

EFFECT OF MICROBIAL ADDITIVES COMBINED WITH AERATION ON REDUCTION OF NUTRIENTS IN SWINE MANURE

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ABSTRACT. *The research presented in this article evaluated a technique using manure additives (Sporzyme and activated sludge) coupled with aeration treatment in reducing nutrients from liquid swine manure. Plastic columns, 91.6 cm in height and 15.3 cm in internal diameter, were each filled with 15 L liquid swine manure. Aeration was provided to each column by an air pump at an airflow rate of 2.0 L/min, controlled by individual airflow meters mounted on the columns. The experiment included five treatments: (1) control (no aeration and no additives), (2) aeration alone, (3) aeration with a microbial additive (Sporzyme), (4) aeration with activated sludge inoculum without anaerobic incubation prior to aeration, and (5) aeration with activated sludge inoculum with three days of anaerobic incubation prior to aeration. The results indicated that total Kjeldahl nitrogen (TKN), ammonia-N, and total soluble phosphorus were reduced by about 42%, 56%, and 72%, respectively, by all the treatments except the control. The reduction of TKN was found to be mainly attributed to the reduction of ammonia because its share of TKN was remarkably reduced at the end of the test. Although Sporzyme significantly increases the aerobic count in the manure, data from this study have showed no advantage of its use (or the activated sludge as seeding) solely for enhancing nutrient reduction in swine manure under aeration treatment since there was little difference found in terms of either nitrogen or phosphorus reduction between treatments with and without additives.*

Keywords. *Aeration, Liquid swine manure, Microbial additives, Nutrient reduction.*

Pig manure containing nutrients such as nitrogen, phosphorus, and potassium at high concentrations has been used for fertilizer for years. With the increasing expansion and consolidation of the swine industry in the U.S., continuing the traditional land disposal of excess manure has become a center of scrutiny because of its potential to harm the environment. To sustain the swine industry and maintain its continued growth, effective methods of controlling nutrient output from manure need to be developed so a healthy environment and a growing swine industry can coexist.

There have been many reports on using advanced techniques to reduce nutrients from swine manure. Osada et al. (1991) and Bicudo and Svoboda (1995) reported on good results in reducing biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen, phosphorus, and metal ions by aeration treatment. However, the high cost of the equipment and energy consumption by aeration has made it difficult to widely promote this technique. Efforts have thus been made to enhance the efficiency, including using a pre-anaerobic process (Gerrish et al., 1975), intermittent

aeration (Osada et al., 1991; Bicudo and Svoboda, 1995), and use of proper aerators (Fallowfield et al., 1994). Gerrish et al. (1975) showed that three days of anaerobic pre-conditioning followed by aeration could increase the rates of COD and total solids reduction and cut the operation time in half, resulting in potential energy savings. However, little has been reported on the effect of such an anaerobic pre-treatment on aeration for nutrient removal in liquid swine manure.

Use of additives to conserve nutrients and control odor for swine manure has been studied in the past. Fenlon and Mills (1980) found that adding lime to fresh pig manure could result in urea conservation and odor control. Barrington and MacKenzie (1989) reported that cement kiln dust containing a considerable amount of potassium and calcium could complement the nutrient value as well as reduce the odor level of swine manure. Many other studies also documented the use of chemicals to treat liquid swine manure for nutrient reduction (Sievers et al., 1994; Zhang and Lei, 1996; Vanotti, et al., 1996; Henriksen et al., 1998). There is no doubt that chemicals can enhance nutrient reduction from swine manure by forming insoluble compounds that precipitate. The downside of this method is not only its high cost in purchasing chemicals but also its potential to cause secondary environmental pollution when the treated manure is land applied. For example, it has been reported that extensive use of aluminum and iron salts may deteriorate soil quality by raising the concentrations of chlorides and sulfates in the soil and reducing the soil pH due to accumulation of unbound SO_4^{2-} and Cl^- ions in the soil receiving the treated liquid (Kox, 1981). It is therefore worthwhile to look at alternative additives to chemicals. One of the possibilities rests with biological additives, which have not been extensively studied from the perspective of nutrient control in liquid swine manure.

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It also has to be pointed out that since most of the treated manure will be eventually land applied (including liquid and solids with concentrated nutrients such as P), caution should be exercised to ensure that the final N:P ratio of the applied manure does not deviate too far from the appropriate ratio that satisfies the needs of crops. In extreme conditions, commercial fertilizers may have to be used to adjust the N:P ratio of the treated manure.

The objective of this project is to examine the effect of biological additives on nutrient reduction from liquid swine manure under aeration conditions. The biological additives tested include a commercial microbial product (Sporzyme) and the activated sludge collected from a municipal wastewater treatment plant. The nutrient components investigated include nitrogen and phosphorus fractions.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Five treatments with two replicates were employed in this study: (1) control (no aeration and no additives), (2) aeration alone, (3) aeration with a microbial additive (Sporzyme), (4) aeration with activated sludge inoculum without pre-aeration anaerobic incubation, and (5) aeration with activated sludge inoculum with three days of pre-aeration anaerobic incubation. Sporzyme is a product consisting of a group of aerobic phosphorus-accumulating organisms with supporting enzymes. Anaerobic incubation prior to aeration is to improve the process of phosphorus reduction in the following aerobic treatment. Using three-day anaerobic treatment was reported to be helpful in improving the subsequent aeration treatment (Gerrish et al., 1975).

A typical aeration apparatus is shown in figure 1, and ten units of such apparatus were built for all the treatments and their duplicates. The columns were made of PVC materials, 91.6 cm in height and 15.3 cm in internal diameter. Aeration was realized by an air pump (Catalog No. 13-875-220, Fisher Scientific, Hanover Park, Ill.), which continuously provided air to columns that needed to be aerated at an airflow rate of 2.0 L/min. The airflow rate for each aerated column was controlled by an airflow meter mounted on that column. Selection of this airflow rate was based on a previous study by Ndegwa et al. (2001) to maximize aeration capacity for the system without producing an overspill of manure from the column. The experiment was performed in a room with temperature maintained at around 25°C throughout the test period, which was 15 days.

The manure used in the experiment was collected from a finishing barn at the University of Minnesota Southern Research and Outreach Center at Waseca and transported to the lab where the PVC columns were set up for the study. The raw manure characteristics are presented in table 1. Each column was filled with 15 L of swine manure, leaving a headspace of around 7.6 cm for accommodating foaming during aeration. The activated sludge used was collected from the Waseca Wastewater Treatment Plant and was added to the columns in treatment 4 and 5 at a dose of 500 mL each immediately after collection. The amount of inoculum used was determined with reference to that used by Obaja et al. (2003). The columns in treatment 5 were then incubated anaerobically for three days before aeration started.

The microbial additive product, Sporzyme, was provided by Semco Laboratories, Inc. (Milwaukee, Wisc.) at a

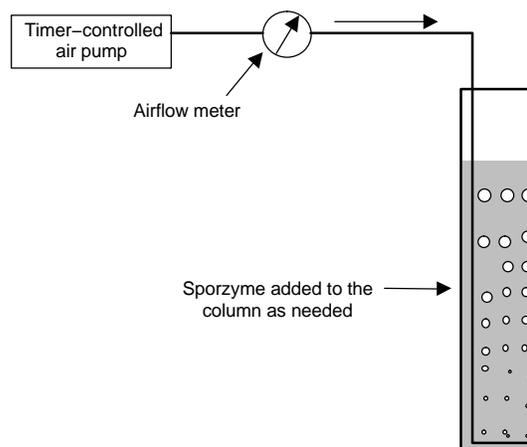


Figure 1. Schematic of the aeration apparatus used in the experiment.

Table 1. The characteristics of manure.

Parameter	Value	Units
pH	7.77	--
TKN	6242	mg/L
Ammonium	3191	mg/L
Total P	1348.4	mg/L
Total soluble P	363.2	mg/L
Aerobic microbes	2.75×10^4	cfu/mL
Anaerobic microbes	1.24×10^6	cfu/mL
TS	46.6	g/L
TVS	12.9	g/L

concentration of 0.5 billion cfu/g. The product is a dry powder made of a group of working microorganisms and assisting enzymes capable of removing nutrients from wastewaters. Each column in treatment 3 received 4.5 g of the additive immediately prior to aeration, a dosage recommended by the company for an initial shock equivalent to 1.5×10^5 cfu/mL. After that, an amount of 0.9 g, equivalent to 3.0×10^4 cfu/mL, was added to each column once a week for maintenance, as recommended by the provider.

MANURE SAMPLING

After aeration started, the manure in each column was sampled every day in the first week and every other day in the following week. Samples of 100 mL each were collected at a depth 40 cm below the liquid surface. In order to obtain uniform samples, sampling was conducted during thorough agitation of manure for 5 min with a motorized paddle-stirrer. The collected samples, if not analyzed immediately, were stored in a freezer at -20°C and thawed immediately before lab analysis.

MEASUREMENTS AND SAMPLE ANALYSIS

The oxidation-reduction potential in each column was measured directly using a digital pH/temp/mV/ORP meter with automatic temperature compensation (Catalog No. 5938-10, Cole-Parmer Instruments, Vernon Hills, Ill.). The pH of the manure samples was measured immediately after sampling using a pH meter (Corion, model 720A, Cole-Parmer Instruments, Vernon Hills, Ill.). The drop count method was used for both aerobic and anaerobic bacterial counts (Collins et al., 1995). The anaerobic condition was realized by placing inoculated petri dishes in an anaerobic jar, which was subsequently cultured in an incubator for three days before counting.

For soluble phosphorus, 10 mL of manure was centrifuged and the supernatant was used for the measurement. The P fraction that was reactive directly with molybdate was considered to be the soluble inorganic P, while total soluble P was determined after digestion with ammonium persulfate (APHA, 1998). Soluble organic P was computed as the difference between total soluble P and soluble inorganic P.

For phosphorus contained in manure solids, the fractionation of P was performed by a sequential extraction process according to the method of Jing et al. (1992), which was used in the fractionation of P in activated sludge from a biological phosphorus removal system. A 10 mL sample of raw liquid manure was centrifuged and the supernatant was discarded. The remaining solid was washed twice with distilled water and used for P analysis. This information was used for calculating total P to determine total insoluble P.

The ammonium analysis was conducted according to the Nesler's method (Adams, 1990). Total Kjeldahl nitrogen was measured using the Kjeldahl method, while organic nitrogen was determined by subtracting ammonium nitrogen from the total Kjeldahl nitrogen measurements.

When needed, statistical t-tests were employed in data comparisons throughout the experiment at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

OXIDATION-REDUCTION POTENTIAL, pH, AND BACTERIAL COUNTS

The means based on two replicates of oxidation-reduction potential (ORP) during the experiment are presented in figure 2. For all the treatments except the control, the ORP increased drastically from around -400 mV to about 250 mV in the first day of aeration and then gradually decreased thereafter. Despite the decrease, the ORP levels in all the aerated columns remained in the positive domain throughout the testing period, indicating the establishment of an aerobic environment in the liquid manure. In addition, little difference appears in ORP readings among the four different treatments, which means that the aeration efficiency was not affected by the manure additives studied.

The average pH of manure (fig. 3) was significantly increased by aeration from around 7.8 to 8.8 within the first two days. This phenomenon was also observed by other researchers (Stevens and Cornforth, 1974; Zhu et al., 2001;

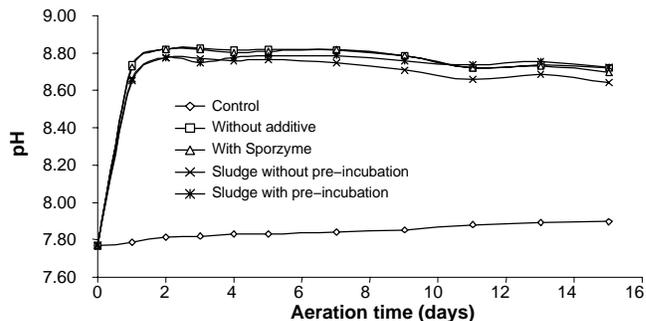


Figure 3. pH variation during aeration for all treatments.

Luo et al., 2002). In the rest of the aeration period, the pH stayed almost constant with only a little fluctuation between 8.7 and 8.8 . This pH change, caused by aeration, is associated with the conversion of $\text{NH}_4^+ - \text{N}$ into ammonia. Stevens and Cornforth (1974) reported that pH increased to between 8 and 9 by purging the dissolved CO_2 out of a solution, which formed NH_4^+ bicarbonate that kept the pH neutral.

Figures 4 and 5 show the means of bacterial counts for both aerobes and anaerobes during the aeration process. First, the magnitude of aerobic bacterial counts is several orders higher than that of anaerobic bacterial counts for all treatments with aeration (approx. 10^8 vs. approx. 10^5). Second, the treatment with Sporzyme demonstrates a consistently higher aerobic count than the rest of the aeration treatments. The two treatments with the activated sludge also indicate higher aerobic counts than that without additives. These observations reveal that addition of external sources of aerobic bacteria is able to increase the number of aerobes in the manure under the identical aeration scheme. Third, for anaerobic bacteria, since the manure environment was always maintained in the positive territory in terms of ORP, the anaerobic bacterial growth was apparently suppressed, with apparent difference found in bacterial counts between the aeration treatments and the control. In addition, there appears little difference in anaerobic count between different aeration treatments.

TOTAL SOLUBLE P, TOTAL INSOLUBLE P, SOLUBLE ORGANIC P, AND SOLUBLE INORGANIC P

A significant decrease (approx. 72%) in total soluble P is obtained after only one day of aeration (fig. 6a), which is

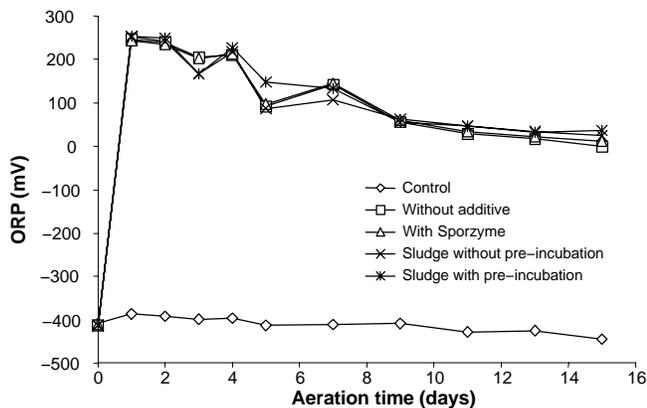


Figure 2. ORP variations during aeration for all treatments.

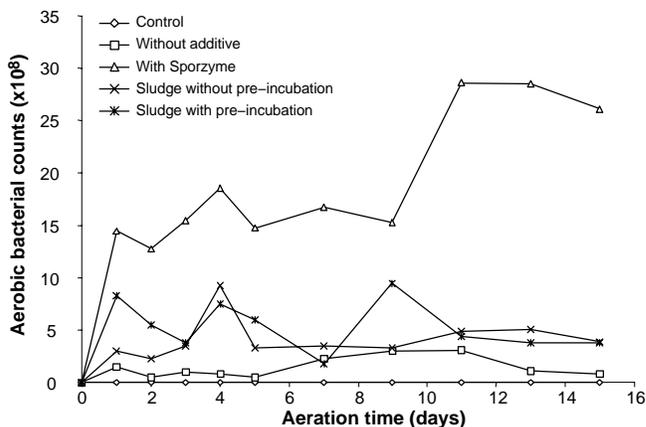


Figure 4. Total aerobic bacterial counts for all treatments.

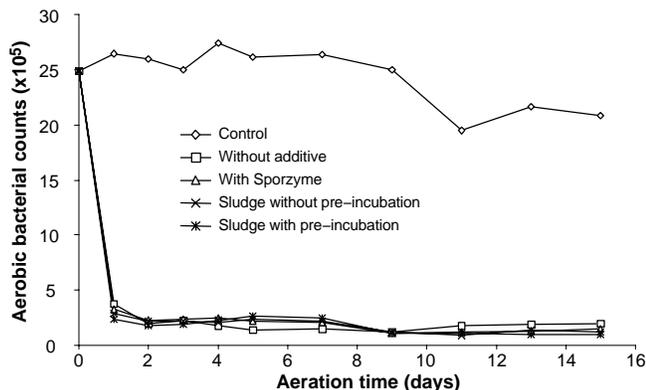


Figure 5. Total anaerobic bacterial counts for all treatments.

consistent with the findings reported by Zhu et al. (2001). This reduction in soluble P is primarily attributed to the formation of insoluble phosphate compounds due to an increase in manure pH. Therefore, it may be implied that if removal of soluble P is the treatment goal, then aerating liquid manure for only one day, followed by solid-liquid separation, will suffice. On the other hand, the decrease in total soluble P is reflected by the increase in total insoluble P (fig. 6b). Although fluctuations are present for the total insoluble P during the aeration process, they are all increased as compared to the control, indicating a possibly continued process of converting soluble P into insoluble P through chemical and biological processes over the treatment period.

If the total soluble P further breaks down to soluble inorganic P (SIP) and soluble organic P (SOP), it is found that the reduction in total soluble P is composed of reductions from both SIP and SOP (fig. 7). A slight decrease in SOP

should be caused by the bacterial uptake of readily available organic P in the liquid for their metabolic activity since SOP is usually not affected by chemical precipitation. In addition, since the amount of SOP is relatively small as compared to SIP (only about 10% of SIP as shown in fig. 7), it is reasonable to assume that the reduction in total soluble P in the liquid manure by short-term aeration is mainly brought about by the precipitation of soluble inorganic P.

One other thing that may deserve some discussion is the significance of soluble P reduction by the added aerobes. The results from this study indicate that although Sporzyme and the activated sludge both can increase the aerobic bacterial counts (fig. 4), the reduction in total soluble organic P is not remarkable (about 38% on average, as shown in fig. 7a). In addition, there is actually limited difference in terms of either SOP or SIP reduction between the two treatments with additives and the one without. Therefore, the current aeration treatment does not appear to justify the use of microbial additives to improve or enhance total soluble P reduction efficiencies.

Charpentier et al. (1987) stated that for microbial soluble P removal to occur, the ORP in the liquid should be maintained at above 0 mV. As a matter of fact, the ORP in all columns except the control in this study was maintained in the positive region throughout the aeration period (fig. 2). It therefore appears that holding ORP above zero may not necessarily guarantee an environment leading to effective and efficient reduction of soluble phosphorus. This may explain the reason why many researchers have turned to systems that are capable of alternating the oxic condition in the treated liquid, such as aerobic-anoxic-aerobic, to achieve high efficiencies of P removal by aerobic microorganisms based on their biological metabolic mechanisms (Converti et al., 1995).

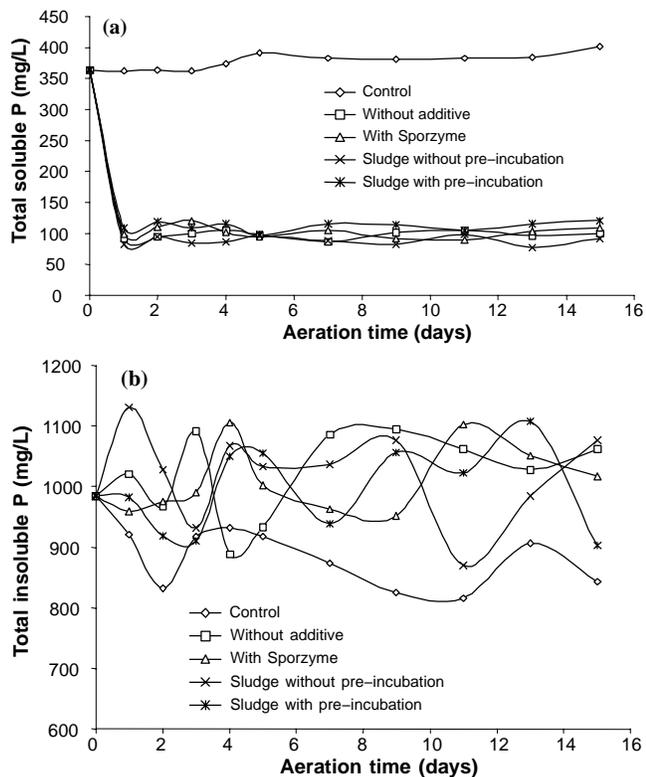


Figure 6. Changes in total (a) soluble and (b) insoluble P for all treatments.

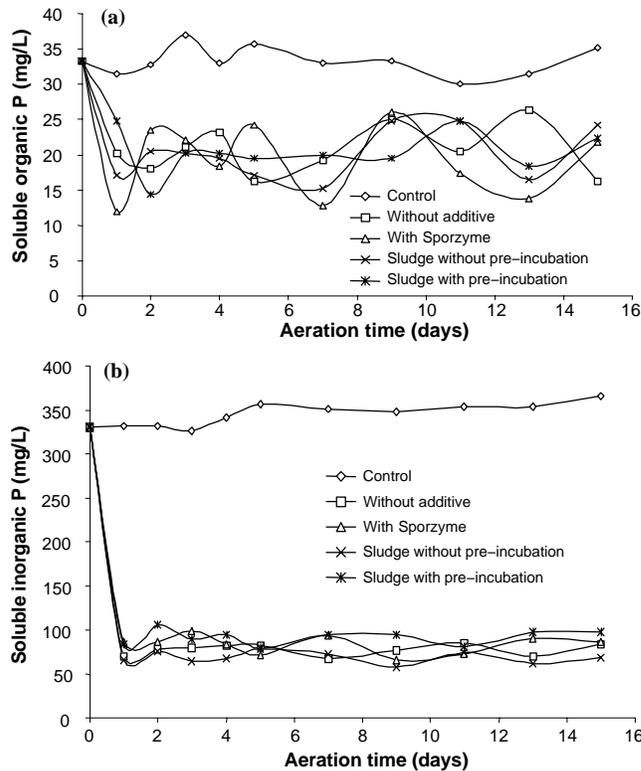


Figure 7. Changes in soluble (a) organic and (b) inorganic P for all treatments.

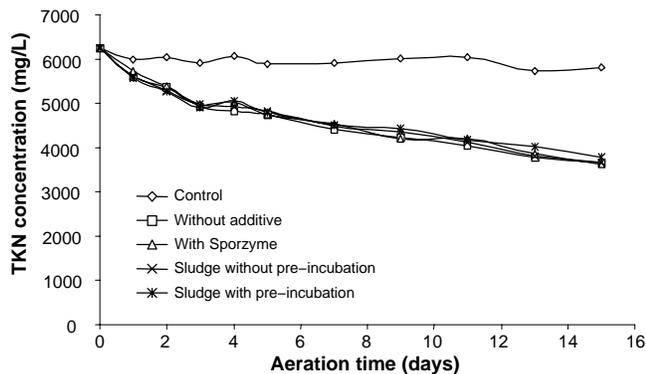


Figure 8. Changes in TKN for all treatments.

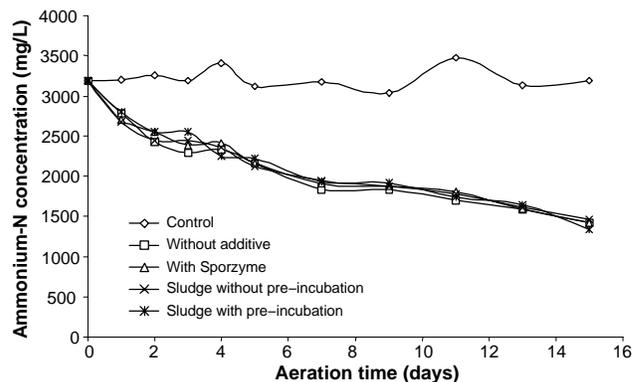


Figure 9. Changes in ammonium-N for all treatments.

TOTAL KJELDAHL NITROGEN AND AMMONIUM NITROGEN

Figure 8 presents the means of total Kjeldahl nitrogen (TKN) for all the treatments. Obviously, the difference is insignificant in terms of TKN concentration among the aerated columns. TKN gradually decreased from 6240 mg/L at the beginning of the aeration period to about 3650 mg/L at the end of the aeration period (a reduction of 41.5%). The reduction of TKN observed in the present study is lower than that reported by Osada et al. (1991), who observed a reduction by 72.2% of total nitrogen from pig wastewater due to aeration. A possible reason for the low removal efficiency of TKN may be due to lack of a significant nitrification/denitrification process. In the report by Osada et al. (1991), intermittent aeration was used (as opposed to continuous aeration in this study), which favored denitrification as well as endogenous respiration of microbial biomass during the anoxic periods, and this could be the reason for higher N reduction. Since there were no efforts made to analyze nitrate nitrogen in this study, the above inference needs to be verified by further research.

It is reasonable to assume that the reduction of TKN is partly because of the reduction in ammonium-nitrogen. Figure 9 presents the changes in ammonium nitrogen for all the treatments during the experiment. As can be seen, the ammonium-nitrogen decreased from 3200 mg/L to around 1400 mg/L in the test period (a reduction of 56.3%). Since ammonium nitrogen accounted for about 51.3% of TKN at the beginning but only about 38% at the end of the aeration period, the loss of TKN can largely be attributed to the loss of ammonium nitrogen by processes including volatilization, nitrification, and microbial assimilation (organic conversion). The increase in aerobic counts for all the aerated columns in this study may provide some explanation for the consumption of ammonium nitrogen due to biosynthesis.

There was little difference in both TKN reduction and ammonium nitrogen reduction among all the aeration treatments (figs. 8 and 9). This means neither the additives nor the pre-aeration conditioning made a noticeable distinction in nitrogen removal as compared to aeration only. Therefore, based on the results from this study, it may be concluded that use of microbial additives and pre-aeration treatment aimed at improving nitrogen removal efficiency may not produce satisfactory results as expected. With the current aeration setting, the indigenous aerobes in the manure apparently can reduce total nitrogen without assistance from external aerobic additives.

CONCLUSIONS

The aeration treatment studied can increase manure pH by about one unit (from 7.8 to 8.8) in the first day of operation and maintain the raised pH thereafter. With the increase in manure pH comes the reduction in total soluble phosphorus by 72%, which is largely caused by chemical precipitation of insoluble phosphates rather than by the microbial-related P reduction processes. This is evidenced by the increase in total insoluble P concentrations. In addition, the additives appear to have caused little improvement in soluble P reduction.

The aeration treatment was able to remove 41.5% of total Kjeldahl nitrogen and 56.3% of ammonium nitrogen. The removal of TKN was found to be mainly attributed to the reduction of ammonia nitrogen because its share of TKN was remarkably reduced at the end of the test. Since neither nitrate nor nitrite were measured in this study, their contributions to TKN removal cannot be quantitatively determined. The same applies to the amount of ammonium nitrogen consumed in aerobic biosynthesis. Excessive ammonia emission may cause undesired environmental problems, so further improvement of aeration treatment should be pursued in order to increase microbial assimilation of ammonia instead of volatilization.

Data from this study show that although the number of aerobic bacteria can be greatly increased by using either Sporzyme or activated sludge as additives, the advantage of such treatments is minimal in terms of enhancing the nutrient reduction in swine manure under aeration treatment since little difference was found in either nitrogen or phosphorus reduction between treatments with and without additives. It may thus be concluded that in aeration treatment of liquid swine manure for nutrient reduction, the additional cost for purchasing microbial additives may be unnecessary, and can thus be saved.

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