Nitrogen removal from digested manure in a simple one-stage process
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ABSTRACT

A process based on partial nitrification and recirculation into the anaerobic digester was studied to remove nitrogen from digested manure and thus reduce enhanced gaseous ammonia emissions due to on-farm biogas production. An anaerobic reactor representing an anaerobic manure digester was fed with a nitrite solution and digested manure liquor. Nitrite was efficiently removed from the influent and ammonium formation was observed first. Ammonium was subsequently eliminated up to a maximum of 90% of the influent concentration, indicating anaerobic ammonium oxidation activity. This activity, however, decreased again and was lost at the end of the 4-month operation period. In a 1.5 L aerobic CSTR that was fed with digested manure liquor, ammonium was efficiently removed from the influent. Nitrite and nitrate formation was observed but mass balances indicated significant N-removal. Accumulation of suspended solids was observed at the end of the experiment suggesting presence of oxygen-free environments. In a second test in a 15 L CSTR where suspended solids sedimentation could be avoided, low N-removal rates were observed in the absence of biofilm carrier elements whereas high N-removal rates were achieved in their presence. A simple one-stage process based on immobilized biomass could therefore be installed downstream of agricultural anaerobic digesters in order to mitigate undesirable gaseous ammonia emissions.

Key words | ammonia emissions, Anammox, biogas, denitrification, partial nitrification

INTRODUCTION

Gaseous ammonia emissions are of increasing environmental concern since they can be responsible for atmospheric acidification, over-fertilization of crops and fragile ecosystems, and they also might result in health impact to humans and animals (Menzi et al. 1997). Anaerobic digestion coupled with biogas utilization is an advantageous agricultural waste management practice. Agricultural biogas production facilities are increasing in number in Switzerland and Europe due to environmental regulations and opportunities in the green energy market. However, while anaerobic digestion improves manure quality and reduces methane emissions, increased ammonium content in digested manure is becoming an issue of concern. Different studies showed that anaerobic digestion of agricultural wastes increases gaseous ammonia emissions by 14–17% due to the mineralization of the organic nitrogen and its volatilization at high pH (Menzi et al. 1997). In Switzerland, agriculture is responsible for up to 90% of the total ammonia emissions, which is equivalent to 46,000 to 51,000 tons per year (Achermann 2004). Others report that livestock is responsible for 64% of anthropogenic NH₃ emissions (Bernet & Beline 2009). In addition, land disposal of digested manure can negatively impact on surface and groundwater due to nitrogen lixiviation and subsequent increasing oxygen demand and eutrophication. Intensification of livestock production and limitation of this production to specific areas in Europe and the United States of America has led to an over-production of liquid livestock effluents and, as a consequence, made it impossible to locally use the produced manure as organic fertilizer (Bernet & Beline 2009). Removal of the valuable nutrient in these effluents is the only option in these areas to minimize adverse environmental impacts.

The conventional strategy for nitrogen removal involves nitrification followed by denitrification. Robustness, easy operation, and cost-effectiveness must be taken into account when a practical application of this kind is proposed for...
agricultural installations. Therefore, one must consider factors such as energy consumption, sludge production, required area, resource recovery and greenhouse gas emissions. The concept proposed for this study was to oxidize ammonium present in digested manure in an aerobic post-treatment bioreactor and recirculate the oxidized effluent in the anaerobic digester for biological nitrogen removal.

Partial nitriﬁcation is an interesting option since it uses less oxygen and less substrate is needed for subsequent deniﬁtrication (Hellinga et al. 1998). It proﬁts from the fact that at temperatures of about 30°C nitrite-oxidizing bacteria grow at slower rates than ammonium oxidizers. At low concentrations of dissolved oxygen (DO), one can achieve nitrite accumulation by simply adjusting dilution rate in a chemostat-like reactor system and thus decreasing nitrite-oxidizers in number by wash-out. Furthermore, higher deniﬁtrication rates can be expected when nitrite rather than nitrate is used as electron acceptor (Abeling & Seyfried 1992).

Simultaneous deniﬁtrication and methanogenesis in one single reactor has been demonstrated as a feasible option for N-removal from piggery wastewater (Bernet et al. 1996; Bernet et al. 2000). Another possibility to eliminate nitrogen from aqueous solutions is anaerobic oxidation of ammonium (Anammox), an autotrophic microbial process which uses nitrite as electron acceptor for ammonium oxidation to produce N2 (Jetten et al. 1998). Coupling Anammox with partial nitrification would lead to even lower oxygen consumption and excess biomass production in the proposed aerobic post-treatment, which implies reduced process energy costs (Strous et al. 1997). If this microbial process can be established in an anaerobic digester, biogas production losses due to consumption of organic matter by deniﬁtrication could also be reduced signiﬁcantly.

The objective of this study was to investigate the feasibility of the proposed aerobic post-treatment of digested manure liquor involving nitritation and recirculation of the effluent, or part of it, into the anaerobic digester, where nitrite is removed by deniﬁtrication or Anammox.

**MATERIALS AND METHODS**

**Substrate source**

Digested cow manure liquor was obtained from a full-scale agricultural biogas facility at Puidoux, Switzerland. The temperature of the 400 m³ digester is kept at 40°C and biogas feeds a combined heat and power 55 kW plant functioning in dual-fuel (biogas-diesel) mode. The digester treats 2,200 tons of cow manure per year and 350 tons of co-substrates such as golf-lawn shearing, slaughterhouse wastes, used oils, and glycerin-rich wastes coming from a biodiesel plant. The liquid fraction of digested manure was collected at the outlet of the dewatering press. The manure liquor was centrifuged in the laboratory at 6,500 × g to minimize clogging problems and to facilitate handling at laboratory scale. The supernatant is referred to as substrate and had the following characteristics: TS = 36.1 ± 0.6 g L⁻¹, VS = 18.7 ± 0.4 g L⁻¹, DOC = 4.5 ± 0.2 g L⁻¹, COD = 14.9 ± 0.6 g L⁻¹, N-NH₄⁺ = 2.5 ± 0.2 g L⁻¹, alkalinity = 1.20 ± 0.2 g CaCO₃ L⁻¹.

**Experimental set-up**

The experimental work was divided into two stages. The first stage investigated the original concept of aerobic-anaerobic post-treatment of digested manure liquor. The second stage aimed at conﬁrming the conclusions and hypothesis from the first stage experiments.

In the first stage, the experimental set-up consisted of two laboratory-scale bioreactors treating digested cow manure liquor (Figure 1). Each reactor was optimized independently and not combined in order to be able to test the feasibility of the anaerobic treatment of the aerobic
reactor effluent independently of the performance of the aerobic reactor.

In a 1.5 L aerated CSTR, partial nitrification of ammonium to nitrite was aimed at. Short hydraulic residence time (HRT) and oxygen-limited conditions were maintained in this reactor to encourage nitrite production and to oxidize only part of the ammonium. Digested manure liquor was downflow fed into the reactor by means of a peristaltic pump. Liquid level was automatically controlled along with pH (7.5) and temperature (30 °C), respectively. Dissolved oxygen (DO) was continuously measured but regulated manually between 1 and 3 mg L⁻¹.

The reactor was inoculated using activated sludge (1.38 g VSS L⁻¹) from a nearby wastewater treatment plant (Morges, Vaud, Switzerland). For the start-up, 10-times diluted digested manure liquor was used, and the reactor was operated at a HRT of 10 days. Volumetric loading rates (VLR) were gradually increased either by reducing HRT or by decreasing substrate dilution.

To test the feasibility of the treatment of the aerobic reactor effluent by denitrification and/or Anammox in the anaerobic digester, an anaerobic 15 L CSTR was set up. It was filled with 90% digested manure and 10% (v/v) of “Anammox” sludge. The “Anammox” sludge came from the rotating biological contactor treating the leachate of a hazardous waste landfill at Koelliken (Switzerland) and in which Anammox activity has been previously shown to occur (Siegrist et al. 1998; Fux et al. 2002). The reactor was fed with diluted digested manure liquor amended with nitrite, simulating the effluent of the aerobic partial nitrification reactor. Continuous feed was guaranteed by means of a peristaltic pump to maintain a HRT of 21 days.

In the second stage, a 15 L aerated CSTR was set up (Figure 1). It was operated like the aerated CSTR of the first stage. After about 100 days of operation in chemostat mode, type K1 biofilm carrier elements developed for the Kaldnes Moving Bed™ Process were added up to 50% fill of the total volume, which resulted in available surface for biofilm formation of 385 m² m⁻³. After batch mode operation for about ten days, continuous feed mode was started again and loading rates stepwise increased.

Chemical analysis

N-NH₄⁺, N-NO₂⁻ and N-NO₃⁻ were measured photometrically using a WTW Photolab S12 spectrophotometer and kits from VWR-MERCK, Dietikon, Switzerland. Chemical oxygen demand (COD) was determined using a HACH DR/2000 direct reading spectrophotometer and kits from HACH-LANGE GmbH, Rheineck, Switzerland. Dissolved organic carbon (DOC) was analyzed with a Shimadzu Total Organic Carbon Analyzer (TOC-5050A). Nitrous oxide (N₂O) was analyzed with a gas chromatograph Varian Star 3400CX equipped with electron capture detector.

**Fluorescence in situ hybridization (FISH)**

Mixed liquor samples of 250 µL from the anaerobic reactor were put into 750 µL of fixative solution (4% formaldehyde in PBS) and incubated at 4 °C for 2 h. Samples were centrifuged, the supernatant removed, and the pellet resuspended in PBS and washed for 5 min. The latter process was repeated once before resuspending the pellet in a 1:1 PBS/Ethanol solution (50:50). Fixed samples were stored at 4 °C. Hybridization was carried out at the Department of Microbiology of Radboud University (Nijmegen, The Netherlands) as described previously (Kuyper et al. 2003).

**RESULTS AND DISCUSSION**

**First stage anaerobic reactor**

In the anaerobic reactor simulating the anaerobic digester, nitrite was efficiently removed right from the beginning (Figure 2). At an early stage, dissimilatory nitrite reduction to ammonium seemed to be responsible for nitrite removal, since ammonium increased. Ahn et al. (2007) reported similar results in a combined anaerobic/aerobic reactor for the treatment of high-strength nitrogen wastewater and linked...
this phenomenon with high influent ammonia concentration.

However, after approximately four weeks, NH₄⁺ started to be removed. The ammonium removal efficiencies steadily increased, suggesting Anammox taking place. NO₂⁻ and NH₄⁺ inlet concentrations were increased simultaneously with no effect on either nitrite or ammonium removal until the end of the second month of operation, when the latter activity reached its peak (Figure 2). Significant nitrogen losses in the treatment of a carbon depleted substrate have also been detected in the system from which part of the inoculum for the anaerobic reactor of this study was collected (Siegrist et al. 1998), and the same biomass has been used for a laboratory-scale Anammox sequencing batch reactor system in which 90% of influent nitrogen load was removed (Fux et al. 2002). Hence, Anammox activity in the anaerobic reactor used here could be expected. FISH analysis on a sample taken on day 105 showed clearly the presence of typical Anammox cells hybridizing with probes specific for the genera Kuenenia and Brocardia. Increasing loading rates from day 65 on apparently affected ammonium degradation without any effect on nitrite removal, and at the end of the experiment no ammonium removal was observed anymore.

First and second stage aerobic reactors

After one month of recurrent pH regulation problems accompanied by two reinculations of the first stage aerobic reactor, ammonium removal efficiencies rapidly increased and stayed between 95 and 100% at a constant loading rate and ammonium concentrations around 200 mg L⁻¹ in the 1.5 L chemostat reactor (Figure 3). Loading rate was then increased, either by shortening HRT or by lowering dilution of the substrate. Ammonium loading rates of up to 600 mg L⁻¹ d⁻¹ were reached, which corresponds to an ammonium concentration of 1,100 mg L⁻¹. At this stage of the experimental study (HRT = 1.5 days, undiluted substrate), reactor performance seemed to be quite sensitive to pH shifts and shock loads.

Approximately nine weeks after reinculation, nitrite production rates became higher than nitrite oxidation rates (Figure 3) and reached maximum values of up to 200 mg L⁻¹ d⁻¹, slightly lower than previously reported rates (Fux et al. 2002). At the final stage of the experimental study, nitrate production was almost negligible, and nitrite accumulation was achieved at maximum ammonium loading rates.

Mass balances showed that a large portion of the influent nitrogen was missing in the effluent, which was taken as nitrogen removal (Figure 3). Hence, the aforementioned production rates were probably underestimates and NO₂⁻ as well as NO₃⁻ were further degraded into a gaseous product. Outlet gases were passed through an acid solution and no pH changes were detected (results not shown). Therefore, ammonia stripping was excluded. Two explanations are proposed to explain this phenomenon of incomplete mass balances. The first one involves production of nitrogen gases by denitrification and/or Anammox in anoxic zones inside the reactor. Upon emptying the reactor, a considerable deposition of suspended solids was observed on the reactor bottom and walls, probably due to insufficient mixing and the high fine solids contents of the digested manure. This deposition certainly allowed biomass retention, creating favorable conditions for slow growing bacteria and anoxic conditions due to oxygen transfer limitations. These conditions would favor either Anammox or classical denitrification, provided that, for the latter option, sufficient biodegradable carbon source is present (Nielsen et al. 2005).

The second explanation involves denitrification in the bulk liquid by facultative heterotrophic bacteria in a process called aerobic denitrification (Thomsen et al. 1993; Robertson et al. 1995) or by nitrifiers under oxygen stress (Blackmer et al. 1980; Poth 1986; Bock et al. 1995). Some facultative heterotrophic bacteria have been shown to be capable of denitrification in the presence of oxygen, using nitrate instead of oxygen as electron acceptor (Robertson et al. 1995), and some of these organisms are also able to perform nitrification. C/N ratio seems to play a major role in occurrence of aerobic denitrification and heterotrophic nitrification. At higher ratios, cultures could become nitrogen limited, whereas lower ratios could lead to exhaustion.

![Figure 3](https://iwaponline.com/wst/article-pdf/63/9/1991/445046/1991.pdf)
of the carbon source (Joo et al. 2005). Dinitrogen production by nitrifiers was successfully implemented in the so-called OLAND system (Oxygen-Limited Autotrophic Nitrification-Denitrification), where 40% of the influent nitrogen was removed as N₂ (Kuai & Verstraete 1998).

In order to discriminate between the two possible explanations, a well mixed 15 L reactor was operated as continuous flow CSTR reactor during the second stage of this study. During operation in the absence of biofilm carrier elements, ammonium was efficiently transformed into nitrite and nitrate at a loading rate of approximately 250 mg L⁻¹ d⁻¹ (Figure 4). After addition of the biofilm carrier elements and batch mode operation for two weeks, nitrogen removal started to increase and reached removal efficiencies of up to 80% at ammonium loading rates of 350–400 mg L⁻¹ d⁻¹ and ammonium transformation efficiencies of 97%. Visual examination of the carrier material clearly showed that a biofilm had formed. These results indicated that the first hypothesis for nitrogen removal involving denitrification in anoxic zones inside the reactor was the explanation for the nitrogen removal observed in the first stage experiments. The results showed in addition that efficient nitrogen removal was possible in a single reactor with a rather short HRT. Hence, depending on the needs for manure post-treatment, a simple CSTR reactor can be installed downstream of the anaerobic digester with or without biofilm carrier material treating part of or all the digester effluent. With biofilm carrier material, nitrogen removal can be achieved; without carrier, ammonium oxidation is achieved without removing the nutrient.

Nitrous oxide (N₂O), a greenhouse gas with high global warming potential (GWP), could be, in some cases, the final product of nitrification and denitrification (Robertson et al. 1995). Reactor off-gas measurements showed that at maximum 1.3% of the ammonium load was emitted as N₂O, which is in the same range as reported for other nutrient removal wastewater treatment systems (Kampschreur et al. 2009). Whether these are acceptable levels from a decision-maker’s point of view is difficult to assess, but it certainly represents a potential drawback of the proposed one-reactor process for nitrogen removal from digested manure.

CONCLUSIONS

The first stage experiments showed that nitritation of ammonium present in digested manure liquor is feasible in an aerobic CSTR and that nitrite added to digested manure liquor was efficiently removed in an anaerobic CSTR without adding additional carbon source. However, not only nitritation occurred in the first stage aerobic CSTR but also nitrogen removal, possibly in anaerobic parts of the reactor. This hypothesis was confirmed in the second stage, where an aerobic moving bed reactor was operated. Ammonium removal efficiencies higher than 97% were obtained and nitrogen removal efficiencies of up to 80% were achieved. Hence, if nitrogen has to be removed from digested manure liquor in order to mitigate gaseous ammonia emissions from agriculture, the anaerobic digester effluent could be treated in a simple one-stage post-treatment process as shown here.

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