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Enhanced retention of deammonification microorganisms for the treatment of psychrophilic anaerobic digestate

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Abstract

This study focused on the treatment of psychrophilic anaerobic digestate from pig slurry digestion through a single-stage Sequencing Batch Reactor (SBR) accomplishing the autotrophic nitrogen removal (ANR). In period 1, the combination of the high sludge retention time (>50 days) and the presence of significant concentrations of biodegradable organic carbon favoured the uncontrolled growth of the denitrifying bacteria (HDB) and the nitrite oxidizing bacteria (NOB), affecting negatively the deammonification (DAM) activity from 14-15 mgN/gMLVSS·h to only 2.2 mgN/gMLVSS·h. In Period 2, the sieving of the suspended biomass at 125 µm was evaluated to enhance the retention time of DAM microorganisms in granular sludge into the SBR. This strategy allowed the recovery of 60%

of the initial specific deammonification activity (sDAA) and to promote the wash-out of the NOB, obtaining a nitrogen removal efficiency of 81%. This method was never applied to increase the robustness and reliability of ANR during the treatment of livestock streams, so it could be considered after optimization as a potential option to increase the sustainability of smaller and local farms.

Keywords

Anammox; anaerobically digested pig slurry; organic content; sequencing batch reactor; selection

List of abbreviations

AD	Anaerobic Digestion	PN/DAM	Partial Nitrification/Deammonification
COD	Chemical Oxygen Demand	PO ₄ -P	Phosphate
sCOD	Soluble COD	sDAA	Specific Deammonification Activity
rbCOD	Rapidly Biodegradable COD	sAUR	Specific Ammonia Uptake Rate
bCOD	Biodegradable COD	SBR	Sequencing Batch Reactor
BCFC	biogas collection floating covers	sNOR	Specific Nitrite Oxydation Rate
FA	Free Ammonia	sNUR	Specific Nitrogen Uptake Rate
HDB	Heterotrophic Denitrifying Bacteria	SRT	Solid Retention Time
HRT	Hydraulic Retention Time	TKN	Total Kjeldahl Nitrogen
MLSS	Mixed Liquid Solids Suspended	TP	Total Phosphorus
MLVSS	Mixed Liquor Volatile Solids Suspended	TS	Total Solids
NH ₄ -N	Ammonia	TSS	Total Suspended Solids
PAS	Psychrophilic Anaerobic Supernatant	vNLR	Volumetric Nitrogen Loading Rate
PN	Partial Nitrification	vOLR	Volumetric Organic Loading Rate

INTRODUCTION

Agriculture and livestock are critical sectors of the EU economy, providing the food, feed, and bioresources that help sustain society. Particularly, the circular management of the livestock can offer many opportunities to make more resource efficient the centralized and decentralized livestock productions and to close the nutrients loop according with the Nitrate Directive 91/676/EEC [1]. In this contest, the application of anaerobic digestion followed by the completely anaerobic nitrogen

removal (ANR) process has been suggested during the last decades as a sustainable solution for the treatment of livestock manure, if the energy consumption and the environmental impact are considered [2-5]. In fact, the combined industrial ammonia production by the Haber-Bosh process and its subsequent removal by the ANR process is less energy intensive compared to the onsite stripping and nitrogen recovery [6]. For small livestock farms, the revamping of the existing storage tanks through their conversion into a psychrophilic anaerobic digester with biogas collection floating covers (BCFC), is often a sustainable solution for the reduction of malodours in the environment by the conversion of volatile organic matter into biogas. However, the anaerobic degradation of more complex organic compounds is not so effective at low temperature, resulting in low biogas production rates and residual biodegradable compounds in the anaerobic effluent. Moreover, the latter could adversely affect the deammonification (DAM) process during the post-treatment since denitrifiers are much more competitive than anammox bacteria at higher organic carbon concentration [7, 8]. ANR have been investigated for the removal of nitrogen from the supernatant of manure digestate under single and two-stage configurations [9-11], which both show advantages and drawbacks. Although the 'two-stage' configuration appeared more robust and might shorten the recovery time after a possible instability of the system, Jaroszynski and Oleszkiewicz [12] underlined the simplicity and the cost-effectiveness of the 'single-stage' configuration using an adequate operational control (dissolved oxygen - DO and pH).

However, such a configuration can be problematic since the DAM process could be adversely affected by the high organic content of the feed effluents, since heterotrophic denitrifying bacteria (HDB) outcompete anammox bacteria at high COD/N ratios [7, 8]. In this sense, the psychrophilic anaerobic digestion process may result in lower performance of the subsequent ANR process due to the higher residual soluble and biodegradable organic carbon which was not converted to biogas. The adsorption/bio-oxidation through high-rate activated sludge process (HRAS) was often reported as an efficient solution for carbon removal and investigated as integrable solution for DAM systems

[13]. However, its application in local and small livestock farms could be difficult because it increases of the complexity of the facilities and the need to handle the sewage sludge produced from the process.

Kindaichi et al. [14] investigated the impact of livestock manure digested liquid on the activity of anammox granules. When real manure digested liquid was fed into an upflow column reactor containing anammox granules, the removal efficiency of ammonia and nitrite decreased by up to 32% and 42% compared to the second reactor fed with synthetic wastewater. Nevertheless, the authors found that still anammox bacteria dominated the interior of the granules.

In suspended sludge system, the selective retention of DAM microorganisms can be possible taking advantage of the different density using a cyclone [15, 36] or the different size using a screen [16]. However, the use of cyclones in the sidestream DAM systems limits the granule size due to high shear force. Recently, Han et al. [17, 35] applied a screen to uncouple the solids retention times of flocs and granules in mainstream DAM for nitrite oxidizing bacteria (NOB) out selection. The authors achieved more than 80% washout of NOB as well as efficient retention of anammox granules from the screen.

In this work, the sieving of suspended biomass was evaluated as physical method to achieve a stable partial nitrification/deammonification (PN/DAM) in a Sequencing Batch Reactor (SBR) for the treatment of psychrophilic anaerobic digestate from the digestion of pig manure. The latter is a special type of effluent which has elevated biodegradable organic matter, rendering the PN/DAM process more challenging. The suspended biomass sieving was used to uncouple and enhance the retention time of the DAM microorganisms in granular biomass, so to better compete with the HDB and NOB in flocculent biomass.

MATERIAL AND METHODS

Psychrophilic Anaerobic Digestion of Pig Manure

On a daily basis up to 100 m³/d of pig slurry, sometimes mixed with residual feeding of pigs (e.g. cheese whey), was fed to a psychrophilic anaerobic digester, having a working volume of 10900 m³, operating at environmental temperature (Figure 1). The bulk of the anaerobic reactor was completely covered with BCFC in order to feed a co-generation unit with an installed electrical power of 170 kW. Due to the absence of insulation, the temperature in the AD reactor was affected by seasonal fluctuations; the minimum registered temperature was 12°C during winter and 18-24°C during summer season. The psychrophilic anaerobic supernatant (PAS) over-flowed from the psychrophilic digester and homogenized in an open tank having a volume of 33000 m³. The PAS of pig slurry was collected every week and was pre-screened through a 125 µm sieve in order to remove 70-80% of the suspended solids and/or inert material which would otherwise accumulate in the SBR. The PAS was stored in a fridge at 4 °C of temperature before its use.

(Figure 1)

Sequencing Batch Reactor

A lab scale SBR was used in a single-stage PN/DAM process for the removal of nitrogen from real PAS of pig slurry. The temperature in the reactor was maintained at 30±1°C through the continuous recirculation of heated water in the external jacket of the SBR. The duration of the SBR cycle was 8 h, while the reaction phases were controlled by a PLC (Programmable Logic Controller) according to the following timing: 0.5-1.0 h of anoxic-feeding, 6 h of micro-aerated-feeding, 0.5 h of anoxic phase (degassing), 0.5 h of settling and 0.5 h of discharge. This sequence and duration was maintained in all the experimental periods. Since the feeding was implemented throughout the whole reaction time, the working volume of the reactor ranged from 20 L during the start of the anoxic-feeding to 26 L at the end of the micro-aerated-feeding. During the aerated feeding, the dissolved oxygen concentration (DO) was controlled at 0.25-0.55 mg L⁻¹ using three blowers which

were set at on/off operation mode depending on the DO concentration. The main reaction phase was the micro-aerobic one in which both the nitrification and the anammox bioreactions occurred. Feeding during the micro-aeration phase was implemented in order to avoid excessive accumulation of nitrite and ammonia in the mixed liquor which could potentially inhibit the anammox biomass.

Inoculum

The SBR was inoculated from a parent lab scale reactor working as a single stage PN/DAM process for the nitrogen removal from the anaerobic supernatant of sewage sludge.

The inoculum was constituted by typical activated sludge flocs with high biological diversity mixed with small red granules embedded in the sludge flocs structure. Although there is no common consensus on the minimum diameter [18], sieves with a diameter of 125 μm have been used to determine the minimum size of granular biomass. Then, the size distribution was measured by an image analyser (Image Analyzer, MeeSoft). A sample of granular biomass was transferred into a petri dish and placed under a stereo microscope (Leica) with a fixed magnification of 7.5X. Around 50 images were recorded and analysed by the image analyser. At the beginning of the experiment and for each experimental period, the Sludge Volume Index (SVI) of the biomass was determined according with the methodology reported in van Loonsdrech et al., (2016). The initial value of the SVI measured at the beginning of Period 1 was 50 ml/gMLSS. Also, the inoculum was characterized based on the DAM activity, the activity of ammonium oxidizing bacteria (AOB) and the heterotrophic denitrifying bacteria (HDB) which were involved in the biological nitrogen removal cycle. The methodologies of each assay were described in the following paragraph.

Evaluation of the biomass activity

The evaluation of the ex-situ specific deammonification activity (sDAA) were conducted using the method developed by Dapena Mora et al. (2007). In this method the gaseous nitrogen emissions are

determined based on pressure differential. The assessment of the specific rates of nitrification (sAUR) were also performed in ex situ tests according with the methodologies reported in van Loosdrecht et al. [19].

Due to the simultaneous occurrence of DAM and heterotrophic denitrification activities, the contribution of the denitrifiers on the nitrogen removal in the SBR was estimated considering the following nitrogen mass balance:

$$\text{Total } N_{\text{removed}} = N_{\text{removed(HDB)}} + N_{\text{removed(DAM)}} = N_{\text{influent}} - N_{\text{effluent}} - N_{\text{waste}}$$

where:

- Total N_{removed} is the nitrogen removed by the activity of HDB and DAM microorganisms;
- $N_{\text{removed(HDB)}}$ is the nitrogen denitrified by HDB;
- $N_{\text{removed(DAM)}}$ is the nitrogen removed by DAM resulted by the actual sDAA and calculated as: $N_{\text{removed(DAM)}} = \text{sDAA} \times \text{MLVSS} \times \text{Reaction time} \times V_r$;
- N_{influent} is the total nitrogen influent in the SBR;
- N_{effluent} is the total nitrogen effluent from the SBR;
- N_{waste} is the removed by the growth of biomass.

The contribution of the DAM and HDB were calculated by the ratio of $N_{\text{removed(HDB)}}$ and $N_{\text{removed(DAM)}}$ respectively by the total N_{removed} respectively and expressed as percentage.

Experimental periods

The experimental period lasted approximately 200 days and included two experimental periods characterized by different strategies to promote the selection of DAM microorganisms. In period 1, the SRT was higher than 50 days for both suspended and granular biomass in order to favour the growth of slow forming anaerobic ammonium oxidizing bacteria. In period 2, bacterial size selection took place driving the wasting of suspended biomass meanwhile retaining in the system granular biomass. This was accomplished during period 2 by the daily sieving the biomass of the

SBR through a 125 μm vibrating screen (Sieving Machine Type AS 200 Control (Retsch GmbH) in order to reduce the SRT at 12-15 days of only the flocculent biomass. The selected sieve pore size was reported to be the optimal one to achieve high retention of DAM microorganisms and limited growth of NOB [20]. The retained biomass from the sieve was recirculated back into the SBR, while the sieved biomass was wasted from the SBR.

Analytical methods

The PAS and the treated effluent from the SBR were analysed in terms of pH, TSS, conductivity, alkalinity, COD, sCOD, TKN, $\text{NH}_4\text{-N}$ and TP according to standard methods [21]. Moreover, the fractionation of the COD of the PAS in terms of biodegradable and readily biodegradable COD (bCOD and rbCOD) was carried out according to the methodology of van Loosdrecht et al. (2016). $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were measured by ion chromatography (Dionex ICS-900 with AG14) in samples that were first filtered through 0.45 μm Whatman membranes. The activated sludge was analysed for MLSS, MLVSS, COD (% TS), N (% TS) and P (% TS) according to standard methods (APHA, AWWA, WEF, 1998).

RESULTS AND DISCUSSIONS

Inoculum characteristics

The results from the biological activity test were presented in Table 1. The initial sDAA of the inoculum was found to be 14.3 $\text{mgN/gMLVSS}\cdot\text{h}$ at 30°C. The sAUR was also high (16.8 $\text{mg N/gMLVSS}\cdot\text{h}$ at 20°C, Figure 3), while the nitrite oxidation rate was very low ($< 1 \text{ mgN/gMLVSS}\cdot\text{h}$), showing the prevalence of AOB over NOB. The nitrogen removal rate by heterotrophic biomass was also very low. Based on the above observations, it was concluded that the anammox and AOB were prevalent in this biomass and thus it was a suitable inoculum to be seeded into the SBR. Moreover, microscopic observations revealed that the average size of

anammox granules were in the range between 200-250 μm of diameter. The size is similar to the one reported by Wett. et al., [22] and Han et al., [17]

Psychrophilic anaerobic digestate of pig slurry

The PAS was characterized by an average $\text{NH}_4\text{-N}$ concentration of 1174 ± 221 mgN/L (Table 2), which represented around 88% of the total nitrogen. The molar ratio of alkalinity/ $\text{NH}_4\text{-N}$ = 1.74 mol/mol was sufficient to accomplish nitrogen removal via PN/DAM pathway without the need of external alkalinity.

The average tCOD concentration was 2611 ± 352 mgCOD/L ratio (Table 2), resulting in a tCOD/TN ratio of approximately 2 kgCOD/kgN . The stoichiometric ratio for denitrification via-nitrite pathway is 1.72 $\text{kgCOD/gNO}_2\text{-N}$ while, if the growth of heterotrophic biomass is considered, the observed COD/N ratio increased up to the range 2-2.5 [4, 23, 24], which is considered appropriate to remove nitrogen via nitrite. However, PAS presented up to around 50% of the tCOD as biodegradable COD (bCOD) and thus as carbon source available for denitrification, which could potentially affect the growth of DAM microorganisms. In fact, the low temperature of the anaerobic digester resulted in significant residual concentration of biodegradable COD in the PAS of 741 ± 55 mgCOD/L (Table 2). Many authors observed that DAM microorganisms are no longer able to compete with heterotrophic denitrifying bacteria at C/N ratio above 1 or an organic matter concentration above 300 mgCOD/L [7, 37]. On the other hand, Molinuevo et al., [25] observed a complete cease of DAM with a COD concentration above 290 mg/L treating partially oxidized pig manure effluent.

(Table 2)

Operating Conditions of the Sequencing Batch Reactor

The operating parameters applied to the SBR were reported in table 3, which indicated that the SRT

was the only parameter that changed among period 1 and period 2. In period 1, the SRT was maintained at 50 days for both suspended and granular biomass during period 1, while in period 2 the sieved biomass was discharged in order to maintain an SRT approximately at 12-15 days.

This change was implemented in order to test whether the growth of DAM microorganism could increase by providing a competitive advantage due to size. In period 1, transient conditions existed (days 0-63), while in period 2 after day 150 pseudo-steady state conditions were maintained.

Effect of the sieving on the biomass properties

Figure 2 shows the profile of the MLSS and the relative volatile fraction (%MLVSS/MLSS) monitored in the Period 1 and Period 2. During Period 1 (days 0-63), the concentration of the MLSS increased significantly between days 0-25 and then stabilized at 7.7 g/L during days 26-63 (Figure 2). At the end of the Period 1, the MLSS were characterized by a low fraction of volatile solids (around 65% based on dry matter), mainly due to the high SRT applied (> 50 days) and the accumulation of inert material in the SBR. Nevertheless, the MLVSS concentration in the SBR increased more than 2 g/L because of the contemporary growth of HDB, as explained in the following paragraph.

However, the increase of the MLSS concentration above 7 g/L in the SBR negatively affected the settling properties of the biomass. The transition concentration depends more on the flocculation state of the particles [26]. At the end of Period 1 (day 63), the SVI was 195 ml/gMLSS (Figure 2), which indicated poor sedimentation capacity and longer settling phase would be required to avoid losses of biomass in the treated effluent.

In experimental Period 2, the discharge of the flocculent biomass sieved at 125 μm resulted in lower and more active biomass in the reactor. At day 64, the retained biomass (> 125 μm) represented around 38% based on dry matter. After 3 times the SRT (days 108-200), the MLSS concentrations stabilized at around 3.15-4.95 g/L, while the volatile fraction was restored close to 80% which was

similar with the values found in Period 1 (Figure 2). Despite the continuous recycle of the retained biomass after the sieving into the reactor, the profile of the MLSS concentration did not increased. The reason was attributed to the shear forces present in the reactor that was sufficient to reduce the size of the retained biomass without compromising its integrity [27]. During the days 108-200, the fraction of biomass retained by 125 μm of pore size was around 65% (based on dry matter), which was 41% more to the one found in Period 1. The increase of the abundance of more granular biomass in the reactor had a significant benefit on the settling capacity of the biomass, as confirmed by the reduction of the SVI to 78 ml/gMLSS (Figure 2).

(Figure 2)

Biomass activity and nitrogen removal

Period 1. During the first 8 days of operation (days 0-8), high sDAA with ex situ test were obtained (14.3 ± 0.1 mgN/gMLVSS·h, Figure 3) in combination with an efficient ex situ sAUR (13.0 ± 2.7 mgN/gMLVSS·h, Figure 3) which resulted in a low concentration of ammonium (17.7 ± 17.2 mgN/L) and nitrite (1.1 ± 0.9 mgN/L) in the effluent (Figure 4). Also, the accumulation of nitrate in the treated effluent increased up to 135.5 mgN/L at day 7 (Figure 4) which corresponded 11% of the ammonia removed indicating the effective metabolism of DAM biomass [28]. The latter was in agreement with the mass balance around the system which showed that the nitrogen was removed through DAM process (Figure 5) Therefore, during the first week of operation, the combination of the simultaneous PN and DAM processes were effective as confirmed by the observed nitrogen removal efficiency close to 82%. After the first 8 days of operation, a significant decrease in the sDAA was observed. Specifically, the sDAA decreased to 8.3 mgN/gMLVSS·h on day 20 and further to 5.1 and 3.1 mgN/gMLVSS·h on days 22 and 42, respectively (Figure 3). In period 1, the DAM activities did not completely cease with the presence of high concentration of biodegradable

COD, but longer exposure could have negative effect as observed by Molinuevo et al. [25]. Similar results were observed by Figuera et al., [39] operating with a CANON system which was not negatively effected even though an inlet COD/N ratio that ranged between 2.4 and 0.5 g COD/g N was applied. Recently, Kindaichi et al. [14] observed that anaerobic digestate is fed to an anammox reactor, the outer layers of the granules are covered with organic matter and/or coexisting bacteria, but DAM activity still is maintained.

During the days 8-40, the nitrate concentration in the SBR sharply decreased to 10 mgN/L due to the fast conversion to nitrite by HDB which used the residual biodegradable COD contained in the PAS (Figure 4). The presence of organic matter and nitrite in the PAS promoted the growth of the HDB which out-competed with DAM microorganisms as observed by the decrease of sDAA and resulting in reduction of the nitrogen removal performances [7]. In period 1, the maximal sNUR of the inoculum increased from 5.2 to 35.1 mgN/gMLVSS·h (Figure 3). The decrease of sDAA with the presence of non-toxic organic matter was also documented by other authors being the change of the metabolic pathway of DAM microorganisms using organic matter rather than ammonium and nitrite as a substrate [29, 30]. Although in period 1 the nitrification rates were maintained at relatively high values (> 9.5 mgN/gMLVSS·h), the occurrence of the DAM activity decrease resulted in a contextual increase of the ammonium concentration in the treated effluent from 1.2 mgN/L to 311.6 mgN/L (days 8-64, Figure 4). However, it should be stressed that the sAUR were determined in batch experiments with high DO concentration (>2 mgO₂/L), while in the SBR the DO concentration and the resulting kinetics were much lower (<0.5 mg/L). At the end of period 1 (days 42-64), the value of the sDAA (4.4 mgN/gMLVSS·h) was less than 40% of the maximum sDAA, which cannot justify the increase of the nitrate concentrations (up to 257 mgN/L) observed in the treated effluent with the stoichiometry of DAM metabolism (Figure 4). Actually, only 65% of the nitrates observed in the effluent were produced directly by the metabolism of DAM microorganisms, while the remain fraction were produced by the oxidation of nitrite by NOB at

around 5 mgN/gMLVSS·h, which was promoted by the high SRT applied (50 days). As shown in Figure 5, the contribution of the nitrogen removal observed in the SBR due to the HDB increased up to 40%, while the nitrogen removal efficiency decreased drastically to around 52% (days 42-63).

(Figure 3)

(Figure 4)

Period 2. The unstable operation of the system due to the detrimental effect of the biodegradable COD in the PAS claimed the adoption of a different strategy to recover the sDAA against the HDB without further affecting the PN. Specifically, the effect of the enhanced retention of the DAM microorganism through the sieving was observed by the gradual increase of the sDAA registered during days 64-108. From Figure 5, the contribution of the nitrogen removal accomplished by DAM microorganisms was 64% on average, obtained during the more stable period (days 109-200).

However, the sDAA stabilized at 8.4 ± 1.0 mgN/gMLVSS·h during days 109-200 (Figure 3) and the complete recovery was never achieved. The reason could be explained by the long-term exposure of free ammonia at level higher than 30 mg/L at the end of period 1, that could have influenced the metabolism of the DAM microorganisms [31]. Another reason could be explained by the still high activity of the HDB (Figure 5) in the background that balanced with the activity of the DAM microorganisms [37].

(Figure 5)

In fact, the contribution of the HDB for the nitrogen removal was still significant between days 109-200, according with a range of 8 to 28% (Figure 5). However, it is important to highlight that also the relative high conductivity of the PAS could result in high osmotic pressure and so, to have a negative effect on the DAM microorganisms [7, 32]

In the same period, the relative nitrate production per ammonium removal was around 8% during days 109-200, which indicated the almost absent activity of NOB in the reactor. The combination of the constant high level of free ammonia (FA > 6 mg/L) in the SBR, the high temperature (30±1 °C), the low value of DO (<0.5 mg/L) and the applied SRT resulted in a almost total inhibition of the NOB anabolism and contemporary promoting the growth of AOB [24, 33, 34]. The observed nitrogen removal efficiency during day 109-200 was around 81% (average) which could be consider a satisfactory result.

CONCLUSION

The anaerobic digestate of pig slurry originating from a psychrophilic anaerobic digestion has still an high content of bCOD which may affect the stability of further ANR processes in single-stage PN/DAM SBR. In Period 1, the activity of sDAA decreased by 85% of its initial value due to the simultaneous growth of the HDB and the affected PN due to the high SRT applied. In Period 2, the sieving of the suspended biomass at 125 µm and the recycle of the retained granules, resulted in 60% recovery of the initial sDAA value. At steady state conditions, the average nitrogen removal efficiency was 81%. Therefore, the sieving of the suspended biomass was successful and resulted in a better balance of DAM microorganisms and HDB despite the high residual biodegradable COD of the PAS.

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REFERENCES

- [1] EEC, D. (1991). Concerning the protection of waters against pollution caused by nitrates from agricultural sources, Off. J. Eur. Commun. L, 375, 1-8.
- [2] Karakashev, D., Schmidt, J. E., & Angelidaki, I. (2008). Innovative process scheme for removal of organic matter, phosphorus and nitrogen from pig manure. *Water Research*, 42(15), 4083-4090.
- [3] Scaglione, D., Tornotti, G., Teli, A., Lorenzoni, L., Ficara, E., Canziani, R., & Malpei, F. (2013). Nitrification denitrification via nitrite in a pilot-scale SBR treating the liquid fraction of co-digested piggery/poultry manure and agro-wastes. *Chemical engineering journal*, 228, 935-943.
- [4] Malamis, S., Katsou, E., Di Fabio, S., Bolzonella, D., & Fatone, F. (2014). Biological nutrients removal from the supernatant originating from the anaerobic digestion of the organic fraction of municipal solid waste. *Critical reviews in biotechnology*, 34(3), 244-257.
- [5] Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H., & van Loosdrecht, M. C. (2014). Full-scale partial nitrification/anammox experiences—an application survey. *Water research*, 55, 292-303.
- [6] Maurer, M., Schwegler, P., & Larsen, T. A. (2003). Nutrients in urine: energetic aspects of removal and recovery. *Water Science and technology*, 48(1), 37-46.
- [7] Jin, R. C., Yang, G. F., Yu, J. J., & Zheng, P. (2012). The inhibition of the Anammox process: a review. *Chemical Engineering Journal*, 197, 67-79.
- [8] Zhang, Z., Li, Y., Chen, S., Wang, S., & Bao, X. (2012). Simultaneous nitrogen and carbon removal from swine digester liquor by the Canon process and denitrification. *Bioresource technology*, 114, 84-89.
- [9] Qiao, S., Yamamoto, T., Misaka, M., Isaka, K., Sumino, T., Bhatti, Z., & Furukawa, K. (2010). High-rate nitrogen removal from livestock manure digester liquor by combined partial nitrification–anammox process. *Biodegradation*, 21(1), 11.
- [10] Hwang, I. S., Min, K. S., Choi, E., & Yun, Z. (2005). Nitrogen removal from piggery waste using the combined SHARON and ANAMMOX process. *Water science and technology*, 52(10-11),

487-494.

[11] Scaglione, D., Ficara, E., Corbellini, V., Tornotti, G., Teli, A., Canziani, R., & Malpei, F. (2015). Autotrophic nitrogen removal by a two-step SBR process applied to mixed agro-digestate. *Bioresource technology*, 176, 98-105.

[12] Jaroszynski, L. W., & Oleszkiewicz, J. A. (2011). Autotrophic ammonium removal from reject water: partial nitrification and anammox in one-reactor versus two-reactor systems. *Environmental technology*, 32(3), 289-294.

[13] Jimenez, J., Miller, M., Bott, C., Murthy, S., De Clippeleir, H., & Wett, B. (2015). High-rate activated sludge system for carbon management—Evaluation of crucial process mechanisms and design parameters. *Water research*, 87, 476-482.

[14] Kindaichi, T., Awata, T., Mugimoto, Y., Rathnayake, R. M., Kasahara, S., & Satoh, H. (2016). Effects of organic matter in livestock manure digester liquid on microbial community structure and in situ activity of anammox granules. *Chemosphere*, 159, 300-307.

[15] Wett, B. (2007). Development and implementation of a robust deammonification process. *Water Science and Technology*, 56(7), 81-88.

[16] De Clippeleir, H., Jimenez, R., Giraldo, E., Wett, B., Dockett, N., Riffat, R., & Murthy, A. A. O. S. (2013). Screens as a method for selective anammox retention in single stage deammonification processes. *WEF/IWA Nutrient Removal and Recovery*. Presented at the *WEF/IWA Nutrient Removal and Recovery*, Canada.

[17] Han, M., Vlaeminck, S. E., Al-Omari, A., Wett, B., Bott, C., Murthy, S., & De Clippeleir, H. (2016). Uncoupling the solids retention times of flocs and granules in mainstream deammonification: a screen as effective out-selection tool for nitrite oxidizing bacteria. *Bioresource technology*, 221, 195-204.

[18] Bathe, S., de Kreuk, M. K., McSwain, B. S., & Schwarzenbeck, N. (Eds.). (2005). *Aerobic granular sludge*. IWA Publishing.

- [19] van Loosdrecht, M. C., Nielsen, P. H., Lopez-Vazquez, C. M., & Brdjanovic, D. (Eds.). (2016). *Experimental methods in wastewater treatment*. IWA publishing.
- [20] Volcke, E. I. P., Picioreanu, C., De Baets, B., & Van Loosdrecht, M. C. M. (2012). The granule size distribution in an anammox- based granular sludge reactor affects the conversion— Implications for modeling. *Biotechnology and bioengineering*, 109(7), 1629-1636.
- [21] APHA, AWWA, WEF, 1998. *Standard methods for the examination of water and wastewater*. American Public Health Association, American Water Works Association, Water Environment Federation, 20th ed. Washington, DC, 1998.
- [22] Wett, B., Podmirseg, S. M., Gómez-Brandón, M., Hell, M., Nyhuis, G., Bott, C., & Murthy, S. (2015). Expanding DEMON sidestream deammonification technology towards mainstream application. *Water Environment Research*, 87(12), 2084-2089.
- [23] Malamis, S., Katsou, E., Frison, N., Di Fabio, S., Noutsopoulos, C., & Fatone, F. (2013). Start-up of the completely autotrophic nitrogen removal process using low activity anammox inoculum to treat low strength UASB effluent. *Bioresource technology*, 148, 467-473.
- [24] Frison, N., Katsou, E., Malamis, S., Bolzonella, D., & Fatone, F. (2013). Biological nutrients removal via nitrite from the supernatant of anaerobic co-digestion using a pilot-scale sequencing batch reactor operating under transient conditions. *Chemical engineering journal*, 230, 595-604.
- [25] Molinuevo, B., García, M. C., Karakashev, D., & Angelidaki, I. (2009). Anammox for ammonia removal from pig manure effluents: effect of organic matter content on process performance. *Bioresource Technology*, 100(7), 2171-2175.
- [26] De Clercq, J., Nopens, I., Defrancq, J., & Vanrolleghem, P. A. (2008). Extending and calibrating a mechanistic hindered and compression settling model for activated sludge using in-depth batch experiments. *Water research*, 42(3), 781-791.
- [27] Tay, J. H., Liu, Q. S., & Liu, Y. (2001). The effects of shear force on the formation, structure and metabolism of aerobic granules. *Applied microbiology and biotechnology*, 57(1-2), 227-233.

- [28] Strous, M., Heijnen, J. J., Kuenen, J. G., & Jetten, M. S. M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied microbiology and biotechnology*, 50(5), 589-596.
- [29] Kartal, B., Rattray, J., van Niftrik, L. A., van de Vossenberg, J., Schmid, M. C., Webb, R. I., ... & Strous, M. (2007). Candidatus "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. *Systematic and applied microbiology*, 30(1), 39-49.
- [30] Kartal, B., Van Niftrik, L., Rattray, J., Van De Vossenberg, J. L., Schmid, M. C., Sinninghe Damsté, J., ... & Strous, M. (2007). Candidatus 'Brocadia fulgida': an autofluorescent anaerobic ammonium oxidizing bacterium. *FEMS microbiology ecology*, 63(1), 46-55.
- [31] Dapena-Mora, A., Fernandez, I., Campos, J. L., Mosquera-Corral, A., Mendez, R., & Jetten, M. S. M. (2007). Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. *Enzyme and Microbial Technology*, 40(4), 859-865.
- [32] Jin, R. C., Zheng, P., Mahmood, Q., & Hu, B. L. (2007). Osmotic stress on nitrification in an airlift bioreactor. *Journal of hazardous materials*, 146(1-2), 148-154.
- [33] Vadivelu, V. M., Keller, J., & Yuan, Z. (2007). Effect of free ammonia on the respiration and growth processes of an enriched Nitrobacter culture. *Water Research*, 41(4), 826-834.
- [34] Gu, S., Wang, S., Yang, Q., Yang, P., & Peng, Y. (2012). Start up partial nitrification at low temperature with a real-time control strategy based on blower frequency and pH. *Bioresource technology*, 112, 34-41.
- [35] Vlaeminck, S. E., Terada, A., Smets, B. F., De Clippeleir, H., Schaubroeck, T., Bolca, S., ... & Verstraete, W. (2010). Aggregate size and architecture determine microbial activity balance for one-stage partial nitrification and anammox. *Applied and environmental microbiology*, 76(3), 900-909.
- [36] Wett, B., Omari, A., Podmirseg, S. M., Han, M., Akintayo, O., Brandón, M. G., ... & Nyhuis, G. (2013). Going for mainstream deammonification from bench to full scale for maximized

resource efficiency. *Water Science and Technology*, 68(2), 283-289.

[37] Van Hulle, S. W., Vandeweyer, H. J., Meesschaert, B. D., Vanrolleghem, P. A., Dejana, P., & Dumoulin, A. (2010). Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chem. Eng. J.*, 162(1), 1-20.

[38] Scaglione, D., Rusalleda, M., Ficara, E., Balaguer, M. D., & Colprim, J. (2012). Response to high nitrite concentrations of anammox biomass from two SBR fed on synthetic wastewater and landfill leachate. *Chemical engineering journal*, 209, 62-68.

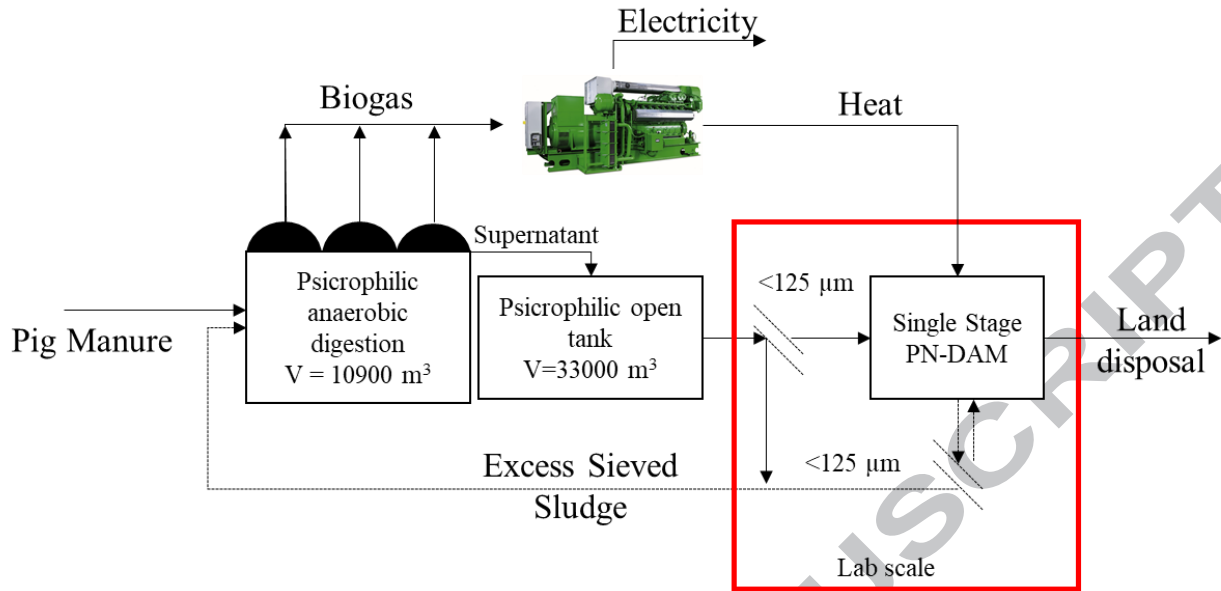


Figure 1. Representation of the integrated PN/DAM for the treatment of PAS

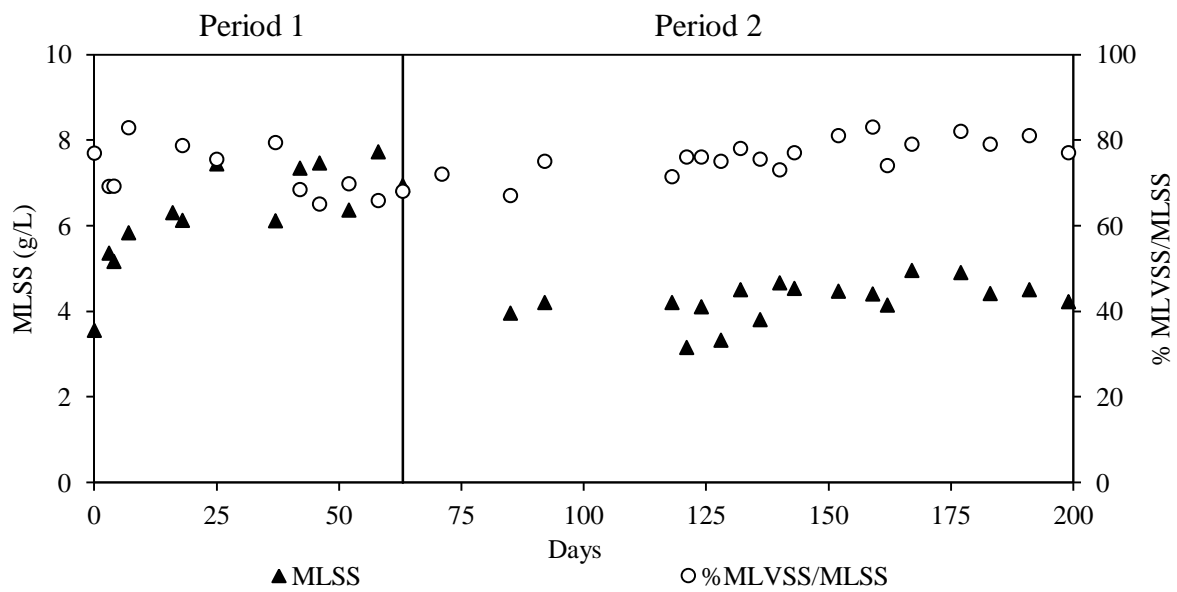


Figure 2 Variation of the MLSS concentration and the MLVSS/MLSS ratio during the SBR operation

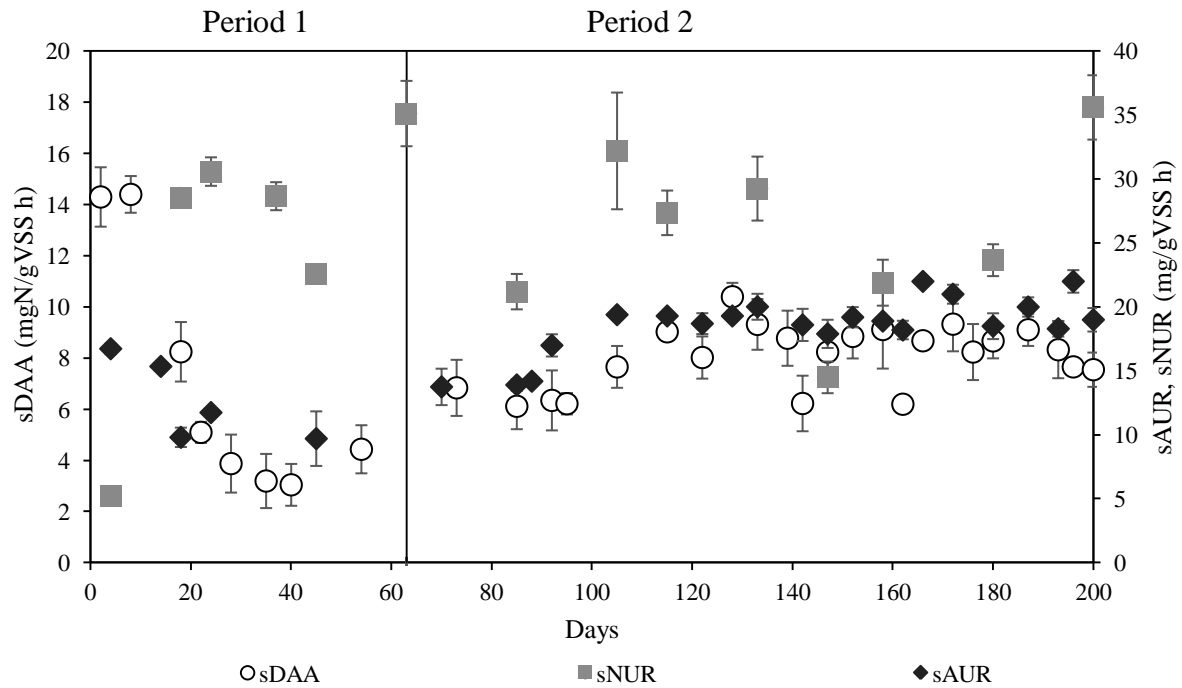


Figure 3: Variation of the nitrification and anammox activity throughout the operation of the SBR

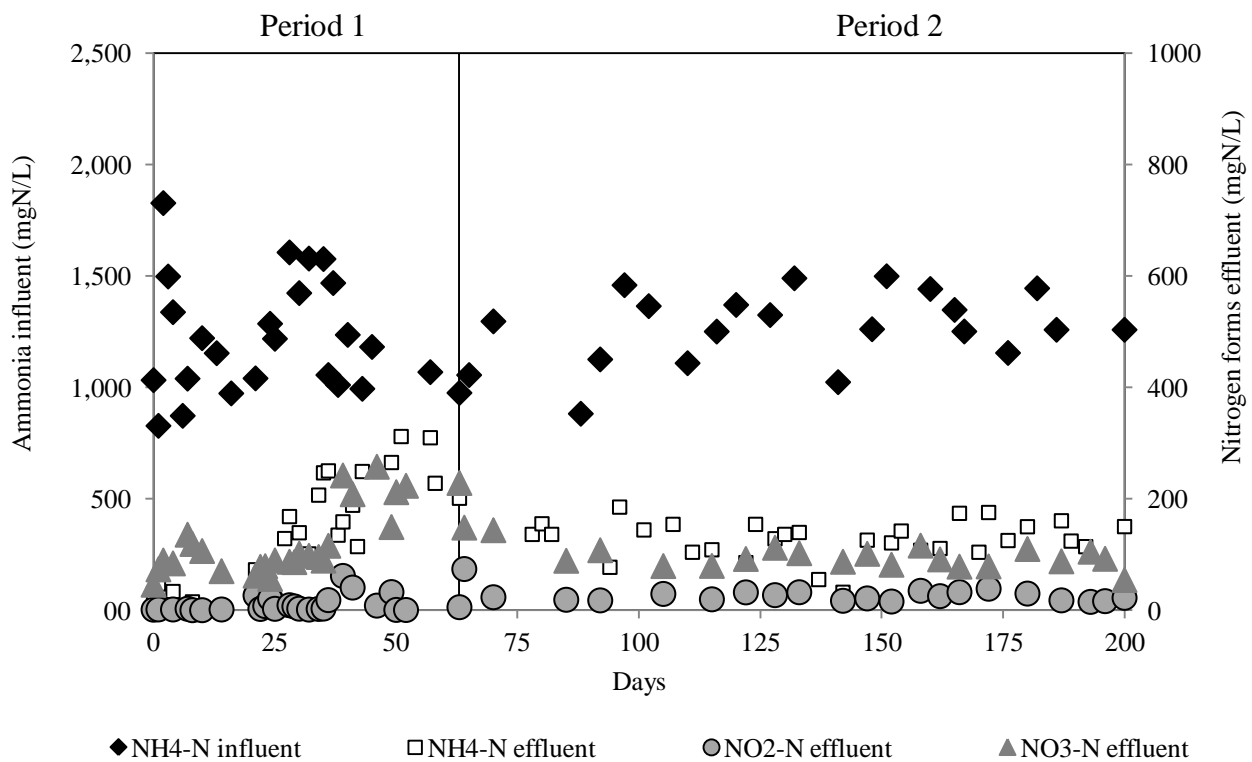


Figure 4 Influent ammonium and treated effluent ammonium, nitrite and nitrate concentrations during the SBR operation for the treatment of pig slurry supernatant

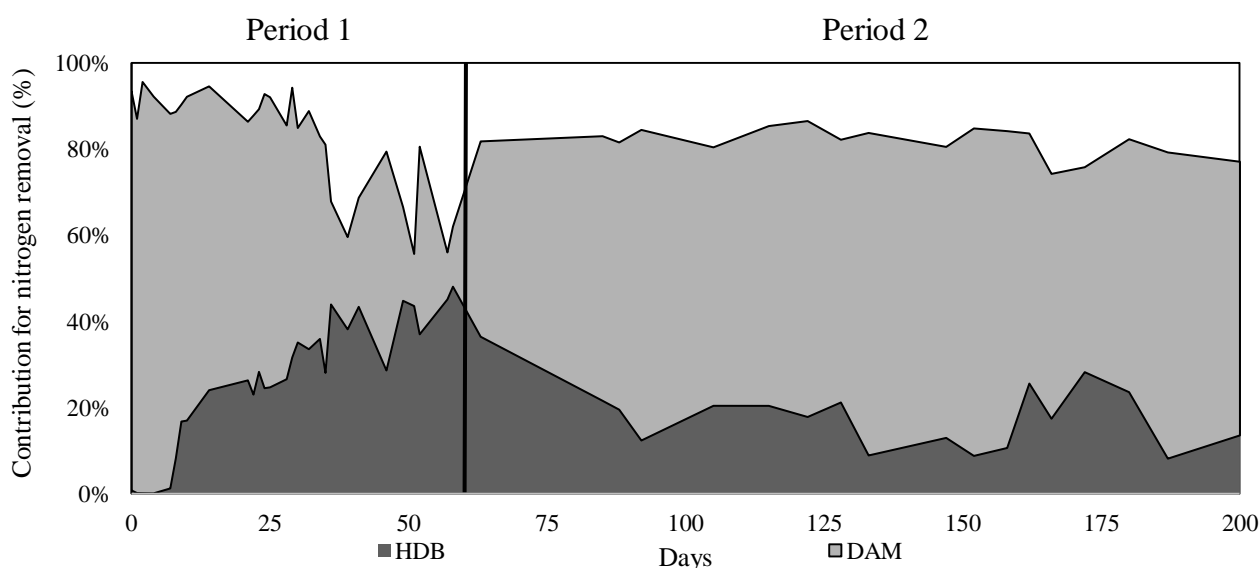


Figure 5: Contribution of the HDB and DAM microorganisms to nitrogen removal observed in the SBR.

Table 1. Initial biological nitrogen removal activity of the inoculum

Activity	Type of process	Unit	Average	Standard Deviation
Deammonification activity (sDAA, 30°C)	Autotrophic	mgN/gVSS h	14.3	0.5
Ammonia uptake rate (sAUR, at 20°C)	Autotrophic	mgN/gVSS h	16.8	1.2
Nitrite oxidation rate (sNOR, at 20°C)	Autotrophic	mgN/gVSS h	0.57	0.02
Nitrogen uptake rate (sNUR, at 20°C)	Heterotrophic	mgN/gVSS h	1.02	0.2

Table 2. Characteristics of the PAS (number of samples = 25)

Parameter	Unit	Average	Standard deviation	Min	Max
pH	-	8.1	0.2	7.8	8.3
Partial alkalinity	mgCaCO ₃ /L	9530	124	7510	9950
Total alkalinity	mgCaCO ₃ /L	14575	223	13250	15240

Total suspended solids (TSS)	mg/L	285	24	230	345
Total COD (tCOD)	mgCOD/L	1752	115	1210	1885
Soluble COD (sCOD)	mgCOD/L	1421	225	1025	1525
Biodegradable COD (bCOD)	mgCOD/L	741	55	541	855
Readily biodegradable COD (rbCOD)	mgCOD/L	112	35	40	160
Total Nitrogen (TN)	mgN/L	1327	330	839	1896
Ammonia (NH ₄ -N)	mgN/L	1174	221	827	1826
Total Phosphorus (TP)	mgP/L	27	5	12	38
Ortho-phosphate (PO ₄ -P)	mgP/L	14	4	11	16
Conductivity	mS/cm	16	4	11	22

Table 3: Operating conditions of the SBR during the two experimental periods

Parameter	Unit	Period 1	Period 2
Days	-	0-63	64-200
vNLR	kgN/m ³ d	0.74±0.16	0.71±0.13
vOLR	kgCOD/m ³ d	1.34±0.15	1.46±0.07
HRT	d	1.7±0.5	1.5±0.03
SRT	d	50 d	12-15 day with recycling of retained sludge

Highlights

- Recovery of heat and electrical energy by psychrophilic anaerobic digestion of pig slurry for local farm;
- Psychrophilic anaerobic digestate inhibited deammonification microorganisms due to the presence of biodegradable COD;
- The sieving of excess sludge at 125 μm promoted the retention of deammonification microorganisms;
- Almost 80% of the nitrogen were removed through deammonification microorganisms