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Operational window of a deammonifying sludge for mainstream application in a municipal wastewater treatment plant

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Abstract

The present work aimed to study the mainstream feasibility of the deammonifying sludge of side stream of municipal wastewater treatment plant (MWWTP) in Kaster, Germany. For this purpose, the deammonifying sludge available at the side stream was investigated for nitrogen (N) removal with respect to the operational factors temperature (15–30°C), pH value (6.0–8.0) and chemical oxygen demand (COD)/N ratio (≤1.5–6.0). The highest and lowest N-removal rates of 0.13 and 0.045 kg/(m^3 d) are achieved at 30 and 15°C, respectively. Different conditions of pH and COD/N ratios in the SBRs of Partial nitritation/anammox (PN/A) significantly influenced both the metabolic processes and associated N-removal rates. The scientific insights gained from the current work signifies the possibility of mainstream PN/A at WWTPs. The current study forms a solid basis of operational window for the upcoming semi-technical trails to be conducted prior to the full-scale mainstream PN/A at WWTP Kaster and WWTPs globally.

KEYWORDS
anammox, mainstream, nitrogen removal, partial nitritation, wastewater

1 | INTRODUCTION

In recent years, a new pathway for nitrogen (N)-removal, which means ammonium (NH_4-N)-removal, has been discovered and studied for its potential engineering application, which is known as deammonification or partial nitration/anammox (PN/A) process. PN/A process integrates ammonium-oxidizing bacteria (AOB) that convert a portion (55%) of ammonium (NH_4-N) to nitrite (NO_2-N) aerobically during partial nitritation phase, with anaerobic ammonium-oxidizing, or anammox bacteria, that anaerobically oxidizes the remaining 45% NH_4-N together with the formed NO_2-N to nitrogen gas N_2 during anammox phase. Oxygen demand during partial nitritation phase is approx. 60% lower than during nitrification/denitrification (N/DN) as only a portion (55%) of NH_4-N should be oxidized (Qiu et al., 2021). Further, the PN/A process does not consume carbon during the anammox phase as DN does (Zekker et al., 2021). PN/A rather allows the carbon to remain available in sludge for more biogas production in the anaerobic digester of the WWTP at the latter stage, contributing to higher self-sufficiency of the WWTP (DWA, 2017). PN/A has already been well established in the side stream of municipal wastewater treatment plants (MWWTP) to treat high-strength or reject water from the dewatering of sludge after anaerobic treatment, which have a typical NH_4-N concentration of up to 1.8 kg/m^3 (Lackner et al., 2014). Further development in transferring it to the mainstream biological treatment promises significant energy savings due to a lower electricity demand for N-elimination.

Typical mainstream conditions include temperatures <20°C, chemical oxygen demand (COD)/N ratios >10 and NH_4-N loads below

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0.2 kg/m^3 (Gustavsson et al., 2020). Although the laboratory and pilot-scale research on PN/A under mainstream conditions are available, the reported N-removal performance is so far differed (de Clippeleir et al., 2013; Hoekstra et al., 2019; Laureni et al., 2016; Lotti et al., 2015). The previous research results cannot be generalized and exactly applicable for all the available deammonifying sludges in MWWTPs as the process performance expected to change over time. Some MWWTPs have suspended deammonifying sludge and others granular or biofilm carriers. The performance of deammonifying sludge moreover differs from one to another plant. Process stability in treating low-strength wastewater is therefore individual for each deammonifying sludge.

Major challenge of mainstream PN/A is to establish the stable PN and anammox phases under low temperatures and at high ratios of chemical oxygen demand to N (COD/N) (Gustavsson et al., 2020). Its application for low-strength wastewater in the mainstream is therefore still questionable. PN/A is further encountered by several inhibitory factors during the wastewater treatment. The activity of anammox bacteria can decrease at too high NO_2-N concentrations especially during the start-up of PN/A because of the faster growth of AOB than anammox bacteria (Feng et al., 2019; Lackner et al., 2014). In the study of Wett (2007) at a MWWTP in Strass (Austria), NO_2-N has an inhibitory effect on the anammox process at concentrations as low as 0.005 kg/m^3. It is difficult to get anammox bacteria active in the mainstream as they have their maximum activity between 30 and 35°C with the optimum at 43°C (Sobotka et al., 2016). Further, nitrite-oxidizing bacteria (NOB) compete for nitrite (NO_3-N) with the anammox bacteria. Anammox bacteria grow approximately 10 times slower than nitrifiers (Grismer & Collison, 2017). The conditions for nitrifying organisms are generally more favourable in mainstream wastewater under low temperatures and hinder the anammox process from taking place. It was reported in previous studies that pH in the wastewater influences the anammox process. A higher pH value in the anammox reactor can lead to destabilizing conditions for the anammox bacteria, and under these conditions, even slight changes in pH value can reduce the N elimination rate (Puyol et al., 2014). This can be attributed to the fact that as pH increases, a higher proportion of N is present as ammonia (NH_3). If this results in higher NH_3 concentrations of about 0.01–0.15 kg/m^3, this has an inhibitory effect further on AOBs. Thus, not enough NO_2-N can be formed by the AOB for an efficient anammox process. Enough heterotrophic bacteria are also present in deammonifying sludge systems and provide immediate turnover when higher COD is present in wastewater. It could eventually displace anammox with DN process. These studies demonstrate that it is challenging to implement PN/A process in the reactor and adapt it to mainstream conditions due to its metabolic sensitivity to minute operational disturbances and biological parameters of wastewater.

Since 2013, the WWTP Kaster, Germany, is been operating PN/A in the side stream of the plant in a sequence batch reactor (SBR) at approximately 32°C to treat high-strength reject water (up to 1.5 kg NH_4-N/m^3). The impact of mainstream conditions like low temperatures and high COD/N ratios on the deammonifying sludge of side stream of the MWWTP Kaster is unknown for a large-scale implementation in its mainstream. A stable operation of PN/A is essential in the mainstream to meet the requirements of discharged effluent concentrations according to the Waste Water Ordinance, Germany. Therefore, the N-removal efficiency of deammonifying sludge of MWWTP Kaster treating low-strength wastewater must be investigated with respect to the operational factors prevailing in the mainstream.

This study aimed to determine the operational window and mainstream flexibility of deammonifying sludge available in the side stream of MWWTP Kaster with respect to the mainstream relevant operational parameters (Figure 1). For this purpose, the investigation was divided into three phases.

I. Establishment of single-stage PN/A process in low-strength wastewater at the laboratory-scale plant using deammonifying sludge of MWWTP Kaster.
II. Investigation of selected range of operational parameters temperature, pH value and the COD/N ratio with respect to the N-removal performance of PN/A.
III. Determination of operational window of the deammonifying sludge for the estimation of PN/A feasibility in the mainstream of MWWTP Kaster using the results of phase-II.

2 | METHODS

2.1 | Set-up of laboratory-scale plant

The plant used for conducting the batch tests in this work was a SIXFORS® laboratory-scale plant (Infors® HT, Switzerland) as shown in Figure 2. The plant consisted of six parallel reactors, each 0.0005 m^3, which were each equipped with a temperature sensor (pt100) and control, pH sensor (Redox electrode, Mettler Toledo), dissolved oxygen sensor (polarographic electrode, Mettler Toledo), magnetic stirrer, aeration units and a common U-Direct Digital Control (U-DDC) operating panel. Each reactor had a total surface area of 0.047 m^2 and a total volume of 0.0006 m^3, respectively, accounting for a filling degree of 83% and a filling volume of 0.0005 m^3. Each stirrer unit consisted of three impellers (two Rushton at the top and one Marine at the bottom) with a 30 mm distance between each, as determined from preliminary trials. The 'Iris' software, which connected the laboratory-scale plant to a desktop, controlled all the operational parameters of the laboratory-scale plant, which included process control and data logging, analysis and export.

2.2 | Reactor operation

The six parallel reactors of the laboratory-scale plant were run as a SBR in the current work. One SBR cycle refers to a single batch test in this study. SBRs were operated in a 20-h SBR cycle (8-h aeration/12-h mixing) for a given ammonium (NH_4-N) start

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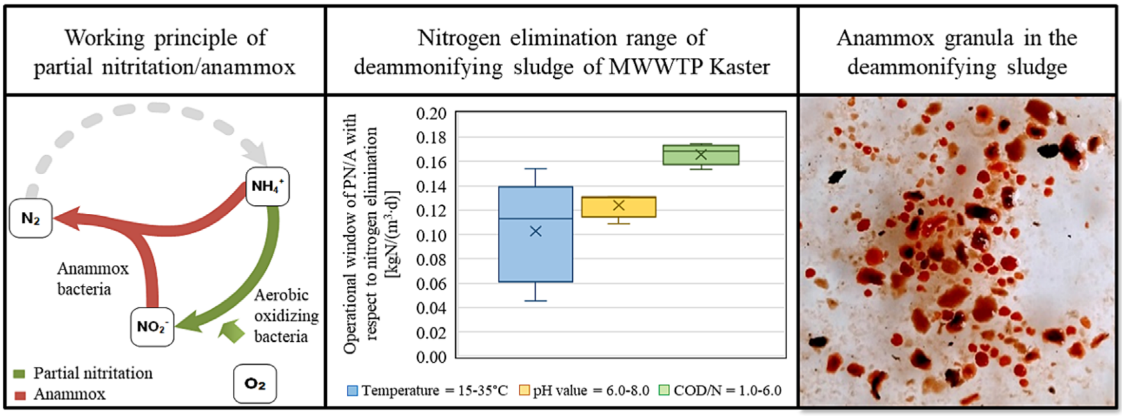

FIGURE 1 Nitrogen elimination range of deammonifying sludge from the side stream of MWWTP Kaster.

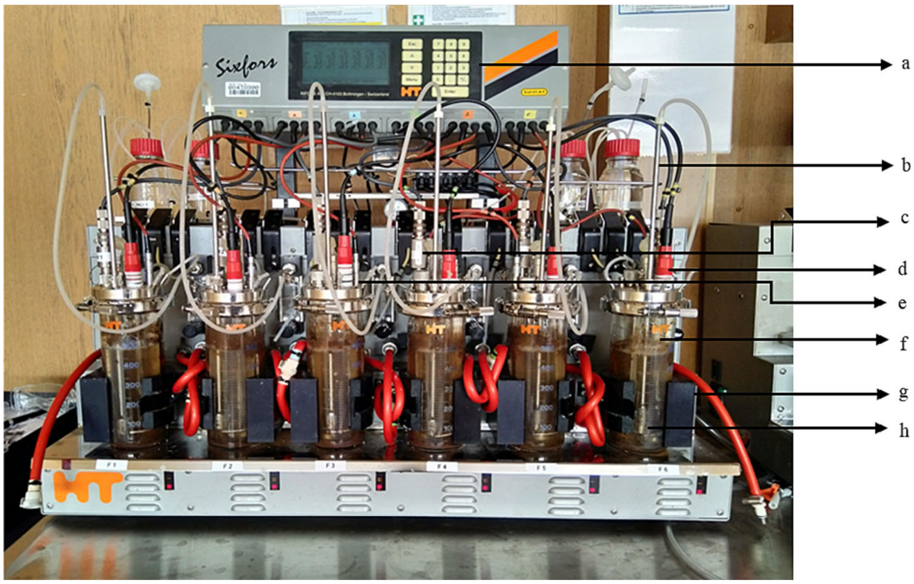

FIGURE 2 Laboratory-scale SIXFORS plant (Infors® HT): (a) U-DDC control panel and display, (b) inlet of aeration unit, (c) dissolved oxygen sensor, (d) pH sensor, (e) temperature sensor, (f) reactor containing deammonifying sludge of MWWTP Kaster, (g) thermostat, (h) air diffuser.

TABLE 1 Parameters tested in the batch tests at laboratory scale during phase I to implement the PN/A and during phase II to investigate the optimal operational parameters.

Phase	SBR cycle [h]	Aeration/non-aeration phase [h]	Type of aeration	DO [kg/m^3]	Start NH_4-N Conc. [kg/m^3]	T [°C]	pH value [–]	COD/N ratio
I	20	8/12	Cont.	≤0.003	0.14–0.27	30 ± 2	7.4 ± 0.4	≤1.5
II	20	8/12	Cont.	≤0.003	0.15 ± 0.02	15–35	8	≤1.5
						35	6–8	≤1.5
						35	8	1.5–6
III	20	8/12	Cont.	≤ 0.003	0.15 ± 0.02	15–35	6–8	≤1.5

Abbreviations: Cont., continuous; DO, dissolved oxygen; T, temperature.

concentration of 0.15 ± 0.1 kg/m^3 as a part of PN/A implementation and optimization during phase I. A relevant range of operational parameters temperature, pH value at the start of the SBR cycle and COD/N ratio were investigated in duplicate determination during phase II. Each operational parameter in the selected range was tested while keeping the other two parameters throughout constant. During phase III, both temperature and pH were varied in the selected ranges keeping COD/N ratio constant. The pH mentioned in the present

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TABLE 2 Average compositions of the inoculum (single-stage SBR) from WWTP Kaster, centrate (sludge dewatering) from WWTP Aachen Brand and Jülich tested in the batch tests at the laboratory-scale plant.

	MWWTP	TN_b [kg/m^3]	NH_4-N [kg/m^3]	NO_2-N [kg/m^3]	NO_3-N [kg/m^3]	PO_4-P [kg/m^3]	COD [kg/m^3]	TSS [kg/m^3]	VSS [kg/m^3]
Inoculum	Kaster	0.285	0.189	0.006	0.088	0.045	0.647	0.37	0.1
Centrate	Jülich	0.69	0.771	0	1.1	0.007	2	9×10^{-6}	5×10^{-6}
Centrate	Aachen	0.554	0.554	0	0	25.5	0.178	0	0

work was the pH provided to SBRs at the beginning of the SBR cycle using HCl or NaOH. COD concentrations were adjusted in the SBR at the start of batch tests using acetic acid to achieve desired COD/N ratios for batch tests. Respective ranges of each process parameter during phases I, II and III are listed in Table 1.

2.3 | Inoculum and treated wastewater

Deammonifying seeding sludge from the side stream of MWWTP Kaster (Germany) was collected from the single-stage PN/A reactor. Centrate was preferred over synthetic wastewater to investigate the PN/A process closer to reality. Centrate produced after the sludge dewatering from the municipalities of Aachen Brand (Germany) and Jülich (Germany) was used to increase NH_4-N concentration in the batch tests. The characteristics of deammonifying seeding sludge and centrate are presented in Table 2.

2.4 | Analytical methods

The concentration of total nitrogen (LCK 338, acc. to EN ISO 11905-1), NO_2-N (LCK 342, acc. to DIN 38405 D10) and COD (LCK 114, acc. to DIN 38409-H41-H44) were measured photometrically using cuvette test kits (Hach Lange®, Germany). The error of proximity in the measurement is assumed to be 8%. The concentration of NH_4-N (Art. Nr. 100683) and NO_3-N (Art. Nr. 101842) were also analysed spectrophotometrically using test kits (Merck®, Germany) according to EN ISO 9001. The samples were prepared by centrifugation at 13 000 RPM for 2 min, and the supernatant was filtered using a 0.45-mm filter (Macherey-Nagel) before analysis. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to DIN EN 872 (H33) and DIN 38409 (H1), respectively. Free ammonia (FA) is calculated according to Sinha and Annachhatre (2007) using a formula (Data S1).

2.5 | Overall volumetric nitrogen removal rates in the PN/A reactors

The overall volumetric N-removal rate is defined as the amount of bound total nitrogen (TN_b) removed per reactor volume and day (kg TN_b/(m^3 d)). Wastewater samples at the start, after aeration phase and at the end of each batch test were taken.

3 | RESULTS AND DISCUSSION

This section is presented according to the respective phases of investigation. Implementation of the PN/A process in the low-strength wastewater in the laboratory-scale plant during phase I using deammonifying sludge of MWWTP Kaster is presented in Section 3.1. The N-removal performance of deammonifying sludge in a selected range of temperature, pH value and COD/N ratio during phase II is discussed in Section 3.2. The PN/A feasibility of deammonifying sludge for the mainstream application in MWWTP Kaster during phase III is estimated in Section 3.3 using the results generated in phase II.

3.1 | Phase I: Implementation of PN/A process at laboratory scale

With the characteristics of inoculum biomass and centrate from Table 2, continuous aeration of 8 h and a mixing phase of 12 h during an overall SBR cycle of 20 h for a given NH_4-N start concentration of 0.15 kg/m^3 were found to be promising to implement PN/A process at laboratory scale after a series of batch tests for optimization. However, a single operational set-point for the overall PN/A optimization does not exist (Leix et al., 2017). Nevertheless, several settings that meet the desired process performance could be defined using the process parameters. The desired stoichiometric reactions of PN/A occurred during the SBR cycle of 20 h (8-h aeration: 12-h mixing) is considered as the optimized reactor operation of PN/A. Figure 3 shows a view of process behaviour of PN/A during the optimized batch test.

The stoichiometric data are one of the deciding factors of PN/A performance. The mechanism of the PN/A process is based on the stoichiometric reactions of a combined partial nitritation (PN) and anammox (A). Ideally, the NH_4-N is almost completely converted to elemental N during the PN/A process. In PN, the AOB group of bacteria oxidizes about half of the NH_4-N present in the wastewater with oxygen to NO_2-N (Equation 1). In the second process step, the remaining NH_4-N with the NO_2-N formed is converted by anammox bacteria to about 90% elemental N and 10% NO_3-N. The anammox process takes place under anoxic conditions (Equation 2). The mechanism of the processes involved are described by the following stoichiometric reaction equations (Mao et al., 2017):

$$NH_4^+ + 1{,}14\,HCO_3^- + 0{,}85\,O_2 \rightarrow 0{,}43\,NH_4^- + 0{,}57\,NO_2^- + 1{,}14\,CO_2 + 1{,}71\,H_2O \tag{1}$$

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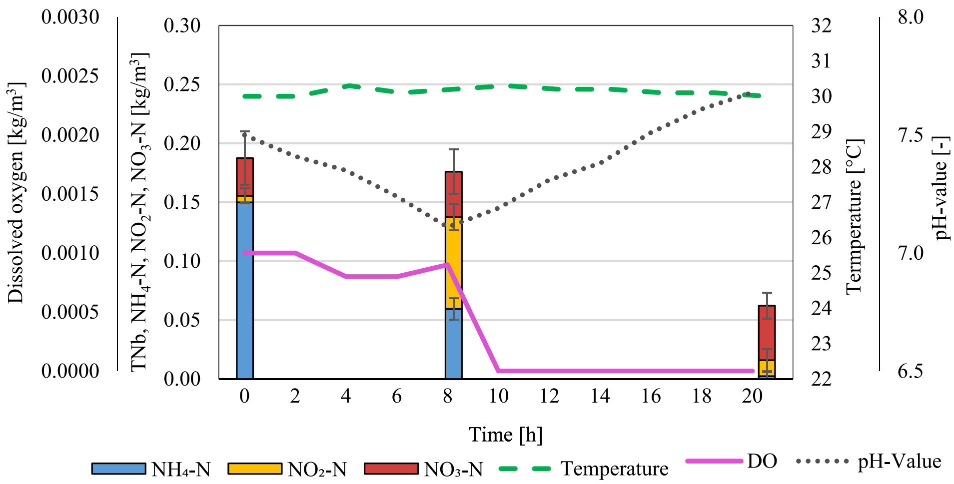

FIGURE 3 Implemented PN/A process at continuous aeration with a start concentration of 0.15 $kgNH_4$-N/m^3 at the laboratory-scale plant as a part of process optimization. Study of N-parameters of an optimized PN/A at three different stages (start, after 8 h aeration and end of batch test) at a temperature of 30°C, starting pH value of 7.4 and DO ≤0.003 kg/m^3 during aeration. Total inorganic nitrogen is the sum of NH_4-N, NO_2-N and NO_3-N compounds.

$$NH_4{}^+ + 1{,}32\,NO_2^- + 0{,}066\,HCO_3^- + 0{,}13\,H^+ \rightarrow 1{,}02\,N_2 + 0{,}26\,NO_3^- + 0{,}066\,CH_2O_{0{,}5}N_{0{,}15} + 2{,}03\,H_2O \qquad (2)$$

In order to be able to establish the successful biological mechanism of PN/A process in the reactors, the evaluation of the NO_2-N/NH_4-N stoichiometric ratio occurred during the wastewater treatment is most essential. In the current work, for a 0.15 $kg_{NH4\text{-}N}/m^3$ NH_4-N start concentration, a 0.086 ± 0.01 $kg_{NH4\text{-}N}/m^3$ was oxidized to NO_2-N during the 8-h aeration or PN phase as shown in Figure 3. The NO_2-N/NH_4-N ratio after the PN phase was found to be 1.312 ± 0.013, which was close to the standard theoretical stoichiometric ratio of 1.32 from Equation (2). The available NO_2-N/NH_4-N ratio determines the efficiency of the following anammox process. The overall PN/A process has eliminated an average TN_b of 0.142 ± 0.026 kg/(m^3 d) during the 20 h SBR cycle, attaining a 68% N-removal rate. A small amount of 0.008 kg/m^3 NO_2-N and 0.003 kg/m^3 NH_4-N accumulations were nevertheless noticed in the effluent (Figure 3).

Though it was continuous aeration, the average NO_3-N production was less than 1% of the NH_4-N-start concentration, which was significantly less in a 20-h SBR cycle compared to intermittent aeration (24%–37% NO_3-N of NH_4-N-start concentration) in a 6-h SBR cycle (0.75-h aeration: 0.25-h mixing) operated at the side stream deammonification of WWTP Kaster. However, the average ratio of $NO_3\text{-}N_{production}/NH_4\text{-}N_{conversion}$ was found to be 0.096 ± 0.077, which did not exactly match the standard stoichiometric ratio of 0.11 (suggested by Strous et al., 1999). The strategy of continuous aeration imposes the risk of favouring NOB growth at longer aeration periods, which necessarily needs to be suppressed as NOBs compete with anammox bacteria for the substrate NO_2-N. For this reason, the dissolved oxygen besides the NO_3-N production needs to be kept at the lowest possible concentration (below 0.001 kg/m^3) and should be carefully monitored during the process (Leix et al., 2017). Similarly, the WWTP Kaster maintains the dissolved oxygen concentrations in the side stream reactors of deammonification at ~0.0006 kg/m^3.

On the other hand, the stoichiometry of NH_4-N and NO_2-N consumption strongly indicated a typical anammox process in the SBR, which replicated the previous molecular biological results that showed anammox bacteria to be dominant with a relative abundance of about 32% (Strous et al., 1999). The pH curve shown in Figure 3 was explained as a reflection of alkalinity consumption caused by NH_4-N oxidation during aerobic periods combined with alkalinity generated by anaerobic ammonium oxidation. Therefore, the pH would decline during aerobic ammonium oxidation and increase during the non-aerated period when anaerobic ammonium oxidation was active. During the initial 0.1 h of the SBR cycle, no change in pH was observed at the aeration phase, which could not be justified within the time span of this study and was assumed as the lag phase of AOB (typically less than 24 h) based on the literature (French & Bollmann, 2015). Possible assumption is however it might be occurred by oxidation of the NH_4-N to ammonia ($NH_4{}^+$) with immediate CO_2 degasification. If there are carbonated species in wastewater, the raise of pH is might be due to the fact of carbonate together with the $NH_4{}^+$ ion is converted to hydro carbonate ion and then further to CO_2 gas and OH^- ion, which can be presented in form of Equation (3).

$$CO_3{}^{2-} + NH_4{}^+ \rightarrow HCO_3{}^- \rightarrow CO_2\,(g) + OH^- \qquad (3)$$

3.2 | Phase II: Role of operational parameters on PN/A

3.2.1 | Impact of temperature at a constant pH 8

The current study showed that the temperature reductions in SBRs influenced the N-removal performance of PN/A in low-strength wastewater negatively. The temperature decrease from 35 to 15°C resulted in a marked drop in the overall mean N-removal rate from 0.13 to 0.045 kg/(m^3 d) (Figure 4). The induced temperature drop from 35 to 15°C further affected the average NH_4-N oxidation from 0.066 to 0.041 kg/m^3 during the PN phase. The average NO_2-N accumulation in the effluent at the end of batch tests of 35 and 30°C was

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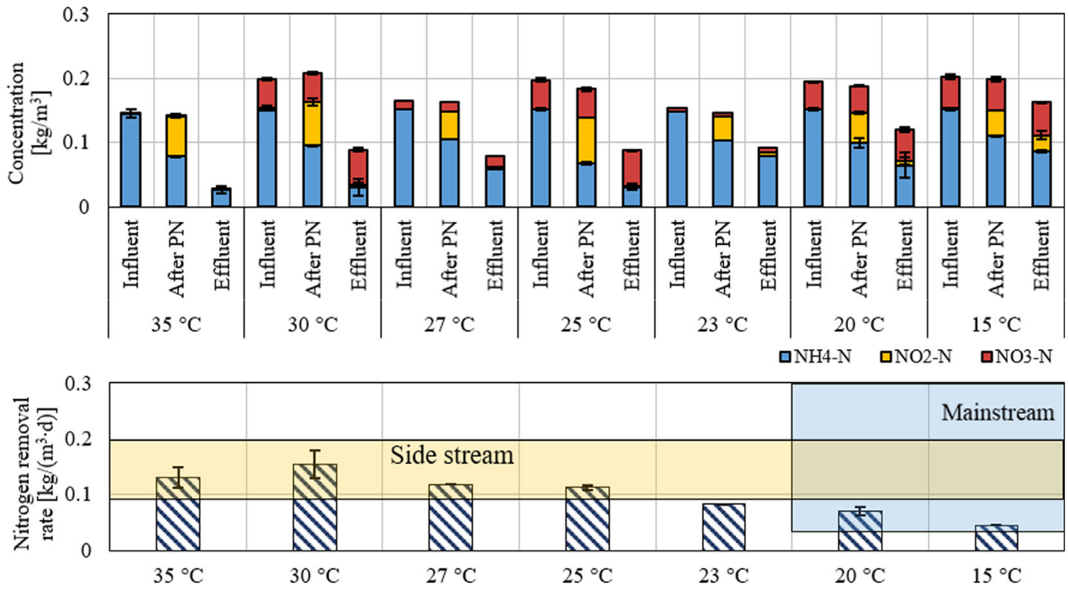

FIGURE 4 Nitrogen parameters during batch tests at temperature reductions at laboratory scale for an SBR cycle of 20 h at a given influent NH_4-N concentration of 0.15 ± 0.02 kg/m^3, starting pH condition of 8 ± 0.1 and DO ≤0.003 kg/m^3 during aeration. Nitrogen removal rates in terms of kg/(m^3 d). Total inorganic nitrogen is meant as the sum of NH_4-N, NO_2-N and NO_3-N compounds.

predominantly 0 kg/m^3, while at temperatures 27, 25, 23, 20 and 15°C contained 0.003, 0.001, 0.005, 0.007 and 0.022 kg/m^3, respectively. The temperature increase from 30 to 35°C largely did not favour the NO_3-N production except in the cases of SBRs, which already contained certain amounts of NO_3-N at the start (influent) of batch tests. The lowest NO_3-N production of 0.003 kg/m^3 was found to be at 15°C. It explains that at 15°C, not only the activity of AOBs and anammox bacteria but also nitrite-oxidizing bacteria (NOB) seemed to be largely inhibited. Nevertheless, N-removal of 0.045 kg/(m^3 d) still occurred at 15°C. A similar observation was found in the study of Gilbert et al. (2014), which reported that N-removal rate under 15°C is still possible. He further showed that the PN/A-process still runs at 10°C, but it is unstable when there is a large abundance of deammonifiers.

According to Trela et al. (2014), the PN/A process comes to a halt, when it is run lower than 10°C. A study by Isaka et al. (2008) revealed a temperature dependency between the anammox process and the deammonifying biomass containing several kinds of planctomycetes. As there is limited information available regarding these mechanisms, both the adaptation of anammox to colder conditions and the takeover of different anammox remain possible. Additionally, low temperatures affect the growth rates of AOB more than those of NOB (Gilbert et al., 2014). When the PN/A is operated at temperatures <15°C, a longer SBR cycle than the practised is preferred (Gilbert et al., 2014; Laureni et al., 2016).

The WWTP Kaster operates the side-stream deammonification at temperatures of ~32°C at which 0.134 kg NH_4-N/(m^3 d) is eliminated in high-strength wastewater (centrate) on large scale. In the current laboratory batch tests, 0.141–0.144 kg NH_4-N/(m^3 d) was eliminated in the low-strength wastewater at 30 and 35°C, respectively, which is in line with the large-scale deammonification. However, the PN/A performance does not yield the same for every large-scale side-stream deammonifying sludge with respect to the temperature. A following study of Cheenakula et al. (2023) on PN/A performance by various side-stream deammonifying sludges of local MWWTPs in Germany found that the TN elimination of various sludges ranged between 0.05 and 0.38 kg/(m^3 d) at 30°C, while the TN elimination drops below 25°C and above 35°C. Some of the side-stream deammonifying sludges in that study came to halt at 15°C, and some yielded yet the TN elimination below 15°C but significantly low.

3.2.2 | Impact of pH at a constant temperature of 35°C

The raw wastewater in the mainstream of WWTP Kaster usually fluctuates between pH 6.5 and 7.5. While it is unusual to have permanent pH changes, pH surges can occur. Therefore, the batch tests were conducted to investigate the pH influence in such a way that the pH values were set at the start of each batch test and the cycle was carried out without further pH control but monitoring. PN/A at different pH conditions from 6.0 to 8.0 at the start of the SBR cycle resulted in a low rise of overall average N-removal rate at laboratory scale from 0.108 to 0.130 kg/(m^3 d) (Figure 5a).

The statistical analysis in batch tests by Puyol et al. (2014) led to the conclusion that in weakly alkaline conditions, the pH value is the main inhibiting factor for PN/A. A pH value above 8.0 in the SBR can lead to destabilizing conditions for the involved bacteria in the PN/A process, and under these conditions, even slight changes in pH value can reduce the turnover rate of PN/A. This can be attributed to the fact that with increasing pH, a higher proportion of N is present as free ammonia (FA) or NH_3. According to Liu et al. (2019), AOB begin

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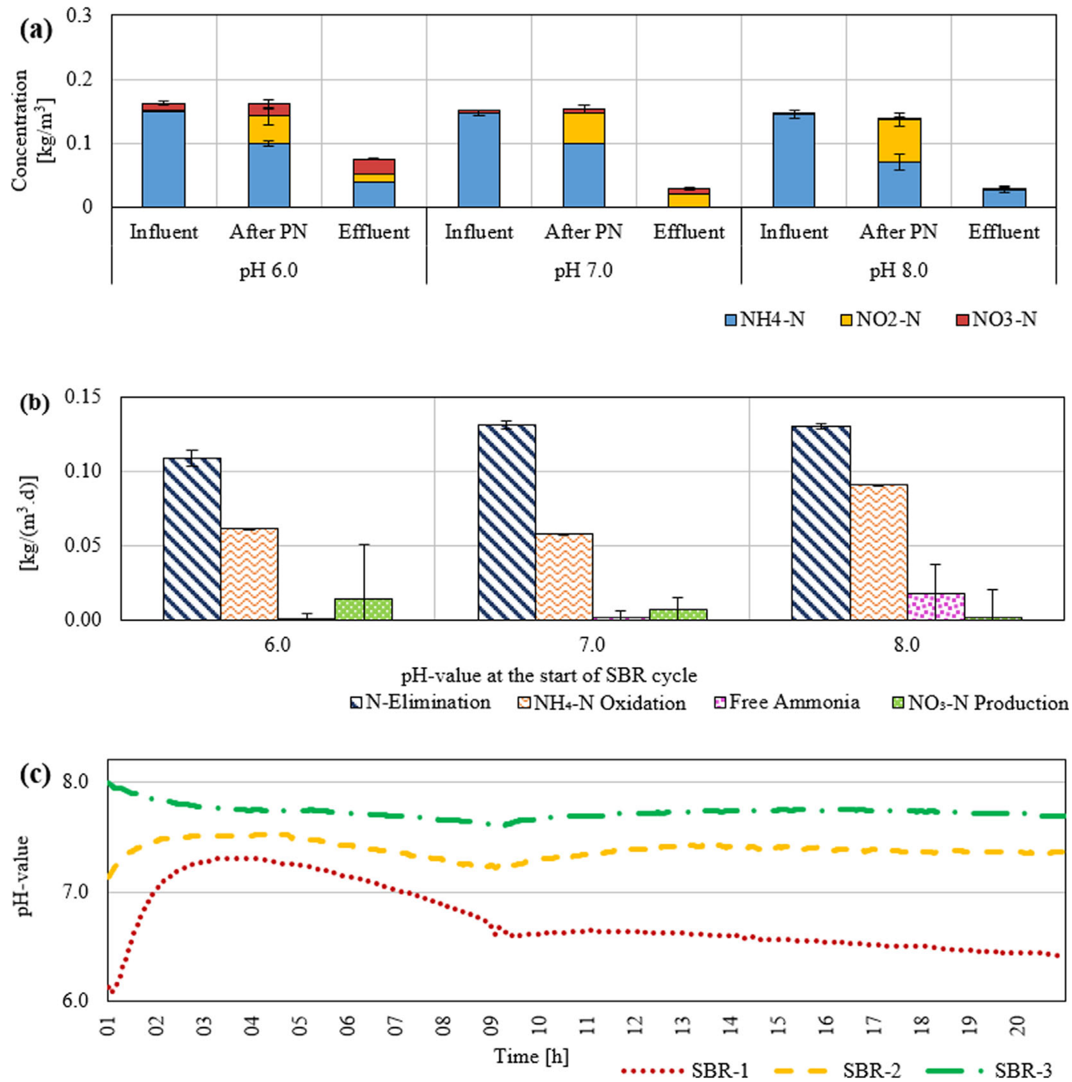

FIGURE 5 (a) Nitrogen parameters during batch tests at starting pH conditions of SBR at laboratory scale. (b) Comparison of N-removal, NH_4-N oxidation, free ammonia and NO_3-N inhibition at three different starting pH conditions at laboratory scale. (c) Study of pH curve during a 20-h SBR cycle among three SBRs, where pH of 6.0, 7.0 and 8.0 are starting conditions of SBRs, respectively, at a constant temperature of 35 ± 1°C, given influent NH_4-N concentration of 0.15 ± 0.01 kg/m^3 and DO ≤0.003 kg/m^3 during aeration.

to be inhibited at an FA concentration of 0.01 kg FA-N/m^3, while complete inhibition of AOB occurs at 0.15 kg FA-N/m^3. These results were slightly different from the observations in this study. The maximum NH_4-N oxidation of 0.075 kg/m^3 (by AOBs) occurred in the SBR of pH 8.0 having the highest FA concentration of 0.15 kg FA-N/m^3 (Figure 5b). The threshold value of AOB inhibition at the laboratory scale seemed to be significantly higher than the findings of Liu et al. (2019). This could be due to the origin of biomass, operation of the SBR cycle and the DO concentration used.

Lower NH_4-N oxidation occurred at pH 7.0 (0.0017 kg FA/m^3) than at pH 6.0 (<1 × 10^{-4} kg FA/m^3), which was 0.048 and 0.051 kg/m^3, respectively. A moderate AOB inhibition might have occurred at 0.0017 kg FA/m^3 (pH 7.0) based on the amount of NH_4-N oxidized in the SBR. NOB activity starts becoming inhibited at 0.12 × 10^{-3} kg FA-N/m^3 and is completely inhibited at higher FA concentration of 0.001 kg FA-N/m^3 as per Liu et al. (2019). These results were comparatively closer to the findings in this study. The higher the pH the higher the FA concentration must be presented. There must be significantly less NOB production at pH 8.0 (0.015 kg FA-N/m^3) in the present study according to the literature and likewise a lower production of NO_3-N (2.0 × 10^{-4} kg/m^3) was noticed in the SBR of pH 8.0 than of pH 6.0 (0.014 kg NO_3-N/m^3). However, a 55% NOB inhibition was also observed in the SBR of pH 7.0 (1.7 × 10^{-3} FA-N/m^3) that led to an average production of 7.0 × 10^{-3} kg NO_3-N/m^3. The highest NO3-N production of 0.014 kg NO_3-N/m^3 was noticed in the SBR of pH 6.0 having lowest FA concentration of 1.0 × 10^{-4} kg FA-N/m^3.

Further, FA-N concentration has an inhibition effect on anammox. The inhibiting concentration of FA to anammox is generally above 5.0 × 10^{-3} kg FA-N/m^3 (Wang & Gao, 2016). At a higher FA concentration at pH 8.0, the NO_2-N/NH_4-N stoichiometric ratio after the

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aeration phase did not match the predetermined theoretical stoichiometric rate of 1.32 but was below 1.0. Unfavourable stoichiometry of NO_2-N/NH_4-N affects the efficiency of following anammox process. It shows that FA concentration is a crucial inhibitory factor of AOB, NOB and anammox. AOB can tolerate tenfold higher concentration of those two kinds of substrates than NOB can (Wang & Gao, 2016). Therefore, the determination of a suitable FA concentration corresponding to the pH value of the influent may also be an effective strategy to enrich the AOB population in activated sludge with simultaneous washout of NOB from the system (Liu et al., 2019).

The favourable pH range for AOB to be oxidized to NO_2-N is assumed to be at pH 8.0 as the pH started to drop immediately in SBR-3 during the aeration phase, indicating the NH_4-N conversion (Figure 5c), while the pH of SBR-1 and SBR-2 increased initially during the aeration. The increased pH value could be explained by bacterial activity and by a stripping effect. Often, the wastewater with NH_3/NH_4-N also contains a significant concentration of CO_2. If the initial pH is not too high, CO_2 naturally degasses during aeration. This causes an increased pH. Biomass activity in SBR-1 and SBR-2 might have started its metabolism when the alkalinity reached 7.5. The highest NH_4-N oxidation took place in SBR-3 at pH 8.0, yielding the maximum N-removal rate.

Hydrazine hydrolase, one of the two key enzymes in anammox metabolism, is known to have an optimal pH range of between 7.5 and 8.0 but is known to be stable between 6.0 and 9.0 (Shimamura et al., 2007), which suggests that it might contribute to the NO_2-N accumulation at the low pH as found in SBR-1 (pH 6.0). Hydrazine hydrolase is less well studied, and its activity as a function of pH is not known; therefore, its role in defining an optimum functional pH range is not known.

The WWTP Kaster operates the side-stream deammonification at a pH of 8.0 at which 0.134 kg NH_4-N/(m^3 d) is eliminated on large scale. In the current laboratory batch tests, 0.141 kg NH_4-N/(m^3 d) was eliminated in the low-strength wastewater at pH of 8.0, which is again in line with the large-scale deammonification. Similar to the impact of temperatures, the PN/A performance does not yield the same for every large-scale side-stream deammonifying sludge with respect to the pH. In the subsequent work of Cheenakula et al. (2023) on PN/A performance by various side-stream deammonifying sludges revealed that the highest TN elimination of various sludges lied between 0.04 and 0.38 kg/(m^3 d) at the optimum pH of 7.0, while the TN elimination decreased very significantly below pH 6.0 and above pH 8.0.

3.2.3 | Significance of the COD/N ratio for the metabolic processes of PN/A

An increase of COD/N ratio in the SBRs of PN/A significantly influenced both the metabolic processes and the associated N-removal rates (Figure 6). The average NO_2-N production at COD/N ratio 6.0 (COD 1.048 kg/m^3) and COD/N ratio 3.0 (COD 0.567 kg/m^3) after the 8-h aeration phase was found to be 0.01 and 0.005 kg/m^3. It was a significantly lower production for a given NH_4-N start concentration of 0.15 kg/m^3 ± 0.02 compared to the batch tests without external carbon increase conducted in the previous tests of temperature (Section 3.2.1) and pH (Section 3.2.2). An average NO_3-N of 0.005 and 0.008 kg/m^3 and a COD concentration of 0.396 and 0.429 kg/m^3 were consumed during the non-aerated phase at COD/N ratios of 6.0 and 3.0, respectively, which indicates a typical denitrification metabolism. Although the COD concentrations in SBRs were very low for several months, the denitrifying bacteria could still survive and denitrify using NO_3-N generated by NOBs and COD from the decayed bacteria (Liang et al., 2016). It was observed that the sludge at high carbon content in the batch tests tended to nitrify and denitrify in parallel during the PN/A process. As a result, the N-elimination rate does not decrease even at higher COD/N ratios, but a different metabolism is adjusted accordingly. On the other hand, the oxygen saturation could not be set precisely enough in the laboratory experiments in this study and had to be run slightly over-stoichiometric. Therefore, the conversion to N/DN could not be inhibited via oxygen or airflow control during the batch tests.

On the other hand, neither NO_3-N nor COD consumption occurred at COD/N ratio ≤1.5, where initial COD concentration in SBRs was kept at 0.248 kg/m^3.

In the study of Azari et al. (2020), 90% of N-elimination occurred at a COD/N ratio of 2.23 when the SBRs with integrated fixed-film activated sludge were operated at 23°C and pH 6.7 using synthetic wastewater. During this period, AOBs doubled in 7–8 h, while NOBs doubled in 10–13 h. Anammox activity decreased sharply at COD concentrations greater than 0.0997 kg/m^3. This limit value of COD concentration was found to be higher in the present study based on the NO_2-N and NH_4-N degradation during non-aerated phase. The degradation of NO_2-N and NH_4-N was not affected at COD concentration of 0.248 kg/m^3 in the SBRs (COD/N ratio ≤1.5). At COD concentrations above 0.5 kg/m^3 in the SBRs, NO_2-N and NH_4-N degradation during anammox phase were partially affected. When COD concentration reached values above 1 kg/m^3 in SBRs, more than 50% of PN/A was replaced by other metabolic processes, suspecting N/DN, as shown in Figure 6.

To be able to assess whether the measured N-elimination was through PN/A or N/DN in this study, carbon depletion was used as a parameter. A stable PN/A was noticed at COD/N ≤ 1.5 (Figure 6), where initial COD was 0.248 kg/m^3. According to this, the COD/N ≤ 1.5 seemed optimal for the anammox process. At higher COD/N ratios above 1.5, the presence of PN/A could not be confirmed and the likelihood of N-removal via N/DN increased, because the competition between anammox bacteria and denitrifiers developed favourably for the denitrifiers by raising BOD levels. A similar observation was found by Kartal et al. (2007).

Chen et al. (2009) observed that AOB were mainly present in the aerobic biofilm region, accompanied by some aerobic heterotrophic bacteria. Anammox bacteria and denitrifying bacteria are mainly found in the region of anoxic biofilm. The cooperation between these bacteria was considered responsible for the simultaneous N and COD removal. The findings of Chen et al. (2009) resembles the

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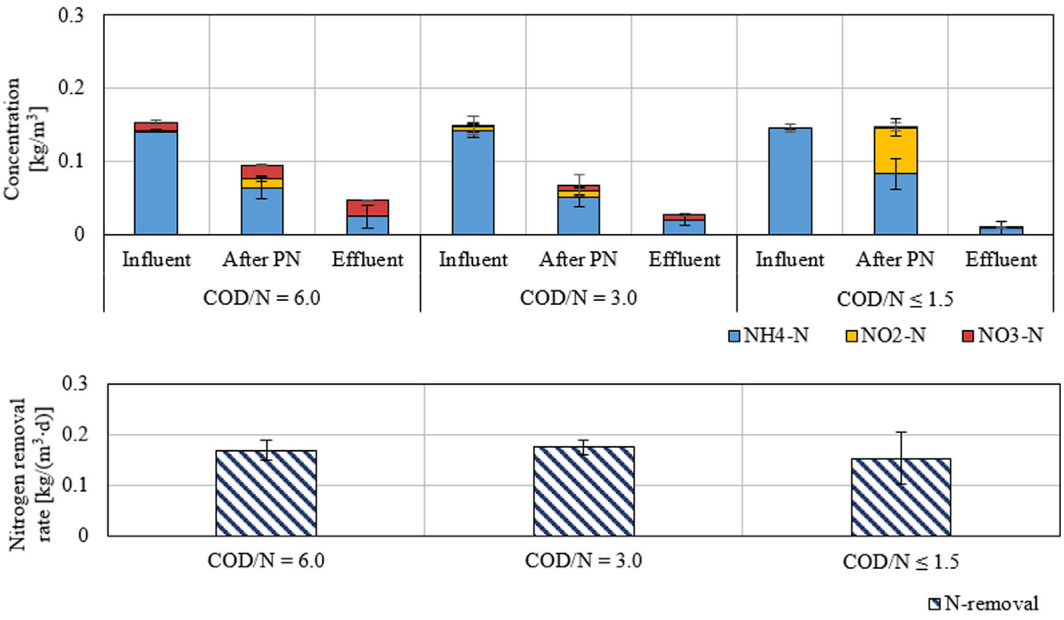

FIGURE 6 Nitrogen parameters during batch tests at stepwise COD/N ratio reductions from 6.0 to ≤1.5 at laboratory scale for an SBR cycle of 20 h at a given NH_4-N start concentration of 0.15 ± 0.02 kg/m^3, temperature at 35 ± 1°C, starting pH condition of 8 and DO ≤0.003 kg/m^3 during aeration. Nitrogen removal rates in terms of kg/(m^3 d). Total inorganic nitrogen is meant as the sum of NH_4-N, NO_2-N and NO_3-N compounds.

behaviour of N-removal rates in SBRs of COD/N ratios of 3.0 and 6.0 in the present study. However, the occurrence of behaviour was noticed at different COD/N ratios in the study of Chen et al. (2009). He reported a decreased N-removal from 79% to 52% when the COD/N was increased from 0.5 to 0.75. In contrast, the N-removal rate in the present study was increased from 75% to 82% when the COD/N ratio was increased from 1.5 to 6.0. However, it must be considered that the removal of pre-existed NO_3-N in the SBRs of COD/N ratios of 3.0 and 6.0 contributed to the higher N-removal rates.

The WWTP Kaster operates the side stream deammonification at a COD/N ratio of 1.0 at which 0.134 kg NH_4-N/(m^3 d) is eliminated on large scale. In the current laboratory batch tests, 0.164 kg NH_4-N/(m^3 d) was eliminated in the low-strength wastewater, which is again in line with the large-scale deammonification. Similar to the impact of temperature and pH of wastewater, the PN/A performance does not yield the same for every large-scale side-stream deammonifying sludge with respect to the COD/N ratios. In the subsequent study of Cheenakula et al. (2023) on PN/A performance by various side-stream deammonifying sludges revealed that the highest TN elimination of various sludges ranged between 0.04 kg/(m^3 d) and 0.4 kg/(m^3 d) at the optimum COD/N ratio of 6.0. Moreover, the COD/N ratio decrease up to 1.0 did not affect the TN elimination in the most of the cases of investigated sludges. Some of the sludges showed a stable TN elimination even at lower COD/N ratios below 6.0, while the others dropped their TN elimination to 0.04 kg/(m^3 d).

3.3 | Phase III: Estimation of mainstream PN/A at MWWTP Kaster based on the operational window of deammonifying sludge of side stream

The MWWTP Kaster is designed for 66 000 P.E. As of 2015, the plant Kaster is loaded with approx. 48 500 P.E. Daily water volume for the treatment at the plant accounts for 10 560 m^3/d (Schäpers & Kasper, 2018). The plant Kaster consists of a mechanical pre-treatment stage and a three-stage cascade (anaerobic/anoxic/aerobic) for P-elimination through Bio-P and N-elimination through N/DN. A simplified flow diagram of the plant Kaster is shown in the Supporting Information. It was assumed that the required reactor volume at MWWTPs in Germany is currently between 0.15 and 0.25 m^3 per inhabitant. Typically, 0.001 kgN/(E·d) of the inhabitant-specific load of 0.011 kgN/(E·d) is eliminated in the primary treatment (mechanical stage). Approx. 0.0075 kgN/(E·d) must be eliminated in the biological stage at a N-elimination rate of 75% in the mainstream. Without taking into account inlet fluctuations and shock loads, this corresponds to at least 0.002 kgN/(m^3 h) as N-removal rate. To be able to implement PN/A in the mainstream of plant Kaster, the PN/A should fulfil the minimum requirement of N-elimination rate of 0.00208 kgN/(m^3 h).

According to the laboratory batch tests in the present study, the deammonifying sludge from the side stream of the plant Kaster could achieve N-removal rates from 0.0008 to 0.0064 kgN/(m^3 h) as shown in Figure 7. It is clear that the favourable temperatures are mostly in the range of 25–35°C at pH 8 and thus in a favourable range for

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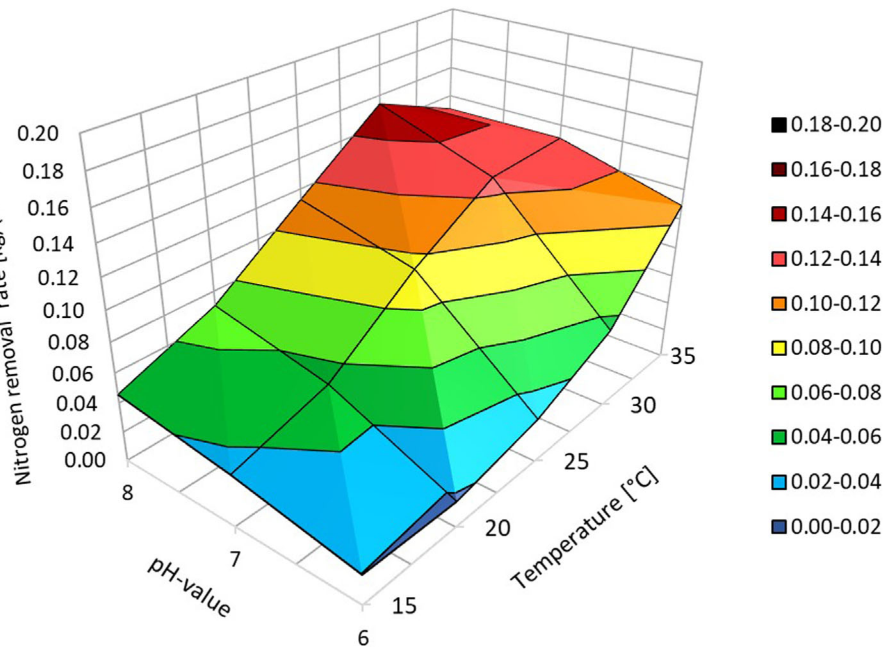

FIGURE 7 Operational window of the deammonifying sludge from the side stream of MWWTP Kaster. Performance of PN/A in terms of N-removal rate in kg/(m^3·h) at temperatures from 15 to 35°C and at pH value from 6 to 8. '*' denotes the fulfilment of the minimum requirement of N-turnover rate of PN/A to implement in the mainstream.

PN/A. If the plant Kaster is regularly operated at temperatures below 20°C, the pH should be between 7 and 8 to achieve the minimum requirement of N-removal rate in the mainstream. However, an N-removal of 0.0008 kg/(m^3 h) still took place at the lowest temperature of 15°C and at the lowest pH of 6. When the PN/A is operated at temperatures <15°C, a longer SBR cycle than the practised is preferred.

On the other side, anammox granules grown during the batch tests of laboratory Sixfors plant in this study are to be found in the Supporting Information.

Although satisfactory N-elimination rates were achieved in the laboratory-scale SBRs, the operational parameters in the present study were investigated in the short term. Therefore, the same results cannot be expected in full scale on long term. Moreover, a direct transfer of results is immediately not possible to full scale due to the high influent quantities and the size of the reactors in reality. An estimation with possible solutions for the mentioned operating conditions can be however made on the basis of the results from the laboratory batch tests. Nevertheless, the results in the current work form a solid basis for the semi-technical trails on large scale at the WWTP Kaster. Based on the scientific insights gained from the current work, semi-technical trails at mainstream conditions must be performed at WWTP Kaster and at other WWTPs having similar specifications on long term. The semi-technical trails must be conducted initially using reduced loads of wastewater prior to the full-scale implementation of mainstream PN/A.

4 | CONCLUSIONS

The N-removal rates of PN/A process using deammonifying sludge from the side stream of MWWTP Kaster treating low-strength wastewater under mesophilic conditions, at different pH levels, and low to moderate COD/N ratios were studied in this study. The current work is a detailed investigation on transfer of deammonification from side stream to mainstream at the WWTP Kaster. However, the current study serves as the basis for other MWWTPs having the similar specifications. The investigation shows the expected clearly high influence of the temperature on the overall process. Decreases in N-removal rates were evident at temperatures ≤20°C. The N-removal efficiency dropped to approx. 35% at 15°C comparatively at 35°C. The difference of N-removal rates between pH 6 and pH 8 were not large, but the N-removal at pH 6.0 was found to be 20% lower than at pH 8.0. Reduction in N-elimination can probably be explained by the inhibition of microorganisms at unfavourable FA concentrations due to the pH in reactor. The metabolism of PN/A was largely affected at COD/N ratio of 6.0. Although N/DN was suspected to disturb PN/A at higher COD/N ratio, no compromise in N-elimination was observed. A transfer of the results to the implementation of PN/A in the mainstream cannot be carried out due to the strongly fluctuating NH_4-N influent loads, so the experimental investigations of the deammonifying sludge are necessary with correspondingly varied influent conditions.

However, the deammonifying sludge of MWWTP Kaster fulfilled the minimum requirement of N-elimination rate (0.049 kgN/(m^3 d)) at temperatures ≤20°C, at pH 7.0–8.0, which leads to a comparable reactor volume to MWWTP Kaster. Large-scale implementation of mainstream PN/A cannot yet be recommended in full stream based on these results as operational parameters were investigated in the short term. Nevertheless, the results in the current work form a solid basis for the semi-technical trails on large scale at the WWTP Kaster. Based on the scientific insights gained from the current work semi-technical trails at mainstream conditions must be performed at WWTP Kaster and at other WWTPs having similar specifications on

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long term. The semi-technical trails must be conducted initially using reduced loads of wastewater prior to the full-scale implementation of mainstream PN/A.

AUTHOR CONTRIBUTIONS

Conceptualization: Dheeraja Cheenakula, Svea Paulsen, Fabian Ott. *Methodology*: Dheeraja Cheenakula. *Formal analysis and investigation*: Dheeraja Cheenakula. *Writing—original draft preparation*: Dheeraja Cheenakula. *Writing—review and editing*: Dheeraja Cheenakula, Svea Paulsen, Fabian Ott, Markus Grömping. *Funding acquisition*: Weesbach-Stiftung. Resources: FH Aachen, Institute NOWUM-Energy. *Supervision*: Markus Grömping. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [D.C.], upon reasonable request.

ETHICS STATEMENT

Dheeraja Cheenakula, as the corresponding author, gives consent for the publication of identifiable details, which can include photograph(s) and/or details within the text to be published in *Water and Environment*.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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