



# Is the CANON reactor an alternative for nitrogen removal from pre-treated swine slurry?

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## ABSTRACT

The use of the completely autotrophic nitrogen removal over nitrite (CANON) process as a post-treatment for nitrogen removal from anaerobically pre-treated swine slurry is studied in the present work. The ammonium removal, under oxygen-limited conditions, in a system with anammox bacteria mainly in the form of granules and aerobic ammonium oxidizing bacteria mainly as dispersed biomass was researched in an air pulsing sequencing batch reactor operated at room temperature. The achieved nitrogen removal rate was of 0.46 kg N/(m<sup>3</sup> d) treating 300 mg NH<sub>4</sub><sup>+</sup>-N/L with values of nitrogen removal efficiencies around 75%.

The presence of slowly or non-biodegradable organic matter (from 260 to 45 mg COD/L) did not affect the operation of the process. By means of the FISH technique, *Nitrosomonas* were detected as the majority of ammonia oxidizing bacteria in the sample, and *Candidatus "Brocadia fulgida"* and *Candidatus "Brocadia anammoxidans"* as the anammox bacteria.

The comparison of this aerobic process with other post-treatments for effluents from anaerobic digesters showed that the CANON process is a promising alternative to remove nitrogen from effluents generated in pig farms.

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## 1. Introduction

Nowadays, pig farming is carried out in intensive units located in concentrated spots distributed in many regions in Europe. The excess of swine wastes spread on land surfaces causes severe pollution problems and forced the authorities to strengthen and increase the number of the environmental regulations, concerning the application of the produced manure as direct fertilizer on agricultural land, which forced the pig farms to treat part or all of their produced wastewater.

Energy policies of the European Union are turning to the application of anaerobic digestion for the treatment and energy recovery of wastes from the pig farms characterized by high pollutants concentrations. With these regulations, the aim of reducing emissions of volatile organic compounds, controlling odors, mineralizing nutrients, improving its fertilizing properties and the recovery of energy by the biogas production can be achieved. Therefore, the anaerobic digestion is widely used to remove organic matter from high strength wastewaters like swine slurry with relatively low sludge production and energy needs [1]. This process can be carried out

either in reactor systems operated at mesophilic conditions or in deep earthen outdoor basins (anaerobic lagoons covered for recovery of greenhouse emissions). The drawback of this process is that the organic nitrogenous compounds contained in the waste, such as proteins, aminoacids or urea, are mainly converted into ammonium which is not further degraded in anaerobic conditions.

Currently, European Nitrate Directive (EC/91/676) has established the maximum load of nitrogen to be spread by year on lands as 170 kg of nitrogen per hectare. Since the amount of land to spread the N-rich effluent generated in anaerobic digesters is limited, it is necessary to establish a treatment for nitrogen removal [2]. In this way, the percentage of reduction of nitrogen content is the percentage of reduction of the acreage necessary if the load limits are not exceeded.

Thus, sustainable solutions regarding nitrogen removal from swine effluents are under study. For example, anammox (anaerobic ammonium oxidation) based processes that were proposed as an alternative to remove ammonium from high nitrogen loaded wastewater with low organic matter content in both full and laboratory scale reactors [3]. A partial nitrification step, where 55% of the ammonium content is oxidized into nitrite, is needed in these processes. This can be performed using two different reactors (e.g. SHARON process (single reactor system for high activity ammonium removal over nitrite) for partial nitrification plus enriched

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anammox reactor) or using a single one reactor by means of the so-called CANON process (completely autotrophic nitrogen removal over nitrite) with a co-culture of ammonia oxidizing bacteria (AOB) and anammox bacteria.

Partial nitrification and anammox treatment in a two-units configuration has been successfully carried out treating swine slurry from anaerobic digesters, working at temperatures that ranged between 30 and 40 °C [4,5] since the optimal temperature for anammox bacteria is 35 °C [6]. Albeit, it was recently shown that the anammox and CANON processes could be operated at temperatures around 20 °C [7]. This fact opens the possibility of applying CANON technology for the treatment of swine slurry at moderate temperature (e.g. as a post-treatment of the effluents from anaerobic lagoons).

Therefore, the objective of this work was to study the feasibility of the application of the CANON process at moderately low temperature as a post-treatment to remove nitrogen from swine slurry characterized by a low C/N ratio. The performance of the process in an air pulsing sequencing batch reactor will be evaluated in terms of nitrogen removal efficiency, paying special attention to the characteristics of the biomass and the effect of remaining organic matter.

## 2. Materials and methods

### 2.1. Analytical methods

Analytical determination of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), conductivity and pH was carried out according to the standard methods [8]. Soluble chemical oxygen demand (COD) was determined by a semi-micro method [9]. The morphology and size distribution of the granules were measured regularly by using an image analysis procedure with a stereomicroscope (Stemi 2000-C, Zeiss).

Bacterial populations were identified and quantified by the Fluorescence in situ Hybridization (FISH) technique according to the protocol described by Daims, [10]. Biomass samples from the reactor were collected, disrupted and fixed with 4% paraformaldehyde solution. Hybridization was performed with probes: EUB338mix, NSO190, NEU653 (for AOB identification), PLA46, Amx820, BAN162 and BFU613 (for anammox identification). Details on oligonucleotide probes are available in Table S1 (Supporting information). The used probes for in situ hybridization were 5' labelled with the fluorochromes FITC, Cy3 and Cy5. Fluorescence signals were observed under an epifluorescence microscope (Axioskop 2, Zeiss) and a confocal laser scanning microscope (TCS-SP2, Leica).

### 2.2. Reactor description, operational conditions and feeding composition

A laboratory scale air pulsing sequencing batch reactor with a working volume of 1.5 L and a volumetric exchange ratio fixed at 50% was used (detailed description in Supporting information and Fig. 1S). Air was supplied by a diaphragm pump (Laboport N86, KNF) with an electrovalve that controlled the pass of air at a constant frequency of 0.09 1/s, meaning that air was supplied in repeated cycles of 1 s of flow and 10 s without flow. A ceramic air diffuser located at the bottom of the reactor promoted the formation of small bubbles. The air pulses provided good mixture inside the reactor and maintained a suitable dissolved oxygen (DO) concentration to carry out the CANON process. The DO was measured periodically with a sensor (Oxi 330i, WTW) and was regulated manually by changing the air volume injected in each pulse and keeping the frequency of pulsation constant. The air flow rate ranged between 2.0 and 4.2 L/min during the experiment.

The reactor was operated at room temperature (18–24 °C) and without pH control, that was, inside the reactor, around of  $7.7 \pm 0.2$ .

Operational cycles of 360 min were distributed as follows: 345 min of feeding and aeration, 10 min of settling and 5 min of effluent withdrawal. The hydraulic retention time was fixed at 0.5 d with a feeding flow rate of 2.18 mL/min.

In previous works (data not shown) the reactor was fed during 350 d with the effluent from the anaerobic sludge digester of the urban wastewater treatment plant of Lugo (Spain); the operational data and conditions are gathered in Vázquez-Padín et al., [11]. The influent was changed (represented from day 0 on in the figures) and the system was fed with diluted swine slurry previously treated by an aerobic granular sludge reactor to remove organic matter [12]. The pre-treated swine slurry was collected and settled daily and kept refrigerated at 4 °C. The composition in terms of nitrogen and organic matter was the following:  $245 \pm 16 \text{ mg NH}_4^+ \text{-N/L}$ ,  $4.1 \pm 1.2 \text{ mg NO}_2^- \text{-N/L}$ ,  $3.3 \pm 0.8 \text{ mg NO}_3^- \text{-N/L}$  and  $420 \pm 60 \text{ mg COD/L}$ .

## 3. Results and discussion

### 3.1. Nitrogen removal

A conservative operational strategy was applied to introduce the pre-treated swine slurry as feeding media and avoid the inhibition events of the anammox bacteria by oxygen and/or nitrite concentrations. Therefore, the efficiency of this CANON system was always limited by the amount of air supplied and the dissolved oxygen concentration in the bulk liquid was maintained between 0.2 and 0.4 mg  $\text{O}_2/\text{L}$ . The pulsed air volume was used as a control parameter to maintain the suitable DO concentration in a similar way as proposed by Wett [13]. Moreover, the application of a pulsating flow would decrease the aeration costs compared with a continuously aerated CANON system [7].

While ammonium was left in the effluent and since nitrite concentration (lower than  $4 \text{ mg NO}_2^- \text{-N}$ ) was far away from the inhibitory levels for this compound ( $30\text{--}150 \text{ mg NO}_2^- \text{-N}$  [14]), the pulsed air volume was increased to favor the ammonium oxidation. This strategy did not cause an increase of DO and nitrite concentrations in the liquid media because of its fast consumption by AOB and anammox bacteria, respectively, but raised the nitrogen removal rate. In this way the ammonium concentration in the effluent was reduced with the increase of the air flow rate in the air pulses (Fig. 1).

Ammonium concentration in the influent of the CANON system was around  $250 \text{ mg N/L}$  and the nitrogen removal rate (NRR) was of  $0.41 \text{ kg N}/(\text{m}^3 \text{ d})$  around day 50 of operation (see Supporting information for calculations). At this point, the ammonium concentration in the influent was of  $241 \pm 3 \text{ mg NH}_4^+ \text{-N}$  and the concentration in the effluent was of  $16.2 \pm 1.0 \text{ mg NH}_4^+ \text{-N/L}$ ,  $2.5 \pm 0.3 \text{ mg NO}_2^- \text{-N/L}$  and  $22.3 \pm 0.3 \text{ mg NO}_3^- \text{-N/L}$ . Hence the removal efficiencies were of 93% and 83% as ammonium and total nitrogen, respectively.

A failure in the aeration electrovalve on day 56 caused an increase of the dissolved oxygen concentration up to  $5 \text{ mg O}_2/\text{L}$ . This caused the inhibition of the anammox bacteria and, therefore, the accumulation of nitrite ( $30 \text{ mg NO}_2^- \text{-N/L}$ ) inside the reactor. In order to recover the system, the ammonium concentration, by dilution with tap water, and the volume of the pulsed air were diminished from days 57 to 63. The previous operational conditions were restored in 30 d and the nitrogen removal capacity increased continuously until the end of the experiment.

At the end of the operational period, the NRR was around  $0.46 \text{ kg N}/(\text{m}^3 \text{ d})$  when treating  $300 \text{ mg NH}_4^+ \text{-N/L}$ , with nitrite and nitrate concentrations in the effluent lower than 3 and  $10 \text{ mg N/L}$ , respectively and removal efficiencies of 80% and 77% as ammonium

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