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Article

Creating Process Stability in Partial Nitritation: A Pilot-Scale Case Study on the Deammonification of Pig Slurry

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ABSTRACT

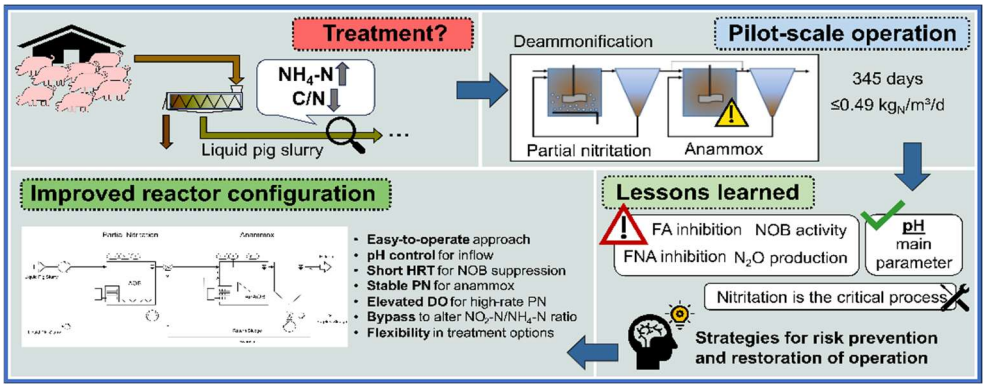
With a worldwide increase in animal protein demand, the treatment of animal slurries gains significance. At times, the conventional nitrification/denitrification used for nitrogen elimination is not applicable because of a low ratio of biodegradable carbon to nitrogen in the slurry, calling for less carbon-dependent treatment options. The autotrophic deammonification (comprising partial nitritation [PN] and anaerobic ammonium oxidation [anammox]) is a well-established process for the treatment of municipal sludge liquor and has been promisingly studied for the treatment of anaerobically pretreated pig slurry. This study investigates the feasibility of the deammonification of raw liquid pig slurry (2.33 ± 0.29 g/L NH_4-N and CODs/NH_4-N ratio of 2.4 ± 0.7) by operation of a two-stage pilot-scale deammonification plant. The focus lies on risk assessment, process control, and instabilities of the PN. Operational strategies are derived, and a risk assessment for the anammox is provided, as the anammox reactor in this study failed because of PN process instabilities. The pH was identified as the main parameter to predict process instabilities such as the inhibition of ammonia-oxidizing bacteria by free ammonia and free nitrous acid. To prevent these inhibitions, a pH-based feed control is proposed. Nitrite accumulation could be resiliently achieved at a hydraulic retention time of 2.2 days at 25 °C. With this, PN was stably operated with a NH_4-N loading of 0.49 kgN/m^3/d. With the results of this study and by contextualizing the findings within current literature, a two-stage reactor configuration with operational strategies for the deammonification of raw pig slurry is proposed.

KEYWORDS: pig slurry treatment; process stability; operational strategies; partial nitritation; deammonification; AOB inhibition; NOB suppression

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Graphical Abstract

ABBREVIATIONS

Anammox, anaerobic ammonium oxidation; AnAOB, anaerobic ammonium-oxidizing bacteria; AOB, ammonia-oxidizing bacteria; COD, chemical oxygen demand; CODb, biodegradable chemical oxygen demand; CODs, soluble chemical oxygen demand; CODt, total chemical oxygen demand; CSTR, continuous stirred-tank reactor; DO, dissolved oxygen; EC, electrical conductivity; FA, free ammonia; FNA, free nitrous acid; HRT, hydraulic retention time; MBBR, mixed bed biofilm-reactor; NH_4-N, ammonium-nitrogen; NO_2-N, nitrite-nitrogen; NO_3-N, nitrate-nitrogen; NOB, nitrite-oxidizing bacteria; PN, partial nitritation; SAD, simultaneous anammox and denitrification; SD, standard deviation; SRT, sludge retention time; TA, total alkalinity; TS, total solids; TSS, total suspended solids; VLR, volumetric loading rate; VS, volatile solids; VSS, volatile suspended solids

INTRODUCTION

Although animal manure is traditionally regarded as a valuable organic fertilizer containing phosphorous and nitrogen, inefficient application and overuse lead to nitrogen leaching into the groundwater body, causing a contamination with nitrate [1–3]. In Germany, this resulted in a 2017 amendment of the ordinance redefining best practice in the application of fertilizer, further restricting the application of nitrogen to agricultural soil. Therefore, the management and treatment of animal manures and slurries gained significance, with a focus on pig manure and slurry in northwest Germany. Still, the treatment of pig slurries is a topic of international concern with a rising concentration in the swine business and fewer growers, increasing the local impact on the environment, especially in China, the United Sates, and the European Union [4].

While the utilization of bioresources is preferable, a safe and direct use of pig slurry is not always possible due to the presence of pollutants such as heavy metals or antibiotics [5,6]. A recovery of nitrogen by processes such as air stripping or membrane processes is technically and economically challenging [7]. Thus, a treatment aiming at the elimination of nutrients, mainly nitrogen, must be considered if a safe use is not

possible. For the conventional nitrification/denitrification to be technically feasible, a ratio of biodegradable chemical oxygen demand (CODb) to ammonium-nitrogen (NH_4-N) of at least 5.8 is necessary, and often an external carbon source is added to improve the denitrification efficiency [8,9]. Pig slurry is usually characterized by an NH_4-N content of 1.5–4 g/L (up to 5.5 g/L), a pH of 7–8.8 (seldom as low as 6.5), and a soluble COD (CODs) of usually <30 g/L (in some cases, up to 60 or 63 g/L) [10–13]. Because CODb is seldom measured in studies concerning pig slurry, CODs is used as an approximative substitute to assess different treatment options. Treating CODs as CODb is methodologically not sound, as particulate organic matter that is excluded from CODs may contribute to CODb, while certain soluble organic substances, as part of CODs, may not be biodegradable. By using CODs as a surrogate parameter, the biodegradability of the COD is likely underestimated. Nevertheless, this approximation is deemed acceptable considering the lack of available data on CODb/NH_4-N ratios in pig slurries impeding a conclusive overview.

In pig slurries, the CODs/NH_4-N ratio is often found to be greater than 3 and can at times reach up to 12.4, making it suitable for a treatment via nitrification/denitrification [10,14]. However, there are reported cases where CODs/NH_4-N lies below 2 and down to 0.1, requiring a less carbon-dependent treatment option [10,13,15,16].

Autotrophic deammonification is a biological process comprising partial nitritation (PN) and anaerobic ammonium oxidation (anammox). Its carbon independency makes it well-suited for the treatment of wastewaters high in nitrogen and low in organic carbon with a CODb/NH_4-N ratio of 2 or lower [9]. In the aerobic PN, only a part of the ammonia (NH_3) present in the wastewater is oxidized to hydroxylamine (NH_2OH) before being oxidized to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), consuming 0.14 mmol of alkalinity for every mg of NH_4-N oxidized [17] (Because of the relatively recent discovery of ammonia-oxidizing archaea (AOA) in wastewater systems by Park, Wells et al. (2006) [18], their affinity towards low NH_3 environments and their uncertain role and function in wastewater treatment, the nitritation process in this study is attributed solely to AOB). The anammox is performed by certain obligatory anaerobic bacteria in the *Planctomycetes* phylum, summarized as anaerobic ammonium-oxidizing bacteria (AnAOB). Here, NO_2^- is reduced to nitric oxide (NO), which reacts with NH_4^+ to hydrazine (N_2H_4) to be finally oxidized to N_2. A byproduct of this metabolic pathway is nitrate (NO_3^-), which is generated in small amounts [19]. The key operational challenge for the deammonification is achieving nitrite accumulation to supply the AnAOB with substrate. For this, nitrite-oxidizing bacteria (NOB) that naturally coexist with AOB must be suppressed, which can be achieved by exploiting the difference in resilience of AOB and NOB to harsh conditions, exploiting their different kinetics, or using competition for NOB suppression as summarized by Su et al. (2023) in their study on nitrite oxidation in wastewater [20]. If readily degradable carbon is

available in conditions of oxygen depletion, the AnAOB also stand in direct competition for nitrite with denitrifying heterotrophic microorganisms [21]. Additionally, AOB and AnAOB may be inhibited by their own substrates. AOB are susceptible to their own substrate in the form of free ammonia (FA) and the associated acid of their metabolic product as free nitrous acid (FNA), both related to temperature and pH [22]. AnAOB are also inhibited by FA and nitrite [23].

Deammonification has already been promisingly studied for anaerobically treated pig manure and anaerobically stored pig slurry as well as conceptually discussed for the treatment of raw liquid pig slurry [10,24–31]. However, the deammonification of raw liquid pig slurry has not yet been tested. AOB inhibition tests are indicating a well-functioning nitritation if common inhibitors such as FA and FNA are kept in check [10]. To test the feasibility of the deammonification process for the treatment of raw liquid pig slurry, a pilot-scale deammonification plant was operated over the course of 345 days, employing different operational modes. The main focus of the operation was on the identification of critical process conditions as a result of the inherent process risks resulting from the slurry's characteristics (high concentrations of FA as well as an elevated pH), concentrating on PN, as process instabilities in this case study resulted in a failure of the anammox reactor beyond recovery. With the results of the pilot-scale operation of the PN reactor and insights from current literature, a reactor configuration and operational strategies (process control, risk prevention, and process restoration) for the deammonification of liquid pig slurry are proposed, aiming at an easy-to-operate approach.

MATERIALS AND METHODS

To test the deammonification of raw pig slurry, a pilot-scale two-stage deammonification plant was operated over the course of 345 days and supplied with liquid pig slurry as substrate, situated onsite a small piggery operation in northern Germany. The parameters measured for substrate characterization and process monitoring include ammonium nitrogen (NH_4-N measured as NH_4^+ + NH_3), total and soluble chemical oxygen demand (CODt, CODs), electrical conductivity (EC), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), pH, total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), and total alkalinity (TA). Additionally, FA and FNA were calculated according to Anthonisen et al. (1976) [22]. The corresponding methods are listed in Table 1. The sensors installed in the experimental plants are described in the corresponding paragraph.

Table 1. Parameters measured or calculated in this study and their corresponding method.

Parameter	Method
TA	Cuvette test HACH LCK 362
CODs	Cuvette test HACH LCK 314/514/1414 filtered (0.45 µm)
CODt	Cuvette test HACH LCK 314/514/1414
EC	Digital handheld multimeter (HQ40D)
FA	Calculated according to [22]
FNA	Calculated according to [22]
NH_4-N	Cuvette test HACH LCK 303
NO_2-N	Cuvette test HACH LCK 342/343
NO_3-N	Cuvette test HACH LCK 339
pH	Digital handheld multimeter (HQ40D)
TS	According to DIN 38409-1
TSS	According to DIN 38409-1 (paper filtered, <8 µm)
VS	According to DIN 38409-1
VSS	According to DIN 38409-1 (paper filtered, <8 µm)

Liquid Pig Slurry

The slurry for the experimental operation originated from a small piggery operation in northern Germany. As part of the piggery operation, the slurry was collected in a large storage tank onsite, from which the slurry for the experimental operation was pumped into a 300 L settling tank. The supernatant (further referred to as liquid pig slurry) was subsequently collected in a 1 m^3 storage tank, from which it was supplied to the experimental plant. The tank could also be used to dilute the pig slurry to achieve any NH_4-N concentration desired. The liquid pig slurry was regularly analyzed for NH_4-N, NO_3-N, pH, EC, TA, TS, and VS. The slurry's main characteristics are summarized in Table 2 as average concentrations and their standard deviation (SD). The measured values can be found in Table S1.

Table 2. Main characteristics of the liquid pig slurry after sedimentation used in this study, expressed as mean concentrations and their SD.

	NH_4-N g/L	CODt g/L	CODs g/L	pH -	EC mS/cm	TA mmol/L	TS g/kg	VS g/kg	CODs/NH_4-N -	TA/NH_4-N mmol/mg
Mean	2.33	13.14	5.37	7.82	22.41	291.00	14.70	7.40	2.40	0.13
SD	0.29	4.47	1.70	0.10	3.93	65.00	2.10	1.60	0.70	0.03
n	30.00	26.00	24.00	30.00	30.00	30.00	7.00	7.00	24.00	30.00

Note: Mean and SD of the ratios were calculated by using the ratios of the individual samples recorded in the monitoring.

Technical Setup

The experimental plant consisted of two continuous stirred-tank reactors (CSTRs) with a volume of 500 L each and two subsequent clarifiers of 160 L. Both reactors were equipped with a stirrer and an electric heating rod. While the first reactor (PN) was uncovered and aerated using a rotary vane compressor and two membrane tube aerators, the second reactor (anammox) was covered. The liquid pig slurry from the storage tank was fed to the PN reactor using a peristaltic pump. The feeding tube was heated

with a trace heater during winter operation to avoid freezing. The settled sludge of both clarifiers could be recycled to the reactors, and surplus sludge could be manually wasted. The supernatant of the first clarifier was supplied to the anammox reactor by overflow. In addition, an automated ball valve was installed to cut the overflow to the anammox reactor and discharge the effluent of the PN reactor directly. Both reactors were equipped with a pH sensor measuring pH and temperature. In the PN reactor, an optical sensor for dissolved oxygen (DO) was additionally employed, while the anammox reactor was equipped with a spectral probe measuring NO_2^-. For additional process monitoring, the reactors' effluents were analyzed for NH_4-N, NO_2-N, NO_3-N, CODt, and CODs every third to fifth day during operation. The measurements are listed in Table S2. To limit excessive foaming, a known phenomenon when aerating animal slurries [32], an anti-foaming agent (antischaum®) was added to the PN using a small dosing pump. The technical scheme of the experimental plant is presented in Figure 1.

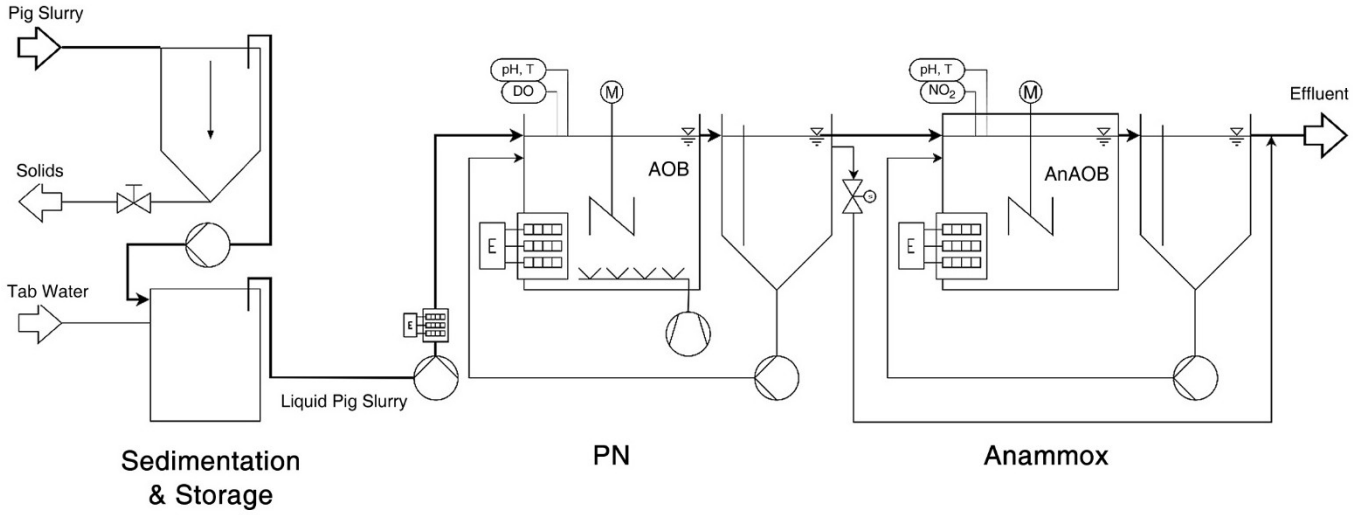


Figure 1. Technical scheme of the pilot-scale experimental plant.

The PN reactor was initially inoculated with 100 L of activated sludge originating from a single-stage deammonification plant for municipal sludge liquor treatment, which already contained few NOB. The anammox reactor was inoculated with 100 L of the same activated sludge. For the start-up of the PN reactor, 130 L of slurry and 240 L of tab water were added and aerated for 48 h at 25 °C. Thereafter, the reactor was supplied with 0.1–0.3 kg/m^3/d NH_4-N, increasing the volumetric loading rate (VLR) over time. The DO was alternating between 0.5 and 5 mg/L. In both reactors, the settled biomass was returned from the corresponding clarifier with a volumetric flow rate of 130 L/d.

Because of the premature failure of the anammox reactor, the operation was mainly focused on the process stability of PN. After the start-up, the PN was operated with sludge recycling and an intermittent aeration for NOB suppression for 247 days, slowly raising VLR by

increasing NH_4-N feed concentration. Thereafter, sludge retention (i.e., sludge recycling) was halted, and the hydraulic retention time (HRT) was reduced to 2.2 days, resulting in a sludge retention time (SRT) equal to the HRT, aiming for NOB washout. Because NOB were suppressed by exploiting their slower growth rate and not by low DO levels, the DO concentration was set to 3 mg/L to allow higher nitritation rates. With an HRT of 2.2 days, a stable nitrite accumulation was reached, so a further shortening to one day, as recommended for SHARON-type reactors [33], was not realized.

The operation can be divided into three phases. The two-day start-up period is considered phase 1 (duration: 48 h; mean feed concentration: 1710 mg/L NH_4-N; VLR: 222 $gN/m^3/d$), followed by the operation with sludge recycling (duration: 245 d; mean feed concentration: 400–1070 mg/L NH_4-N; VLR: 100–300 $gN/m^3/d$) considered phase 2, and the final operation without sludge retention (duration: 98 d; mean feed concentration: 1204 mg/L NH_4-N; VLR: 300–490 $gN/m^3/d$) considered phase 3. A detailed overview of the operational phases can be found in the accompanying article by Reiter et al. (2026) [34].

RESULTS

Failure of the Anammox Reactor

Even after sedimentation, the liquid pig slurry contained significant amounts of finely dispersed solids, which led to regular blockages of the peristaltic pumps (small tube diameters because of the scale of the experimental plant), resulting in irregular feed and occasional shock loadings to the PN in the first weeks of operation. This problem also presented itself in the recurring inhibition of AOB by FA (see Section “Considerations on AOB inhibition by FNA and FA”). During this period, a stark decrease and ultimately a stop of nitrogen elimination in the anammox reactor could be observed, likely caused by the inhibition of AnAOB via different possible mechanisms, as discussed below.

Within the first two months of operation, the PN reactors’ effluent exhibited high FA concentrations of 85–260 mg/L over a period of 3–4 weeks, resulting in FA concentrations of up to 250 mg/L at pH 8.7 in the anammox reactor. The inhibition of AnAOB by FA may start at concentrations as low as 2 mg/L [35], and even highly adapted AnAOB show a 10% decrease in activity at 190 mg/L [36]. Thus, it can be inferred that the high FA concentration in the PN’s effluent caused a notable inhibition of AnAOB activity. Additionally, the high FA concentrations in the PN reactor resulted in an inhibition of AOB, therefore decreasing oxygen uptake and nitrite production in the PN reactor. Apart from an insufficient supply of substrate for AnAOB, namely nitrite, this resulted in a drag of DO into the anammox reactor and subsequently to concentrations of 0.5–1.7 mg/L over 3–4 weeks. As AnAOB are strictly anaerobic bacteria, even small amounts of oxygen can impair the

anammox. DO concentrations of up to 2% air saturation (equal to 0.17 mg/L at 25 °C and 1 atm) can reversibly inhibit AnAOB, while concentrations higher than 18% air saturation (equal to 1.49 mg/L at 25 °C and 1 atm) irreversibly damage the AnAOB, as summarized by Jin et al. (2012) in their review on anammox inhibitions [37]. As the anammox activity stopped beyond recovery, it can be assumed that, while high FA concentrations and low nitrite supply most likely slowed anammox activity, the high DO concentration ultimately caused the breakdown of the anammox reactor.

To address this type of problem, the pilot-scale plant was equipped with a bypass to allow for the effluent of the PN reactor to be discharged directly, protecting the anammox reactor against unfavorable conditions such as high FA, high DO, or especially high nitrite concentrations. However, the electric ball valve responsible for opening the bypass was faulty and could not be replaced quickly enough. This emergency strategy could therefore not be implemented in this study. The failure of the anammox reactor highlights the importance of a stable PN operation in a two-stage deammonification system for the successful treatment of challenging wastewaters. Nevertheless, a bypass of the anammox reactor is advisable to prevent beforementioned reactor failures, if a safe discharge or collection of the PN's effluent is possible.

PN Reactor Performance

For 245 days, the PN was operated with sludge recycling for biomass retention (phase 2) before shifting to the operation without sludge retention and a short HRT of 2.2 days for another 98 days (phase 3), while increasing the VLR in both phases. The key performance indicators of the operational phases 2 and 3 are presented in Table 3 and can be found in detail in Table S3. As expected with a shift in operation from an active biomass recycling to an SRT equal to HRT of only 2.2 days, the TSS and VSS in the reactor decreased dramatically from 9.6 (±2.3) to 0.6 (±0.1) g/L. The specific NH_4-N turnover rate increased by a factor of 16 after the shift to phase 3, maintaining the overall NH_4-N turnover rate and raising it with an increase in VLR. This increase in specific turnover rate does not directly translate to an equal increase in AOB activity, however, as slurry-derived solids also contribute to VSS and were accumulated during sludge recycling in phase 2. The accompanying research article published independently by Reiter et al. (2026) [34] addresses the adaptation of the biomass to pig slurry as a substrate in terms of sludge performance, showing an increase in AOB activity by a factor of 2.1, an increase in FA tolerance, and changes in the bacterial community.

To ensure an NH_4-N/NO_2-N ratio of 1:1.32, optimized for a complete anammox [38], an NH_4-N turnover of at least 57% is necessary. Practically, a higher NH_4-N turnover is necessary, as part of the NO_2-N may be oxidized to NO_3-N and some NH_4-N may leave the system via NH_3 stripping. In this study, the high TA of the pig slurry theoretically allowed for an average

turnover of 90% (±19), which fits well with the literature, where, based on TA availability, a theoretical oxidation of up to 80% NH_4-N is possible [10,39]. The high TA harbors the potential for excessive nitrite production and must addressed in the operational design (see Section "Further insights derived for the deammonification of pig slurry"). In this study, NH_4-N/NO_2-N ratios of up to 1:39.07 were reached in phase 2, while in phase 3 an NH_4-N/NO_2-N ratio of up to 1:4.03 was reached, showing a surplus of nitrite in the PN's effluent when considering a full deammonification.

Inhibition of AOB by FA or FNA was a phenomenon experienced several times over the course of operation and is thematized in the Section "Considerations on AOB inhibition by FNA and FA."

Table 3. Performance indicators of phases 2 and 3 of the experimental operation (SD in parentheses).

Performance Indicator *	Phase 2	Phase 3
VLR in kgN/m^3/d	0.1–0.3	0.3–0.49
Mean TSS in g/L	9.6 (±2.3)	0.6 (±0.1)
Mean VSS in g/L	7.1 (±1.8)	0.5 (±0.1)
NH_4-N turnover rate in kgN/m^3/d	0.06–0.27	0.28–0.32
Specific NH_4-N turnover rate in gN/gVSS/d	0.01–0.04	0.56–0.64
Mean NH_4-N turnover	82% (±18)	66% (±15)

* Outliers due to process instabilities removed.

NOB Suppression and Nitrite Accumulation

To illustrate nitrite and nitrate production as well as overall NH_4-N turnover, NO_2-N, NO_3-N, and NH_4-N are displayed in Figure 2 as their proportion of total inorganic nitrogen in the PN reactor.

Initially, NOB activity in the PN reactor could be successfully suppressed by intermittent aeration in phase 2. Comparing NO_2-N and NO_3-N in the PN reactor shows a well-functioning nitritation, with up to 99% of their sum (NO_x-N) being NO_2-N in the first few weeks and a nitrite production of up to 0.13 kgNO_2-N/m^3/d. After 35 days, however, a decrease in NH_4-N turnover and a shift in the composition of NO_x-N occur with a stark increase in NO_3-N. The increase in NOB activity and high FA concentrations of up to 83.66 mg/L ultimately ended nitrite accumulation by day 51, with 97% of inorganic nitrogen being present as NH_4-N and up to 97% of NO_x-N being NO_3-N (see Figure 2, A). This phase of AOB inactivity lasted for several days. To restart the PN, mixing was halted, and the sludge was allowed to settle. Afterward, supernatant was drawn and replaced with fresh water to quickly lower FA concentrations. To suppress NOB activity, the DO was constantly lowered to < 1 mg/L, changing from a regime of intermittent aeration to a regime of low DO concentration (see Figure 2, B). With this, nitrite accumulation could be restored with only occasional increases in NO_3-N, corresponding to a lower nitrite production of up to 0.08 kgNO_2-N/m^3/d.

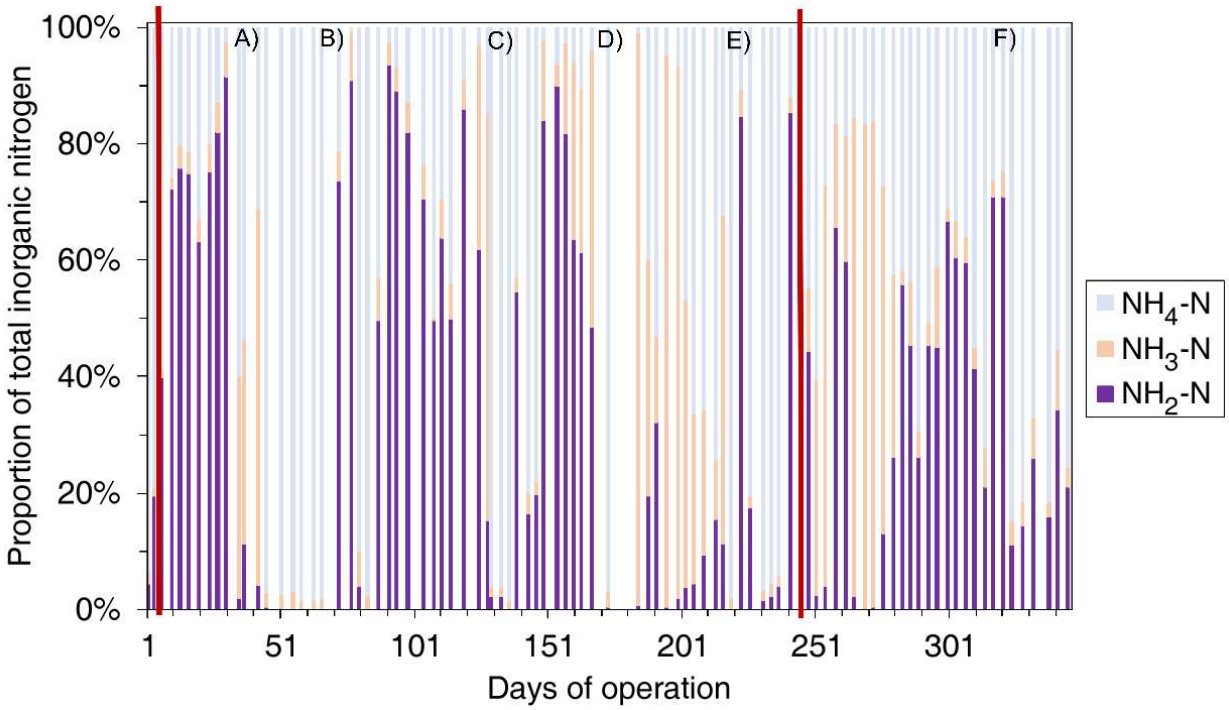


Figure 2. NO_2-N, NO_3-N, and NH_4-N in the PN reactor as a proportion of total inorganic nitrogen over the course of the experimental operation with major process interventions marked as A–F. The red lines indicate the start of phases 2 and 3.

To mitigate high FA concentrations of 50–108 mg/L as a result of an unexpected shock loading around day 135 (see Figure 2, C) the aeration was again set to 5 mg/L DO to quickly restore AOB activity (see Figure 2, D) which ultimately supported NOB activity, leading to another phase of predominantly nitratation from day 157 to day 219 with 66%–99% of NO_x-N being NO_3-N, translating to an NH_4-N turnover of up to 0.16 kgN/m^3/d. Changing back to a regime of low DO concentrations (<1 mg/L; see Figure 2, E) somewhat restored nitrite accumulation, followed by a period of activity loss due to high FNA concentrations (0.43 mg/L), before substantially changing the mode of operation for the start of phase 3 at day 248.

Directly after changing to phase 3, NOB activity increased, leading to nitrate accumulation for 24 days, before nitrite accumulation was restored at day 283, corresponding to a nitrite production of up to 0.23 kgNO_2-N/m^3/d. The increase in NOB activity after the shift to phase 3 can be attributed to the increase in DO (3 mg/L), from which the former suppressed NOB profited. With time, a decrease in NOB activity could be observed, gauged by the increase in the proportion of NO_2-N of NO_x-N. This observation suggests a successful washout of NOB due to the reduced HRT. This assumption could be substantiated in the accompanying article detailing the bacterial community in the PN reactor (see [34]). At the end of operation, the amount of NOB in the living biomass was reduced to only 0.1%. The fact that nitrite accumulation seems less stable at the end of the

operation than in the beginning is most likely due to the comparatively fast increase in VLR from 0.3 to 0.49 $kg_N/m^3/d$ (see Figure 2, F).

Considerations on AOB Inhibition by FNA and FA

For the considerations on AOB inhibition by FA and FNA, the PN reactor's performance was used to evaluate AOB activity, inferring an inhibition of AOB. Event-specific activity tests were not executed. In most cases, an inferred inhibition of AOB by FA was caused by an inadequate aeration or an overload of NH_4-N impairing the ability of AOB to sufficiently oxidize NH_4-N and gradually increasing pH and FA. Interestingly, a process failure due to an FNA inhibition as a result of high AOB activity and an accelerated nitrite accumulation was the more relevant process risk in this study, as it often preceded FA inhibition. The accelerated oxidation of NH_4-N leads to a sudden drop in pH due to the consumption of alkalinity and a stark increase of NO_2-N, conditions, resulting in elevated levels of FNA. If unchecked, this can cause FA inhibition because of the continuous supply of NH_4-N becoming too high for the reduced AOB activity, thereby increasing pH and FA. Such an inhibition event, as inferred from the reactor performance, is illustrated in Figure 3. The event was part of operational phase 2, with a mean VLR of 0.225 $kgN/m^3/d$ over the 20 days in question. DO concentration was measured between 0.4 and 2.25 mg/L.

After a shock loading as a result of the restart of a former blocked pump, the sudden oxidation of NH_4-N led to a sharp drop in pH (day 223). The pH decreased from 8.65 to 6.81 over a short period due to an almost complete nitritation of incoming NH_4-N (48 mg/L NH_4-N and 382 mg/L NO_2-N in the reactor), resulting in 0.43 mg/L HNO_2, inhibiting AOB, and impeding nitritation. With continuous inflow for the next three days, pH and NH_4-N in the reactor rose to 8.62 and 351 mg/L, respectively. Still, some AOB activity was observable, for NO_2-N was found to be 76 mg/L. At this point, 81.62 mg/L NH_3 was present in the reactor, consequently stopping nitritation. After five more days, NH_4-N in the reactor measured 464 mg/L (influent concentration at that time was 484 mg/L), while NO_2-N decreased to 7 mg/L and FA reached 87.50 mg/L NH_3. Nitrate measurements showed concentrations of 6.94–21.00 mg/L following the pattern of the nitrite concentration.

To mitigate the risk of FA and FNA inhibitions, monitoring the pH is paramount. A sudden drop in pH indicates an accelerated consumption of alkalinity and production of nitrite, favoring conditions for high FNA concentrations (indicated by an approximated pH threshold of 7 in this study). To prevent FNA inhibition, increasing the inflow can help balance pH, alkalinity, and the supply of NH_4-N. The results of Wang et al. (2020) suggest that an additional increase in aeration might also help restore AOB activity [40]. From a state of stable operation, a rising pH indicates a decline in AOB activity. In an onset of FA inhibition (indicated by an approximated pH threshold of 8 in this study), reducing the inflow can

quickly recover AOB activity and restore the aspired reactor performance. This approach was successfully employed after prolonged periods of FA inhibition and is also confirmed by Magrí et al.'s (2012) experiments [28]. In cases of severe FA inhibition in this study, stopping the inflow while increasing aeration reduced FA concentration and restored AOB activity in a matter of days (e.g., from 159.3 mg/L NH_3 to 1.5 mg/L NH_3 in six days) but may lead to increased NOB activity as well. In circumstances with high FA concentrations where an elevated aeration rate is employed to facilitate ammonia-oxidation, the stripping of ammonia also must be considered. The stripping might help to reduce FA concentration in the reactor and restore AOB activity more quickly, albeit with considerable NH_3 emissions.

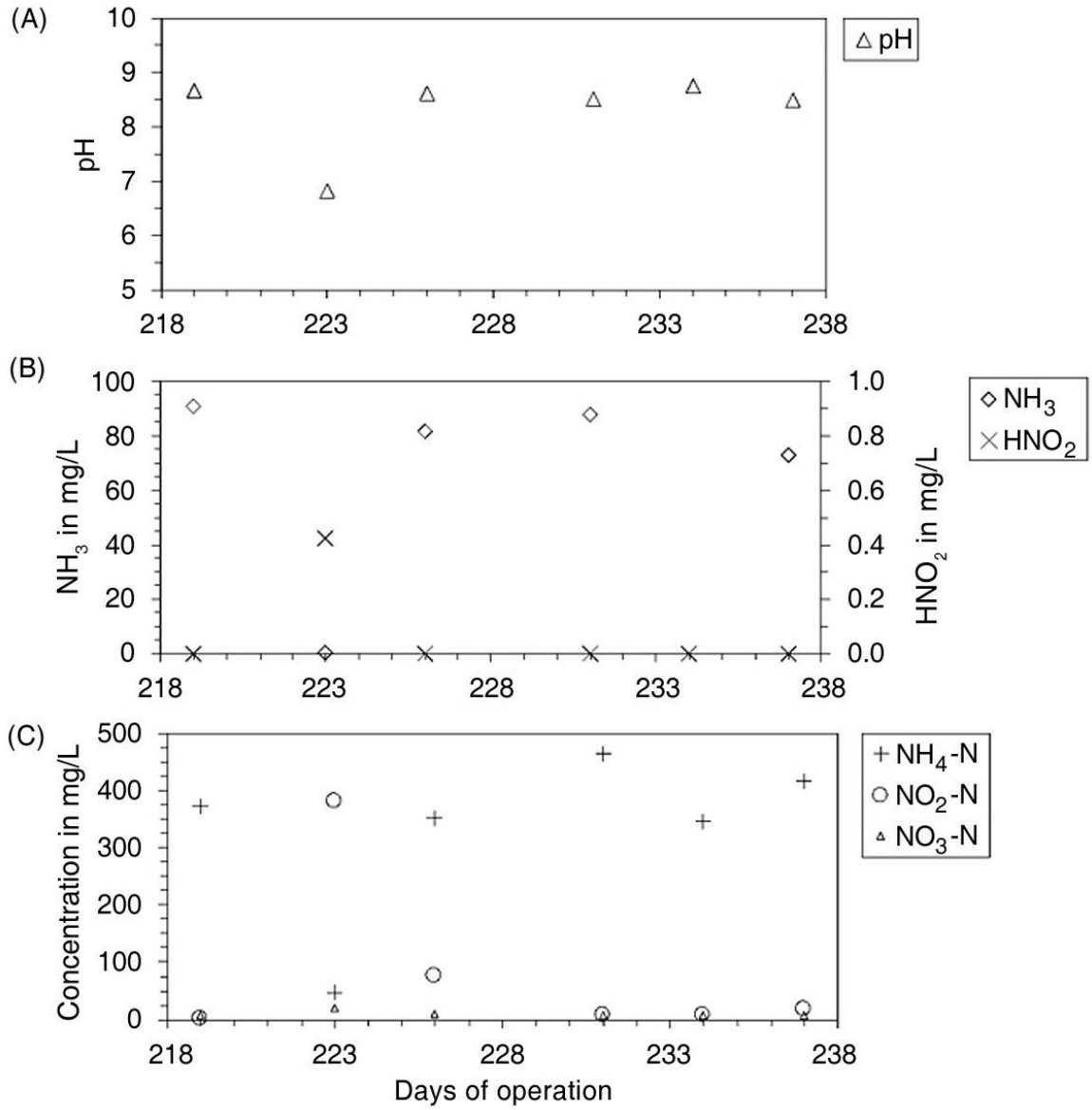


Figure 3. Different parameters measured (pH, NH_4-N; NO_2-N) or calculated (NH_3, HNO_2 according to [22]) in the PN reactor within a time period characterized by FNA and FA inhibition of AOB with **(A)** pH, **(B)** concentrations of NH_3 and HNO_2, and **(C)** the concentrations of NH_4-N and NO_2-N.

With these considerations, a pH-based inflow control is suggested to be evaluated for further operations. Derived from the experience of the pilot-scale operation, a reactor temperature of 25 °C and a pH window between 7 and 8 creates the conditions in which the risk for both FA and FNA inhibition in PN is minimized while creating an effluent with a pH well-suited for the anammox reactor [37]. A suitable pH in the PN reactor should therefore be realized by increasing the inflow at a pH threshold of 7 and decreasing inflow at a pH threshold of 8. In terms of pH safeguarding, over- or undershooting pH by 0.5 (6.5–8.5) seems tolerable [37,41].

Further Insights Derived for the Deammonification of Pig Slurry

The pilot-scale operation confirmed the difficulties associated with the biological treatment of wastewater with a particularly high concentration of NH_4-N (e.g., inhibition of the organisms involved by FA or FNA and the resulting process instabilities). Nevertheless, a stable nitrite accumulation was eventually obtained, and valuable insights into process design and operational design were gained. With these, a preliminary process design aiming at the deammonification of raw liquid pig slurry is derived and proposed for further investigation and validation. The design is based on the assumption that the anammox is not negatively affected by factors other than FA, FNA, temperature, and DO. It is assumed that a stable PN effluent leads to a stable anammox. Problematic substances in pig slurry (e.g., heavy metals and antibiotics) are discussed in the Section “Process design and operation.”

The PN was therefore identified as the critical step in the applied two-stage deammonification system, as process instabilities in the PN reactor may easily impair anammox performance. Operating the PN with an SRT and HRT of 2.2 days at 25 °C and 3 mg/L DO yielded the best results in this study and is therefore the basis for the proposed deammonification system. A temperature of 25 °C can be considered low for both PN and anammox, as the highest ammonium conversion and removal rates are typically achieved between 30 and 39 °C and 30 and 40 °C, respectively, but for PN, a temperature of 25 °C is usually considered sufficient when balancing energy input and nitrogen conversion rates in large-scale operations [37, 41]. For the anammox, there are cases of a successful operation at even lower temperatures, as summarized by Tomaszewski et al (2017); the reduced AnAOB activity may be addressed by increasing VSS in the anammox reactor [42]. Thus, the anammox reactor must be equipped with the means for biomass retention. Additionally, lower temperatures drastically decrease the risk for FA inhibition, thereby stabilizing the process. Because of the suppression of NOB by washout, a DO concentration of 3 mg/L is considered favorable for heightened ammonia conversion rates. This may also help to mitigate the risk of N_2O emissions from PN as low DO concentrations are directly linked to an increased N_2O production. This considerably elevates the risk of N_2O emissions, as concentrations above 2 mg/L DO drastically reduce N_2O formation in

comparison with 0.5 mg/L, as shown in a modeling study investigating, among others, a single-stage as well as a two-stage deammonification plant [43]. While operating the PN reactor at higher DO concentrations supposedly reduces N_2O emissions, the energy demand for the aeration increases. This energy demand, however, may be met by the use of renewable energy, resulting in a net benefit in terms of CO_2e emissions. To decrease the stripping of NH_3 at high DO concentrations, a low-/no-bubble aeration should be implemented.

As mentioned in the Section "PN reactor performance," a pH-based feed control is suggested, as it may help to prevent FA and FNA inhibition events in PN and produces a stable input pH for the anammox. The pH can also be used as an indicator of AOB activity. In cases of lower AOB activity, pH in this study ranged between 7.9 and 8.2. In instances of heightened AOB activity, the pH ranged between 6.7 and 7.1, which fits well with the findings of Magrí et al. (2012), where pH ranged from 6.2 to 8.5 depending on VLR and aeration setting, influencing AOB activity [28].

As shown in the Section "PN reactor performance," the ammonium conversion in the PN in this study exceeded the aspired 57% for an optimal anammox, oxidizing a mean 82% and 66% in operational phases 2 and 3, respectively. Magrí et al. (2012) suggest using the DO concentration to alter the NO_2-N/NH_4-N ratio in the PN's effluent, as it has a direct influence on AOB activity [28]. By prioritizing the mitigation of N_2O formation and higher conversion rates by employing higher DO concentrations, this strategy cannot be employed. Magrí et al. (2012) also suspect the control of the effluent's pH could be used for manipulating the NO_2-N/NH_4-N ratio [28]. The observation of different pH levels at various VLR in this study supports this suspicion, but before suggesting this mechanism as a technical solution, it must be further studied and practically verified. Alternatively, the NO_2-N/NH_4-N ratio for the anammox reactor may be manipulated by bypassing the PN reactor and supplying the anammox reactor with fresh liquid pig slurry to supplement NH_4-N in the case of a nitrite surplus (this could not be tested in this study because of a technical issue). This suggestion, which is to be tested in future experiments, may impair anammox performance, as it augments the COD/NH_4-N ratio in the anammox reactor's feed. Hypothetically, overall nitrogen elimination should not be impaired, as heterotrophic denitrifiers can use the carbon for nitrogen elimination, effectively combining anammox and denitritation (simultaneous anammox and denitrification [SAD]). Depending on the COD of the liquid pig slurry, this approach may shift the treatment from a true deammonification to a PN/SAD system. With an easy-to-operate approach in mind, this shift in the community of organisms involved in nitrogen elimination may be acceptable, as it reduces the need for elaborate process control strategies to enforce a strict anammox.

Additionally, because of the relatively complex handling of ion-selective electrodes and the cost of optical NO_2 probes, the online

measurement of NH_4^+, NO_3^-, or NO_2^- is not part of this operational design, considering the application in a rural environment. The daily use of NH_4^+ and NO_2^- quick tests may be sufficient for process monitoring if pH is used as the main control parameter. This low-instrumentation strategy is set for further validation since it could not be systematically tested in this study's operational timeframe.

With these considerations, the experimental process design of this study was enhanced, proposing a slightly different reactor configuration for further investigation and testing of the deammonification of raw liquid pig slurry. The technical scheme is presented in Figure 4. With this proposal, the foundation has been lain for further investigation and validation. When the proposed concepts are validated, a further step toward upscaling can be taken.

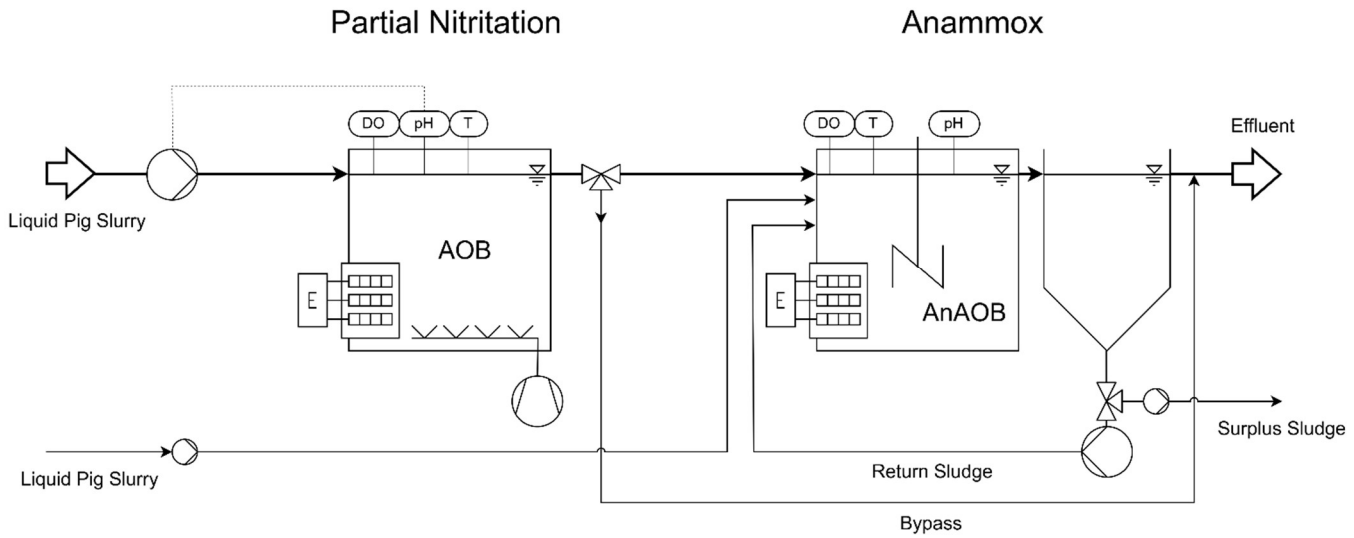


Figure 4. Technical scheme of the proposed reactor configuration for the deammonification of liquid pig slurry.

DISCUSSION

System Performance

Process comparison can be addressed on the level of biomass performance and overall process performance. In terms of biomass performance, the comparison of the PN with current literature on the treatment of pig slurry is difficult, as many studies use different reactor types (e.g., mixed-bed biofilm reactor [MBBR]) or do not elaborate on VSS, making a comparison of sludge performance virtually impossible. Only one study was found to be comparable. Magrí et al. (2012) used untreated swine slurry that was anaerobically stored for two months and achieved a partial nitritation in a batch reactor with suspended biomass using high-performance nitrifying sludge [28]. With 0.51 gN/gVSS/d, this sludge showed a comparable, albeit slightly lower, specific NH_4-N turnover rate than the specific turnover rate registered in phase 3 of this study (0.56–

0.64 gN/gVSS/d). In terms of relative NH_4-N turnover, the PN reactor in this study performed similarly to the 70% achieved by Magrí et al. (2012) [28]. Hwang et al. (2006), who did not indicate reactor VSS, established stable nitritation in a SHARON-type reactor treating anaerobically pretreated swine manure with an NH_4-N turnover of 67% [31]. In an MBBR treating livestock manure digester liquor, Qiao et al. (2010) registered an NH_4-N turnover of 55% [30].

This study's PN reactor performed comparatively low, with a VLR of 0.49 kgN/m^3/d and an NO_2-N production rate of 0.27 kgN/m^3/d at the end of operation. Hwang et al. (2006) operated their SHARON-type reactor stably at a VLR of 0.97 kgN/m^3/d with an NH_4-N turnover of 0.68 kgN/m^3/d, which translates to an NO_2-N production rate of 0.52 kgN/m^3/d, considering the proportion of NO_2-N to NO_3-N in the reactor's effluent [31]. Magrí et al. (2012) achieved an NO_2-N production of 0.91 kN/m^3/d with a VLR of 1.47 kgN/m^3/d [28]. In their MBBR, Qiao et al. (2010) even achieved an NO_2-N production of 2.1 kgN/m^3/d at a VLR of 3.8 kgN/m^3/d [30]. While this study seems to exhibit a comparatively low-performing PN reactor despite its high-performing sludge, it must be taken into account that the reactor temperature was noticeably lower than in this case, with 25 °C compared with 30 °C [30], 32 °C [28] and 35 °C [31]. Because of time restrictions on the operation, the VLR could not be increased more than 0.49 kg_N/m^3/d. It is assumed that a further increase in VLR may lead to an increase in NH_4-N turnover. Nevertheless, the temperature of 25 °C impairs maximum AOB activity.

For the failure of the anammox reactor, the high FA concentrations (up to 250 mg/L NH_3) in the reactor most certainly led to a stark decrease in AnAOB activity, just as Fernández et al. (2012) reported a decrease of AnAOB activity of 50% at 46 mg/L NH_3 and of 80% at 121 mg/L NH_3 [44]. Even the adapted biomass used by Aktan et al. (2012) showed a 10% activity loss at 190 mg/L NH_3 [36]. However, the activity loss imposed by the FA inhibition seems to be reversible [44]. The inhibition by DO, on the other hand, does not [37]. The results of this study support the hypothesis of Joss et al. (2011), who suggest that a drag of DO into the anammox is one of the main reasons for process instability and activity loss in the anammox regarding deammonification [45]. PN effluent characteristics are therefore the main parameters to consider when assessing possible anammox performance.

Process Design and Operation

While the foaming in the PN reactor is a phenomenon to be expected in a large-scale operation, the regular blockage of the pumps is mainly due to the pilot-scale application and the corresponding low diameters of the peristaltic pump's tubes. Nevertheless, the high TSS of pig slurry must be considered even in large-scale operations. Impairments of the process due to heavy metals or antibiotics in the pig slurry could not be quantified in this study. Still, the presence of heavy metals and antibiotics in pig slurry

may pose a serious risk to the process in certain cases [5,46] but cannot generally be implied [6].

The proposed HRT for NOB suppression in the PN in this study (2.2 days) can be considered comparably high, as in similar PN operations such as the SHARON process typically treating reject water from the dewatering of anaerobically treated sewage sludge, the HRT is usually set to a maximum of one day, albeit at temperatures between 30 and 40 °C [33,47,48]. Nevertheless, van Kempen et al. (2001) reported a successful suppression of NOB in the SHARON reactor at the wastewater treatment plant in Utrecht at an HRT of 2.5 days, similar to this study, but also at higher temperatures [48]. The successful NOB suppression at 2.2 days and 25 °C in this study is especially significant in the treatment of wastewaters that do not have the same temperature level as wastewaters resulting from an anaerobic pretreatment.

Several studies focus on the use of a two-stage deammonification system for the treatment of pig wastewaters [28,30,31,49], though newer studies tend to focus on a single-stage approach [24–26,50]. Similar to this study, Prá et al. (2021) used a low-cost and easy-to-operate approach to develop their one-stage reactor configuration, NITRAMMOX®, addressing common drawbacks [24]. However, one of the estimated advantages of the two-stage reactor configuration proposed in this study is the possibility to adjust reactor configuration and operation for AOB and AnAOB separately to accommodate their specific needs (e.g., presence of DO, intense versus slow mixing), which is difficult to achieve in a single-stage setup, as argued by Prá et al. (2021) [24]. Its versatility regarding different treatment options is also deemed favorable, as it can strengthen the robustness of nitrogen elimination.

Although its primary use case is the deammonification comprising PN and anammox for the treatment of pig slurry with a low ratio of CODb/NH_4-N, it is nevertheless possible to operate the second reactor for simultaneous anammox and denitrification, a pathway already explored for the treatment of pig wastewaters containing relevant amounts of COD [51–55]. By separating the PN and the anammox in two reactors, each reactor is mainly characterized by its mode of operation (aerated, nonaerated) and therefore can accommodate several different biological processes. The proposed reactor design thus acknowledges the limits of the deammonification process while simultaneously highlighting the versatility of biological nitrogen elimination.

Nonetheless, the results of this study only lay the foundation for further research on the deammonification of raw liquid pig slurry, as some of the proposed concepts must be systematically tried and validified to ultimately be tested on a large scale in a rural implementation.

CONCLUSIONS

As shown in this pilot-scale application of the deammonification for the treatment of raw liquid pig slurry, the overall process is prone to

instabilities due to the high NH_4-N content of the slurry. The PN was identified as the process's critical step. Nevertheless, a stable PN was obtained by employing an HRT and SRT of 2.2 days at 25 °C. A pH-based feed control is proposed to mitigate the risk of FA and/or FNA inhibition of AnAOB, as the pH was identified as the main parameter to consider for process control. Not only does it allow for gauging FA and FNA concentrations, but it is also closely linked to AOB activity, allowing for a qualitative monitoring of AOB activity. The pH window most suitable for PN in this study was identified as 7–8.

As proposed, the separation of PN and anammox in the two-stage system may allow the operation of each process independently, reducing the need for complex process control. It can also allow for process flexibility and ensures nitrogen elimination, as the anammox reactor may be operated as a SAD reactor if the CODb/NH_4-N ratio shifts toward CODb. This is particularly useful in the rural use case, where broad process technological knowledge cannot be implied. The results of the operation indicate that the deammonification of raw liquid pig slurry may be possible on a large scale, though the proposed operational design and control strategies (e.g., pH-based feed control, bypassing the PN to alter NO_2-N/NH_4-N in the anammox's influent) must be further studied and validified before applying the system on a larger scale.

SUPPLEMENTARY MATERIALS

The following supplementary materials are available online, Table S1: Measurements of the pig slurry monitoring, Table S2: Measurements in the PN reactor over the course of operation, Table S3: PN reactor performance over the course of the operation.

DATA AVAILABILITY

All data generated from the study are available in the manuscript or supplementary files.

AUTHOR CONTRIBUTIONS

Conceptualization, JR and MB; methodology, JR and MB; investigation, JR; validation, MB; writing—original draft preparation and editing, JR; writing—review and editing, MB; visualization, JR. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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