and respective affinity constant values for FNA (K_{S,FNA_NOB}) of 8.723E-4 and 5.4E-5 mgN/l (the first set of values were applied in the model of Trojanowicz et al. [11] and the second pair of values were presented as the feature of K-strategists NOB by Winkler et al. [20]). In each of the considered variants, AnAOB bacteria have competitive advantage over NOB in biofilm ($r_{N\_AnAOB}/r_{N\_NOB} > 1$). It means that AnAOB bacteria are able to utilize more nitrites than NOB in the same time. However, we could take fully advantage of those interrelations only when DO does not suppress AnAOB growth. Due to this, a biofilm zone with permanent anoxia is needed (even while a bioreactor is aerated), as well as application of time intervals without aeration (fully anoxic conditions).

Predicted concentration of free ammonia is lower by one order of magnitude than half saturation coefficient of AOB for this key substrate (Figure 2(B)). At the same time, it is two orders of magnitude higher than FA affinity constant for AnAOB. Due to this, it is not the limiting factor for AnAOB growth. That can be recognized as another proof that AOB growth is the limiting step of the PN/A process. In other words, if only AOB could generate

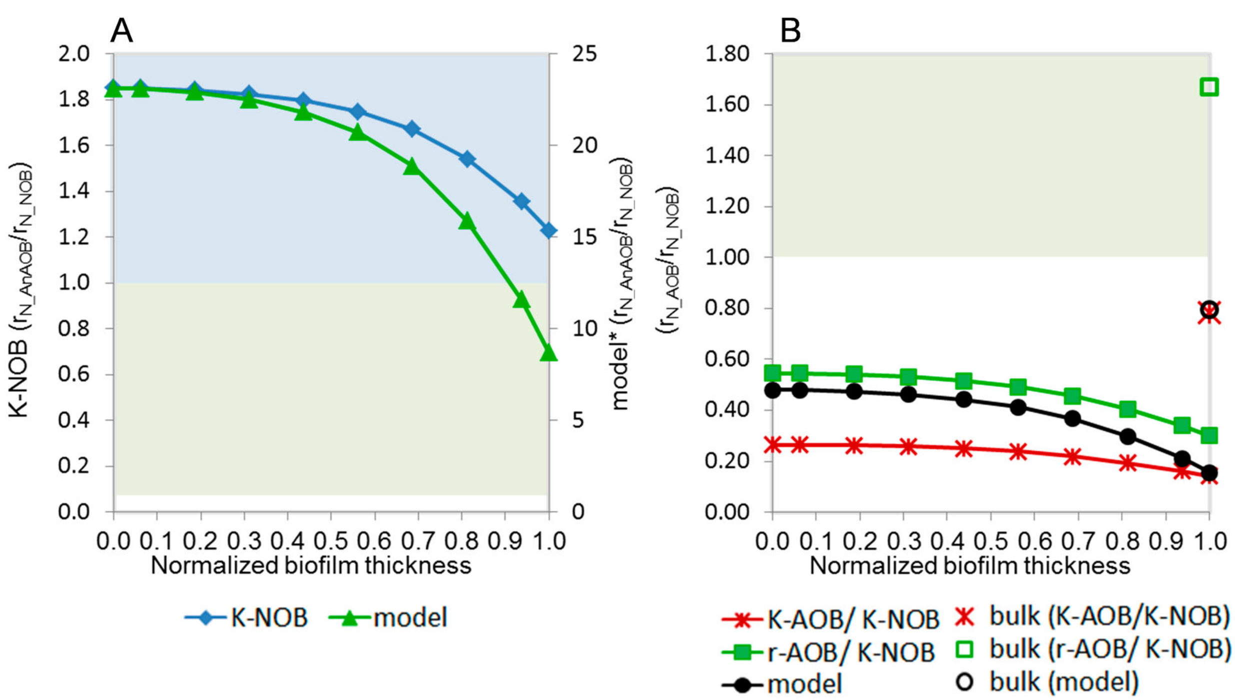

Figure 3. (A) Values of ratio between nitrogen removal rate of AnAOB and NOB (r_{N_AnAOB}/r_{N_NOB}) in the biofilm. (B) Values of ratio between nitrogen removal rate of AOB and NOB (r_{N_AOB}/r_{N_NOB}) in the biofilm and bulk liquid (activated sludge).

*model–scenario, in which NOB bacteria are characterized by maximum growth rate (μNOB) of 0.6 and affinity constant value for FNA (K_{S,FNA_NOB}) of 8.723E-4 (applied in the PN/A model of Trojanowicz et al. [11])

higher flux of N–NO_2 than NOB could utilize in the same period of time (aerated phase), then entire surplus load of N–NO_2, which was not consumed by NOB, would be available for AnAOB. Because of this, the most important factor in the PN/A system probably is the ratio between nitrogen removal rates of AOB and NOB (r_{N_AOB}/r_{N_NOB}). The higher the ratio, the easier to outselect NOB in the system. Results of the conducted theoretical analysis, displayed in Figure 3(B), indicate that the nitrogen removal rate of AOB (at 15°C) is always lower than that of NOB in the biofilm reactor's compartment, regardless of K-AOB or r-AOB is considered ($r_{N\_AOB}/r_{N\_NOB} < 1$). Therefore, NOB growing in a biofilm would have the capacity to utilize the total amount of nitrites produced by AOB (during aerated phase, in an aerobic zone of biofilm where AnAOB are suppressed by oxygen) and by this decrease amount of N–NO_2 available for AnAOB. It explains why it is so hard to remove NOB from biofilm. But in the case of activated sludge (Figure 3(B)), in one of three scenarios: assuming r-AOB as a dominant group of this kind of bacteria in this reactor compartment, the 'overplus production of N–NO_2' (surplus nitrites accumulation, which cannot be utilized by NOB) is possible ($r_{N\_AOB}/r_{N\_NOB} > 1$). As a result, the route to major elimination of NOB from the system could be opened. The ratio (r_{N_AOB}/r_{N_NOB}) depends mainly on the amount of AOB in the bioreactor and concentration of ammonium nitrogen (FA) in the bulk liquid. High bulk N–NH_4 not only increases AOB growth rate but also creates conditions for selection of fast growing r-AOB in the bioreactor [19]. In IFAS, we can increase the concentration of AOB by rising concentration of activated sludge and therefore the hybrid system has prodigious advantage over MBBR. The other, important problem is the concentration of ammonium nitrogen in the bulk liquid zone of the bioreactors for mainstream PN/A.

Nitrogen removal rates and specific growth rates of autotrophs in the function of temperature and at different N–NH_4 and VSS concentrations in IFAS bioreactor

In accordance with the legal regulations, total nitrogen (TN) concentration in the treated wastewater must be lower than 10 mgN/l (large-scale wastewater treatment plants). As a consequence, ammonium nitrogen in the effluent from PN/A should be below 10 mgN/l. In the case of fully mixed, continuous-flow bioreactors (when bulk liquid nitrogen concentration is close to its concentration in the effluent) it will have a negative impact on the kinetics of ammonium oxidation process (r_{N_AOB}) due to the AOB growth limitation by low substrate concentration. As a result, we can expect lowering of ammonium–nitrogen removal rate and discussed above the ratio of (r_{N_AOB}/r_{N_NOB}), as well as increase of nitrate production by NOB. In Figures 4 and 5, theoretically estimated values of nitrogen removal rates (r_N) and specific growth rates (μ) of AnAOB, AOB and NOB in IFAS in the function of temperature are shown. Two cases were taken into consideration: (A) assumed bulk N–NH_4 of 5 mgN/l and (B) assumed bulk N–NH_4 of 1 mgN/l. Additionally, for each scenario, the impact of activated sludge concentration (assumed VSS: 1000 and 4000 mg/l) was examined. Nitrite–nitrogen concentration in the bulk liquid was set as the constant value of about 0.1 mgN/l, what is consistent with the recorded values of those nitrogen species in the pilot-scale IFAS [4,5,34,35]. In the first analysis (Figure 4) kinetic parameter values of autotrophs, consistent with applied in PN/A model [11], were used. In turn, the comparison between nitrogen removal rates of r- and K-strategists AOB with K-NOB is presented in Figure 5. 'K-strategist' bacteria are characterized by low half saturation coefficients for substrates (K_S) and low maximum growth rate (μ_{max}), so they are adapted to the low substrate concentrations in the environment, whereas 'r-strategists' grow faster with lower substrates affinity (high K_S) usually in the conditions of high substrate concentrations.

Both nitrogen removal rates (r_{N_AOB}) and specific growth rates values of AOB (μ_{AOB}) are higher when bulk N–NH_4 is 5 mgN/l (Figures 4 and 5). What is important r_{N_AOB} is higher than $r_{N\text{-}NOB}$ when assumed N–NH_4 is 5 mgN/l and at the temperatures over 16°C. In contrast, when bulk N–NH_4 of 1 mgN/l is considered, the nitrogen removal rate of AOB is lower than that of NOB in the temperature range (10–25°C). Because of that, in the first case (N–NH_4 of 5 mgN/l) it would be possible to gradually eliminate NOB from the system down to a temperature of 16°C. In the second scenario, out-selection of NOB would be difficult in the whole temperature range (since $r_{N\_AOB} < r_{N\_NOB}$). Remarkably, a threshold temperature of 16°C (under which values of r_{N_AOB} are lower than those of r_{N_NOB}) derived from the current, theoretical analysis is consistent with the results obtained by Sultana et al. [9], in which nitrogen removal capacity by AOB and NOB levelled out at 16°C. Gilbert et al. [17] also reported 16°C as a threshold temperature for biomass growth in activated sludge in PN/A bioreactor, below which N–NO_3 accumulation occurred. We can note that the increase in the concentration of VSS in the system has a positive impact on nitrogen removal rate by AOB, what is important since it could be applied as the measure of keeping high deammonification capacity under lower temperatures (Figure 4(A1–

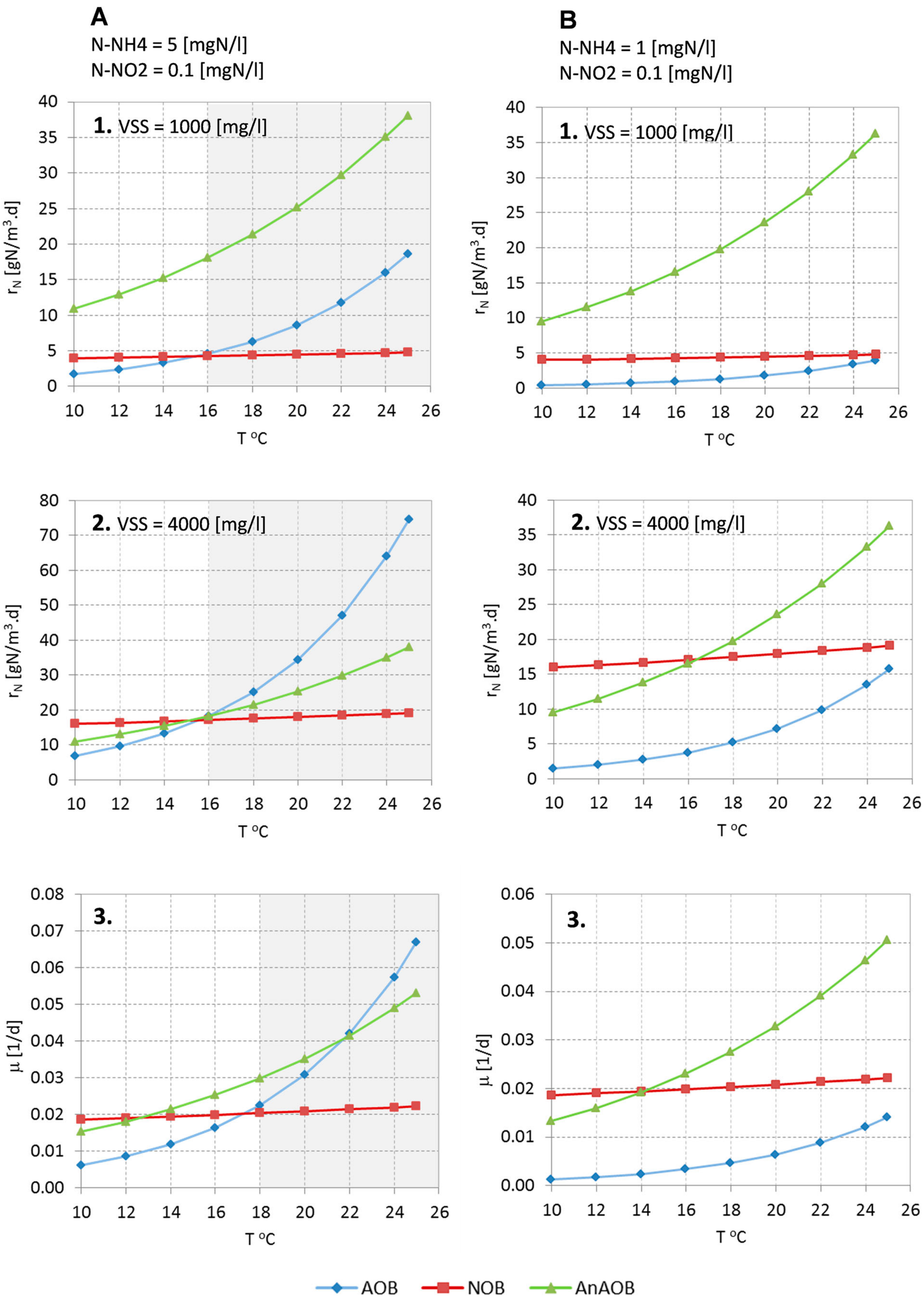

Figure 4. Analysis of temperature impact on nitrogen removal rate and specific growth rate of autotrophic microorganisms under realistic substrate concentrations in IFAS system.

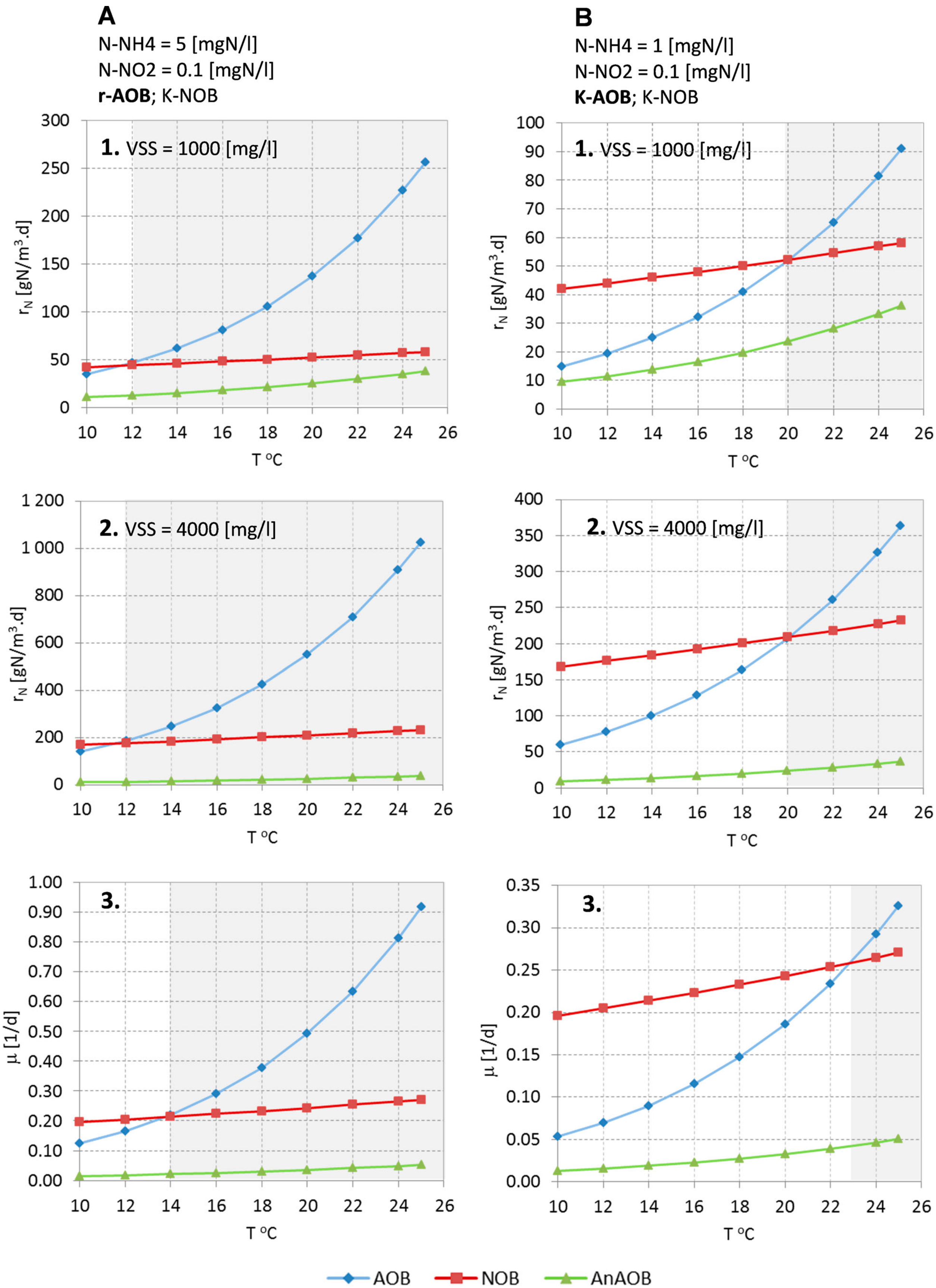

Figure 5. Analysis of temperature impact on nitrogen removal- and specific growth rate of autotrophic microorganisms under realistic substrate concentrations in IFAS system. Growth of r-AOB (under high N–NH_4 concentration in the bulk liquid), K-AOB and K-NOB (under low N–NH_4 concentration in the bulk liquid) were taken into account.

2,B1–2)). An increase in VSS does not influence the ratio of 'r_{N_AOB}/r_{N_NOB}' (essential for NOB elimination from the system). However, from the comparison of specific growth rate values of AOB and NOB (Figure 4(A3,B3)) we can conclude that NOB could be selected out from the activated sludge by applying sludge age (SRT) as another controlling factor. It would be possible at temperatures higher than 18°C and under N–NH_4 of 5 mgN/l (or more) in the bulk liquid, the specific growth rate of AOB is higher than that of NOB (so the minimum SRT of AOB would be lower than that of NOB), hence NOB could be eliminated by shortening SRT. In the situation, in which bulk N–NH_4 was assumed as 1 mgN/l there is no opportunity to apply SRT as a tool for NOB elimination due to lower specific growth rate of AOB than that of NOB in the whole temperature range.

Influence of growth strategy on nitrogen removal rates and specific growth rates of AOB and NOB

Impact of existence of better adapted to low substrate conditions K-AOB and K-NOB in the bioreactor (case B), as well as enrichment of r-AOB in the system when bulk N–NH_4 is higher (case A), on calculated 'r_N' and 'μ' values is presented in Figure 5. Higher nitrogen removal rates than it was earlier described (Figure 4) of better adapted strains of AOB and NOB to the environmental conditions in each of the analysed scenarios were predicted (Figure 5(A1–2,B1–2)). Furthermore, when bulk N–NH_4 of 5 mgN/l is assumed, higher nitrogen removal rates of r-AOB than K-NOB can be expected in a wider temperature range (from the threshold temperature of 12°C to 25°C) than in the earlier discussed case (Figure 5(A1–2). Application of SRT as a control tool for NOB elimination from the system could be utilized at lower temperatures, down to threshold point of 14°C (at which specific growth rates of r-AOB and K-NOB are equal).

Impact of environmental conditions in IFAS on growth rate and nitrogen removal by AnAOB

Considering the nitrogen removal rate by AnAOB, in some of the scenarios being analysed (Figure 4(A1,B1) it is higher than that of AOB and NOB. In such a case, if only r_{N_AOB} is higher than r_{N_NOB} (Figure 4(A1)) then all available nitrites could be consumed by AnAOB. The higher the flux generated by AOB, the better the growth conditions of AnAOB, since in the situation being considered, there is a potential for a decay of anammox cells due to lower nitrite supply than AnAOB need for their growth. Nitrate production would probably occur as a result of NOB activity in the aerated phase. However, the existence of an anoxic phase in biofilm (by setting of proper DO and keeping the right biofilm thickness) would decrease the amount of nitrites being oxidized by NOB during aeration. If r_{N_AnAOB} is lower than r_{N_AOB} and simultaneously r_{N_AOB} is higher than r_{N_NOB} (Figures 4(A2) and 5(A1–2,B1–2)) then nitrite accumulation can occur. However, if only surplus nitrite concentration is kept below the inhibition level of anammox bacteria, then extension of non-aerated phase will allow AnAOB utilize them completely. The beneficial consequence would be an increase of AnAOB abundance in biofilm, rise of anammox process capacity and gradual elimination of NOB from activated sludge (by decay). The worst situation would occur in the case of higher r_{N_NOB} than r_{N_AOB} (for example when low bulk N–NH_4 reduces AOB growth rate and by this decreases nitrogen removal rate) (Figures 4 and 5 (B1–2)). Then out-selection of NOB would be much harder or even impossible, what in turn could result in the reduction of amount of AnAOB in the system due to lower N–NO_2 supply and endogenous respiration.

SRT separation in IFAS between biofilm and activated sludge

Because AnAOB mostly grow in biofilm, their SRT is much longer and independent from the activated sludge age. That is another great advantage of IFAS since the elimination of NOB from activated sludge by decreasing SRT can be done without influencing the negative quantity of AnAOB biomass in the system. The beneficial effect of SRT separation of different bacterial groups in the same bioreactor was discussed by other authors [10,23]. Moreover, on the basis of the conducted analysis it can be concluded that the increase in VSS in the IFAS is beneficial to AnAOB, since higher N–NO_2 load is accessible to them.

Impact of IFAS type on PN/A process performance

The discussed results clearly indicate high significance of N–NH_4 concentration in the system as the factor influencing growth rate and nitrogen removal rate by AOB (since operating the PN/A under higher value of ammonium–nitrogen reduces AOB growth rate constraints). However, at the same time low effluent ammonium–nitrogen concentration is required. This contradiction could be overcome by running the mainstream PN/A process in an 'IFAS sequencing batch reactor' or an 'IFAS plug-flow system'. In those systems we could keep N–NH_4 above 10 mgN/l for some period of time, after which it decreases to the low values compliant with regulations. It would not be possible in the case of fully mixed, continuous-flow IFAS bioreactors, in which N–NH_4 must be kept continuously at low level (below 10 mgN/l). Another option for maintaining high bulk N–NH_4 is the separation of partial nitritation from anammox (two-stage process). Because only about half of ammonium–nitrogen load has to be oxidized to nitrites, high residual bulk N–NH_4 in PN reactor would be assured. There are a body of evidence that operating the system under higher N–NH_4 allows to enrich biomass in r-AOB bacteria [19,45] what was shown in the current analysis as truly beneficial for efficient mainstream PN/A (Figures 3(B) and 5(A1–2)). The advantage of running the process at high N–NH_4 was indicated as well by Malovanyy et al. [34] and by Hoekstra et al. [15]. Thus in the case of a continuous-flow system, keeping residual N–NH_4 in the bulk liquid would be a necessary factor for efficient PN/A process running.

Comparison with results from empirical studies

The results published by other authors confirm presented earlier theses regarding mechanisms of PN/A in IFAS system. Comparisons of the mainstream PN/A process performance operated under different reactor configurations, influent ammonium nitrogen concentrations (from 14 to 80 mgN/l) and temperatures (from 10.4°C to 32°C) are presented in Table 3. Usually higher capacity (from 65.6 to 223 gN/m^3) and efficiency (from 75% to 95%) of the process was gained in SBR or plug-flow hybrid systems (biofilm or granular biomass mixed with activated sludge) and under conditions of residual N–NH_4 in the bulk liquid more than 2 mgN/l [4,18,26,28,30,34,40]. Those reactors type must have allowed keeping higher N–NH_4 bulk concentrations over the substantial period of time (after starting aeration cycle in SBR). As explained above, the positive effect of such conditions on nitrogen removal rate by AOB was possibly reinforced by high residual N–NH_4 [17,18,25,28,30,34,39]. There are proofs that biomass growing in the PN/A systems can be enriched with AOB by the application of high nitrogen loading rate [25]. Some studies presented bioaugmentation of the bioreactors with 'AOB-rich' biomass as the measure for improving nitrogen removal efficiency and NOB's outcompeting [5,18,34]. Moreover, improved elimination of NOB was supported by shortening of biomass age (SRT) in the bioreactor [19,28,30]. Applied dissolved oxygen concentrations in the efficient PN/A systems were in the wide range from 0.17 to 2.0 mgO_2/l (Table 3). It seems that more crucial is to create conditions in the bioreactor, under which oxidizer (DO) is the limiting substrate of the AOB and NOB's growth instead of reducer (N–NH_4) [45]. Therefore, setting of DO concentration should be correlated with the concentration of N–NH_4 in order to assure the competitive advantage of AOB. It could be easier gained at higher ammonium concentrations in the bioreactor. The possibility of keeping high N–NH_4 in the system has another effect related with the selection of r-AOB in the PN/A system, thereby increasing ammonium oxidation rate and easier elimination of NOB [19,46–48]. Winkler et al. [20] demonstrated that under both low substrate

Table 3. Comparison of mainstream anammox system's configurations, selected operational parameters and gained capacity and efficiency of nitrogen removal via partial nitration/anammox processes.

Lp.	Bioreactor type[a]	AnAOB form of growth[b]	DO [gO_2/m^3]	T [°C]	In[c] N–NH_4 [gN/m^3]	(COD/N)in [gCOD/gN]	Bulk N–NH_4 [gN/m^3]	NRR [gN/m^3d]	E%	Ref.
1.	IFAS-SBR (laboratory scale, R)	BF	0.17	15.5	23(±6)	2	1.9 (±0.5)	79 ± 16	88	[28]
2.	SBR (laboratory scale, S)	AS	0.33 0.21	28.8	263.1 103.3	0.4 0.5	~ 90.5 46.2	740 160	65.6 2	[29]
3.	SBR (laboratory scale, S)	BF	0.8-1.1	30	50	1	<5	~ 90	>90	[25]
4.	IFAS, plug flow (laboratory scale, R)	BF	0.15-0.36	22–25	40	1.3	>3	100	82	[30]
5.	Upflow PN/A system (laboratory scale, R)	GR	0.6	25	28(±5)	1.8	3.3 (±1.6)	81	82.7–87.5	[31]
6.	SBR-IFAS (laboratory scale, R)	GR	0.4-0.6	32	37–80	~1	10–70 (>10)	~75	70	[18]
7.	SBR-IFAS (laboratory scale, R)	GR	0.8-1.2	30	33.0–76.9	2.5 1.1	>5.5 >15	77.3 30.8	94.7 38.9	[26]
8.	SBR (pilot scale, R)	GR	2.0 (±1.4)	12–18	17(±6)	1.5-4.4	5.22 (±2.7)	33.5	52	[32]
9.	PACKED BED (laboratory scale, R)	BF	0.5	20–35	20(±7)	1.8	0	210	38	[33]
10.	GRANULARfully mixed (pilot scale, R)	GR	n.a.	10.4–24.7	14.2–33.0	0.6–2.5	5	223 (23°C) 97 (13.4°C)	n.a.	[15]
11.	IFAS fully mixed, continuous flow (pilot scale, R)	BF	1.0	25	45.5 ± 2.3	1.8 ± 0.1	5	55 ± 6	70 ± 4	[4]

Abbreviations: [a]'R' – real wastewater, 'S' – synthetic wastewater; [b]'BF' – biofilm, 'AS' – activated sludge, 'GR' – granules; [c]'In' – influent.

concentration and short SRT, r-NOB (instead of K-NOB what could have been expected) was selected, so maybe the same strategy could be applied for AOB's growth, what would be useful for the PN/A process. It is consistent with the results presented in Figures 3(B) and 5(A1–2). Those results could lead us towards another part of explanation why is PN/A process more efficient in IFAS than MBBR reactors? In biofilm, conditions would be more preferential for selection of K-bacteria due to the stratification of substrate concentrations. In the environment of more even distribution of substrate concentration, as in activated sludge flocks, r-AOB are easier to be selected. Moreover, it indicates that enrichment of r-AOB in SBR and plug-flow system (due to cycles of high substrate concentration), are more probable than in continuous flow bioreactors (steady, low concentrations of substrates).

Conclusions

On the basis of the presented analysis and discussed literature results, we can indicate some important points regarding the mechanism of efficient PN/A in hybrid bioreactors (IFAS).

- The limiting process in PN/A systems is ammonium oxidation to nitrites by AOB. Achieving high nitrogen removal rate of AOB is a pass to the efficient mainstream PN/A.
- Nitrogen removal rate by AOB (r_{N_AOB}) depends on the amount of active AOB in the system, substrate concentration (bulk $N–NH_4$, DO and inorganic carbon), temperature and intrinsic, genetically determined features of ammonium oxidizers (described and explained by kinetic factors and the theory of r- and K-selection of organisms).
- Sufficient supply of nitrite–nitrogen assures AnAOB biomass retention in a bioreactor and as the effect a satisfactory nitrogen removal rate.
- In IFAS, it is possible to increase the nitrite–nitrogen production (value of r_{N_AOB}) by enriching the system with AOB biomass. It is conducted by retaining suspended solids originated from the detachment of outer layers of biofilm/granules or by bioaugmentation with activated sludge from conventional bioreactors. The effect is a 'surplus nitrites' flux from activated sludge into biofilm, which cannot be transformed by NOB and is available to AnAOB.
- The efficacy of NOB out-selection depends on the ratio between nitrogen removal rate of AOB and NOB (r_{N_AOB}/r_{N_NOB}). If the ratio is higher than one ($r_{N\_AOB}/r_{N\_NOB} > 1$), then NOB elimination would be possible.
- In hybrid reactor (IFAS), AOB can grow in activated sludge under conditions of less diffusional limitations of substrates and product transfer. In turn, slow growing AnAOB are protected in biofilm against washing out and toxic factors like oxygen.
- In intermittently aerated reactors, optimal conditions for 'surplus nitrites' utilization by AnAOB are created by periodically stopping oxygen transfer into biofilm and by this growth of NOB. Then a constant decrease in NOB abundance in the system can occur. At the same time, the increase in the amount and activity of AOB and AnAOB can take place.
- In hybrid systems, biomass age can be applied as another control parameter for NOB out-washing; however, its application is limited to some T range (with threshold temperature at which specific growth rates of AOB and NOB get even).
- Since AOB growth rate depends also on bulk ammonium concentration, 'IFAS-SBR' or 'IFAS-plug flow' reactors will be advantageous system layouts. It is because higher $N–NH_4$ could be kept for certain time in repeating cycles in SBR or continuously in the initial zones of plug-flow systems. In the case of fully mixed, continuous flow systems a residual effluent ammonium is required in order to sustain high nitrogen removal rate by AOB.

Disclosure statement

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