(P8) Treatability of digested piggery/poultry manure by anammox bacteria

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Abstract
The liquid fraction of digested material is rich in ammonium and may require nitrogen removal. The aim of this research was to evaluate the applicability of the anammox process for the biological N removal from a supernatant coming from the anaerobic digestion of a mixture of piggery manure, poultry manure, and of agro-wastes. The supernatant was pre-treated in a partial nitritation pilot-scale reactor located at the farm. A batch procedure for testing the short term effect of high-strength wastewaters on anammox activity is presented. The anammox process was successfully applied for the first time to undiluted digestate, and the average N removal efficiency achieved during 350 days of experimentation in a SBR lab-scale reactor was 91%.

Keywords
Anammox, activity test, digestate, manometry, piggery manure, poultry manure

INTRODUCTION

Anaerobic digestion (AD) can effectively treat livestock wastewater and produce renewable energy as biogas. The liquid fraction of the digested material is rich in ammonium and its disposal on agricultural soil is regulated by the European directive on nitrates (91/676/CEE). Therefore N removal may be required in intensive breeding farms. This aspect has prompted attention toward advanced biological processes such as the nitritation-denitritation process (Scaglione et al. 2013), and the completely autotrophic process (partial nitritation and anammox) that are cost effective thanks to lower aeration and external carbon source requirements (Van Hulle et al. 2010). Most studies regarded digestates from piggery manure (Dosta et al. 2008) or diluted wastewater in lab-scale reactors (Yamamoto et al. 2008). Literature documented the treatment of neither digestates from mixed animal wastes nor undiluted agricultural digestate (Magrì et al., 2013). This work is part of a two-years project (BRAIN) funded by the Italian Ministry for Agriculture, whose aim was to evaluate autotrophic N removal processes applied to digested liquid fraction coming from the anaerobic digestion of a mixture of agro-wastes, mainly piggery and poultry manure. In this paper some lab-scale results are presented, namely: (i) activity batch tests, to assess anammox activity of digestate supernatant after partial nitritation stage and the effect of different salinity concentrations; (ii) report the results of a 350-day operation of an SBR treating digested supernatant.

MATERIALS and METHODS

Characteristics of the supernatant

The supernatant from a full-scale anaerobic digester (solid/liquid separation by centrifugation), located in a piggery farm in Northern Italy, was used. The digester was fed on thickened piggery manure, poultry manure and agro-wastes. The supernatant showed a high variability in its chemical composition during the course of the experimentation (pH =8.0±0.1, Conductivity =14.2 ± 2.1 mS cm^{-1}; NH_4^+-N =1151 ± 251 mg L^{-1}; COD = 2634 ± 1178 mg L^{-1}; TSS = 430 ± 220 mg L^{-1}; Alkalinity = 7128 ± 2932 mg$CaCO_3$ L^{-1}). The supernatant was treated in a partial-nitritation 650-L SBR. The NO_2/NH_4 molar ratio of the effluent from the partial nitritation pilot plant was slightly adjusted to remain within 1.25±0.25, thus suitable for feeding the subsequent anammox reactor.
Soluble COD concentration in partial-nitrified supernatant was 853±257 mg/L while BOD_5 and BOD_{20} were 25±15 mg/L and 49±26 mg/L respectively.

Manometric batch tests

The assessment of the N_2 production rate from the anammox reaction was performed by means of an OxiTop® Control system. This is a manometric device consisting of a pressure transducer and data logger located inside a measuring head that is mounted on a glass bottle of 1140 ml volume, filled with anammox biomass suspension. The bottle has got two lateral openings; one is sealed by a rubber septum and is used for substrate injections, the second one is sealed by a teflon airtight valve for gas discharge. During the course of the batch test, the overpressure due to N_2 production by the anammox reaction is automatically registered by the measuring heads. Manometric determinations of Anammox activity were performed according to the following procedure. Each bottle was filled with 30 ml of granular anammox biomass and 700 ml of either mineral medium or a blend of real-wastewater and mineral medium, depending on the test type. Sodium bicarbonate (1g/L) was added and the headspace was flushed with 95%N_2 and 5% CO_2 to ensure anaerobic conditions and to keep the pH around 7.8. Bottles were thermostated at 35±0.5°C and stirred by a magnetic mixer. Substrates were added by spike injections through the rubber septum. Then, the gas volume of cumulatively evolved N_2 was calculated from overpressure data according to the ideal gas law. At the end of the test, concentrations of ammonium, nitrite and nitrate were measured. For each test, the maximum N_2 production rate, the maximum specific Anammox activity, SAA_{max} ($mgN_2/gVSS/h$) and the nitrogen mass balance was verified.

Lab-scale anammox reactor

To test the stability of the anammox process, a thermostated (34-36°C) 3-L lab-scale reactor was used. It was equipped with time-controlled feeding/discharging pumps, mechanical mixing, and with a pH control unit. The reactor was operated in a SBR mode with a 8 h cycle (5 h of FILL, 2.5 h of REACT, 0.5 h of SETTLE/DRAW).

RESULTS AND DISCUSSION

Manometric batch tests

A first set of manometric tests were performed to evaluate the effect of exposing granular anammox biomass sample to the effluent of a partial nitritation reactor. However, the high concentration of nitrite in this wastewater (around 500 mgN/L) would have caused anammox inhibition. Therefore, the wastewater was first pretreated by adding activated sludge and a stoichiometric amount of sodium acetate to remove nitrite by heterotrophic denitrification. After 24h of reaction, the supernatant was centrifuged and the clarified fraction used, either as such or after dilution with a mineral medium, for testing its effect on anammox biomass. The ammonium concentration was yet high (around 190 mgN/L) so that only nitrite was spiked to achieve an initial concentration of 50 mgN/L. Soluble COD concentration was 1018 mg/L while BOD_{20} and BOD_5 were 80 and 30 mg/L respectively. The first set of activity tests aimed at evaluating the response of a non-acclimated anammox biomass to the pre-denitrified digestate. To this purpose, a granular biomass sample was used, having a specific activity of 0.11 $gN_2/gVSS/d$. Results are reported in Figure 1. No inhibition was observed when the wastewater was blended with 50% mineral medium, while activity showed an 85% decrease after 24h exposure to a blend containing 75% wastewater. For the last two samples, anammox activity recovered with time, more significantly for the less concentrated blend. The results confirmed that the inhibition increases with the fraction of wastewater in the blend and that a long term adaption may take place.

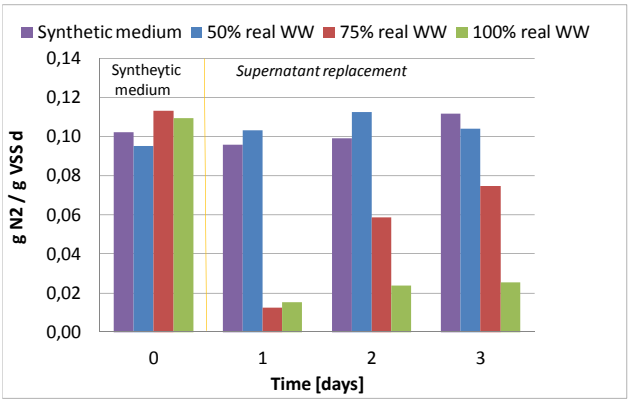

Figure 1: Results of the first set of inhibition tests

Table 1: Results of the second set of inhibition tests

	Activity loss		
Days	Unacclimated biomass	Acclimated biomass	
	7 g/L NaCl	7 g/L NaCl	13.5 g/L NaCl
1	0%	0%	0%
2	62%	57%	60%
3	57%	80%	87%
4	86%	66%	77%

A second set of tests were used to evaluate whether the previously observed anammox inhibition could be due to the high salinity of the digested supernatant. In these tests, salinity of the mineral medium was modified by NaCl addition. The NaCl concentrations tested were 7 and 13.5 g/L, while salinity of the digested supernatant corresponded to around 5 g/L as NaCl. As inoculum, two type of biomass were used: (1) the unacclimated granular sludge, and (2) an acclimatised sample drawn from the anammox. Results are reported in Table 1. For the unacclimated sludge the activity decreased during time with a final inhibition of 86%. At the same salinity concentration, the acclimatized sludge showed a faster adaptation with an activity recovery trend after 3 days of incubation. By doubling the salinity level a slightly higher inhibition was observed.

The inhibition level obtained are slightly higher than the one reported by Dapena Mora et al. (2007) who found a IC50 value of 13.5 g/L of NaCl in similar manometric batch tests on granular anammox biomass.

Lab scale anammox SBR

The SBR reactor was inoculated with granular anammox sludge coming from a full-scale anammox reactor located in Rotterdam (NL). The SBR was operated at an HRT of 2 d. A synthetic feed was used initially, at an average nitrogen loading rate (NLR) of 0.5-0.6 gN L^{-1} d^{-1}, kept constant during the experimentation. Then a blend with increased fractions of real wastewater were used (10%, 25%, 33%, 50%, 70%, 100%), adjusting the NLR and the NH_4/NO_2 molar ratio by $NaNO_2$ and NH_4Cl additions. The reactor was operated for 350 days; during a 40 days stop in summer, the biomass was kept at 4°C. Batch tests were periodically performed to assess the maximum anammox activity (NRR_{max}). In figure 2-right influent and effluent nitrogen compounds are reported. The average N removal efficiency was 91±10% with an average nitrite removal efficiency higher than 97%. NO_2-N/NH_4-$N_{removed}$ ratio was 1.28±14% while NO_3-N/NH_4-$N_{removed}$ ratio was 0.10±72%. In Figure 2-left, the NRRmax is plotted together with the percentage of supernatant in the feed. During the initial

start-up and restart after the first break, the activity increased from values between 1 and 2 kgN/m^3/d up to values between 3 and 4 kgN/m^3/d in about 30 days, suggesting an increase of the active biomass. In contrast, after each increase in blending real wastewater in the influent, the activity decreased, suggesting that real wastewater had an inhibitory effect on the anammox bacteria, similarly to what had been observed in the manometric batch tests. While working at 75% of wastewater in the feed and also during the first days of operation with undiluted wastewater, a minimum value of 0.14 kgN/m^3/d was achieved. In these low-activity phase, temporary but significant increase in nitrite concentration in the effluent was observed. After 30 days of operation with undiluted wastewater, the anammox activity began to recover and continued to rise until the end of the experimentation, achieving values greater than 1 kgN/m^3/d. The trend observed in the last two months of operation at 100% real wastewater suggests the ability of anammox bacteria to adapt to inhibitory substances that may be present in the agricultural digestate. Three particle-size tests were carried out at day 0, 260 and 350 of operation, and showed that the average diameter of granules increased from 775µm of day 0 to 1003µm and 1033µm of day 260 and 350 respectively.

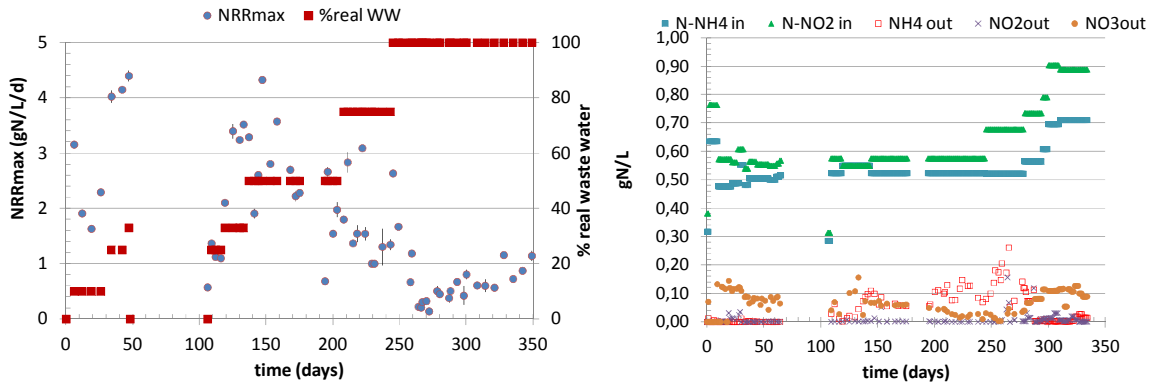

Figure 2: Maximum anammox activity in the SBR reactor during the experimentation and fractions of real wastewater blended in the feeding (left) and influent and effluent nitrogen compound (right).

CONCLUSIONS

The following main conclusions can be drawn:

- a procedure to perform short-term batch tests to assess inhibition of anammox activity in presence of high-strength wastewaters was developed and tested successfully;
- inhibition in presence of undiluted digestate supernatant from piggery/poultry manure processing with not acclimatized biomass; was as high as 85%;
- salinity concentration of 7 gNaCl/L caused 68% activity reduction in the test with acclimated biomass and 86% in the test with non- acclimated biomass;
- in the first days of operation with undiluted supernatant, the maximum anammox activity in the SBR decreased sharply, but recovered afterwards, suggesting that biomass acclimated to the wastewater;
- in the long term, the anammox SBR proved successful in treating undiluted supernatant, with N removal efficiencies higher than 90%.

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