Development of a New Generation Low Cost Treatment of Ammonia for Livestock Effluents Using Anammox and Nitritation

Airton Kunz[1], Ricardo Steinmetz[1], Matias Vanotti[2], Ariel Szogi[2] and Hugo Moreira Soares[3]
Embrapa Swine and Poultry, Concordia, SC, Brazil. airton@cnpsa.embrapa.br
[2] USDA-ARS Coastal Plains Research Center, Florence, South Carolina, USA.
[3] Federal University of Santa Catarina, Florianopolis, SC, Brazil

Introduction

The environmental problems due to confined animal feeding operation (CAFOs) are under concern around the world in the last years. Among CAFOs, the swine production wastewater are very significant due to its high nutrient concentration, mainly phosphorous and nitrogen (Kunz et al., 2005). Specifically for nitrogen, its high concentration in water, contribute to the toxicity of life organisms, depletion of dissolved oxygen, risks to public health and, associated with phosphorous, to the eutrophization phenomena (Bitton, 2005).

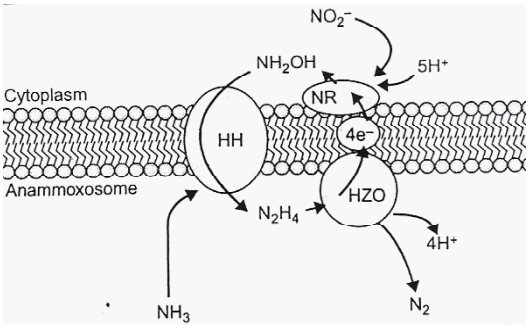
Based in these facts, when it is no sufficient soil to use it as a fertilizer, the necessity of treatment is necessary. The main problem is that nitrogen is an specie that is very difficult to remove from swine manure by classical treatment processes. Nitrification/denitrification processes, have some limitations to be used to swine manure, as an inhibition by some species that are formed during the degradation process needing a high control of nitrification/denitrificaton.

New paths to remove nitrogen from effluents have been developed in last years, as the process of anaerobic ammonia oxidation (ANAMMOX). In this process ammonio (NH_4^+) are directly oxidized to gaseous nitrogen (N_2) and nitrite (NO_2^-), is the final electron acceptor. The process is autotrophic and use CO_2 as a carbon source. Nitrate (NO_3^-) is the by-product and the process global stechiometry is described bellow (Strous et al. (1998)):

$$1NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0,5}N_{0,15} + 2.03H_2O$$

In this metabolic rote, the nitrite is reduced to hydroxylamine that react with ammonium producing gaseous nitrogen. Figure 1 presents a suggestion of anammox reactions in cytoplasm. Part of this reactions occur inside the bacteria cellular compartment named Anammoxosome (Jetten et al. 2000).

Figure 1: Ammonium anaerobic oxidation mechanism. NR= nitrite reducing enzyme; HH= hydrazine hydrolase; HZO= hydrazine oxidacing enzyme (AHN, 2006)



It were identified sludges with anammox activities around the word, (Den Camp et al, 2006, Vanotti et al., 2006) showing a development characteristics of microrganisms in a different habitats.

The big challenge to anammox good activity is to supply ammonium and nitrite in the stoichiometric microorganism necessity. Anammox bacteria use ammonia and nitrite. Therefore, nitrification as the preceding treatment step of Anammox should be partial in that only about 50% of the ammonia needs to be oxidized, and the oxidized N should be mostly nitrite (by preventing the conversion of nitrite to nitrate). Although various approaches have been proposed for achieving partial nitritation, there is still a need for more effective and economical processes. The SHARON process is an effective partial nitritation process. It is based on the higher growth rate of ammonia oxidizing bacteria (*Nitrosomonas* sp.) relative to nitrite oxidizing bacteria (*Nitrobacter* sp.) obtained at high process temperatures (> 30 ^{o}C) and the subsequent disappearance or washout of *Nitrosomonas* sp. with short retention times. But heating of the water is very expensive and may be impractical in many situations. Another approach for partial nitritation has been the use of low DO concentrations and the higher affinity of *Nitrosomonas* sp. for oxygen leading to accumulation of nitrite. However, maintaining stable nitrite production using the low DO approach has been difficult to achieve in wastewater treatment plants. Our approach for partial nitritation was the inhibition of Nitrobacter to free ammonia using pH control.

One mechanism by which pH affects the rate of nitrification has been proposed by Anthonisen et al. (1976). They proposed that nitrifying bacteria are inhibited by the un-ionized rather than the total ion concentration of ammonia and nitrite. Their studies showed that NH_4^+ oxidation by *Nitrosomonas* sp. is usually inhibited at concentrations of un-ionized nitrous acid (HNO_2) of 0.2 to 2.8 mg L^{-1} and concentration of un-ionized ammonia (NH_3) of 10 to 150 mg L^{-1}, while NO_2- oxidation by *Nitrobacter* sp. is inhibited with NH_3 concentrations in the range of 0.1 to 1.0 mg L^{-1}. I studies of nitrification of swine wastewater, Vanotti and Hunt (2000) obtained effective inhibition of *Nitrobacter* sp. when NH_3 exceeded 2.0 mg L^{-1}. These inhibitory conditions should be relatively easy to obtain in practical treatment of livestock effluents or other high-ammonia effluents because for example a liquid with a total NH_4-N concentration of 230 mg L^{-1} and a pH of 8.3 contains 38.7 mg NH_3 L^{-1}.

Material and Methods

Anammox

Inocculum. The sludge for this study was collected from the bottom of an inactive anaerobic lagoon in experimental swine manure treatment lagoons at Embrapa's Swine and Poultry, Concordia, SC, Brazil.

Acclimation. The sludge was screened, washed with water, and kept in a KNO_3 (100 mg L^{-1}) solution until the denitrification process stopped, that is a good indication that the organic carbon was consumed. After this initial acclimation, the sludge was inoculated in an anammox reactor.

Reactor set-up

The sludge was inoculated at 4 g/L TSS in a 2 L reactor. The process was kept at 35 ^{0}C and pH 7. The feeding medium was prepared according to Shierholt Neto et al. (2006). To remove the interference of oxygen in the process, N_2 was bubbled until dissolved oxygen concentration was 0.5 mg/L. The Hydraulic Retention Time (HRT) was adjusted between 18.5 h and 24 h.

Partial nitrification

Reactor set-up

The reactor consisted of a 5-L aeration tank containing 600 mL of porous PVA beads (Kurarey Co., Japan) used for attachment of the nitrification bacteria. The reactor had a divider to allow up and down circulation of the beads. A 1-mm screen was placed before the outlet port to separate the beads and free cells, and retain the beads inside the reactor. Air was supplied from the bottom of one half of the tank at a flow rate of 0.8 L min^{-1} to ensure full fluidization of immobilized pellets. The diffuser consisted of an aquarium porous stone that provided fine bubble aeration. A variable flow peristaltic pump was used in the continuous flow experiments to feed the wastewater to the nitrification tank. The NH_4-N loading rate was adjusted by varying the flow rates from 2500 to 5000 mL d^{-1}. A pH controller and an injection pump was used to control the process pH using 2 mol L^{-1} NaOH at set points of 7.5, 8.5, 8.7 and 8.9. The reactor was inoculated with 70 mL of nitrifying sludge acclimated to livestock wastewater and operated first under batch mode during approximately 20 days using a fill-and-draw technique to facilitate attachment and growth of a nitrifying biofilm on the surface of the beads. The reactor was then operated under continuous flow with a synthetic medium (Vanotti and Hunt, 2000) containing 700 ppm of NH_4-N. The process pH was increased gradually in the range of 7.5-8.9 to study the pH effect on partial nitritation at low temperatures (22 °C).

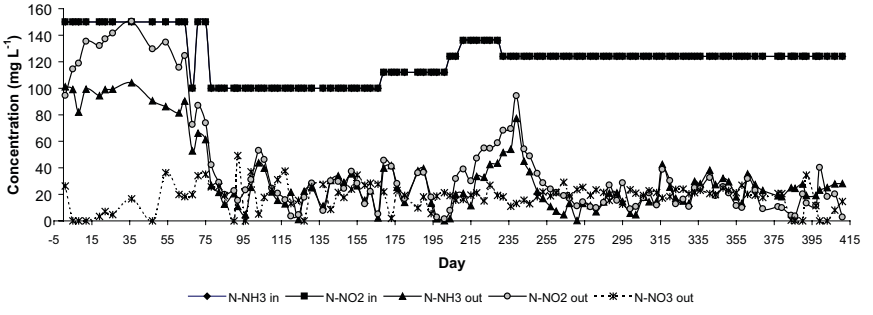
Chemical analyses

All chemical analyses were done according to APHA (1995). Liquid samples from the reactor (in and out) were taken twice a week and analyzed for $N\text{-}NH_3$, $N\text{-}NO_2$, $N\text{-}NO_3$ using Flow Injection Analysis (FIALAB INSTRUMENTS). Alkalinity was determined by titration with H_2SO_4 0.02 mol/L until pH 4.5.

Results and Discussion

The anammox reactor (Figure 2) took around 75 days to start to develop a anammox activity with a fast decrease of nitrite and ammonia concentration in the reactor effluent. The system is working in a very stable conditions for around a year, excepting around the day 235 when an anammox inhibition activity was developed, probably due to the effect of increase in nitrite concentration (Dapena-Mora et al. 2006).

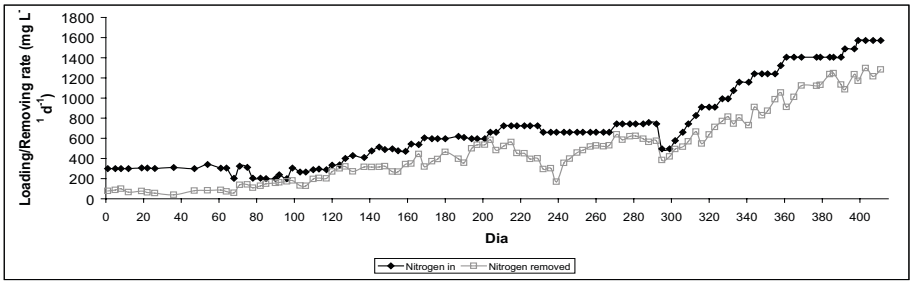
Figure 2: Nitrogen conversion in anammox reactor during the operation time



A high nitrogen removal capacity of anammox reactor was reached (Figura 3) mainly after 300 days of operation when the reactor was feeding with a nitrogen loading rate around 1600 mg L^{-1} d^{-1}. The nitrogen removal efficiency of anammox system is very high when compared to classical N removal system as nitrification and denitrification reactors. This

make possible to conclude that we can have smallller anammox reactors working with high efficiency reducing the cost with reactor constructions.

Figure 3: Nitrogen Loading/removing rate in the anammox reactor



The partial nitrification reactor was stabilized in a few weeks during a batch experiment establishing first of all the complete nitrification process. After this the reactor was operated as a continuos form and the pH was increased. A desired partial nitrification, 50 % ammonia oxidation was reached between pH 8.5 –8.9. This is a important result and make able a connection between anammox and a partial nitrification reactor.

Acknoledgements
This research was part of Embrapa project "Development of nitrogen removal systems in residues with high nutrient load" and USDA-ARS National Program 206: Manure and Byproduct Utilization, ARS Project 6657-13630-003-00D "Innovative Animal Manure Treatment Technologies for Enhanced Environmental Quality." Funding provided by USDA - Foreign Agricultural Service, Scientific Exchange Program is gratefully acknowledged.

References

Ahn, Y (2006). Susteinable nitrogen elimination biotechnologies: A review. Proc. Biochem., vol 41, p. 1709-1721.

APHA. (1995) Standart Methods – For the examination of water and wastewater, 19 ed.

Anthonisen, A.C., Loehr R.C. Prakasam, TB.S, Srinath, E.G. (1976). Inhibition of nitrification by ammonia and nitrous acid. J. WPCF, 48: 835-852.

Bitton, G. Wastewater microbiology. New York: Willey-Liss, (2005).

Dapena-Mora, A et al. (2007). Evaluation of activity and inhibition effects on anammox process by batch tests based on the nitrogen gas production. Enz. Microb. Technol., 40: 859-865.

Den Camp, H. et al. (2006). Global impact and application of the anaerobic ammonium oxidazing (anammox) bacteria. Biochemical Society Transactions. 34: 174-178.

Kunz, A.; Higarashi, M.M.; Oliveira, P.A. (2005). Management and treatment technologies for swine manure studied in Brazil. Cadernos de Ciência e Tecnologia, Brasília, DF, 22: 651-665.

Jetten, M.S.M. et al (1999) The anaerobic oxidation of ammonium. FEMS Microbiol Rev. 22: 421 - 437.

Schierhold Neto, G. F.; Kunz, A.; Vanotti, M.B.; Soares, H.M.; Mattei, R.M. (2006) Aclimatação e acompanhamento da atividade de lodos de efluentes de suinocultura para remoção de nitrogênio pelo processo de oxidação anaeróbia de amônia (anammox). In Proc. XXX Congreso Interamenricano de Engenieria Sanitaria y Ambiental.. BR 04327 219-232. Punta del Este, Uruguay. AIDIS.

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. Appl Microbiol Biotechnol. 50: 589-596.

Vanotti, M. Hunt P.G. (2000). Nitrification treatment of swine wastewater with acclimated nitrifying sludge immobilized in polymer pellets. 43(2): 405-413

Vanotti, M.; Szogi, A.; Kunz, A.; Gonzalez, M.C.G. (2006). Development of anaerobic ammonium oxidation (Anammox) technology using immobilized biomass from swine manure. Proc. 12th Ramiran Int. Conf. Vol. I, Aarhus, DK. 143-146.