

Article

Transitioning Deammonification from Sidestream to Main-Stream Treatment: Long-Term Comparison of Integrated Fixed Film Activated Sludge and Moving Bed Biofilm Reactors with Polyurethane Foam Carriers at Lab-Scale

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Abstract

Deammonification, which is based on partial nitrification and anammox (PN/A), is a well-established sidestream treatment for nitrogen removal. However, transferring deammonification to mainstream wastewater treatment remains challenging due to low temperatures, the need to retain slow-growing anammox bacteria (AnAOB), and their competition for nitrite with nitrite-oxidizing bacteria (NOB) and heterotrophic denitrifiers. This work investigates cubic polyurethane foam carriers to promote growth and retention of AnAOB. A moving bed biofilm reactor (MBBR) and an integrated fixed-film activated sludge (IFAS) reactor were compared over a three-year experimental period at lab-scale. The feasibility of the biofilm carriers for deammonification was first evaluated under sidestream conditions, followed by a stepwise transition to mainstream operational conditions. The impact of operational parameters, including dissolved oxygen concentration, pH value, and aeration strategy, was evaluated with respect to the activity of aerobic ammonium-oxidizing bacteria (AOB), NOB, and AnAOB, as well as nitrogen removal rates. Deammonification reached nitrogen removal rates of $0.04\text{--}0.12\text{ kg N m}^{-3}\text{ d}^{-1}$ (IFAS reactor) and $0.02\text{--}0.28\text{ kg N m}^{-3}\text{ d}^{-1}$ (MBBR) at subphases with reactor bulk concentrations above $60\text{ mg NH}_4\text{-N L}^{-1}$. Highest nitrogen removal degrees of $77 \pm 6\%$ (IFAS) and $76 \pm 5\%$ (MBBR) were achieved at reactor bulk concentrations of $96\text{ mg NH}_4\text{ L}^{-1}$ and $97\text{ mg NH}_4\text{ L}^{-1}$, respectively. Lower concentrations triggered NOB activity in both reactors, leading to an increase in nitrate concentration up to $22\text{ mg NO}_3\text{-N L}^{-1}$. AOB and AnAOB activities were on average 6-fold higher on the carriers compared to suspended biomass throughout all experimental phases, demonstrating the feasibility of using cubic polyurethane foam carriers for deammonification. This was also confirmed by fluorescence in-situ hybridization (FISH) measurements. Median nitrogen removal rates over all experimental phases of $0.07\text{ kg N m}^{-3}\text{ d}^{-1}$ for the IFAS reactor and $0.05\text{ kg N m}^{-3}\text{ d}^{-1}$ for the MBBR were achieved, which are comparable to conventional activated sludge systems performing nitrogen removal via nitrification–denitrification. While at lower nitrogen concentrations, the IFAS reactor yielded superior nitrogen removal rates, peak nitrogen removal rates of $0.28\text{ kg N m}^{-3}\text{ d}^{-1}$ were measured in the MBBR configuration. However, controlling NOB activity at lower temperatures and concentrations remains a challenge in MBBR and IFAS configurations. In our study, in the IFAS reactor NOB activities were visible on fewer days than in MBBR. At mainstream-like conditions, higher nitrogen removal rates of IFAS ($0.09\text{--}0.12\text{ kg N m}^{-3}\text{ d}^{-1}$) were achieved compared to the MBBR ($0.06\text{--}0.09\text{ kg N m}^{-3}\text{ d}^{-1}$). This demonstrates the advantage of the IFAS reactor



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in treating mainstream wastewater via deammonification. As an autotrophic nitrogen removal process, the implementation of deammonification in the mainstream of municipal wastewater treatment plants enables enhanced recovery of biogas from sewage organic matter. The latter would otherwise be consumed during the conventional nitrification-denitrification pathway. Consequently, the overall energy balance for wastewater treatment can be improved, contributing to a more environmentally sustainable process.

Keywords: wastewater treatment; anammox; MBBR (moving-bed biofilm reactor); IFAS (integrated fixed-film activated sludge); polyurethane foam carrier; anammox activity; nitrogen removal

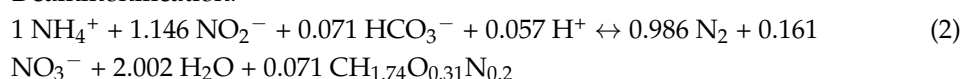
1. Introduction

Biological nitrogen removal in the mainstream of municipal wastewater treatment plants (WWTPs) commonly relies on nitrification-denitrification. Nitrification is performed by aerobic ammonium (NH_4^+) oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), which metabolize NH_4^+ to nitrite (NO_2^-) (nitritation) and NO_2^- to nitrate (NO_3^-), respectively. Under anoxic conditions (absence of dissolved oxygen), heterotrophic denitrifying bacteria (HB) reduce the produced NO_2^- or NO_3^- to elemental nitrogen (N_2) through enzymatically mediated reduction steps using organic carbon as the electron donor (referred to as chemical oxygen demand: COD). Deammonification combines partial nitritation with the anammox (anaerobic ammonium oxidation) process (Equations (1) and (2) [1]). Partial nitritation aims to convert only a part of NH_4^+ into NO_2^- , providing both required substrates for the anammox bacteria (AnAOB). In the anammox process, NH_4^+ and NO_2^- are converted to N_2 , with a smaller fraction oxidized to NO_3^- , without the consumption of COD [2,3].

Nitritation:



Deammonification:



In municipal wastewater treatment, deammonification is well established in the sidestream treatment of the effluent resulting from dewatering of anaerobic digested sludge (sludge dewatering effluent—SDE), which is rich in NH_4^+ but poor in readily biodegradable COD. By 2014, over 100 full-scale sidestream treatment plants employing deammonification had been implemented worldwide [4], and number of installations has continued to increase since then. The application of the anammox process in the mainstream line of wastewater treatment, as an alternative to conventional nitrification/denitrification, has also been a focus of research in recent years [5–8]. While reducing COD demand for nitrogen removal, deammonification would enable increasing the COD load removed in primary clarification and sent to anaerobic sludge digestion, enhancing methane production. The estimated higher methane production would account for approximately 4.5 kg COD per kg N removed, compared to conventional nitrification-denitrification [9]. Thus, deammonification would help establish an energy-positive wastewater treatment concept.

Deammonification as a mainstream treatment faces some challenges, compared to sidestream application, including performing partial nitritation at low concentrations and lower temperatures, seasonal fluctuations, and the enrichment of the slow-growing AnAOB [8,10]. For stable deammonification, only about half of the NH_4^+ needs to be oxidized to NO_2^- . In sidestream applications, the alkalinity in the SDE is typically consumed when approximately 50% of NH_4^+ is converted, and the drop in pH value inhibits further

nitritation [11,12]. In mainstream applications, higher alkalinity may be available; therefore, partial nitritation cannot be reached with limited alkalinity alone and must be maintained through a tailored aeration strategy [13,14].

One of the biggest challenges in mainstream applications is to establish stable nitritation on a long-term, suppressing the further NO_2^- oxidation step to NO_3^- . This can be achieved either by applying short sludge age to promote NOB washout and/or by inhibiting NOB growth [15,16]. Reduced sludge age leads to a selective wash out of NOB, as NOB have a lower specific growth rate compared to AOB at temperatures higher than 18 °C and 5 mg $\text{NH}_4\text{-N L}^{-1}$ (0.91–1.31 d^{-1} for NOB, 1.05–1.4 d^{-1} for AOB, tested with a sludge retention time of 2–5 days) [15–17]. This is effective in sidestream treatment but not at the lower temperatures of the mainstream. In addition, controlling NOB growth only by lowering the sludge age is difficult in one-stage systems, as AnAOB require a high sludge age to be enriched in the system. When biofilm carrier materials are used to support AnAOB growth and retention, further operational measures are required to limit NOB growth and activity. These methods [16] include dosage of inhibiting chemicals, hydrazine [18], FA (NH_3 , free ammonia) or FNA (HNO_2 , free nitrous acid) shock treatment [19–21], and decreasing dissolved oxygen (DO) availability by aeration strategy combined with pH control [4,6,22,23]. In mainstream systems, the lower FA and FNA concentrations compared to sidestream conditions reduce the inhibitory effect on NOB, making effective NOB control more challenging. In MBBR (moving bed biofilm reactor) systems, a FA shock treatment solution for suppression of NOB has already been successfully tested on AnoxKaldnes K5 carriers [21]. Also, combined inhibition strategies with low DO, periodical activated sludge exposure to high FNA, as well as operation at high residual NH_4^+ concentration (about 8 mgN L^{-1}) have already been applied in a pilot-scale IFAS (integrated fixed-film activated sludge) deammonification system with K5 carriers [24].

Literature evidence indicates that the first biofilm carrier-based full-scale deammonification systems under mainstream conditions still face further challenges beyond controlling NOB activity, as interference by heterotrophic bacteria, augmentation of activities of AnAOB and AOB under low N/COD ratio, seasonal temperature variations, and final polishing of effluents [25,26]. In previous investigations, mainly flat biofilm carriers were employed. In the present study, a substantially different carrier type, cubic polyurethane foam carriers, was used. Due to their three-dimensional structure, pronounced DO gradients can be expected within the carrier matrix, with lower DO concentrations in the center of the carrier, potentially creating favorable conditions for AnAOB over NOB. Additionally, AnAOB are protected from shear forces inside the cubic carrier, supporting adhesion and retention of the bacteria in the biofilms. Mainstream deammonification at lab-scale has been successfully tested with synthetic wastewater in different reactor configurations like upflow membrane-aerated biofilm reactor [5] and with cubic polyurethane foam carriers in plug flow fixed-bed [27–29] or anaerobic sequence batch biofilm reactors [30]. In the present study, both an MBBR and an IFAS process were employed. The short sludge age of the suspended biomass in MBBRs may be useful to select bacteria that can grow on the carriers and potentially control NOB enrichment in the system. In contrast to the MBBR, the IFAS process combines biofilm growth on carriers with suspended activated sludge that is returned to the reactor from the secondary clarifier. By providing adequate sludge age for AOB growth in suspended flocs, while allowing AnAOB to develop in the biofilm carriers, process conditions for AOB and AnAOB growth can be optimized [31,32]. One of the research questions was to elucidate whether controlling the sludge age may support AOB activity on the flocculent sludge and help control NOB growth.

Another challenge to be faced is the different temperature dependency of AnAOB compared to AOB, with decreasing temperatures leading to a higher AOB to AnAOB

ratio. DO adjustment may balance NO_2^- production by AOB with the NO_2^- uptake by AnAOB. Lotti et al. also found that AnAOB cultivated in biofilm systems seems to be less influenced by decreasing temperature than AnAOB grown in suspension [33]. Biofilm suitability for mainstream treatment facing low temperatures was shown by Laureni et al. [34]. Laureni et al. demonstrated over 5 months process stability in 12 L sequencing batch reactors (MBBR and Hybrid-MBBR) at mainstream conditions at low temperatures around 15 °C with removal rates and effluent quality similar to conventional treatment for biological nutrient removal [34]. Statistical analysis by Li et al. showed temperature as one of two factors (the other one being the N/COD ratio) influencing the nitrogen removal pathway [35]. Their study was based on experiments of an upflow double-blanket filter filled with cubic polyurethane carriers and fed with synthetic and synthetic-municipal wastewater mix [35].

The presence of biodegradable COD in mainstream applications, compared to sidestream systems, increases process complexity. Moderate COD concentrations (high N/COD ratios) may support the control of NOB activity, since moderate heterotrophic activity may reduce DO concentration and AOB exhibit a higher affinity for DO than NOB. However, heterotrophic bacteria may also compete with AnAOB for NO_2^- in anoxic niches. At low N/COD ratios, heterotrophic activity increases and may dominate over anammox activity as heterotrophic bacteria grow faster than AnAOB [31,35]. For a CANON (completely autotrophic nitrogen removal over nitrite) process in membrane bioreactors, Zhang et al. found that below a threshold of 0.59 N/COD ratio, AnAOB were suppressed and nitrogen removal decreased [36]. In mainstream deammonification, COD concentration is higher than in sidestream, and therefore N/COD needs to be considered to establish a stable deammonification in mainstream over a long time period. This aspect was a key focus of the present study, in which different N/COD ratios were obtained by mixing real municipal wastewater with SDE.

To summarize, the major challenges for the application of deammonification in mainstream are low water temperature, low ammonia (NH_3) and NO_2^- concentrations (not favorable to control NOB activity), low N/COD-ratio in the influent, as well as the enrichment and retention of AnAOB biomass [37,38].

The objective of the study was to investigate the feasibility of cubic polyurethane foam carriers to support deammonification under mainstream conditions in an MBBR and in an IFAS configuration, while examining how different reactor configurations influence the growth and competition of AOB, AnAOB, and NOB in these systems.

Cubic polyurethane foam carriers were chosen for their three-dimensional structure, which promotes pronounced DO gradients. The study aimed to assess whether these oxygen gradients within the cubic carriers provide suitable conditions for AOB and AnAOB growth and help to outcompete NOB. This was also demonstrated by fluorescence in-situ hybridization (FISH) measurements.

Polyurethane foam carriers have already been studied in previous studies with synthetic wastewater in upflow anaerobic sludge blanket and sequencing batch reactors [39,40]. The novelty of the present work also relies on (i) the use of real wastewater instead of synthetic water, and (ii) the experimental approach with stepwise decrease of the influent NH_4^+ concentrations in SDE, therefore transitioning deammonification from sidestream to mainstream conditions. This approach allowed us to identify threshold concentrations for NOB activity. In the second half of the experimental period, real municipal sewage was added to the influent to evaluate process operation changes due to a lower N/COD ratio and the impact on nitrogen removal performance.

A key strength of the study is also the extended duration of the continuous lab-scale investigations over 3 years, providing a robust data set that is suitable for understanding the dynamics of biofilm systems.

The results provide valuable insights into the process dynamics and operational challenges, representing an important contribution toward the further development of treatment technologies aimed at achieving stable deammonification performance in the mainstream of municipal WWTPs.

2. Materials and Methods

2.1. Experimental Set-Up

Two lab-scale set-ups (behrotest[®] KLD 4N, Behr Labor-Technik GmbH, Düsseldorf, Germany) were operated in parallel over 1000 days, one configured as MBBR, the other as an IFAS process (Figure 1). In each system, the influent wastewater was fed by a peristaltic pump in a cylindrical aerated tank (4 L) filled with cubic polyurethane foam carriers (Linpor[®], Strabag SE, Wien, Austria): 40 pores per Inch, totally open porous, 20% volumetric filling degree. The Linpor[®] carriers had been pre-cultivated in a lab-scale deammonification MBBR treating the effluent of anaerobic sludge dewatering (SDE) of a real municipal WWTP and were transferred to the aerated tanks at experimental day 0. Depending on the experimental phase, the feeding strategy relied either on a set pH value in the aerated tank or on fixed time intervals. From the aerobic tank, the water flow over a connecting pipe into a cylindrical settler (2.5 L), where treated water and activated sludge flocs were separated. In the IFAS configuration, the settled sludge was pumped back to the aerated tank with a recycling ratio of about 200%. Sludge age ranged from 5 to 8 days and was adjusted by taking excess sludge out of the mixed tank reactor three to five times a week. In the MBBR configuration, excess sludge was disposed of regularly. SDE provided by a municipal wastewater treatment plant (190,000 PE) was used as influent, in some experimental phases diluted with tap water, in others mixed with real pre-settled municipal sewage. In this way, the N (sum of NH₄-N, NO₂-N, and NO₃-N) and COD concentrations could be changed in the experimental phases. The composition of both the SDE and the mixture with municipal sewage is provided in Table 1. Influent concentrations of N and COD were quantified at least once per week. NH₄-N, NO₂-N, and NO₃-N concentrations in the effluent were analyzed with a minimal sampling frequency of two days a week. COD, N, total suspended solids (TSS), and volatile suspended solids (VSS) were measured on a regular basis as well. The temperature in the tanks was not controlled and varied with room temperature between 21 and 27 °C (10% and 90% percentile). Temperature (WTW FDO[®] 925, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany), pH value (HI1230B, Hanna Instruments GmbH, Graz, Austria), and oxygen concentrations (WTW FDO[®] 925, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) were measured online in each aerated tank. In addition to the regular analytical program, ex-situ activity tests for aerobic (AOB) and anaerobic (AnAOB) bacteria and FISH (fluorescence in situ hybridization, Nitri-VIT[®], VIT[®] Anammox, vermicon AG, Hallbergmoos, Germany) measurements were conducted.

2.2. Experimental Phases, Feeding and Aeration Strategies

The experiment was conducted in three phases, A (marked red in tables and figures), B (marked yellow), and C (marked blue), each containing subphases (A1–A3, B1–B3, and C1–C3, see Table 2). Throughout the whole experiment, the reactors were operated without pause. Switching from one phase to the next one was achieved by adjusting the operational conditions as described in Table 2. Phase A investigated the influence of decreasing influent N concentration (from 590 to 100 mg NH₄⁺ L^{−1}) with the question at which N concentration from side-to-mainstream NOB activity will occur. Due to increased NOB activity in phase A3, in subphase B1, reactors were emptied, cleaned, and refilled with new carriers from the same lab-scale sidestream MBBR deammonification reactor on day 472

(MBBR) and 473 (IFAS), respectively. To remove NOB completely, suspended sludge was discharged, and reactors were filled with new influent wastewater. As a pH-based feeding strategy in Phase A (pH upper limit 7.5–7.7) led to some load reduction, starting with Phase B, a time-based feeding program was used to guarantee a constant influent load. An upper pH limit of 7.9 also stopped influent dosage during the time-based feeding strategy. Furthermore, decreased N/COD-ratios from 1.7 to 0.3 of the influent and the effect thereof on nitrogen removal and activity of bacteria groups was investigated in Phase B. In Phase C, influent N concentration was further decreased, and N/COD-ratio decreased to reach mainstream-like conditions.

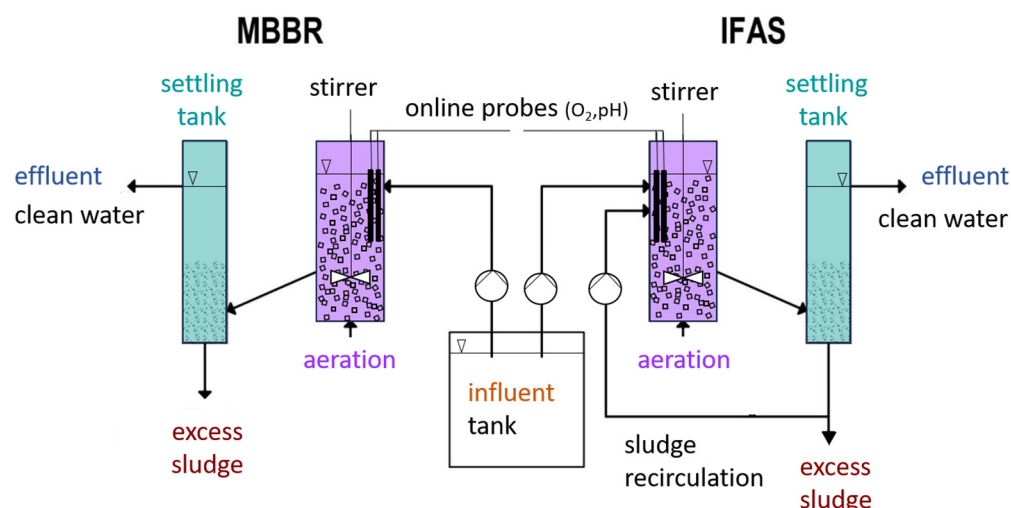


Figure 1. The cylindrical aerated tank with stirrer, used as MBBR or IFAS reactor, had a volume of 4 L, the cylindrical settler 2.5 L. Tanks are made from glass, and peristaltic pumps for the influent, recirculation, and excess sludge were used. Online probes for oxygen and pH were integrated in a process control system.

Table 1. Composition of influent wastewater within the experimental phases (mean values).

Matrix	Phase	mg COD L ⁻¹	mg NH ₄ -N L ⁻¹	N/COD
Undiluted SDE	A1-C1			
	before dilution	439	1017	2.3
Diluted SDE * mixed with sewage	B2 IFAS	434	289	1.0
	B2 MBBR	463	293	1.0
	C1 IFAS & MBBR	113	294	2.6
	C2 IFAS	119	112	1.2
	C2 MBBR	130	115	0.9
	C3 IFAS	264	70	0.5
	C3 MBBR	203	70	0.4

Note: * SDE diluted with tap water.

In phases A and B1, the aeration strategy relied on time-based aeration and non-aeration periods with a maximum oxygen concentration in the aerated phase, where aeration was stopped. Starting with phase B2, we established a fixed non-aerated period of 15 min with an oxygen concentration below 0.3 mg O₂ L⁻¹. This supports AOB over NOB, as AOB resumes activity faster than NOB (shorter lag phase in the nitrogen metabolism) after an anoxic phase of at least 15 min [22]. In phases with sewage addition to the influent, the aerated phases were prolonged to cope with higher loadings of biodegradable COD.

Table 2. Experimental phases and subphases.

Subphase	Experimental Days		Aeration Strategy		Feeding Strategy			Influent Composition
	IFAS	MBBR	Aeration * [min]	Pause [min]	IFAS	Lower and Upper pH Limit	MBBR	
A1	11–121	5–71	15	15		6.0–7.7, later 6.0–7.5		
A2	122–224	72–154	15	15		6.0–7.5		
A3-I	225–252	155–252	15	15		6.0–7.2		
A3-II	257–380	257–379	15, 20, 5	30, 40, 45		6.0–7.5		
A4	381–458	381–465	5	45		7.52		
B1	466–704	481–704	5	25	15 min every 1.5 h, later every 1.25 h	7.90 **	15 min every 2 h, later every 1.75 h	SDE
B2-I	705–751	709–751	10	15 < 0.3 mg O ₂ L ⁻¹	15 min every 1.25 h		15 min every 1.75 h	SDE mixed with sewage
B2-II	752–800	752–795	30	15 < 0.3 mg O ₂ L ⁻¹			15 min every 1.75 h	SDE
B3	801–844	801–844	30, 45	15 < 0.3 mg O ₂ L ⁻¹	15 min every 1.25 h		15/10 min every 1.75/1.00 h	SDE mixed with sewage
C1	863–945	863–945	45	15 < 0.3 mg O ₂ L ⁻¹	15/20 min every 1.25/1.42 h		20/30 min every 1.00 h	
C2	946–984	946–983	45	15 < 0.3 mg O ₂ L ⁻¹	25/40 min every 55/65 min		20 min every 35 min	
C3	985–1004	984–1004	45	15 < 0.3 mg O ₂ L ⁻¹	45 min every 60 min			

Notes: * oxygen concentration of 2 mg O₂ L⁻¹ was aimed for during aerated periods. ** only upper pH-limit.

2.3. Analytical Methods and Process Control

A HI1230B pH probe (Hanna Instruments GmbH, Graz, Austria) was used to measure pH. Oxygen concentration and temperature were measured by a WTW FDO[®] 925 probe (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany). At a filling ratio of 20%, the carriers floated freely in the reactor, and comparing measurements at different points in the reactor yielded constant oxygen concentrations throughout the reactor. All probes were integrated into a process control system (Siemens LOGO! Soft Comfort V8, Siemens Aktiengesellschaft, München, Germany), and values were stored in a 30 s interval in a database. The analytical methods for COD, N, TSS, VSS, NH₄-N, NO₂-N, and NO₃-N are displayed in Appendix A Table A1.

2.4. Aerobic Activity Test

Ex-situ aerobic batch activity tests were performed in 300 mL cylindrical reactors in triplicate according to Svoldal et al. (1997) [41]. 1 L suspension (without carriers) from MBBR and IFAS reactor was taken and shaken to enrich oxygen saturation. The cylindrical reactor was completely filled with the oxygen-saturated suspension and closed hermetically. Oxygen concentration was monitored by an online oxygen probe (WTW FDO[®] 925) while stirring. In phases with SDE only activity of AOB (AOB_{act}) was estimated as oxygen consumption (mgO₂(t) – mgO₂(0)) per liter and hour (Equation (3)). This test was performed without carriers for the activity in the suspended biomass and with 27 cubic carriers for the activity with biomass on the carriers. ATH dosage to distinguish between AOB/NOB and HB was not possible, as carriers were given back into the reactors (MBBR and IFAS) after the experiment. Hence, in phases with NOB activity and in the ones with sewage dosage, these ex-situ activity tests only allow an estimation of overall aerobic activity.

$$\text{AOB}_{\text{act}} = (\text{mgO}_2/\text{L}(t) - \text{mgO}_2/\text{L}(0))/ (t - 0) \quad (3)$$

2.5. AnAOB Activity Test

Ex-situ AnAOB batch activity tests were conducted in 500 mL reactors in duplicates according to [42]. 1 L suspension of each the MBBR and IFAS reactor was taken, and 30 mg NO₂-N was added before the suspension was filled into the small batch reactors. N₂ was bubbled through the batch test reactors to guarantee an oxygen-free environment. While shaking at 160 U min⁻¹, grab samples were taken every 15 min over a time span

of one hour and analyzed for NH_4^+ , NO_3^- , and NO_2^- as described above. These tests were carried out without carriers for activity measurement of suspended biomass and with 25 cubic carriers for activity measurement of biomass on carriers. AnAOB activity was calculated for NH_4^+ and NO_2^- separately according to Equations (4) and (5).

$$\text{AnOB}_{\text{act},\text{NH}_4} = (\text{mgNH}_4^+ \cdot \text{N/L}(t) - \text{mgNH}_4^+ \cdot \text{N/L}(0)) / (t - 0) \quad (4)$$

$$\text{AnOB}_{\text{act},\text{NO}_2} = (\text{mgNO}_2^- \cdot \text{N/L}(t) - \text{mgNO}_2^- \cdot \text{N/L}(0)) / (t - 0) \quad (5)$$

2.6. Calculations

NOB activity was estimated in-situ in each reactor as NO_3^- -ratio of theoretically produced (via anammox in the deammonification process) to actually produced NO_3^- as given in Equation (6). As stoichiometric coefficients, the ones given above in Equation 2 are used [1]. If more NO_3^- is produced as AnAOB would stoichiometrically do while removing nitrogen, the ratio will be below 1. At a ratio below 0.5, more than half of the produced NO_3^- cannot be explained via nitrogen removal of anammox, and therefore it is assumed to be produced by NOB. In this study, NOB activity describes a NO_3^- ratio below 0.5, according to Wu et al. (2025) [6]. Values for N_{in} , N_{out} , $\text{NO}_{3,\text{in}}$ and $\text{NO}_{3,\text{out}}$ were measured in mg N L^{-1} and inserted in this calculation in mg N L^{-1} . If the ratio is above 1, denitrification is present.

$$\text{NO}_3\text{-ratio} = ((N_{\text{in}} - N_{\text{out}}) / (2 \times 0.986) \times 0.161) / (\text{NO}_{3,\text{out}} - \text{NO}_{3,\text{in}}) \quad (6)$$

Removal efficiency was calculated in two different ways. Nitrogen removal degree in percentage of influent nitrogen is calculated according to Equation (7) ($N_{\text{rem},\%}$). The difference between influent nitrogen (N_{in}) and effluent nitrogen concentration (N_{eff}) is related to N_{in} . Equation (8) calculates the volume-specific nitrogen removal rate (N_{rem} in $\text{kg N m}^{-3} \text{ d}^{-1}$). Nitrogen influent and effluent concentrations (mg L^{-1}) are multiplied by influent flowrate (Q_{in} as L d^{-1}) and divided by the reactor volume (V_{reactor} in L).

$$N_{\text{rem},\%} = (N_{\text{in}} - N_{\text{eff}}) / N_{\text{in}} \times 100 \quad (7)$$

$$N_{\text{rem}} = (N_{\text{in}} - N_{\text{eff}}) \times Q_{\text{in}} / (1000 \times V_{\text{reactor}}) \quad (8)$$

FA and FNA concentrations were calculated according to Anthonisen et al. [43] as shown in Equations (9) and (10), respectively. T represents temperature in Kelvin.

$$\text{NH}_3 = \text{NH}_4^+ \times 10^{\text{pH}} / (e^{(6344/T)} + 10^{\text{pH}}) \quad (9)$$

$$\text{HNO}_2 = \text{NO}_2^- / (e^{(-2300/T)} \times 10^{\text{pH}}) \quad (10)$$

2.7. FISH—Fluorescence In-Situ Hybridisation

FISH analysis was carried out in suspended sludge, on the carrier itself, including the biofilm attached to the carrier and in sludge washed out of the pores of the carrier (“carrier-sludge”). Measurements were carried out at least every 6 months with VIT[®] ANAMMOX test kits (Nitri-VIT[®], VIT[®] Anammox (AMX SE2), vermicon AG, Hallbergmoos, Germany). The visual comparison of samples from the same day was carried out via fluorescence microscopy to identify where AOB, NOB, and AnAOB were located.

3. Results and Discussion

To assess whether deammonification can be implemented as an IFAS or MBBR process configuration in the mainstream treatment line of municipal WWTPs, investigations at

lab-scale have been performed with real wastewater over an extended temporal scale of 1000 days. The experimental approach was to start at NH_4^+ concentrations typical for the sidestream and then reduce them stepwise to mainstream levels to determine the lowest concentration at which the deammonification process remains stable. The impact of reactor design (MBBR or IFAS) on NOB activity, nitrogen removal efficiency, and process stability was observed on a long-term basis.

3.1. Impact of Influent Composition

The experiment was structured into three distinct operation phases, A, B, and C, as outlined in Section 2. Measured influent concentrations are presented in Figure 2, while effluent concentrations are depicted in Figures 3 and A1 (Appendix A).

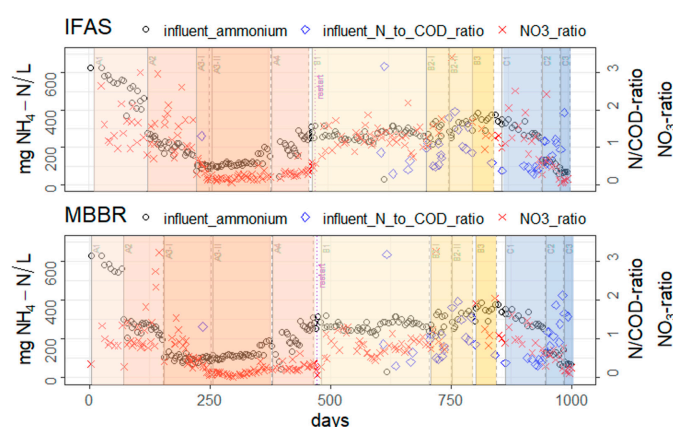


Figure 2. Influent NH_4^+ concentration of MBBR and IFAS reactor, as well as N/COD ratio in influent and NO₃-ratio in the reactors, is shown over 1000 days of operation. In subphases A1 to A3 (red), influent NH_4^+ concentration was varied by diluting SDE. In subphases B1 to B3 (yellow), different N/COD ratios in the influent have been applied by adding sewage to diluted SDE, and in phase C (blue), the influent with fixed SDE-to-sewage ratio was diluted stepwise to mainstream-like conditions.

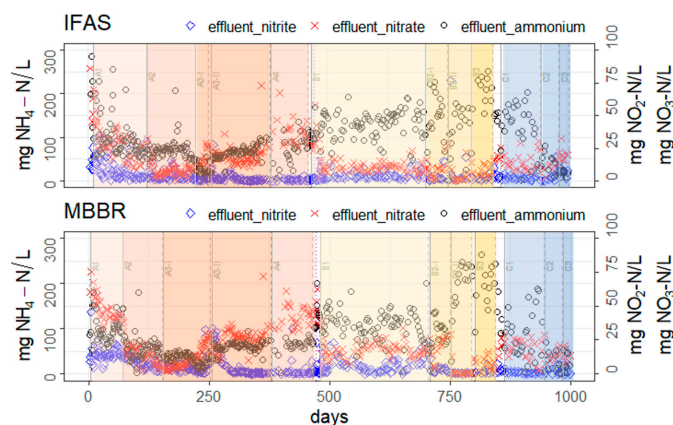


Figure 3. Effluent NH_4^+ concentration of MBBR and IFAS reactor, as well as pH in the reactors, is shown over 1000 days of operation. Mean values of each subphase are given in Table 3. Experimental phases are marked with colors: A (red), B (yellow), C (blue).

Table 3. Average values for pH, nitrogen removal rate, influent and effluent concentrations of subphases.

Subphase	pH		Oxygen Concentration ** [mg O ₂ L ⁻¹]		Nitrogen Removal Rate [kgN m ⁻³ Reactor d ⁻¹]		Influent Concentration [mg NH ₄ -N L ⁻¹]		Effluent Concentration [mg NH ₄ -N L ⁻¹]		Influent N/COD Ratio	
	IFAS	MBBR	IFAS	MBBR	IFAS	MBBR	IFAS	MBBR	IFAS	MBBR	IFAS	MBBR
A1	7.6	7.7	1.9 ± 1.7	3.0 ± 2.1	0.12	0.28	509	573	96	97	NA	NA
A2	7.5	7.6	1.9 ± 1.1	2.9 ± 2.0	0.07	0.16	209	255	71	58	NA	NA
A3-I	7.4	7.4	2.3 ± 1.1	2.3 ± 1.2	0.03	0.05	98	99	26	34	0.9	0.9
A3-II	7.5	7.5	0.8 ± 0.9	0.9 ± 1.0	0.04	0.02	121	122	66	61	NA	NA
A4	7.4	7.4	0.3 ± 0.4	0.4 ± 0.4	0.06	0.04	210	209	71	64	NA	NA
B1	7.7	7.6	0.5 ± 0.5	0.6 ± 0.7	0.07	0.05	264	263	127	106	1.8	1.8
B2-I	7.7	7.5	1.2 ± 1.9	0.9 ± 0.8	0.09	0.07	277	283	148	97	1.3	1.3
B2-II	7.6	7.7	0.9 ± 0.7	0.8 ± 0.5	0.12	0.06	303	304	117	190	0.7	0.6
B3	7.6	7.7	0.7 ± 0.4	0.8 ± 0.6	0.11	0.07	352	352	196	195	1.8	1.8
C1	7.5	7.3	0.9 ± 0.8	0.8 ± 0.5	0.11	0.09	294	294	124	89	2.6	2.6
C2	7.1	7.2	0.8 ± 0.8	0.6 ± 0.3	0.09	0.12	112	115	39	26	1.2	0.9
C3	6.9	7.3	0.7 ± 0.4	0.7 ± 0.6	0.05	0.06	70	70	19	32	0.5	0.4

Notes: NA: not available. ** Average in aerated phases.

During the initial experimental phase (Phase A), diluted SDE sourced from a full-scale WWTP was used as influent. Influent NH₄⁺ concentrations were stepwise reduced from side-stream to main-stream conditions (Figure 2). The influent load was controlled in phase A by setting upper limits for the pH value in the reactors, leading in phase A1 to average values of 7.6 and 7.7 in IFAS and MBBR respectively. The highest dilution step in phase A yielded reactor concentrations around 30 mg NH₄-N L⁻¹ with average oxygen concentrations of 2.3 ± 1 mg O₂ L⁻¹ in the aerated phases (subphase A3-I, Table 3). Subsequent reduction of the upper pH limit resulted in lower loading, which led to lower reactor concentrations (26 ± 11 mg NH₄-N L⁻¹ in the IFAS reactor and 33 ± 10 mg NH₄-N L⁻¹ in the MBBR). Due to the low NH₄⁺ concentration, NOB activity increased in subphase A3. As a result, N removal rates decreased from above 75% in subphase A1 to about 30% in subphase A3.

The pH-values within both reactors ranged from 7.4 to 7.7, aligning with the typical operational range of AnAOB (6.8 to 8.3) and AOB (e.g., for *Nitrosomonas eutropha* and *Nitrosomonas europaea* species 7.4 to 7.8) [44–46]. Alkalinity, biological activity, and the concentrations of NH₄⁺ and NO₂⁻ ions in SDE determine the pH value in the reactor. At higher pH-values, the dissociation equilibrium shifts from NH₄⁺ to FA and H⁺, potentially increasing inhibition by FA, especially on NOB. According to literature, the inhibiting threshold for FA on AOB is higher (*Nitrosomonas*: 10 to 150 mg FA L⁻¹) compared to NOB (*Nitrobacter*: 0.1 to 1.0 mg FA L⁻¹) [43], and AOB recover faster than NOB from high FA concentrations [21]. In subphase A3-I, it is argued that the lower FA concentrations of 0.4 ± 0.3 mg L⁻¹ (IFAS) and 0.6 ± 0.3 mg L⁻¹ (MBBR) allowed for increasing NOB activity with NO₃-ratio below 0.5 (Figure 2). In subphase A3-II, an elevated upper pH limit resulted in slightly higher pH values and elevated FA concentrations of 1.2 ± 0.4 mg L⁻¹ (IFAS) and 1.0 ± 0.5 mg L⁻¹ (MBBR). This adjustment failed in decreasing NOB activity as NO₃-ratio stayed below 0.5 during the whole subphase A3-II. It is plausible that the seed sludge had acclimated to higher FA concentration, given its origin from a lab-scale sidestream deammonification reactor with mean FA concentration of 1.7 mg L⁻¹. Continued NOB activity points to an adaptation of NOB to the existing FA concentration, as also found in An et al. (2021) [47]. Continuous exposure to FA in An et al. (2021) [47] led to activity loss of NOB of less than 25%, even at concentrations of 11 mg FA L⁻¹. In addition, NOB could have enlarged their population in phase A3-I and thus have a similar activity in phase A3-II, even if partly inhibited.

If not inhibited by FA, NOB could also be inhibited by FNA. FA and FNA are both substances that are present in the system without external chemical dosage. The concentration of FNA rises at decreasing pH values. Notably, at pH-values below 6.5 and NO₂⁻ concentrations below 100 mgN L⁻¹, both NO₂⁻ and FNA exist, which exhibit inhibitory

effects on both AOB and NOB [43]. In subphase A3, despite maintaining a pH above 7 and achieving a concentration of at least $1.2 \text{ NO}_2\text{-N mg L}^{-1}$, the levels of FNA remain below 0.01 mg L^{-1} . Given that a complete inhibition via FNA requires concentrations above $0.02 \text{ mg FNA L}^{-1}$ [19,43,48], the FNA concentration within the reactors was insufficient to induce inhibitory effects.

Notably, in phase A3-I with the lowest NH_4^+ reactor concentration ($26 \pm 11 \text{ mg NH}_4^+ \text{ L}^{-1}$ in IFAS and $33 \pm 10 \text{ mg NH}_4^+ \text{ L}^{-1}$ in MBBR), the time until NOB activity (NO_3 -ratio below 0.5) occurred was longer in MBBR (97 days) than in IFAS (27 days). This might be due to enhanced washout effects in MBBR compared to IFAS. The hydraulic retention time of MBBR in A3-I was lower, with 1.2 days, compared to the IFAS reactor sludge age of 6 days. In subphase C1, hydraulic retention time in MBBR was higher, with 3.7 days (IFAS stayed in the same range as in A3-I with 5.6 days). With similar suspended biomass retention in C1, no difference in NOB activity uptake depending on sludge age was observed.

In phase A4, FA inhibition of NOB was increased by using similar nitrogen influent concentration as in subphase A2, where no NOB activity was observed (refer to Figure 2 and Table 3). However, despite these efforts, NOB remained active. This supports the hypothesis that NOB adapted to the present FA concentration and remained active as described above. This led to a restart of the MBBR and IFAS reactors with new seed sludge and higher influent NH_4^+ concentrations at the onset of subphase B1 (IFAS: day 473, MBBR: day 472). FA levels ranged from $1\text{--}7 \text{ mg L}^{-1}$ in phases B1 to C1.

In subphase B2, municipal sewage was added to the influent to investigate the impact of biodegradable COD on the nitrogen removal efficiency (COD in SDE is predominantly refractory). Higher COD levels could lead to enhanced oxygen uptake by heterotrophs, resulting in lower oxygen concentration in the sludge flocks and biofilm. Lowered oxygen concentration could help improve NOB activity control. At the same time, heterotrophs could compete with AnAOB for the available NO_2^- . To guarantee NO_2^- production, the aerated time was prolonged in phase B2. Mean oxygen concentration stayed below 1 mg L^{-1} . Lowered N/COD ratio had neither an effect on nitrogen removal rate nor NOB activity. In IFAS subphase B2, the NO_3 -ratio is above 1, indicating that denitrification also took place. In MBBR, the NO_3 -ratio is below 1 and therefore does not show signs of denitrification. This is in line with the higher sludge age of 5–8 days in IFAS compared to MBBR with a hydraulic retention time of about 2.5 days, which might be too short to build up a stable heterotrophic bacteria community for denitrification, if not attached to the biofilm carrier. When sewage addition was stopped in phase B3, this did not impact nitrogen removal rate or NO_3 -ratio significantly. In our experiments, variation of N/COD ratio in the range applied had no significant effects on nitrogen removal rate, as also seen in long-term (120 d) experiments with granules by Le et al. (2019) [49].

In phase C, sewage addition was started again, and influent concentrations were lowered in each subphase. The N/COD ratio subsequently decreased from subphase C1 (2.6) and C2 (1.2 ± 0.5 IFAS reactor and 0.9 ± 0.3 MBBR) to C3 (0.5 ± 0.4 IFAS reactor and 0.4 ± 0.2 MBBR). Towards the latter days of phase C2, NOB activity was observed and persisted throughout subphase C3. The stepwise dilution of influent concentrations resulted in lower FA concentrations of 0.4 mg L^{-1} (IFAS) and 0.5 mg L^{-1} (MBBR) and potentially allowed higher NOB activity starting in phase C2 (day 974—IFAS reactor and day 975—MBBR). FA concentrations in phase C2 are similar to those measured in phase A3-I, where the first NOB activity occurred. Nitrogen removal degree decreased from C1 and C2 for IFAS from $57 \pm 17\%$ to $34 \pm 12\%$ and for MBBR from $69 \pm 16\%$ to $38 \pm 20\%$. In subphase C3, nitrogen removal rate declined below $0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$ in activated suspended sludge reactor with nitrification/denitrification [50] in both reactors. This could be attributed to a combination of NOB activity and lower N/COD-ratio, leading to

intensified competition between AnAOB, NOB, and heterotrophic bacteria on NO_2^- . An increased NOB activity was observed also by higher $\text{NO}_3\text{-N}$ concentration in IFAS reactor (C1: $14 \pm 7 \text{ mg L}^{-1}$, C2: $13 \pm 5 \text{ mg L}^{-1}$ to C3: $21 \pm 5 \text{ mg L}^{-1}$). Also, increased $\text{NO}_2\text{-N}$ concentrations were observed, which could be attributed to decreased AnAOB activity, while heterotrophic bacteria also did not completely reduce available NO_2^- . Increased NO_3^- and NO_2^- concentrations coupled with decreased NH_4^+ concentrations resulted also in a lower pH value. These effects could be seen in IFAS more distinctly than in MBBR. In MBBR, a lower removal rate might be an effect of lower retention time compared to IFAS sludge age (9–8 days in C2–C3). In MBBR, hydraulic retention time is even more reduced in subphase C2 (0.7 days) and C3 (0.5 days) due to elevated flow to maintain similar influent loading rate ($\text{kg N m}^{-3} \text{ d}^{-1}$) at lower nitrogen concentration. Lower retention time facilitates biomass washout and thus can support activity loss.

In the IFAS reactor, a NO_3^- -ratio below 0.5 was observed on some days (72 compared to 89). Consequently, the IFAS process is considered more stable in terms of NOB activity. Given that NOB typically exhibit higher nitrogen turnover rates compared to AOB at 15°C , NOB can theoretically exhaust all NO_2^- produced by AOB if NOBs are present in the biofilm, thus limiting the availability of this substrate for AnAOB [17].

In this study, temperatures within the reactors were not controlled and ranged from 21 to 27°C (10% and 90% percentile). AOB growth rate is higher than NOB growth rate above 20°C , whereas between 10 and 20°C , NOB growth rate exceeds AOB growth rate [51,52], thus making it more difficult to control NOB activity below 20°C . The optimal temperature range for AnAOB, with $30\text{--}37^\circ\text{C}$ [11], is even higher than that for AOB. In the present study, the temperature remained above 15°C . Below 15°C , AnAOB activity drops more than for AOB, thus making it more difficult to balance AnAOB and AOB activity [33]. Established relative activity of AOB, NOB, and AnAOB in this study should be stable given the small and relatively high temperature range ($21\text{--}27^\circ\text{C}$).

Aerobic bacteria activity and AnAOB activity were measured in each subphase (Figure 4). Aerobic respiration tests included AOB, NOB, and HB. In phases with low NOB and HB activity (e.g., at high NH_4^+ concentrations and no addition of municipal wastewater), respiration can be mainly attributed to AOB activity. In most subphases, aerobic activity was notably higher in tests involving carriers compared to those without carriers (mean: 600%). However, exceptions were noted in subphases A1 and A4 in the IFAS reactor. In subphase A4, aeration was reduced to favor AOB over NOB, resulting in a decrease in aerobic activity, particularly on the carriers where oxygen diffusion might be limited due to lower oxygen concentration. AnAOB activity on the carriers exceeded AnAOB activity in suspended biomass in all measurements (average MBBR: $2.6 \text{ mg NH}_4\text{-N L}^{-1} \text{ h}^{-1}$, average IFAS: $2.9 \text{ mg NH}_4\text{-N L}^{-1} \text{ h}^{-1}$). Both active aerobic bacteria and AnAOB were primarily situated on the carrier material, as evidenced by the enhanced activity observed in measurements involving carriers compared to suspended biomass only. Furthermore, the presence of AnAOB on the carrier material was confirmed through FISH measurements (see Figure 5). Microscopic examination consistently revealed a mixture of AOB and AnAOB within the biofilm, whereas NOB visibility coincides with NOB activity (both in bulk and biofilm). Notably, free suspended biomass contained a greater proportion of AOBs compared to AnAOBs, which aligns with expectations considering the higher oxygen concentration typically found in suspended biomass compared to the biofilm.

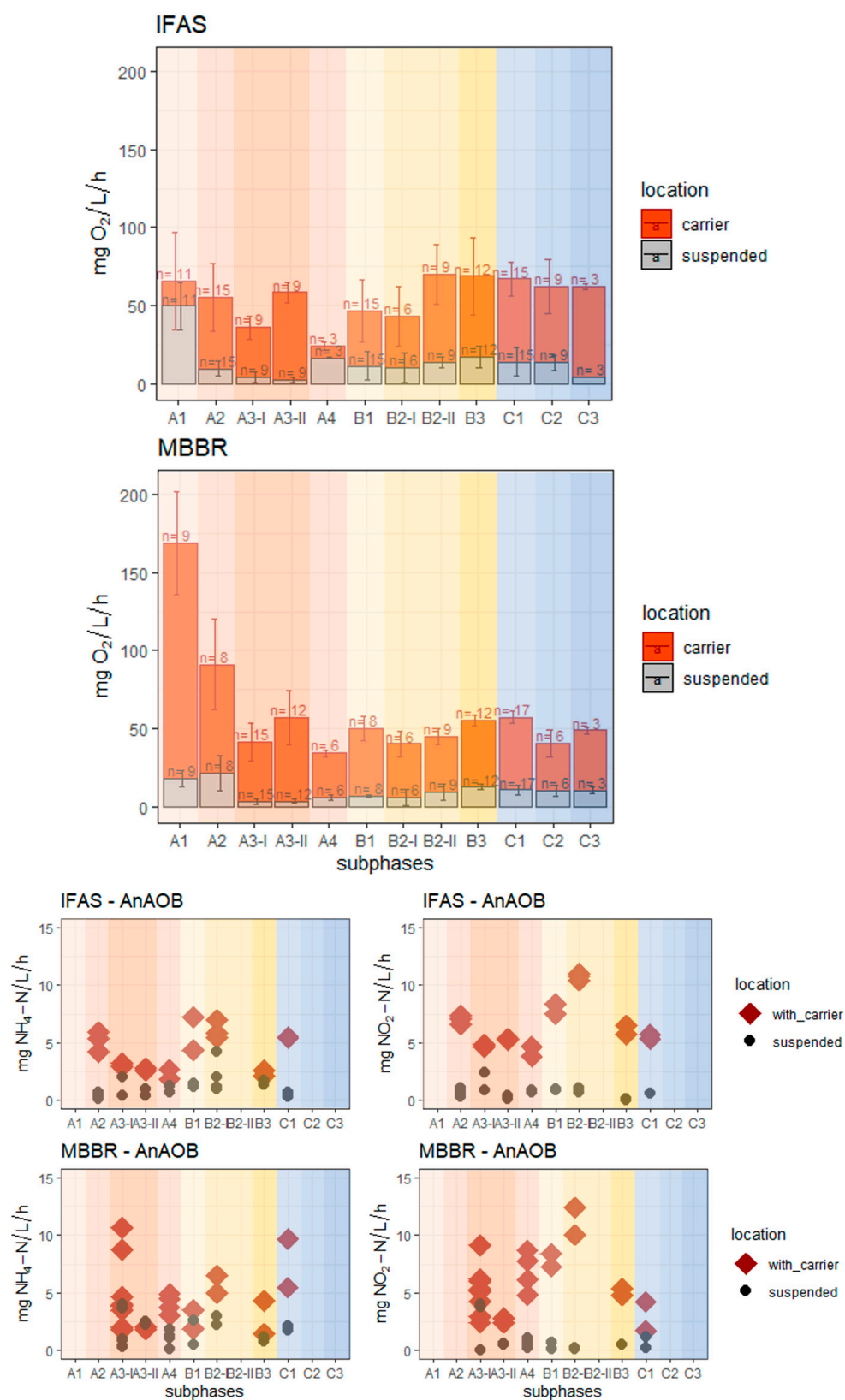


Figure 4. Activity measurements in MBBR and IFAS reactor in suspended biomass and with carrier (filling ratio of 20%) in ex-situ batch tests. Experimental phases are marked with colors: A (red), B (yellow), C (blue).

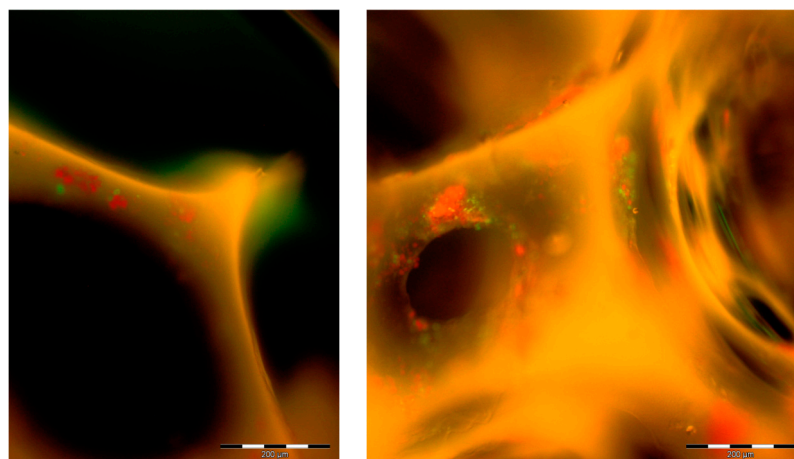


Figure 5. FISH labeled bacteria AnAOB in red and AOB in green on cubic carrier in orange ((left) IFAS reactor, (right) MBBR).

3.2. Nitrogen Removal Efficiency

With conventional nitrification-denitrification, a lab-scale MBBR achieved a nitrogen removal rate of $0.03 \text{ kg N m}^{-3} \text{ d}^{-1}$ at 10% carrier filling ratio and $0.18 \text{ kg N m}^{-3} \text{ d}^{-1}$ at 50% carrier filling ratio [53]. This is similar to activated sludge nitrification-denitrification rates of $0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$ [50]. This study observed a maximum nitrogen removal rate in the MBBR (subphase A1: $0.28 \text{ kg N m}^{-3} \text{ d}^{-1}$ —refer to Figure 6). On average, N removal rate of MBBR and IFAS reactor was $0.07 \text{ kg N m}^{-3} \text{ d}^{-1}$. While MBBR surpasses the rate of $0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$ achieved by conventional one-stage nitrification-denitrification in half of the subphases, IFAS reactor exceeded the nitrogen removal rate of $0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$ in 9 out of 12 phases compared to MBBR. In subphases relevant for mainstream application (B2 and C1) characterized by the absence of NOB activity and lower nitrogen concentrations, the IFAS reactor yielded higher nitrogen removal rates ($0.09\text{--}0.12 \text{ kg N m}^{-3} \text{ d}^{-1}$) compared to the MBBR ($0.06\text{--}0.09 \text{ kg N m}^{-3} \text{ d}^{-1}$).

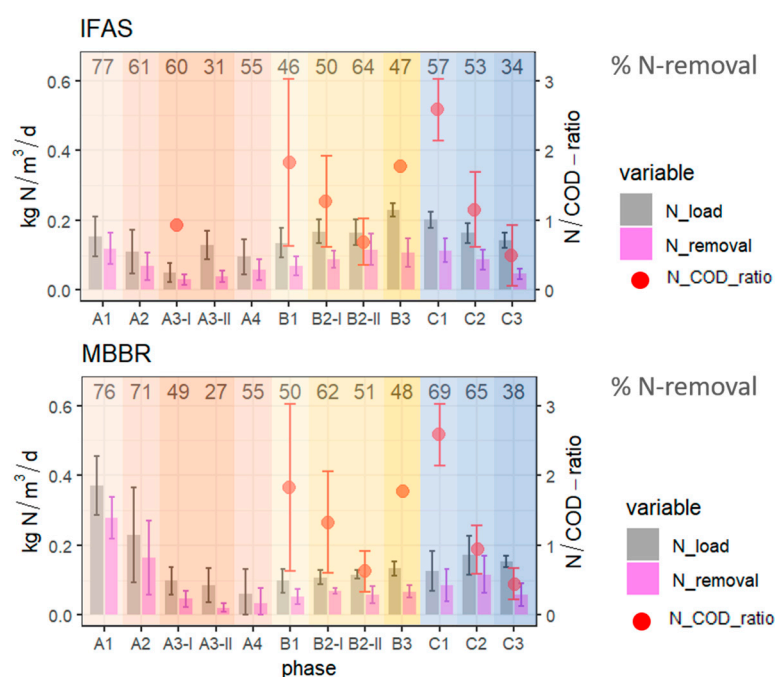


Figure 6. Nitrogen removal rate in $\text{kg N m}^{-3} \text{ d}^{-1}$ for IFAS reactor (top) and MBBR (bottom) over subphases A1–C3. Experimental phases are marked with colors: A (red), B (yellow), C (blue).

Nitrogen removal rates decreased in the IFAS reactor from $0.12 \text{ kg N m}^{-3} \text{ d}^{-1}$ (subphase A1) to $0.03 \text{ kg N m}^{-3} \text{ d}^{-1}$ (subphase A3-I) and in the MBBR from $0.28 \text{ kg N m}^{-3} \text{ d}^{-1}$ (subphase A1) to $0.02 \text{ kg N m}^{-3} \text{ d}^{-1}$ (subphase A3-II) at a temperature range from 21–27 °C (10–90% percentile). Decreased influent load due to pH-controlled influent dosage (as described in Sections 2.2 and 3.1) led to decreased removal rates in phase A1–A3-I while keeping comparable removal degrees. In phase B, time-based influent dosage resulted in increased nitrogen load and a rising nitrogen removal rate.

In a simultaneous nitrification, anammox, and denitrification process, lower N/COD ratios lead to an enhanced competition of aerobic heterotrophic bacteria and AOB. This results in a decreased nitrification performance and thus in less NO_2^- as a substrate for AnAOB. Nitrogen removal efficiency declines with lower N/COD ratios starting from 0.5 downwards and limited oxygen availability. In addition, anoxic heterotrophic bacteria outcompeted AnAOB at higher concentrations of biodegradable COD (lower N/COD ratios) [54]. In our study, nitrogen removal rate and nitrogen removal degree declined with a N/COD ratio of 0.6 ± 0.3 in the MBBR (Figure 6) at a reactor concentration of $190 \pm 62 \text{ mg NH}_4\text{-N L}^{-1}$. With an elevated N/COD ratio in phase B3, nitrogen removal rate and nitrogen removal degree stayed low, probably due to the enhanced NOB activity (explained in Section 3.1). In IFAS reactor, nitrogen removal decreased even at higher N/COD-ratios in subphase C2 of 1.2 ± 0.5 at lower nitrogen concentrations compared to B2-II in MBBR. Subsequently, in subphase C3, nitrogen removal rates declined for both reactors to below $0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$ at N/COD-ratios of 0.5 ± 0.4 (IFAS) and 0.4 ± 0.2 (MBBR), similar to decreasing nitrogen removal rates found in literature [54]. The nitrogen removal degree declined from 53% (IFAS), 65% (MBBR) at N/COD-ratios above 0.5 (subphase C2) to 34% (IFAS), 38% (MBBR) at N/COD-ratios of below 0.5 (subphase C3). This trend is also similar to a reported study of a bench-scale sequencing bed reactor fed with synthetic wastewater, where N removal decreased from 79% to 40% (NH_4^+ removal efficiency dropped by 85%) with decreasing N/COD ratio from 1 to 0.5 [55]. These findings underscore the necessity of utilizing influent with a high N/COD ratio for anammox processes, as advocated in the literature [56].

3.3. MBBR vs. IFAS Configuration

Wastewater treatment in the mainstream treatment line requires stable nitrogen removal to ensure compliance with effluent limit values. Average nitrogen removal over all phases was $0.07 \text{ kg N m}^{-3} \text{ d}^{-1}$ for both reactors. Median values were $0.07 \text{ kg N m}^{-3} \text{ d}^{-1}$ for IFAS and $0.05 \text{ kg N m}^{-3} \text{ d}^{-1}$ for MBBR. The IFAS reactor demonstrated greater operational performance and stability, regarding nitrogen removal rates and a lower number of days with NOB activity shown as NO_3^- -ratio below 0.5 (Figure 7). The more days NO_3^- -ratio is below 0.5, the more days NOB compete with AMOX for NO_2^- , which is also seen in a sinking removal rate (Figure 6). Particularly in subphases A3-I, throughout phase B, and C1, IFAS achieved higher removal rates in $\text{kg N m}^{-3} \text{ d}^{-1}$. Only subphase C2 showed higher days of NOB activity in IFAS reactor compared to MBBR (Figure 7). The simulation of an IFAS and MBBR system in Trojanowicz et al. (2021) [17] supports the advantage of IFAS over MBBR systems for AnAOB. The simulation of 1 mm biofilm systems showed elevated concentration in the bulk of FNA compared to MBBR due to greater amounts of NO_2^- produced by AOB in activated sludge. Due to higher affinity to FNA of AnAOB compared to NOB, AnAOB could use more of the substrate NO_2^- in the biofilm if anaerobic zones are present.

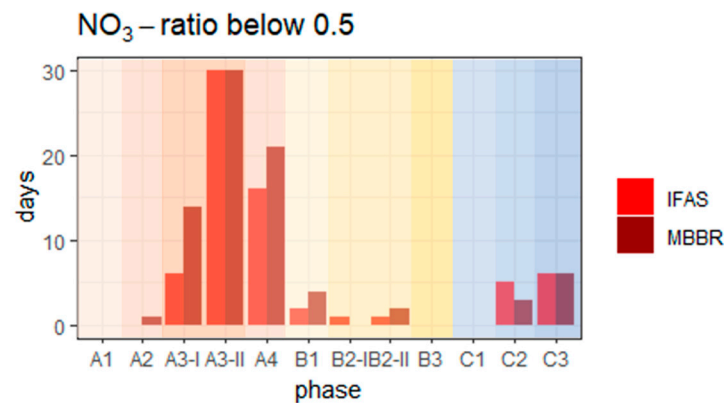


Figure 7. Number of days with NO₃-ratio below 0.5. Phases are marked with colors: A (red), B (yellow), C (blue).

4. Conclusions

This study provides new insights into the comparison of an IFAS reactor and an MBBR for the use of deammonification in the transition from sidestream to mainstream. Due to a high sludge retention in biofilm systems, at least half a year of study is necessary to gain reliable results. Lab-scale reactors in this study were operated for over 1000 days. Our research underlines the importance of FA inhibition on NOB when transitioning from sidestream to mainstream. In our study, NOB activity manifested in experimental phases with low nitrogen concentrations. IFAS configuration exhibited greater stability compared to the MBBR, with fewer days of NOB activity. In addition, at mainstream-like temperature and concentration conditions, IFAS reactor yielded higher nitrogen removal rates compared with MBBR. This may be due to higher flexibility in operation of IFAS reactors resulting from the presence of suspended biomass in addition to the biofilm on the carriers. The deammonification process with the newly tested cubic polyurethane carriers in both the MBBR and IFAS reactor achieved stable nitrogen removal rates. AnAOB were predominantly situated on the carriers rather than in suspended biomass in both reactors, as indicated by ex-situ activity and FISH measurements, underscoring the ability of carriers in retaining AnAOB within the system. The present study has only explored lab-scale conditions. Results obtained need to be confirmed and further improved, e.g., in pilot-scale applications.

Given the low in-situ inhibition of FA at reactor concentrations below 50 mg NH₄-N L⁻¹ (1 mg FA L⁻¹) shown in our study, we recommend maintaining a reserve biomass for restarting or inoculating deammonification in the mainstream, if NOB should take over. An important issue to address is the long-term impact of biomass adaptation to operational conditions. Future research should focus on the development of an appropriate operational framework for mainstream anammox with an approach of in-situ NOB inhibition, preferably without external inhibition substances. Exploring selective suppression strategies and optimizing aeration control are key approaches to further assess the potential of IFAS reactors.

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Abbreviations

The following abbreviations are used in this manuscript:

AOB	Ammonium oxidizing bacteria
AnAOB	Anaerobic ammonium oxidizing bacteria—anammox bacteria
COD	Chemical oxygen demand
DO	Dissolved oxygen
FA	Free ammonia
FISH	fluorescence in-situ hybridization
FNA	Free nitrous acid
IFAS	Integrated fixed film activated sludge
MBBR	Moving bed biofilm reactor
N ₂	Elemental nitrogen
NH ₄ ⁺	ammonium
NH ₄ -N	Nitrogen contained in ammonium
NO ₃ [−]	Nitrate
NO ₃ -N	Nitrogen contained in nitrate
NO ₂ [−]	Nitrite
NO ₂ -N	Nitrogen contained in nitrite
NH ₃	Ammonia
NOB	Nitrate oxidizing bacteria
SDE	Sludge dewatering effluent
N	Nitrogen as sum of NH ₄ -N, NO ₂ -N and NO ₃ -N
TSS	Total suspended solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

Appendix A

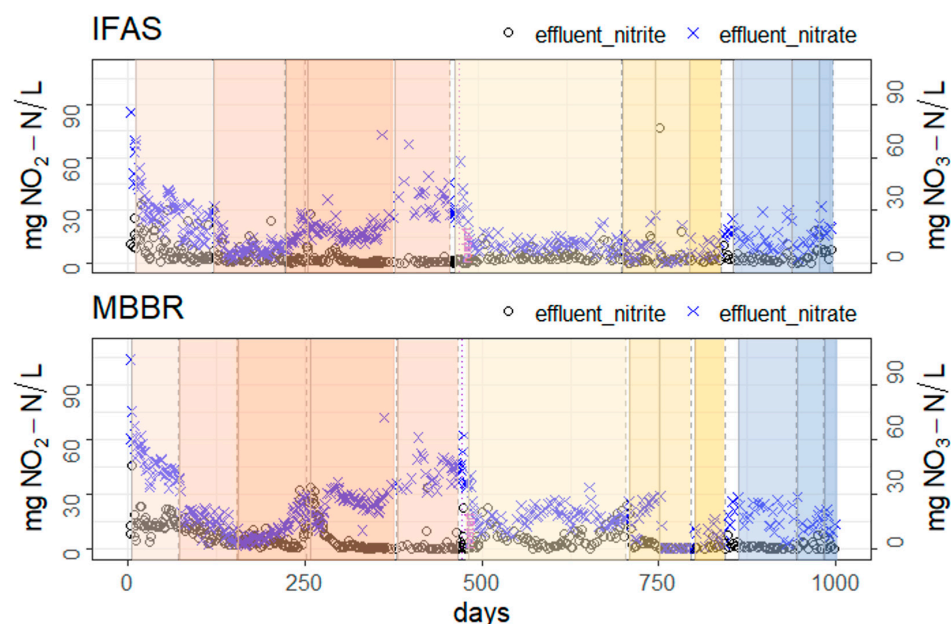


Figure A1. Effluent NO_2^- and NO_3^- concentration of MBBR and IFAS reactor over 1000 days of operation. Phases are marked with colors: A (red), B (yellow), C (blue).

Table A1. Analytical methods used for COD and nitrogen concentrations.

Analyte	Method
COD	photometrically according to ÖNORM ISO 15705 [57]
NH_4^+	continuous flow analysis and photometrical detection (Skalar, The Netherlands) according to ÖNORM EN ISO 11732 [58]
NO_3^- , NO_2^-	continuous flow analysis and photometrical detection (Skalar, The Netherlands) according to ÖNORM EN ISO 13395 [59]

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