Full-scale simultaneous partial nitrification, anammox, and denitrification process for treating swine wastewater

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ABSTRACT

A full-scale swine-wastewater activated sludge treatment plant that contains naturally enriched anammox biofilms was investigated for 2 years. Red biofilm in this system included Planctomycetes at a maximum of 62.5% of the total bacteria diversity, including Candidatus Jettenia and Candidatus Brocadia. The plant was operated with an influent containing $1,104 \pm 513$ mg/L biochemical oxygen demand (BOD) and 629 ± 198 mg/L total nitrogen (TN) (BOD/N of 1.78 ± 0.58) at a volumetric BOD loading rate of 0.32 ± 0.12 kg/m^3/d. Notwithstanding drastically varying influent concentrations, BOD removal efficiency was stable at $95 \pm 4\%$. However, TN removal fluctuated at $75 \pm 14\%$. Dissolved oxygen (DO) concentrations in the aeration tank were 0.06–2.0 mg/L. DO concentration greatly affected nitrogen removal, e.g. when DO was lower than 0.3 mg/L, total inorganic nitrogen removal was $61 \pm 14\%$ ($\leq$20 °C), $78 \pm 16\%$ (20–30 °C), and $75 \pm 12\%$ ($\geq$30 °C), whereas at higher DO concentrations, removal rates were $47 \pm 13\%$, $55 \pm 16\%$, and 68%, respectively. As BOD concentration in the influent was limited compared to nitrogen concentration, nitrogen was likely removed by simultaneous nitrification, anammox, and denitrification (SNAD) under microaerobic conditions. Maintaining low DO concentrations would therefore be a simple method to improve nitrogen removal during SNAD processes for swine-wastewater treatment with fluctuating influent.

Key words | activated sludge, anammox, nitrogen removal, partial nitrification, SNAD, swine wastewater

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INTRODUCTION

Livestock waste is a major cause of nitrogen pollution in public water bodies globally (Steinfeld et al. 2006). In Japan, livestock waste generates 700,000 t of nitrogen per year, exceeding that generated by chemical fertilisers (400,000 t per year). Livestock waste therefore contributes significantly to nitrogen contamination in rivers, lakes, and groundwater. Japanese law stipulates the upper limit of nitrogen concentration in purified wastewater discharged to public water bodies from livestock farms. The most general discharge standard for livestock farms in July 2019 was 500 mg N/L (NO_2^--N + NO_3^--N + $0.4 \times NH_4^+$-N). This value has been reduced in line with the discharge standard
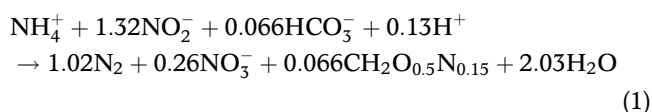
currently applied to other activities (100 mg/L). Therefore, improved nitrogen removal at wastewater treatment facilities on livestock farms is essential.

Swine farms commonly have wastewater treatment facilities because swine, unlike cattle and poultry, excrete more urine than faeces, which generates large amounts of wastewater. Wastewater treatment with activated sludge is an important method for treating swine wastewater, and the effluent is discharged to public water bodies. Pre-treated swine wastewater arriving as the influent for activated sludge treatment contains $3,500 \pm 2,100$ mg/L biochemical oxygen demand (BOD) and $1,300 \pm 650$ mg/L total nitrogen (TN) (Waki et al. 2010). Since a significant part of the treatment process aims to remove BOD but not nitrogen, treated effluents can contain TN concentrations as high as 430 ± 540 mg/L, even with BOD concentrations as low as 59 ± 83 mg BOD/L.

Conventionally, nitrogen is removed biologically during wastewater treatment. The general nitrogen removal process involves nitrification followed by denitrification. The nitrification process oxidises ammonium to nitrite or nitrate by autotrophic ammonium-oxidising bacteria or nitrite-oxidising bacteria, after which nitrite or nitrate are converted to nitrogen gas by heterotrophic denitrifiers during denitrification. A relatively new process of nitrogen removal, namely anammox, was discovered in the 1990s. Anammox is an autotrophic nitrogen removal process that produces nitrogen gas from nitrite and ammonium without the need for organic carbon sources (Equation (1)) (Strous *et al.* 1998). It has been proposed as a promising way of saving the aeration-energy cost of nitrification and the chemical cost of denitrification.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+$$
$$\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$
$$(1)$$

More than 100 full-scale anammox plants are currently in operation globally (Lackner *et al.* 2014). These plants are used to treat various nitrogen-rich wastewaters, such as reject water at municipal wastewater treatment plants, landfill leachates, and wastewater from industrial plants, including food processing and semiconductor production. Side-stream treatment of municipal wastewater was carried out by 75% of the full-scale anammox plants.

The application of anammox to swine wastewater or its anaerobic digestor liquor has been tested with laboratory-scale reactors in several studies. Generally, anammox treatment can be divided into two systems: one is a two-stage configuration combining a nitrite-production (partial nitritation) reactor followed by an anammox reactor, and the second is a single-stage configuration including both partial nitrification and anammox. Two-stage anammox systems for swine wastewater or its anaerobic digestor liquor have been studied using up-flow anaerobic sludge bed reactors, up-flow fixed bed reactors, or continuously stirred granular anammox reactors (Ahn *et al.* 2004; Hwang *et al.* 2006; Yamamoto *et al.* 2008; Qiao *et al.* 2010). Single-stage anammox systems have been studied using sequencing batch or sequencing batch biofilm reactors (Zhang *et al.* 2012b; Daverey *et al.* 2013). These experiments in anammox treatment for swine wastewater were performed with laboratory-scale reactors at controlled temperatures of 30–35 °C, with only one exception (Daverey *et al.* 2013). Daverey *et al.* (2013) operated a 5 L sequencing batch reactor to treat anaerobic digestate of swine wastewater containing

519 mgN/L, 288 mg/L, and 387 mg/L ammonium, BOD, and chemical oxygen demand (COD), respectively, at ambient temperature. The TN removal rate was 0.123 kg/m³/d in a steady state. Nitrogen removal was carried out by simultaneous nitrification, anammox, and denitrification (SNAD) (Chen *et al.* 2009). Since actual swine wastewater contains high concentrations of both BOD and nitrogen, SNAD is a reasonable choice for its treatment. However, full-scale SNAD plant operation on a working farm under ambient temperature conditions has not been studied to date.

Anammox was unintentionally enriched in biofilm at full-scale swine-wastewater activated sludge plants on three farms in Japan (Suto *et al.* 2017). The concentration of anammox in the biofilm was almost as high as that of anammox in artificial wastewater, with a maximum activity of 295 μmol N_2/g ignition loss (IL)/h and maximum anammox DNA copy numbers of 1.35×10^{12} copies/g IL. The influent at these facilities had high concentrations of organic carbon and nitrogen, with BOD and ammonium concentrations of 400–4,645 mg/L and 465–4,931 mg/L, respectively, and a BOD/N ratio of 0.23–2.76. This suggests that the conditions at these facilities are ideal for nitrogen removal by SNAD, i.e. full-scale SNAD plants on swine farms.

The purpose of this study was to clarify the key factors in nitrogen removal performance at a full-scale SNAD swine-wastewater treatment plant. A full-scale SNAD plant on a swine farm was investigated for 2 years, and the effects of environmental and operational conditions on nitrogen removal were analysed. The long-term operation of such a wastewater treatment plant is reported here for the first time.

MATERIALS AND METHODS

Description of full-scale swine-wastewater treatment plant

The activated sludge treatment facility in this study was constructed in 1989 to treat swine wastewater at the Swine and Poultry Research Center, Shizuoka Prefectural Research Institute of Animal Industry, Japan. It currently treats wastewater produced by about 900 head of fattening swine. A schematic diagram of the treatment plant is shown in Supplementary Figure 1. The main aeration tank (first aeration tank) was operated with a hydraulic retention time of 80 hours and mixed liquor suspended solids of 10,900 ± 2,000 mg/L (average ± s.d.). Influents were continuously fed into the tanks, which were continuously aerated. Most nitrogen removal occurred in the first aeration tank, and not much removal

took place in the second. Therefore, analyses focused on the first aeration tank. Wastewater characteristics were measured for 2 years, from April 2016 to March 2018.

Analytical methods

BOD, suspended solids (SS), and IL were determined following the Japan Sewage Works Association (2012). BOD was determined after an incubation period of 5 days, and the effluent was measured as carbonaceous BOD. Because the number of BOD data measurements was small, if the dates on which BOD and other data were measured were not the same, a gap within 5 days was taken as being the same day for analysis. The volatile suspended solids (VSS) were determined for liquid samples after washing the precipitates from the wastewater twice by centrifuging at 1,500 g, decanting and resuspending in deionised water, and then drying the precipitates at 105 °C and 600 °C. IL was determined for biofilm samples after drying the solids at 105 °C and 600 °C. COD and TN were determined via the closed reflux colorimetric and persulfate digestion methods, respectively, using a DR 2400 spectrophotometer (Hach Co., Loveland, CO, USA).

Total ammonium was determined using ion chromatography (ICS-1100 Dionex, California, USA), as were total nitrite and nitrate (IC20, Dionex). The pH and electric conductivity were measured using a glass electrode (HI98310, Hanna Instruments, Rhode Island, USA). Dissolved oxygen (DO) was measured using a luminescent DO probe (HQ40d and LDO 10105, Hach Co.). Free ammonia (FA) and free nitric acid (FNA) concentrations were calculated through equilibrium as described by Anthonisen *et al.* (Anthonisen *et al.* 1976).

Anammox activity measurement was conducted using floating granules (collected in March 2014 and January 2019) by the tracer method (Yoshinaga *et al.* 2011). Samples were anaerobically incubated in triplicate with ^{15}N-labelled $NaNO_2$ and non-labelled NH_4Cl at 25 °C. We estimated anammox activity from $^{29}N_2$ production during incubation. Net N_2 production by anammox was calculated from gross $^{29}N_2$ concentration by deducting (1) contamination from $^{29}N_2$ in ambient air and (2) $^{29}N_2$ production via denitrification using $^{15}NO_2^-$ and $^{14}NO_2^-$ contamination in the reagent of $^{15}NO_2^-$.

Real-time quantitative PCR analysis of anammox bacteria

Flocs in mixed liquor sampled from the aeration tank in April 2016 were fractionated using sieves with mesh sizes of 2, 1, and 0.5 mm. Total DNA was extracted twice from each floc size using a FastDNA SPIN kit for soil (MP Bio, Tokyo, Japan), bulked, and purified with a QIAEX II gel extraction kit (Qiagen, Hilden, Germany). The abundance of anammox bacterial 16S rRNA genes was determined in triplicate by quantitative polymerase chain reaction (qPCR) amplification using SsoAdvanced SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, CA, USA); qPCR was performed in 96-well optical plates in a real-time PCR detection system (MyiQ2, Bio-Rad) with the anammox-specific primer sets S-*-Amx-0368-a-A-18 and AMX820, according to the method of Suto *et al.* (2017).

Pyrosequencing analysis

Pyrosequencing of 16S rRNA genes was conducted using floating granules (collected in March 2014) and sieved flocs (April 2016), following real-time qPCR analysis, which amplified the forward primer 563F and four mixed reverse primers, R1–4 (Zhang *et al.* 2012a). Each amplification reaction mixture (10 μL) contained 1 ng total DNA, 0.5 U Ex *Taq* Hot Start Version (TaKaRa, Shiga, Japan), 1 μL 10 × Ex buffer, 0.8 mM deoxynucleotide triphosphate, and 0.5 μM of each primer. The following steps were used for PCR amplification: the mixture was kept at 95 °C for 5 min, denatured for 30 cycles at 95 °C for 30 seconds, annealed at 55.8 °C for 30 seconds, and extended at 72 °C for 1 min. This was followed by a final extension at 72 °C for 10 min.
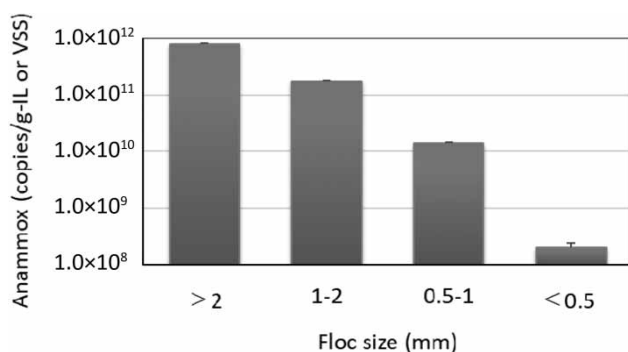
PCR products were purified with an AMPure XP (Beckman Coulter, CA, USA). The purified amplicons were paired-end sequenced on an Illumina MiSeq platform (Illumina, CA, USA) according to the standard protocols. Reads thus obtained were processed using Sickle (https://github.com/najoshi/sickle) to trim bases with quality values of less than 20. Reads of less than 41 bases after quality trimming were discarded with the counterpart paired-end reads. The quality-filtered paired-end reads were deposited in the DDBJ database under BioProject accession number PRJDB8118 and BioSample accession numbers SAMD00165693-SAMD00165696 and SAMD00165699 and were merged via FLASH (Magoč & Salzberg 2011) with the default parameters. Chimeric amplicons were filtered via the UCHIME algorithm (Edgar *et al.* 2011) using the 97% dataset of the Greengenes database. Construction of operational taxonomic units and taxonomical estimation were conducted using a workflow script (pick_de_nobo_otus.py) in QIIME 1 (Caporaso *et al.* 2010) with the default parameters and without reference sequences.
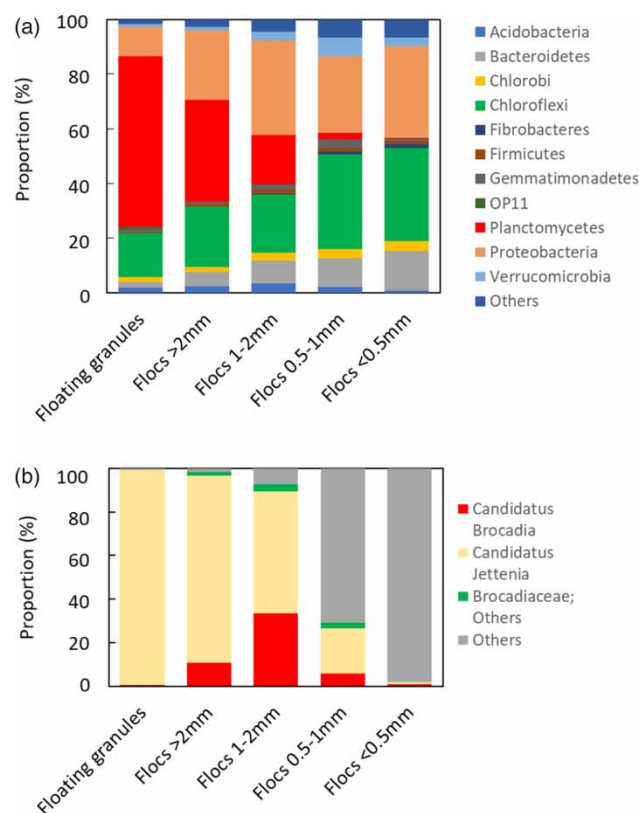
RESULTS AND DISCUSSION

Anammox characteristics

A red biofilm has been observed in the swine-wastewater treatment facility since at least March 2012 (Supplementary Figure 2). During the study period, the red biofilm was present on the wall of the first aeration tank and in later stages. High concentrations of anammox activity have been measured in the red biofilms (Suto *et al.* 2017). A maximum anammox activity of 115 μmol N_2/g IL/h was measured, and anammox DNA copy numbers in the biofilm were 1.2×10^8–2.0×10^{10} copies/g IL (from surveys in October 2014 and February 2015). Red granules floating in the sedimentation tanks were often observed during winter (Supplementary Figure 2(d) and 2(e)). These granules showed anammox activity as high as 339 ± 73 μmol N_2/g IL/h (March 2014) and 439 ± 74 μmol N_2/g IL/h (January 2019). Additionally, small flocs were found in the suspended solids in the first aeration tank. When flocs were fractionated by size into >2, 1–2, 0.5–1, and <0.5 mm groups, the larger flocs showed higher anammox concentrations with a maximum of 7.9×10^{11} copies/g IL (April 2016, Figure 1). The highest anammox DNA copy number was about one-tenth of that in enriched anammox sludge, namely 6.23×10^{12} copies/g VSS (Bae *et al.* 2010).

The pyrosequencing analysis showed that the floating red granules contained Planctomycetes in 62.5% of total reads (Figure 2(a)). Planctomycetes content in fractionated flocs varied from 0.3 to 37.2%, with larger flocs having a higher frequency. In the floating red granules and flocs of >2 or 1–2 mm, 56.1–99.0% of Planctomycetes were identified as *Candidatus* Jettenia and 10.9–33.3% as *Candidatus* Brocadia (Figure 2(b)). In contrast, in the smaller flocs, bacteria unrelated to anammox accounted for most of


Figure 2 │ Relative abundance of (a) predominant bacterial phyla and (b) Planctomycetes genera. 'Others' comprises phyla with <1% abundance or genera other than anammox bacteria. 'Brocadiaceae; Others' comprises other genera in Brocadiaceae.

the Planctomycetes. *Candidatus* Jettenia or *Candidatus* Brocadia are the typical anammox bacteria in wastewater treatment systems (Pereira *et al.* 2017). The red biofilm on the wall, floating flocs, and granules in this study contained a high anammox concentration, and they were present continuously during the study period.

Profiles of water temperature, DO concentration, and pH

The profiles of water temperature, DO concentration, and pH in the first aeration tank are shown in Figure 3(a) and 3(b). The water temperature rose and fell according to seasonal changes in ambient temperature. During the study period, the average temperature was 22.8 ± 6.7 °C (average $\pm$ s.d.), with a maximum of 34.2 °C and minimum of 10.1 °C. As mentioned above, red granules floating in the sedimentation tanks were often observed during the winter (Figure 3(a)). DO concentration in the aeration tank was unintentionally maintained at as low as 0.31 ± 0.39 mg/L

Figure 1 │ Anammox 16S rRNA gene copy numbers in sieved flocs (>2 mm, 1–2 mm, 0.5–1 mm, <0.5 mm).
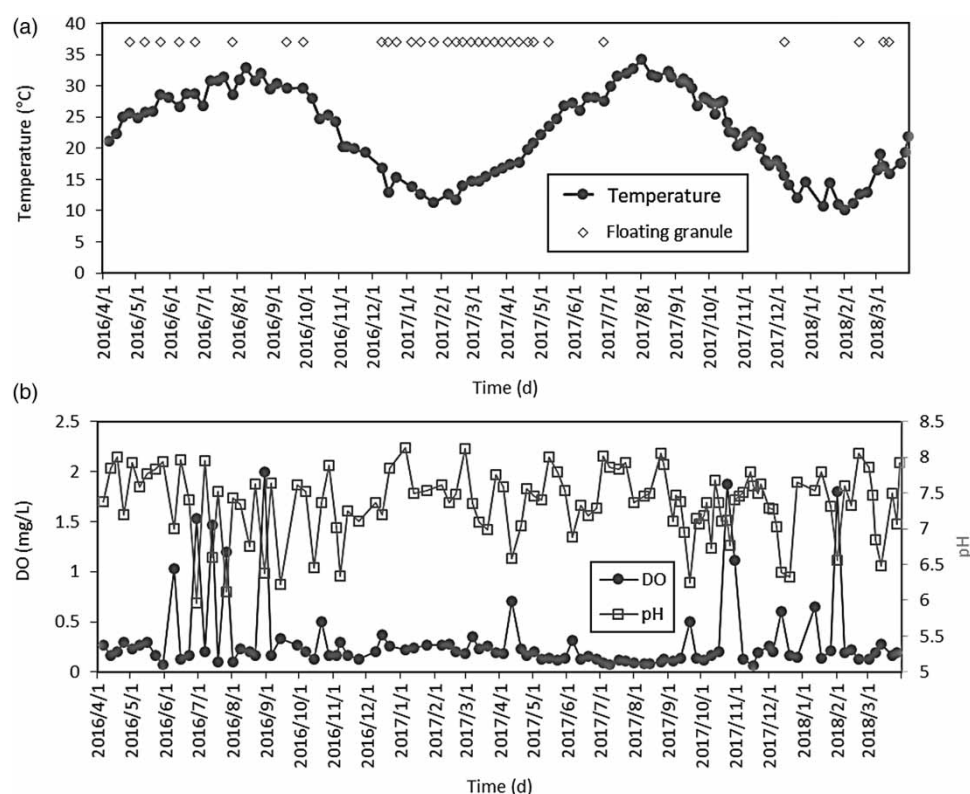
Figure 3 │ Conditions recorded in full-scale simultaneous nitrification, anammox, and denitrification processing tanks. (a) Changes in water temperature in the first aeration tank and floating granules in the first sedimentation tank; (b) dissolved oxygen (DO) concentration and pH in the first aeration tank.

(Figure 3(b)). However, occasional sudden increases to a maximum concentration of 2.0 mg/L were observed; a minimum concentration of 0.06 mg/L was recorded.

The average pH in the aeration tank was neutral, at 7.35 ± 0.49 (Table 1). However, it varied, with minimum and maximum recorded values of 6.0 and 8.1, respectively. The pH value decreased when the DO concentration increased (Figure 3(b)). The high DO concentration likely enhanced ammonium oxidation, producing hydrogen ions, which accounted for the observed pH decrease. The increase in DO concentration was likely caused by a low influent concentration due to factors such as a change in the number of feeding swine or dilution by rain water.

BOD removal

BOD concentration in the influent was 1,104 ± 513 mg/L (Table 1). The influent flow rate was 14 ± 3 m^3/d, and the volumetric BOD loading rate in the first aeration tank was 0.32 ± 0.12 kg/m^3/d, which was within the average range of loading rates for swine-wastewater treatment but still relatively low. Influent BOD concentration varied from 555 to

2,500 mg/L due to changes in the number of feeding swine and varying weather conditions (Figure 4). However, in the final effluent, i.e. the effluent from the second sedimentation tank, a BOD concentration of 44 ± 27 mg/L was recorded, representing a BOD removal efficiency of 95 ± 4%. Despite the fluctuating BOD concentration in the influent, BOD removal was high and stable in this treatment system. Since heterotrophs have a high growth rate and high affinity for DO, it was estimated that the BOD removal rate would be high even in conventional activated sludge processing of swine wastewater with a low DO concentration (Waki *et al.* 2018).

Nitrogen removal

TN and total inorganic nitrogen (TIN) concentrations in the influent were 629 ± 198 and 457 ± 128 mg/L, respectively (Table 1, Figure 5(a)). The volumetric TIN loading rate in the first aeration tank was 0.13 ± 0.03 kg/m^3/d (Figure 5(b)) and the TN concentration fluctuated in a similar way to the BOD concentrations, with a minimum of 290 mg/L and a maximum of 1,070 mg/L. TIN decreased to 146 ± 92 mg/L

**Table 1** │ Wastewater characteristics of the influent and the effluent

	Unit	Influent		Effluent	
		Average ± s.d.	Measurements	Average ± s.d.	Measurements
pH	–	8.36 ± 0.34	111	7.35 ± 0.49	111
BOD	mg/L	1,104 ± 513	30	44 ± 27[a]	23
COD	mg/L	3,984 ± 1,484	19	409 ± 146[a]	19
TN	mg/L	629 ± 198	25[b]	154 ± 92[a]	25[b]
NH_4^+-N	mg/L	457 ± 128	111	91 ± 66	111
NO_2^--N	mg/L	0.01 ± 0.08	111	42 ± 56	111
NO_3^--N	mg/L	0 ± 0.04	111	13 ± 29	111
TIN	mg/L	457 ± 128	111	146 ± 92	111
BOD/TN	mg/L	1.78 ± 0.58	25	0.37 ± 0.24	18
SS	mg/L	1,743 ± 712	23	35 ± 14[a]	5[c]

BOD, biochemical oxygen demand; COD, chemical oxygen demand; TN, total nitrogen; TIN, total inorganic nitrogen; SS, suspended solids.
[a]Data sampled from second sedimentation tank.
[b]Data after sampling period in October 2016.
[c]Data after sampling period in March 2018.
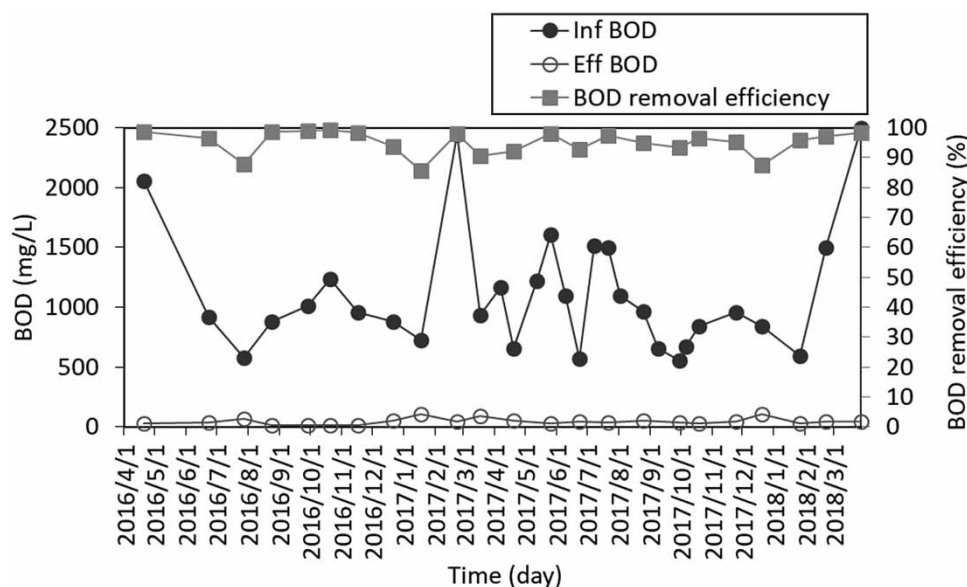
Figure 4 │ Recorded biochemical oxygen demand (BOD) concentrations in the influent (Inf) and the effluent (Eff) and BOD removal efficiency.

in the first aeration tank, and TN decreased to 154 ± 92 mg/L in the second sedimentation tank, with TIN and TN removals of 68 ± 17 and 75 ± 14%, respectively, and a TIN removal rate of 0.09 ± 0.03 kgN/m³/d in the first aeration tank. TIN removal was unsteady, unlike BOD removal, and occurred mostly in the first aeration tank; TIN removal was similar in the first and later aeration tanks, namely 70 ± 16% in the first sedimentation tank, 70 ± 17% in the second aeration tank, and 72 ± 16% in the second sedimentation tank. Inorganic nitrogen compounds in the first aeration tank were ammonium and nitrite in most cases (Figure 5(a)). Concomitant with decreased and increased pH, FNA and FN had concentrations of 0–0.76 mg/L and 0.008–26.7 mg/L, respectively (Figure 5(c)).

The concentration of DO and the ratio of BOD/TN in the influent are important factors for nitrogen
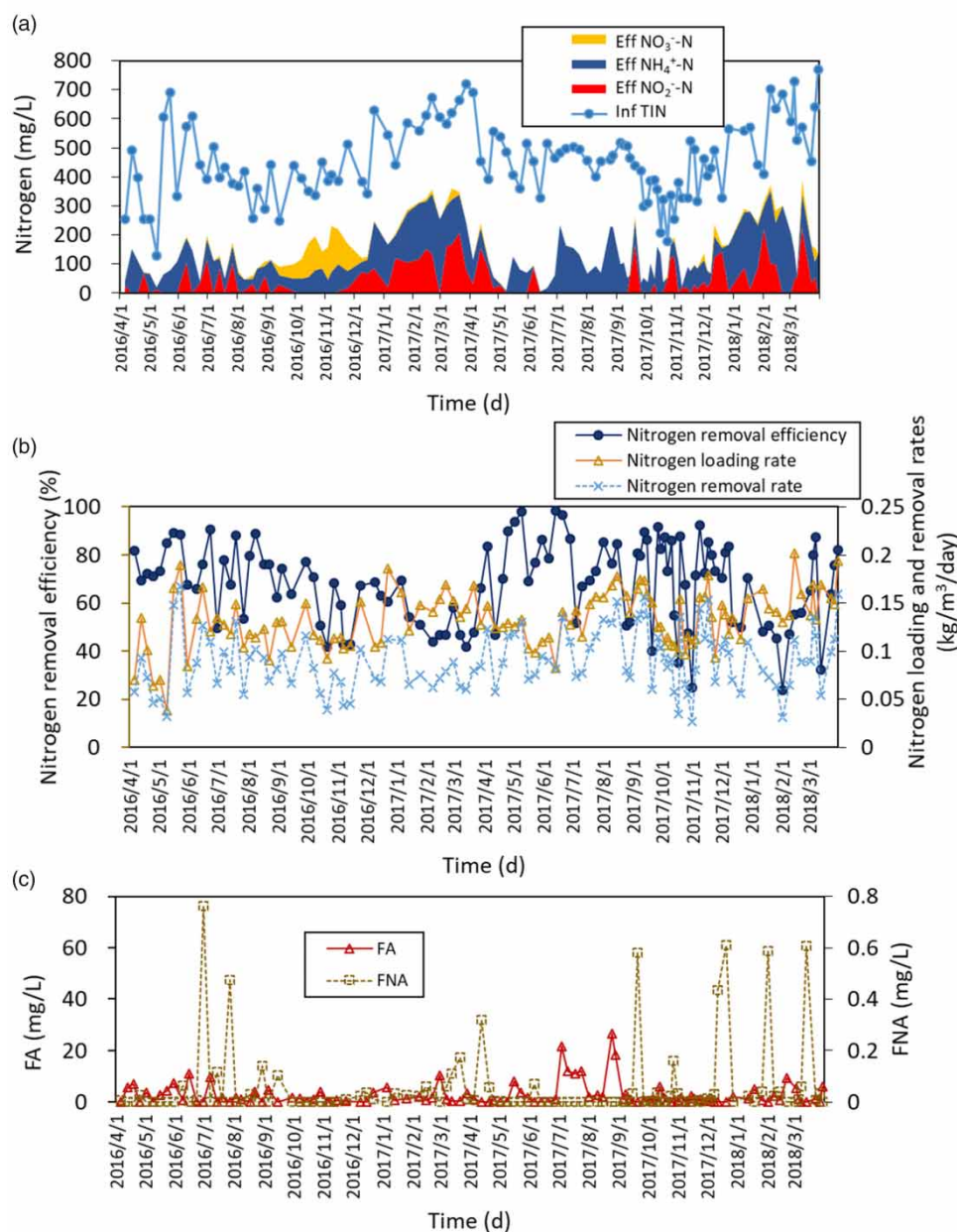
Figure 5 │ Nitrogen measurements. (a) Total inorganic nitrogen (TIN) concentration in the influent (Inf) and inorganic nitrogen concentrations in the first aeration tank (in the effluent (Eff)); (b) TIN removal efficiency, loading rate, and removal rate; (c) free ammonia (FA) and free nitric acid (FNA) concentrations in the first aeration tank.

removal. Figure 6(a) shows the relationship between DO concentration in the aeration tank and inorganic nitrogen removal under each temperature condition (≤20, 20–30, and ≥30 °C), revealing that high nitrogen removal occurred at DO concentrations of 0.3 mg/L or less under every temperature condition. The average nitrogen removal at DO concentrations of less than 0.3 mg/L was 61 ± 14% (≤20 °C), 78 ± 16% (20–30 °C), and 75 ± 12% (≥30 °C), which was higher than at concentrations

of more than 0.3 mg/L, where an average of 47 ± 13% (≤20 °C), 55 ± 16% (20–30 °C), and 68% (≥30 °C) of nitrogen was removed. The average BOD/TN ratio in the influent was 1.78 ± 0.58 (Table 1), but it fluctuated from 1.0 to 3.3. Figure 6(b) shows the relationship between the BOD/TN ratio in the influent and inorganic nitrogen removal under each temperature condition (≤20, 20–30, and ≥30 °C); however, no effect of BOD/TN on nitrogen removal was seen.
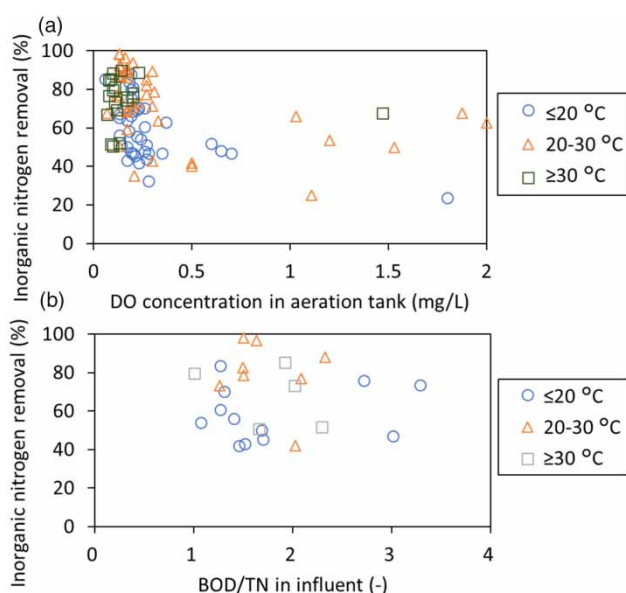
Figure 6 │ (a) Effect of DO concentration on TIN removal in the first aeration tank; (b) relationship between BOD/TN in the influent and TIN removal in the first aeration tank. Three different temperature conditions are shown: ≤20 °C, 20–30 °C, and ≥30 °C.

Accumulation of nitrite (Figure 5(a)) and low DO concentration in the first aeration tank (Figure 6(a)) suggests that simultaneous nitrification and denitrification via nitrite (short-cut nitrification and denitrification) occurred in the first aeration tank, under low DO concentrations (Waki *et al.* 2018). The fact that ammonium was also present in the first aeration tank suggests that the anammox substrates were present in significant concentrations in the first aeration tank. As mentioned already, anammox took place in the facility studied. It is thought that a BOD/TN of more than 3 is required for enough nitrogen removal by conventional nitrification and denitrification, even in sequencing batch reactor processes (Osada *et al.* 1991; Kim *et al.* 1999). Since short-cut nitrification and denitrification can theoretically reduce 40% of organic carbon sources (Sun *et al.* 2010), it is estimated that a BOD/TN of more than 1.8 is required for enough nitrogen removal by short-cut nitrification and denitrification. In Figure 6(b), it could be said that a TIN removal of more than 95% with a BOD/TN of 1.5 and 1.6 was caused not only by short-cut nitrification and denitrification but also by anammox, namely, the SNAD process.

In our study, a higher nitrogen removal was observed at lower DO concentrations in the aeration tank (Figure 6(a)). Therefore, for optimal SNAD operating conditions in swine-wastewater treatment, the DO concentration should be maintained at ≤0.3 mg/L. Furthermore, in a previous laboratory-scale SNAD study on swine-waste anaerobic

digestion liquor, DO concentration was set below 0.5 mg/L (Daverey *et al.* 2013). These processes operated with influents with lower BOD/N ratios than in our study (BOD versus ammonium concentrations of 288 ± 229 versus 519 ± 134 mg/L for Daverey *et al.* (2013) compared to $1,104 \pm 513$ versus 457 ± 128 mg/L for this study). However, the DO concentration in our study was almost the same as the concentrations indicated by the above-mentioned SNAD study, and its importance was reconfirmed.

FA and FNA concentrations were concomitant with high and low pH values, respectively. Various inhibiting FA and FNA threshold concentrations have been proposed, e.g. FA concentrations of 9.7–146, 0.097–0.996, and 57–187 mg/L and FNA concentrations of 0.671–9.4, 0.201–2.786, and 0.006–0.213 mg/L for ammonium-oxidising bacteria, nitrite-oxidising bacteria, and anammox, respectively (Van Hulle *et al.* 2010; Jin *et al.* 2012). In our study, increases in FA and FNA concentrations were also observed (Figure 5(c)). However, during SNAD, low pH values were caused by increased DO concentration, and the negative effect on the TIN removal rate was caused by a combination of conditions, including high DO concentration. As previously stated, maintaining DO concentrations below 0.3 mg/L would avoid the negative effects of low pH and high FNA concentration.

In this study, despite a relatively low average influent BOD/N ratio of 1.78, inorganic nitrogen removal was higher than 80% on 30% of sampling days, with a maximum of 98.5%. These results indicated that successful nitrogen removal could be achieved by SNAD. In the facility studied, anammox biofilm was unintentionally maintained. The presence of naturally occurring anammox suggests that SNAD is suitable for swine wastewater treatment. In the full-scale SNAD process for swine-wastewater treatment, maintaining the DO concentration below 0.3 mg/L is a simple strategy to maintain nitrogen removal. Additionally, in order to obtain a more stable removal ability even under such conditions, it would be necessary to consider not only strict controls for preventing DO from being too low, but also measures against low temperatures in future research.

CONCLUSIONS

In a full-scale activated sludge treatment reactor for swine wastewater with an influent BOD concentration of $1,104 \pm 513$ mg/L, TN concentration of 629 ± 198 mg/L, and BOD/N ratio of 1.78 ± 0.58, an anammox biofilm was consistently present on the walls of the aeration tank over

the 2 year study period. BOD was consistently removed, whereas nitrogen removal fluctuated, with removal efficiencies of $95 \pm 4\%$ and $75 \pm 14\%$, respectively. Lower DO concentrations in the aeration tank increased the TIN removal rate, with a DO concentration of less than 0.3 mg/L being an indication of high nitrogen removal. In the full-scale plant studied, environmental conditions fluctuated, but SNAD for swine-wastewater treatment could be consistently operated by considering DO concentration.

DECLARATIONS OF INTEREST

None

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SUPPLEMENTARY MATERIAL

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