Bioresource Technology 86 (2003) 139–145

Evaluation of Anammox and denitrification during anaerobic digestion of poultry manure

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Received 11 January 2001; received in revised form 25 June 2002; accepted 26 June 2002

Abstract

Two approaches based on new process development and biological nitrogen transformation were investigated in a bench study for removing nitrogen as N_2 gas from poultry waste while stabilizing the wastes. The process, known as "Anammox", was explored in batch anaerobic culture using serum bottles. The Anammox process involves the use of nitrite as an electron acceptor in the bacterially mediated oxidation of ammonia to yield N_2. Studies are described wherein nitrite was added to poultry waste and the effects on ammonium levels were monitored. About 13–22% ammonium removal was observed with the inoculation of returned activated sludge, and the total ammonium reduction was not proportional to the reduction of nitrite, thereby suggesting that Anammox was less competitive under the conditions in our studies. The addition of nitrite and nitrate was not inhibitory to the process based on gas generation and COD reduction. The classical nitrogen removal process of nitrification followed with denitrification offers a more reliable basis for nitrogen removal from poultry wastes.

Keywords: Anammox; Denitrification; Anaerobic digestion; Anaerobic ammonia oxidation; Nitrogen removal; Poultry manure

1. Introduction

Poultry production plays a vital role in the agricultural economy of the southeast United States. Animal densities continue to increase at the farm level, and the consolidation of animal agriculture has created manure-related problems, which are air pollution, water pollution, and disease-causing potential. The main air pollution is ammonia volatilization. These problems are leading to more regulations concerning where and how poultry producers may dispose of wastes.

Direct land application of poultry litter (from broiler production) and lagoon effluent (from layers) is still the least costly and mostly widely used disposal method for poultry manure. However, with consolidation, this alternative is often not feasible because adequate land is not sufficiently available on a timely basis to allow for acceptable N and P application rates.

Variations of composting and anaerobic digestion are the most traditional ways to treat manure. Composting is an accepted way of manure disposal because of the advantages of volume reduction, pathogen inactivation and resulting soil amendments. Nutrient losses and odor problems in composting of poultry manure have always been among the most important and difficult issues. Physical, chemical, and biological approaches have been adapted for addressing the ammonia emission problems with composting (Bernal and Lopez-Real, 1993; Bernal et al., 1993; Witter and Lopez-Real, 1988; Witter and Kirchmann, 1989). Most of these strategies are not prevalent in composting industry due to the unfavorable economics. Anaerobic digestion has less potential for odors and environmental damage because digesters are typically closed systems. Poultry manure, like other animal wastes, contains a high level of organic nitrogen (ASAE, 1997). During anaerobic digestion, the concentration of ammonia-nitrogen rises considerably as protein breakdown occurs. The excess of ammonium can inhibit the decomposition of organic compounds, the production of volatile fatty acids (VFAs), and methanogenesis (Krylova et al., 1997; Kayhanian, 1999).

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0960-8524/03/$ - see front matter
PII: S0960-8524(02)00154-2

Anaerobic ammonia oxidization (Anammox) is a recently discovered anaerobic process where ammonium is oxidized to nitrogen gas using nitrite as the electron acceptor (Jetten et al., 1998). Partial nitrification (Surmacz-Gorska et al., 1997; Yoo et al., 1999) is the accumulation of nitrite by inhibiting the second step of the nitrification. These discoveries enrich the traditional nitrogen cycle, and therefore provide possible alternatives to address the nitrogen issue in both solid and liquid waste disposal.

As air quality issues become increasingly prominent, innovative approaches based on traditional and new biological approaches to removing excessive nitrogen while stabilizing the manure are becoming practical and acceptable alternatives to treat poultry manure. We envisioned two approaches following this concept. The first alternative was based on Anammox. The liquid with high ammonia/ammonium concentration in the anaerobic process would be removed from the bottom. Half of the ammonium in the liquid would be partially nitrified to nitrite firstly and the liquid would subsequently be recirculated to the anaerobic digester. The recirculation operation would maintain a uniformly mixed reaction. It was envisioned that ammonium could be converted to N_2 through Anammox using nitrite as electronic acceptor in the anaerobic digester. Another alternative to be studied was the co-occurrence of denitrification and anaerobic digestion. Ammonium in the leachate would be fully nitrified to nitrite and then nitrite would be converted to nitrogen gas through denitrification in the anaerobic digester, using the organic intermediates of the anaerobic decomposition as the electron donors.

The objectives of this paper were (1) to investigate the possibility of Anammox occurring when inoculating poultry wastewater with a denitrification sludge in anaerobic digestion, where Anammox would be deemed present if decreases in ammonia were accompanied by concomitant decreases in nitrite; (2) to investigate possible inhibiting effects of nitrite and nitrate on the anaerobic digestion of poultry manure; and, (3) to investigate the possible co-occurrence of anaerobic digestion and denitrification processes.

2. Methods

2.1. Material preparation and experimental setup

Returned activated sludge from Wastewater Treatment Plant (Will Hunter Rd, Athens, GA) served as inocula. This source was used because of the difficulty of locating *Planctomycetales* from commercial sources and due to the fact that the organism is most likely to be associated with environments facilitating denitrification. The sludge was homogenized by passing it through a 60 ml syringe. Fresh layer poultry manure was collected in a tray placed for 3–5 days under the layer cages at the University of Georgia's Poultry Complex. The manure was dissolved with deionized (DI) water, filtered or centrifuged to remove particles. For each experiment, the concentrations of NH_4-N and NO_2-N were adjusted to a selected level by dilution, adding $(NH_4)_2SO_4$ or $NaNO_2$.

After substrate and the inoculum were added, graduated serum bottles (500 ml) were flushed with helium gas or inert gas mixture ($N_2 + CO_2$, 1:1) for 10–15 min to establish the anaerobic conditions. The bottles were then firmly closed with red-rubber flange straight-plug stoppers and aluminum seals. The bottles were statically incubated at 37 °C.

2.2. Sampling and analysis

Before sampling, the serum bottles were vigorously shaken and allowed to settle. Syringes (60 ml) with 21G 37 mm needles were used to relieve the pressure and approximate the produced gas volume. Liquid samples were collected using 10 ml disposable syringes with 18G 12 cm needles. The pH value in each sample was measured. The samples were then acidified using 2 ml H_2SO_4/l and stored at 4 °C in a refrigerator until analyses were conducted.

Homogenized samples were then diluted and digested. Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were measured using a TRAACS 2000 Continuous-Flow Analyzer (Bran + Luebbe, Inc., 1025 Busch Parkway, Buffalo Grove, ILL 60089). Supernatant of centrifuged samples (in 1.5 ml tubes 10,000 rpm for 5 min were appropriately diluted for analysis of ammonium/ammonia, nitrate/nitrite using TRAACS.

2.3. Anammox in anaerobic digestion of poultry manure

To meet the first objective, the following hypothesis was tested: with the existence of nitrite in the anaerobic digestion of poultry manure, the ammonium concentration in the system would not decrease significantly with time. Preliminary experimentation of anaerobic digestion of poultry manure showed that the organic nitrogen in the manure liquid was decomposed into ammonium rapidly at the early stage of digestion. The ammonium concentration remained approximately constant in the later stages. In order to avoid the stage of ammonium concentration increasing, anaerobic treated animal wastewater (AnAWW) and aerobically treated animal wastewater (AeAWW) were used as substrates. Concentrated nitrite solutions were fed to each treatment according to their nitrite consumption intermittently. The experimental protocol is shown in Fig. 1. Four treatments were investigated with two replicates.

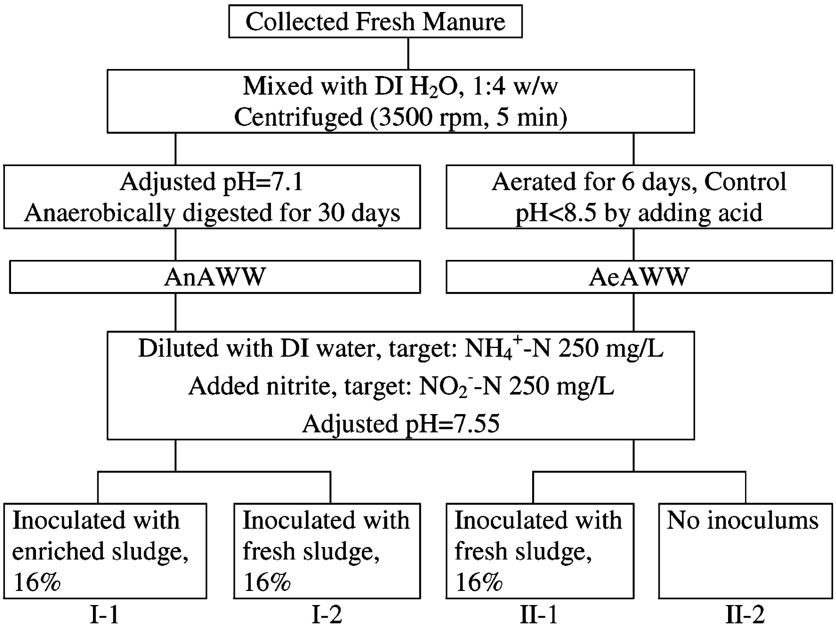

Fig. 1. Experiment design: co-occurrence of Anammox and anaerobic digestion using biologically treated substrates.

2.4. Denitrification in anaerobic digestion of poultry manure

To meet the second objective, an experiment was designed to test NO_x^- feeding frequency and the difference of feeding nitrate vs nitrite. The response variables were COD reduction percentage (%), Total NO_x^- consumed (mg/l), and NO_x^- reduction percentage (%). The COD reduction percentage was used to represent the degree of anaerobic digestion. Total NO_x consumed and NO_x reduction were used to represent the capacity of denitrification. The experimental design is shown in Table 1. There were seven treatments in a completely random design with two replicates.

Initially, the significance of difference between two substrates was tested (using treatments I-1, I-2, II-1 and II-2). In order to facilitate sampling and analysis, more hypotheses were only tested using the supernatant as the substrate. The main effect tested in this group (group II) was H_0: Feeding NO_x^- has no significant effect on COD reduction compared with the 'control'. The following hypotheses were tested for each of the response variables: (1) H_0: there was no significant interaction between feeding rate and feeding solution type; (2) H_0: when feeding nitrate, feeding with high rate was not significantly different from feeding with low rate; (3) H_0: when feeding nitrite, feeding with high rate was not significantly different from feeding with low rate. The P-value used to determine significant difference is 0.05.

2.5. Statistical analysis

Magnitude of error bars in each graph was calculated using Eq. (1).

$$e = 1.96 \times \frac{s}{\sqrt{n}} \tag{1}$$

Table 1
Experimental design: denitrification in the anaerobic digestion of poultry manure

	Concentrated sludge (ml)	Substrate type and volume (ml)[a]	Target initial nitrate/nitrite concentration (mg/l)	Feeding rate of nitrate/nitrite[b]
I-1		Filtrate (500)	0	Control
I-2		Filtrate (500)	Nitrate (200)	Nitrate, high
II-1	20	Supernatant (480)	0	Control
II-2	20	Supernatant (480)	Nitrate (200)	Nitrate, high
II-3	20	Supernatant (480)	Nitrate (200)	Nitrate, low
II-4	20	Supernatant (480)	Nitrite (150)	Nitrite, high
II-5	20	Supernatant (480)	Nitrite (150)	Nitrite, low

Bicarbonate (10 mM) was added, and the bottles were flushed with $N_2 + CO_2$ (1:1), with aim to form a buffer system to keep pH around 7.4. The experiment lasted for 13 days and nitrate was fed during the first eight days. Samples were collected every two days.

[a] Filtrate: filtrate of manure solution through 0.5 mm sieve; supernatant: supernatant of manure solution centrifuged at 4500 rpm for 10 min.

[b] High feeding rate: once every two days; low feeding rate: once every four days.

The interval $(\bar{x} - e, \bar{x} + e)$ is the 95% confidence interval for the mean, where s is the sample standard deviation, n is the sample number, $s/\sqrt{n}$ is the standard error of the mean, and $\bar{x}$ is the sample mean (Dougherty, 1990).

The general linear models (GLM) procedure in the statistical analyses system (SAS) package was used to test the hypotheses in Sections 2.3 and 2.4. The statement CONTRAST, in the SAS GLM procedure, was used for individual contrasts. A contrast takes the form $\psi = \sum_{i=1}^{a} c_i \mu_i$ and is estimated by $C = \sum_{i=1}^{a} c_i \bar{y}_i$, where $\sum_{i=1}^{a} c_i = 0$, a is the treatment number, and N is the total sample number. Null hypothesis H_0: $\psi = 0$ is rejected at significance level α if

$$F = \frac{\left(\sum_i^a c_i \bar{y}_i\right)^2 / \sum_i^a c_i^2 / n_i}{\mathrm{MS}_E} > F_\alpha(1, N - a)$$

where MS_E is the estimate of the variance (Dean and Voss, 1999).

3. Results and discussion

3.1. Anammox in anaerobic digestion of poultry manure

Statistical analysis was performed for each of the four treatments. For each treatment, the first and the last sample were removed because the ammonium concentration had not reached the peak at the beginning, and ammonification occurred possibly in the end, which caused the increase of ammonium concentration (Fig. 2a). The P-values of the one-way ANOVA were 0.0349, 0.0241, 0.0141, and 0.4547 for treatments I-1, I-2, II-1, and II-2 respectively, which suggested that the inoculation of returned activated sludge resulted in the decrease of ammonium concentration. The reduction percentages (based on the highest ammonium concentration) were 16.7%, 22.5% and 13.1% for treatments I-1, I-2, and II-1 respectively. The oxidization of one mole of ammonium through Annamox requires 1.31 mole of nitrite (Jetten et al., 1998). Much more nitrite than required for the oxidization of ammonium was consumed, and the reduction of nitrite must be mainly through denitrification, using VFAs or reduced inorganic compounds as electron donors. It was concluded that Anammox reaction occurred with a level less competitive than denitrification. From Fig. 2b, the AnAWW (I-1 and I-2), whether inoculated with enriched or fresh sludge, supported more nitrite reduction than AeAWW (II-1 and II-2). For treatments I-1 and I-2, with about 700 mg/l nitrite added, 97% reduction was achieved; while for treatments II-1 and II-2, less than 80% of nitrite reduction was achieved with similar addition. The total gas volume was consistent with the nitrite consumption (Fig. 2c).

3.2. Denitrification in anaerobic digestion of poultry manure

The total NO_x consumed (mg/l), NO_x^- reduction percentage (%), and COD reduction percentage (%) for each treatments are shown in Fig. 3. The P-values of different statistical analysis are listed in Table 2. The null hypotheses were rejected at 95% significant level when $P < 0.05$.

The two substrates had significantly different capacities to support nitrate reduction ($P = 0.0001$ and 0.0002 for total and percentage, respectively). Using manure filtrate, 640 mg/l nitrate was consumed, which accounted for 93% of the total nitrate; while using manure supernatant, only 156.4 mg/l was consumed and it accounted for 22.9% of the total nitrate. Manure filtrate was not inoculated with returned activated sludge, so the high nitrate reduction implied the presence of denitrifying organisms.

The test of main effect of feeding nitrate on COD reduction gave a P-value of 0.0201, which suggested that adding nitrate promoted denitrification. This main effect was further tested using manure supernatant as the substrate, and the effect of feeding NO_x was significant ($P = 0.0015$).

For all three response variables, the interactions of feeding rate and feeding solution type can be noted from Fig. 3, and the statistical analysis of interaction gave P-values less than 0.05 (Table 2). Thus, the main effect of feeding rate could not be tested, and the effects of feeding rate could only be tested within each feeding solution group. For the total NO_x^- consumption, when feeding with nitrate, the higher rate resulted in less consumption, while with nitrite, the higher feed rate resulted in more consumption. The differences were significant at 95% level in both groups (P-values are 0.0075 and 0.0094 respectively). Similar evaluations were made for the nitrate/nitrite reduction percentage. When feeding with either nitrate or nitrite, a lower feeding rate led to higher consumption percentage (P-values are 0.0007 and 0.0294 respectively), which is reasonable.

The effect of feeding rate on the COD reduction percentage, i.e., the overall digestion capacity, was tested within each feeding solution group. When feeding nitrate, the high feeding rate was significantly better than the low feeding rate ($P = 0.0376$). However, when feeding with nitrite, the low feeding rate was evidently somewhat better than high feeding rate, though the differences were not significant ($P = 0.1823$).

From the above analysis, the ratio of COD over NO_x^- apparently is very important for the co-occurrence of denitrification and anaerobic digestion. Because denitrifying organisms use organic matter oxidized by the COD procedure, sufficient COD should exist in the substrate to ensure complete removal of NO_x^--N.

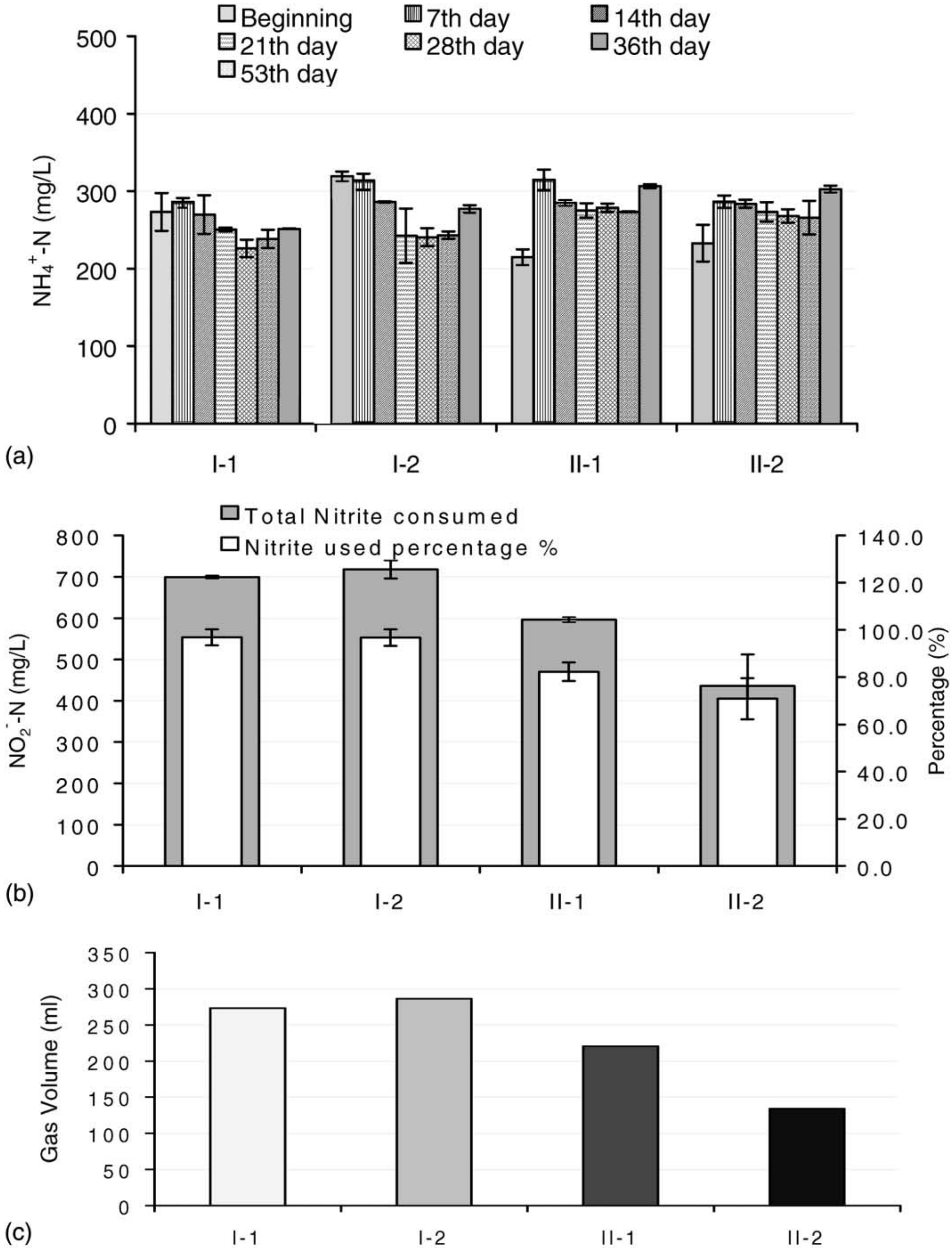

Fig. 2. Profiles of ammonium (a), nitrite consumption (b), and total gas production (c) in the study of Anammox with AnAWW and AeAWW: (1) AnAWW: anaerobically treated animal wastewater; AeAWW: aerated treated animal wastewater, (2) I-1: AnAWW with fresh inoculum; I-2: AnAWW with old inoculum; II-1: AeAWW with fresh inoculum, II-2: AeAWW without inoculum (control)).

The COD of manure filtrate was about 5400 mg/l, while the COD of manure supernatant was about 2200 mg/l; this explains the high nitrate consumption and reduction percentage in the filtrate. However, COD was not the sufficient condition for complete denitrification. Except in the case of filtrate fed with nitrate and supernatant fed with nitrite at low frequency, the reduction of nitrate/nitrite in the other three feeding treatments stopped at the late stage when there were more than 1000 mg/l COD left in the systems. Presumably, this represented the portion of the oxygen demand that was not readily degradable.

Gonzales and Gudgel (1998) described several innovative approaches such as the Bardenpho process and separated nitrification and denitrification schemes for nitrogen removal from wastewater. They were primarily aerobic with some anoxic zones. Our results suggested the possibility of incorporating an aeration zone (for

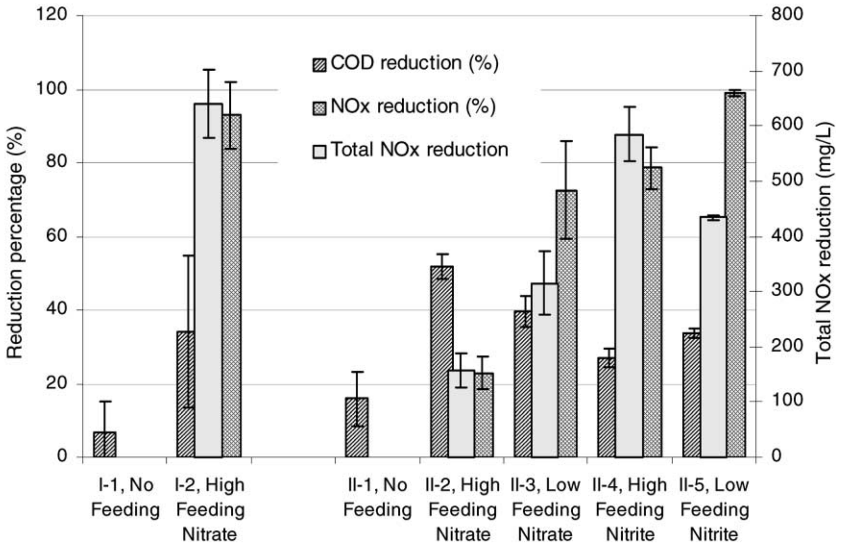

Fig. 3. Total NO_x consumption, consumption percentage, and COD reduction percentage in different substrates with respect to feeding materials and feeding rate (substrate: manure filtrate for I-1 and I-2; manure supernatant for II-1, II-2, II-3, II-4 and II-5).

Table 2
P-value for different statistical tests in investigating denitrification in the anaerobic digestion of poultry manure

Test	Response variables		
	Total NO_x reduction (mg/l)	NO_x reduction (%)	COD reduction (%)
I-2 vs II-2	0.0001	0.0002	0.2107
Main effect			
I-1, II-2 vs I-2, II-2	N/A	N/A	0.0201
II-1 vs II-2, II-3, II-4, II-5	N/A	N/A	0.0015
Interaction			
Feeding rate and feeding material	0.006	0.0502	0.0114
Individual contrasts			
II-2 vs II-3	0.0075	0.0007	0.0376
II-4 vs II-5	0.0094	0.0294	0.1823

nitrification) with an anaerobic digester wherein COD reduction could occur with denitrification. A system utilizing anaerobic digestion is normally operated at a mesophilic temperature (higher than 30 °C). When the filtrate at temperatures exceeding 25 °C is aerated, the second step of nitrification is inhibited, thus nitrite should accumulated (Yoo et al., 1999). When the nitrified effluent is recirculated to a digester, nitrite could be reduced to N_2 via conventional denitrification. Additional results (Dong, 2000) showed that most nitrogen in the solid was in the dissolved form, and most organic nitrogen was decomposed into ammonium by 13 days into the study. This suggests that removing liquid from anaerobic digester for nitrification is feasible given the rapid decomposition of the organic nitrogen. An appropriate nitrate/nitrite feeding rate should be used to maintain the electron and intermediates balances in the system to avoid inhibitory effects.

The sequencing batch reactor (SBR) may be an interesting system for implementing concepts developed in this paper. Bernet et al. (2000) reported the use of the combined anaerobic-aerobic sequential batch reactor (SBR) for the treatment of swine wastewater. Further studies are anticipated with SBR systems.

4. Conclusions

With the addition of nitrite and inoculation of returned activated sludge, significant decrease of ammonium concentration was observed in the anaerobic digestion of poultry waste. However, the reduction of nitrite was much higher than required for the oxidization of ammonium through Annamox. High N_2 concentration was detected in the gas samples from the void space. Anammox is defined as the concomitant decreases of nitrite and ammonium in the anaerobic conditions. Based on the experiment results, Anammox microorganisms evidently developed at a very slow rate, and/or could not compete with the denitrifying organisms in anaerobic digester for nitrite. It is suggested that future studies involve enriched Anammox-causing microorganisms (Strous et al., 1998). In addition, the capacity of Anammox-causing microorganisms to compete for nitrite with denitrifiers needs further study.

Feeding nitrate/nitrite into the digester did not inhibit the poultry waste anaerobic digestion. Feeding nitrate/nitrite with lower rate (about 150 mg/l every four days) increased the nitrate/nitrite reduction percentage, suggesting creative approaches for combining denitrification with anaerobic digestion for nitrogen management.

High solid anaerobic digestion with feeding of nitrite needs more study at different feeding rates.

Acknowledgement

This research was performed with funding from the Georgia Agricultural Experiment Stations.

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