



## Anammox for ammonia removal from pig manure effluents: Effect of organic matter content on process performance

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### ABSTRACT

The anammox process, under different organic loading rates (COD), was evaluated using a semi-continuous UASB reactor at 37 °C. Three different substrates were used: initially, synthetic wastewater, and later, two different pig manure effluents (after UASB-post-digestion and after partial oxidation) diluted with synthetic wastewater. High ammonium removal was achieved, up to 92.1 ± 4.9% for diluted UASB-post-digested effluent (95 mg COD L<sup>-1</sup>) and up to 98.5 ± 0.8% for diluted partially oxidized effluent (121 mg COD L<sup>-1</sup>). Mass balance clearly showed that an increase in organic loading (from 95 mg COD L<sup>-1</sup> to 237 mg COD L<sup>-1</sup> and from 121 mg COD L<sup>-1</sup> to 290 mg COD L<sup>-1</sup> for the UASB-post-digested effluent and the partially oxidized effluent, respectively) negatively affected the anammox process and facilitated heterotrophic denitrification. Partial oxidation as a pre-treatment method improved ammonium removal at high organic matter concentration. Up to threshold organic load concentration of 142 mg COD L<sup>-1</sup> of UASB-post-digested effluent and 242 mg COD L<sup>-1</sup> of partially oxidized effluent, no effect of organic loading on ammonia removal was registered (ammonium removal was above 80%). However, COD concentrations above 237 mg L<sup>-1</sup> (loading rate of 112 mg COD L<sup>-1</sup> day<sup>-1</sup>) for post-digested effluent and above 290 mg L<sup>-1</sup> (loading rate of 136 mg COD L<sup>-1</sup> day<sup>-1</sup>) for partially oxidized effluent resulted in complete cease of ammonium removal. Results obtained showed that, denitrification and anammox process were simultaneously occurring in the reactor. Denitrification became the dominant ammonium removal process when the COD loading was increased.

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### 1. Introduction

Pig farming is a major European Union (EU) agricultural industry. Nowadays, farmers in the EU are confronted with an increasing number of environmental regulations, concerning the application of the manure produced, as direct fertilizer on agricultural land. Phosphorus and nitrogen pollute potable water and cause eutrophication. As the main part of nitrogen in manure, ammonium is readily oxidized to nitrate, which is poorly absorbed by soil colloids, thus facilitating its transfer to surface waters. Manure also contributes to increased greenhouse gas emissions (GHG) (Thorman et al., 2007). Special attention needs to be given to N<sub>2</sub>O gas emissions, which can be minimized by developing a sustainable manure management strategy. Thus, sustainable solutions for pig manure treatment regarding nitrogen removal need to be implemented with respect to environmental and agricultural benefits.

Anaerobic ammonium oxidation (anammox) has received special attention since its discovery, because it is an efficient biological

alternative to conventional nitrogen removal from wastewaters. Under anaerobic conditions, ammonium is oxidized to nitrogen gas with nitrite as the electron acceptor and carbon dioxide is used for growth of the anammox microorganisms involved. In comparison to traditional nitrification–denitrification process, this autotrophic process consumes 100% less biodegradable organic carbon and at least 50% less oxygen (Tal et al., 2006) and has, therefore, a lower operating cost.

The anammox process is suitable for wastewater with low C:N ratios. At C:N ratios above 1, the anammox bacteria are no longer able to compete with heterotrophic denitrifying bacteria (Güven et al., 2005).

The organic loading rate was found to affect the anammox process performance, but the exact inhibitory levels still remain unclear (Sabumon, 2007; Wang and Kang, 2005). An organic matter concentration above 300 mg chemical oxygen demand (COD) L<sup>-1</sup> was previously found to inactivate anammox communities in a UASB reactor fed with fat milk as organic matter source (Chamchoi et al., 2008). Concentrations of 50 mM of acetate resulted in 70% inhibition in the anammox process (Dapena-Mora et al., 2007). Therefore it is necessary to clearly establish the COD levels inhibiting the anammox process.

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Ammonia removal via anammox has been developed for the treatment of many different wastes with low organic matter content (below 1700 mg COD L<sup>-1</sup>), such as water from the secondary clarifier of municipal wastewater treatment plants in a down flow biofilter (Li et al., 2005), nitrous organic wastewater in ASBR reactors (Jing-Ping et al., 2006) and landfill leachate in a continuous reactor (Liang and Liu, 2008). Only a few studies have investigated the possibility of using the anammox process for ammonia removal from animal waste treatment water, which is indeed a residue with high organic matter and nitrogen content (Waki et al., 2007). However, there is still a big gap regarding effect of different pre-treatments (reducing organic and ammonia loads) of the waste streams on anammox process performance.

The aim of the present study is to investigate the performance of the anammox process under different organic loadings in a semi-continuous UASB reactor fed with two pretreated pig manure effluents (UASB-post-digested effluent and partially oxidized effluent) as organic matter source.

## 2. Methods

### 2.1. Substrate characteristics

Three different substrates were used in this study:

#### 2.1.1. Synthetic wastewater (SWW)

Synthetic wastewater was used for the start-up of the lab-scale UASB reactor.

The synthetic wastewater composition used throughout this study was (g L<sup>-1</sup>): NaHCO<sub>3</sub>, 2.6; K<sub>2</sub>HPO<sub>4</sub>, 0.025; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.3; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.00625; EDTA, 0.00625; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.19; NaNO<sub>2</sub>, 0.26; NaNO<sub>3</sub>, 1.22 dissolved in distilled water. Trace element solutions I and II (1.25 ml per litre medium) were also added as previously described (Chamchoi and Nitisoavut, 2007).

#### 2.1.2. Pig manure effluent after UASB-post-digestion (UASB-post-digested effluent)

This effluent was collected after three treatment steps: full-scale anaerobic digestion (AD), decanter separation and post-digestion in a lab-scale UASB reactor for reduction of the residual organic matter (Karakashev et al., 2008).

Full-scale anaerobic digestion was performed continuously in a thermophilic (55 °C) biogas plant (Hegndal biogas plant, Denmark) with hydraulic retention time (HRT) of 15 days and organic loading rate of 4.6 kg COD m<sup>-3</sup> day<sup>-1</sup>. After full-scale AD, the effluent was continuously separated in a decanter centrifuge (Alfa Laval, NX 309B-31, Rodovre, Denmark) operated at 5000g. After centrifugation, digested manure was separated into a solid organic fiber fraction (10–15% wet weight) and a liquid fraction (85–90% wet weight). The liquid fraction was post-digested in UASB to reduce the residual COD. The reactor's operational parameters were: temperature 55 °C, total volume 334 ml, liquid volume 255 ml, HRT 4 days. The reactor was fed 12 times per day with a feeding rate of 2.63 ml min<sup>-1</sup> for 2 min. The reactor was inoculated with 0.05 L of anaerobic granular sludge obtained from a potato factory (Kruiningen, The Netherlands). The average organic loading rate of the reactor was 3.8 g total COD L<sup>-1</sup> day<sup>-1</sup>. Chemical characteristics after this treatment are shown in Table 1.

#### 2.1.3. Pig manure effluent after partial oxidation (Partially oxidized effluent)

A partial oxidation (nitrification) of the UASB-post-digested effluent was carried out to create more favorable conditions for the anammox process through partial removal of COD and partial

**Table 1**

Chemical characteristics of the pig manure effluents.

Parameters	Unit	Average value ± SD*	
		Effluent after UASB	Effluent after partial oxidation
TS	g L <sup>-1</sup>	nd**	nd
VS	g L <sup>-1</sup>	nd	nd
COD	g L <sup>-1</sup>	4.74 ± 1.05	2.42 ± 0.29
N-NH <sub>4</sub> <sup>+</sup>	g L <sup>-1</sup>	3.78 ± 0.46	0.67 ± 0.16
N-NO <sub>2</sub>	g L <sup>-1</sup>	1.7 ± 0.20	0.7 ± 0.24
N-NO <sub>3</sub>	g L <sup>-1</sup>	4.01 ± 0.40	1.65 ± 0.58

\* SD represents standard deviation from a triplicate sampling experiments.

\*\* Not determined.

conversion of ammonium to nitrite. A mixture of nitrifying sludge (20%) (Lundtofte WWTP, Denmark) and 80% of UASB-post-digested effluent was aerated for 30 h with an aeration rate of 1500 ml air min<sup>-1</sup>.

The chemical characteristics of the partially oxidized effluent are presented in Table 1.

### 2.2. Experimental setup

#### 2.2.1. Lab-scale UASB reactor for the anammox process

A lab-scale UASB reactor operated in semi-continuous mode was used. The UASB was inoculated with 40 ml granules from anaerobic granular sludge (potato factory, Kruiningen, The Netherlands) and 40 ml anammox seed sludge (provided by the Laboratory of Microbial Ecology, Ghent University, Belgium). The reactor was operated at 37 °C with a total volume of 334 ml; the liquid volume was 255 ml. The flow rate was set up at 120 ml day<sup>-1</sup> and the HRT was 2.1 d. The UASB reactor was initially fed with synthetic wastewater. The effect of organic matter concentration was tested with two pig manure effluents, after UASB and after partial oxidation. Addition of the effluent to the artificial wastewater was done gradually in increments, 2%, 3% and 5% for UASB-post-digested effluent and 5%, 7%, 10% and 12% for partially oxidized effluent. When no ammonia removal was detected, the experiment was terminated.

#### 2.3. Analytical methods

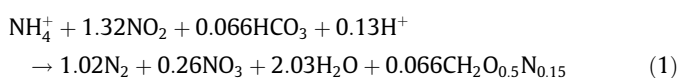
COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, and NO<sub>3</sub><sup>-</sup>-N, were measured according to APHA (Standard Methods for the Examination of Water and Wastewater, 2001).

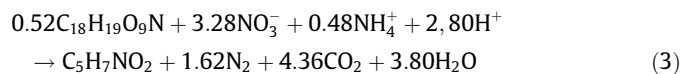
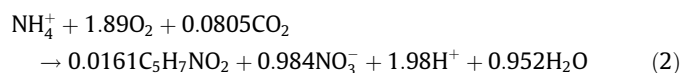
#### 2.4. Fluorescence *in situ* hybridization (FISH)

Fluorescence *in situ* hybridization (FISH) was used to study the anammox community dynamics. An Amx 368 probe targeting all known anammox bacteria was used. Hybridization procedures were followed as given by Hugenholtz et al. (2001). FISH images were analyzed with an epifluorescence microscope and digital image analyzer (Image-Pro Plus 5.1 software).

#### 2.5. Mass balance calculations

Based on nitrogen mass balance over the entire system, taking into consideration the different nitrogen conversion processes possible, the removal of ammonium was established. The nitrogen conversion processes considered were: anammox process (Eq. (1)), autotrophic nitrification (Eq. (2)) and heterotrophic denitrification process (Eq. (3)).





It was assumed that the oxygen concentration was  $8.7 \text{ mg L}^{-1}$ , which is the saturated dissolved oxygen concentration in fresh water at 1 atm,  $22^\circ\text{C}$ .

### 3. Results and discussion

#### 3.1. Ammonium removal from UASB-post-digested effluent

An UASB reactor was chosen in our experiments due to the high stability of this type of reactor for anammox process performance (Jin et al., 2008). The granulation process of anammox sludge was generally evaluated by the particle size distribution. Granules of smaller size (0.3–0.6 mm) dominated the upper portion of UASB sludge bed while the granules of larger size (0.8–2.00 mm) occupied the lower portion of reactor sludge bed. Compared to HRT of different anammox reactor configurations ranged from 3 h for anaerobic biological filtrated reactor, ABF (Isaka et al., 2006), through 12–16 h for UASB and upflow stationary fixed film reactor, USFF (Jin et al., 2008), up to 1 d for sequencing batch reactor, SBR (Third et al., 2005), HRT of our UASB reactor system was chosen to be a bit higher (2.1 d) with respect to treat high strength organic residue. After a starting up period, with synthetic wastewater as feed, UASB-post-digested effluent ( $4.7 \text{ g COD L}^{-1}$ ) was gradually introduced to the feed of the anammox UASB reactor. An addition of 2% (v/v) and 3% (v/v) of UASB-post-digested effluent (organic load of  $95 \text{ mg COD L}^{-1}$  and  $142 \text{ mg COD L}^{-1}$ , respectively) resulted in very high ammonium removal ( $92 \pm 4.9\%$  for 2% (v/v) and  $80 \pm 7.8\%$  for 3% (v/v) effluent addition, respectively). Ammonium removal fell sharply to 0% when 5% (v/v) UASB-post-digested effluent was added (organic load of  $237 \text{ mg COD L}^{-1}$ , corresponding to loading rate of  $112 \text{ mg COD L}^{-1} \text{ day}^{-1}$ ) (Fig. 1). This addition resulted in decrease of the  $\text{NO}_2^- \text{-N}:\text{NH}_4^+ \text{-N}$  ratio, from 1:0.67 to 1:0.33 for addition of 2% and 5% post-digested effluent, respectively. Results obtained indicated that the chemical composition of the UASB-post-digested effluent was not suitable for optimal performance of the anammox process, as the  $\text{NO}_2^- \text{-N}:\text{NH}_4^+ \text{-N}$  ratio for the best anammox process performance obtained by Strous et al. (1999), was 1:1.32. UASB-post-digested effluent had relatively high ammonium levels (Table 3) resulting in an  $\text{NO}_2^- \text{-N}:\text{NH}_4^+ \text{-N}$  ratio much lower than the reported optimum for anammox. As anammox bacteria are often inhibited by sulphide

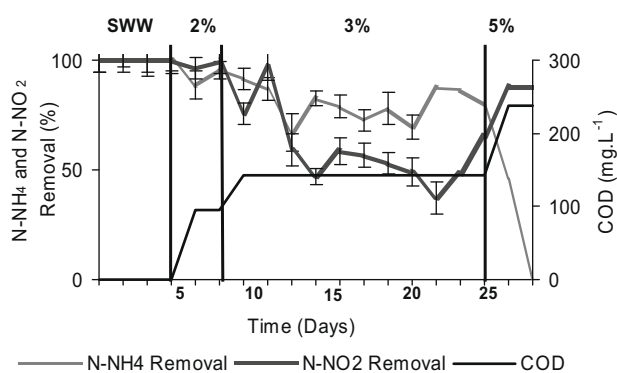


Fig. 1.  $\text{NH}_4^+ \text{-N}$  and  $\text{NO}_2^- \text{-N}$  removal during gradual implementation of the effluent after UASB-post-digestion. Error bars represent standard deviation from a triplicate sampling analysis.

concentrations above 1 mM (Dapena-Mora et al., 2007), another reason for process inhibition could be sulphide formation due to some activity of sulphate reducers naturally presented in pig manure. In respect to organic load, several studies have reported that presence of organic matter combined with high concentration of nitrite, negatively affected anammox bacteria due to the existing competition between anammox bacteria and heterotrophic denitrifiers (Ahn et al., 2004; Chamchoi et al., 2008; Dong and Tollner, 2003; Jianlong and Jing, 2005; Tal et al., 2006). In mixed culture environment anaerobic ammonia oxidizers are always in competition with heterotrophic denitrifiers for nitrite. When enough organic matter is available, anammox bacteria are not longer able to outcompete denitrifiers due to difference in the growth rates for both groups of microorganisms. In our study, mass balance for nitrogen showed that  $19.9 \pm 0.24\%$  and  $11.3 \pm 1.3\%$  of  $\text{NH}_4^+ \text{-N}$  were removed by anammox process when organic loads of UASB-post-digested effluent  $95 \text{ mg COD L}^{-1}$  and  $142 \text{ mg COD L}^{-1}$ , respectively were applied (Table 2). Other process, responsible for removal of the major part of the ammonium, such as heterotrophic denitrification, was also involved (Table 2).  $7 \pm 0.24\%$ ,  $6 \pm 0.96\%$  and  $10 \pm 0\%$  of  $\text{NH}_4^+ \text{-N}$ , was removed via heterotrophic denitrification for organic loads of  $95 \text{ mg COD L}^{-1}$ ,  $142 \text{ mg COD L}^{-1}$  and  $237 \text{ mg COD L}^{-1}$ , respectively.

Anammox bacteria have a very slow growth rate compared to heterotrophic denitrifiers (Strous et al., 1999). According to Kang and Wang (2006), removal of  $\text{NH}_4^+ \text{-N}$  and  $\text{NO}_2^- \text{-N}$  is controlled by the COD concentration in the reactor. Results obtained clearly showed that anammox activity decreased and heterotrophic denitrification increased when the organic loading is increased. Similar findings were previously reported for nitrogen removal from animal waste treatment water (Waki et al., 2007).

Aerobic nitrification (Eq. (2)) was to some extent involved in ammonium removal due to air entering the reactor system during feeding or sampling (data not shown).

Physical processes, such as ammonia volatilization or ammonia stripping, could have been involved. Ammonia stripping at moderate temperature was previously observed. High ammonia removal (more than 90%  $\text{NH}_3 \text{-N}$  reduction) by ammonia stripping was reported by Liao et al. (1995) in swine manure wastewaters at  $20^\circ\text{C}$ .

#### 3.2. Ammonium removal from partially oxidized effluent

A partial oxidation of the UASB-post-digested effluent resulted in a 51% COD reduction, from  $5 \text{ g COD L}^{-1}$  to around  $2.5 \text{ g COD L}^{-1}$ , due to activity of heterotrophic aerobic microorganisms. 83%  $\text{NH}_4^+ \text{-N}$  reduction, from  $3.78 \text{ g NH}_4^+ \text{-N L}^{-1}$  to  $0.67 \text{ g NH}_4^+ \text{-N L}^{-1}$  was also registered.

The addition of 5% (v/v) of partially oxidized effluent (organic load of  $121 \text{ mg COD L}^{-1}$ ) resulted in high ammonium removal (up to  $98.5 \pm 0.8\%$ ), compared with ammonium removal for UASB-post-digested effluent ranged between  $92 \pm 4.9\%$  and

Table 2  
Participation of different processes for ammonium removal from effluent after UASB-post-digestion.

% (v/v) of UASB-post-digested effluent added to SW	Effluent COD ( $\text{mg L}^{-1}$ )	% Ammonia removal $\pm$ SD*			
		Anammox	Denitrification	Nitrification	Other
0	0	$48.20 \pm 9.09$	$2.98 \pm 0.02$	$5.56 \pm 1.04$	$39.84 \pm 11.28$
2	95	$19.98 \pm 0.24$	$7.14 \pm 0.21$	$3.18 \pm 0.19$	$69.70 \pm 0.64$
3	142	$11.32 \pm 1.29$	$6.32 \pm 0.96$	$3.36 \pm 0.62$	$79.22 \pm 2.22$
5**	237	0.00	9.61	5.08	85.31

\* SD represents standard deviation from a triplicate experiment.

\*\* When no ammonia removal was detected the reactor was stopped, so standard deviation was not achieved in this case.

80 ± 7.8% for 2% and 3% (v/v) effluent addition, respectively. As high concentration of free ammonia was previously proven to be inhibitory for anammox reaction (Waki et al., 2007), partial oxidation of ammonia to nitrite in our study definitely facilitate anammox reaction.

The feeding rate was increased gradually corresponding to organic loads of 169 mg COD L<sup>-1</sup>, 242 mg COD L<sup>-1</sup> and 290 mg COD L<sup>-1</sup> (7%, 10% and 12% (v/v) of partially oxidized effluent), small steps to permit the anammox bacteria to adapt to higher organic loads. Ammonium removal ranged between 83% and 86% when 169 mg COD/l and 242 mg COD/l was added. When organic load of 290 mg COD L<sup>-1</sup> (loading rate of 136 mg COD L<sup>-1</sup> day<sup>-1</sup>) was applied, the ammonium removal was totally absent (Fig. 2).

On average, 98.9% of nitrite removal was achieved during all the experiments (Fig. 2). NO<sub>2</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N ratios obtained were 1:1.23, 1:1.16 and 1:1.13 when organic loads of 169 mg COD L<sup>-1</sup>, 242 mg COD L<sup>-1</sup> and 290 mg COD L<sup>-1</sup> were applied, respectively. This ratio was very close to the theoretical NO<sub>2</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N = 1:1.32 for anammox (Strous et al., 1999).

When organic load of 290 mg COD L<sup>-1</sup> was applied, a ratio of 1:2.56 was obtained. In this case the heterotrophic denitrification was the major reaction involved in ammonium removal. 22.5% of NH<sub>4</sub><sup>+</sup>-N was removed by heterotrophic denitrifiers (Table 3). Nitrate consumption was also found to be much higher than in the rest of the experiments (data not shown). Similar findings about dominance of heterotrophic denitrification over the anammox process, were reported previously by Jetten et al. (1999). Results from the mass balance (Table 3) showed that the anammox process performance improved by up to 3 times. 33 ± 1.2% and 41.8 ± 3.4% of NH<sub>4</sub><sup>+</sup>-N was removed by the anammox process for

organic loads of 121 mg COD L<sup>-1</sup>, and 169 mg COD L<sup>-1</sup>, while 11.3 ± 0.3% of NH<sub>4</sub><sup>+</sup>-N was removed for addition of UASB-post-digested effluent with organic load of 142 mg COD L<sup>-1</sup>. Partial oxidation decreased ammonia concentration, which resulted in final NO<sub>2</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N ratios very close to the theoretical ratio for optimal anammox process performance.

As expected, when more partially oxidized effluent was added, the participation of the anammox process in the total ammonium removal decreased from approximately 30–0% (no anammox ammonia removal), when partially oxidized effluent with organic load of 290 mg COD L<sup>-1</sup> (Table 3) was implemented. On the other hand, the denitrification part in total ammonium removal increased from approximately 9.3% for 242 mg COD L<sup>-1</sup> added to 22.5% for 290 mg COD L<sup>-1</sup> of partially oxidized effluent added. When 290 mg COD L<sup>-1</sup> (12% of partially oxidized effluent) was added, no ammonium removal was detected, so the anammox reaction was completely inhibited. Inhibitory level of COD detected in this study was lower compared to inhibitory COD level of 300 mg COD L<sup>-1</sup> previously reported by Chamchoi et al. (2008).

A change in the physiological characteristics of the biomass was observed. A biomass aggregation was registered when a concentration of 290 mg COD L<sup>-1</sup> was introduced to the reactor. The biomass settled and began to turn black, until the ammonium removal disappeared. This finding indicates that anammox communities decreased while denitrifiers increased, due to change in the environmental conditions (in this case the addition of organic matter). Ammonia removal by denitrification was 22.5% from total NH<sub>4</sub><sup>+</sup>-N removal, while ammonia removal via anammox was zero. This may be due to the fact that denitrifiers have a higher growth yield (yield coefficient of heterotrophic denitrifiers; Y = 0.3) compared to anammox bacteria (Y = 0.066 ± 0.01) (Strous et al., 1999). Moreover, denitrification reactions are thermodynamically more favorable than ammonia oxidation reactions (Ahn et al., 2004).

FISH analyses (data not shown) revealed that there was a reduction in the number of anammox bacteria when an effluent with organic load of 290 mg COD L<sup>-1</sup> was applied, while a large amount of anammox cells were found when an organic load of 121 mg COD L<sup>-1</sup> (5% of partially oxidized effluent) was applied.

### 3.3. Organic matter concentration vs. ammonium removal via anammox

Fig. 3 shows the effect of organic loading on ammonium removal for both effluents – UASB-post-digested and partially oxidized. In order to quantify the effect of organic loading on ammonium removal more precisely, a COD inhibitory organic load threshold concentration was defined when ammonium removal dropped to around 80%. Results obtained showed that up to

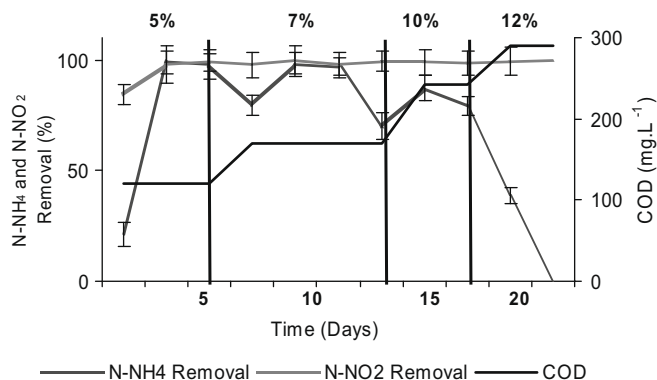


Fig. 2. NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N removal during gradual implementation of the effluent after partial oxidation. Error bars represent standard deviation from a triplicate experiment.

Table 3  
Participation of different processes for ammonium removal from effluent after partial oxidation.

% (v/v) of partially oxidized effluent added to SW	Effluent COD (mg L <sup>-1</sup> )	% Ammonia removal ± SD*			
		Anammox	Denitrification	Nitrification	Other
5	121	33.23 ± 1.23	13.42 ± 4.68	5.74 ± 0.48	47.61 ± 6.39
7	170	41.75 ± 3.35	14.84 ± 2.73	7.13 ± 0.69	36.28 ± 6.77
10	242	29.97 ± 1.07	9.31 ± 0.32	6.24 ± 0.11	54.47 ± 1.5
12**	290	0.00	22.52	13.63	63.85

\* SD represents standard deviation from triplicate experiment.

\*\* When no ammonia removal was detected the reactor was stopped, so standard deviation was not achieved in this case.

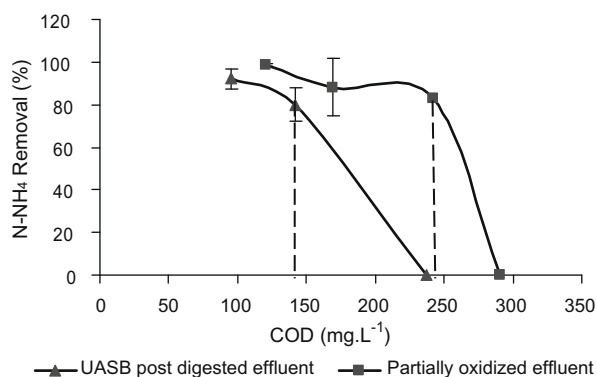


Fig. 3. Effect of organic matter concentration on ammonium removal. Error bars represent standard deviation from a triplicate experiment. Dotted lines show COD threshold inhibitory levels for each effluent.

threshold concentrations of 142 mg COD L<sup>-1</sup> for UASB-post-digested effluent and 242 mg COD L<sup>-1</sup> for partially oxidized effluent, almost no effect of organic loading on ammonia removal was registered as ammonium removal was above 80% (Fig. 3). This finding indicates that although organic matter inhibited the anammox process performance in both cases-post-digested and partially oxidized effluent, a partial nitrification improved the ammonium removal when a high concentration of organic matter is presented in the effluent.

#### 4. Conclusions

Organic loadings negatively affected anammox process performance, as confirmed by low ammonium removal rates obtained. Loading rates above 112 mg COD L<sup>-1</sup> day<sup>-1</sup> for UASB-post-digested effluent and above 136 mg COD L<sup>-1</sup> day<sup>-1</sup> for partially oxidized effluent, inhibited ammonium removal and decreased anammox bacterial numbers, due to denitrifier competition. Anammox and denitrification always occurred simultaneously showing that both processes could coexist in the same environment. So, environmental conditions (COD, nitrite, nitrate, ammonium, pH, and temperature) have to be controlled to get a good balance between anammox and denitrification communities.

In this study we demonstrated that livestock wastewaters can be successfully treated by the anammox process. However, the COD concentration in the wastewaters treated by anammox in full-scale plants determines whether anammox or denitrification would be the dominant route for ammonia removal.

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