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Removal of pathogen and indicator microorganisms from liquid swine manure in multi-step biological and chemical treatment

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Abstract

Concern has greatly increased about the potential for contamination of water, food, and air by pathogens present in manure. We evaluated pathogen reduction in liquid swine manure in a multi-stage treatment system where first the solids and liquid are separated with polymer, followed by biological nitrogen (N) removal using nitrification and denitrification, and then phosphorus (P) extraction through lime precipitation. Each step of the treatment system was analyzed for *Salmonella* and microbial indicators of fecal contamination (total coliforms, fecal coliforms, and enterococci). Before treatment, mean concentrations of *Salmonella*, total coliforms, fecal coliforms, and enterococci were 3.89, 6.79, 6.23 and 5.73 $\log_{10}$ colony forming units (cfu)/ml, respectively. The flushed manure contained 10,590 mg/l TSS, 8270 mg/l COD, 688 mg/l TKN and 480 mg/l TP, which were reduced >98% by the treatment system. Results showed a consistent trend in reduction of pathogens and microbial indicators as a result of each step in the treatment system. Solid–liquid separation decreased their concentrations by 0.5–1 $\log_{10}$. Additional biological N removal treatment with alternating anoxic and oxic conditions achieved a higher reduction with average removals of 2.4 $\log_{10}$ for *Salmonella* and 4.1–4.5 $\log_{10}$ for indicator microbes. Subsequent P treatment decreased concentration of *Salmonella* and pathogen indicators to undetectable level (<0.3 $\log_{10}$ cfu/ml) due to elevated process pH (10.3). Our results indicate that nitrification/denitrification treatment after solids separation is very effective in reducing pathogens in liquid swine manure and that the phosphorus removal step via alkaline calcium precipitation produces a sanitized effluent which may be important for biosecurity reasons.
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Keywords: *Salmonella* spp.; Manure treatment; Pathogen inactivation; Nitrification–denitrification; Phosphorus removal; Confined swine production

1. Introduction

Pathogen reduction aspects of treatment have often been a secondary consideration to nutrient stabilization, volume reduction, and temporary storage benefits. Animal manure and slurries may contain a variety of pathogenic microorganisms, i.e. bacteria such as *Salmonella* species, *Campylobacter jejuni*, and *E. coli* 0157:H7, parasites such as *Cryptosporidium parvum*, and viruses such as enteroviruses (Sobsey et al., 2001). The recycling of these wastes to agricultural land creates the risk of pathogens contaminating the environment, entering the food chain, or infecting livestock (Martinez and Burton, 2003). The usefulness of composting, heat drying, digestion, and alkaline stabilization processes to destroy infectious microorganisms contained in biosolids destined for land application is well known. However, little is known about rates of pathogen reduction in new treatments developed for liquid manure and livestock wastewater effluents (Sobsey et al., 2001; Bohm, 2002).

An alternative system for treatment of liquid swine manure was developed to replace anaerobic lagoon technology commonly used in the USA to treat swine waste (Vanotti et al., 2001). In this manure treatment system, solids and liquid are first separated with polyacrylamide (PAM) polymer and filtration, followed by treatment of the liquid stream using biological nitrogen (N) removal, and then by phosphorus (P) extraction

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using a lime precipitation process. Polymer produces flocculation of suspended particles and enhances separation of solids from liquid swine manure (Vanotti and Hunt, 1999; Zhang and Lei, 1998). Along with the solids, there is a significant capture of organic nutrients and oxygen-demanding compounds associated with the clumping of the small suspended particles that usually escape through screens or clog sand filters. Soluble ammonia and phosphorus levels, which may constitute 35–65% of total N and 15–30% of total P, are mostly unaffected by polymer separation. Biological removal of ammonia through the process of nitrification is regarded as the most efficient and relatively low cost means of removing ammonia from wastewater (Tchobanoglous and Burton, 1991). Once ammonia and carbonate alkalinity concentrations are substantially reduced with nitrification treatment, the subsequent addition of $Ca(OH)_2$ rapidly increases the pH of the liquid above 9, thereby promoting formation of calcium phosphate precipitate with small amounts of chemical added (Vanotti et al., 2001).

The pilot system was successfully tested for two years at the North Carolina State University's Lake Wheeler Rd. Swine Unit (Vanotti et al., 2003a). A full-scale demonstration system was subsequently constructed at a 4400-pig operation in Duplin County, North Carolina, for verification of Environmental Superior Technology under the Smithfield Foods/Premium Standard Foods and North Carolina Attorney General agreement program to replace anaerobic lagoons (Williams, 2001; Vanotti et al., 2003b).

In this paper, we report how each of the process units comprising the total treatment system affected the survival of pathogens in liquid swine manure. The pilot system was used for this investigation.

2. Methods

2.1. Pilot treatment system

The pilot treatment system was set up at the Swine Unit of the Lake Wheeler Rd. Field Laboratory in Raleigh, NC. This was a research farm operated by North Carolina State University (NCSU). The unit contained several swine houses that used under-slat flushing and anaerobic lagoon for treatment and storage of the flushed manure. The houses were flushed with lagoon supernatant.

For the pilot runs, we used the manure that accumulated over a 48-h period under the slatted floors of two finishing houses containing approximately 250 pigs each. Manure flushes were diverted into a 15 m^3 homogenization tank before reaching the lagoon. The collected manure was subsequently treated in the pilot plant consisting of three process units in series: polymer-enhanced solid–liquid separation, biological N removal, and alkaline phosphorus extraction (Fig. 1).

The polymer-enhanced solid–liquid separation unit consisted of an in-line polymer injector and static mixer used to flocculate the suspended solids in the raw manure, and two sand filter beds (6.1 m × 4.9 m) that filtrated and dewatered the flocculated solids. Both the in-line polymer injector-mixer and filter beds were components of the Deskins process (F.D. Deskins Company, Inc., Alexandria, Ind. [1]). Separation treatment was relatively fast. Well mixed raw manure was pumped from the homogenization tank to the in-line flocculation unit at a rate of 500 l/min and applied on the surface of the sand filter beds. The beds received 30 cm depth of liquid manure during each pour. The drainage from the sand filter bed was pumped to a 15 m^3 storage tank at a rate of about 750 l/min. The polymer used was a cationic emulsion (Magnifloc c-1596, Cytec Industries, Inc., West Paterson, NJ). Detailed information and performance evaluation of this separation process unit was provided by Vanotti et al. (2002).

The separated liquid was continuously fed into the nitrogen removal unit that was the second step in the treatment system. Liquid was transferred using a peristaltic pump at a flow rate of 1000 l/day. The nitrogen removal unit used a Biogreen process (Hitachi Plant Construction and Engineering Co., Tokyo, Japan) that biologically converts ammonia into N_2 gas. The process uses nitrifying bacteria immobilized in polymer gel pellets to increase the concentration of bacterial biomass in the nitrification tank and improve nitrification performance (Vanotti and Hunt, 2000). The Biogreen process has a pre-denitrification configuration where nitrified wastewater is continuously recycled to an anoxic tank. In this tank, suspended denitrifying bacteria uses soluble manure carbon contained in the liquid after separation to remove the nitrate and nitrite. The pilot Biogreen unit contained: a 1.3 m^3 anoxic denitrification tank (1) with a mixer and a concentration of ~3 g/l mixed liquor suspended solids (MLSS) to remove soluble manure carbon and NO_3–N, a 0.55 m^3 nitrification tank with a fine-air diffuser and 100 l of polyethylene glycol (PEG) immobilized pellets for conversion of NH_4^+ to NO_3^-, a second 0.63 m^3 tank (2) with methanol injection for post-denitrification, a 20 l oxic tank, and a 0.63 m^3 tank for settling and recycling of suspended solids to the first denitrification tank. Nitrification activity after 60 days of initial acclimation was 790 g N/100 l pellets/day. Nitrified liquid was recirculated to the first denitrification tank at a rate of 2.5 m^3/day, and settled sludge was

[1] Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

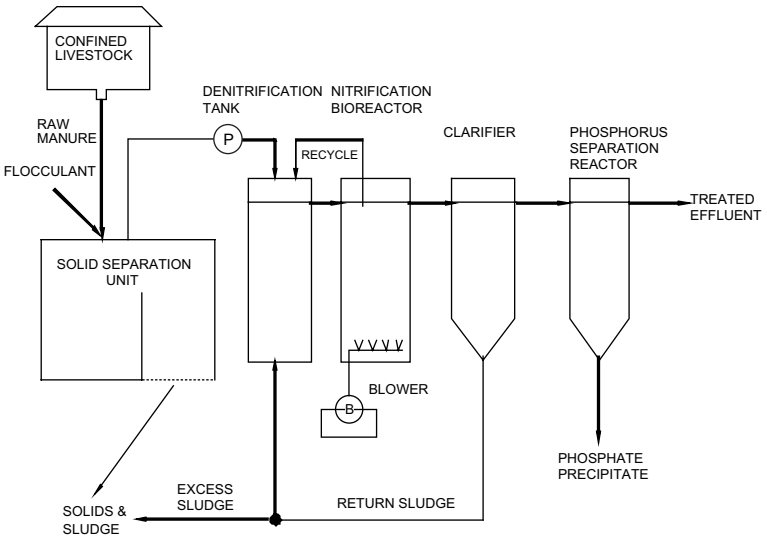

Fig. 1. Schematic drawing of the pilot waste treatment system without lagoon (Vanotti et al., 2001).

recycled to the first denitrification tank at a rate of 0.5 m^3/day. Hydraulic retention time (HRT) of the biological process was 3.1 d.

The third and final step in the treatment system was the phosphorus removal unit using a process developed by USDA-ARS (Vanotti et al., 2001). The effluent from the biological N removal unit was treated with hydrated lime (2% $Ca(OH)_2$) in a stirred tank and subsequently settled in a conic tank where the precipitate was removed from the bottom of the tank. Hydraulic retention time (HRT) of this unit was 1.8 h. Chemical was added to reach a set point pH value of 10.5 that optimized removal of soluble P and formation of calcium phosphate. An average of 280 mg/l of $Ca(OH)_2$ was needed to reach this point. Detailed reactor information and water quality performance evaluation of this process unit was provided by Vanotti et al. (2003a).

2.2. Wastewater sampling

Liquid samples were collected manually as grab samples five times approximately 3 weeks apart from the following points: (1) the lagoon liquid that was used to flush the manure from the barns, (2) the homogenization tank receiving the liquid manure flush from the barns, (3) the effluent of the sand filter after liquid–solid separation (post-separation), (4) the effluent after the nitrification–denitrification step (post-N removal), and (5) the effluent after the phosphorus extraction step (post-P removal). One set of duplicate samples was overnight shipped with cold packs to the ARS Environmental Microbial Safety Laboratory in Beltsville, MD, for microbiological analyses. Another set of duplicate samples was transported on ice to the ARS Coastal Plains Research Center in Florence, SC, for water quality analyses (APHA, AWWA, WEF, 1998).

2.3. Microbiological analyses

Each step of the treatment system was evaluated for its effectiveness in reducing pathogens, by counting total and fecal coliforms, enterococci, and *Salmonella* on selective and differential nutrient media, using the standard protocols for pathogens and indicator microbes for the examination of compost and water. Before microbial analysis, the pH of the post-P removal samples was brought down from >10 to 8.0 using 6N H_2SO_4. Fecal coliforms and presumptive *E. coli* were enumerated by using MacConkey's agar plates containing 100 μg/ml of MUG. Incubation of these plates at 44.5 °C selected for fecal coliforms and a fluorescent blue halo around a colony when exposed to UV light at 365nm was indicative of *E. coli*. Total coliforms were enumerated on MacConkey's agar incubated at 37 °C overnight. Enterococci were enumerated on modified Enterococcus agar incubated at 37 °C overnight. Salmonellae were enumerated by spiral plating on XLT4 agar and incubating the plates at 37 °C. A colony lift immunoassay specific for *Salmonella* serotypes was performed on presumptive *Salmonella* colonies for confirmation of *Salmonella*. Effectiveness of treatment for reducing *Salmonella* and enteric microbial indicators was calculated using mean $\log_{10}$ reductions for each microbe by subtracting $\log_{10}$ converted concentration after each process from the calculated mean $\log_{10}$ concentration in flushed manure influent to the system. Significant differences among treatment means were evaluated using analysis of variance and 5% level Duncan test (Gomez and Gomez, 1984).

2.4. Other analyses

Wastewater analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WEF, 1998). Total suspended solids (TSS) was analyzed per Standard Method 2540 D. Chemical oxygen demand (COD) was determined with the closed reflux, colorimetric method (Standard Method 5220 D). Total P (TP) and Total Kjeldahl N (TKN) were determined using the automated ascorbic acid method (Standard Method 4500-P) and the automated phenate method (Standard Method 4500-NH_3 G), respectively, adapted to digested extracts (Technicon Instruments Corp., 1977).

3. Results and discussion

3.1. Water quality characteristics

Average TSS, COD, TKN, TP, and pH values in the liquid manure before and after passing through each step in the treatment system are shown in Table 1. The operation used anaerobic lagoon recycle to flush the manure from the houses. It contained significant amounts of N and P but low concentrations of TSS and COD compared with flushed manure. The range of total solid (TS) obtained in the flushed manure (7.2–19.2 g/l, data not shown) was consistent with values of 5–20 g/l described for flush systems in the USA (Chastain et al., 1999).

The treatment system was developed to provide an alternative to lagoon-sprayfield swine waste management systems in North Carolina. Data in Table 1 show the primary function and contribution of each step to the total treatment system (sampling points 3–5). Average separation efficiencies of 98% TSS, 84% COD, 77% TP, and 66% TKN were obtained in the solid–liquid separation step (Table 1). By capturing the suspended particles, most of the oxygen-demanding organic compounds were removed from the liquid stream. Instead of oxygen being used to break down organic compounds, it was used in the subsequent aeration treatment to more efficiently convert ammonia. This is important because the separated liquid contained significant amounts of N (232 mg/l), mostly soluble ammonia. The biological N removal step eliminated 95% of the TKN and used most (78%) of the remaining COD after separation for denitrification. It also reduced bicarbonate alkalinity (from 1381 to 338 mg/l) which, in turn, affected the succeeding P separation step. Reduction of carbonate and ammonium buffers during nitrification substantially reduces the $Ca(OH)_2$ demand needed for optimum P precipitation and removal at high pH (Vanotti et al., 2003a).

3.2. Microbial reduction by treatment

Results showed a consistent trend in reduction of *Salmonella*, total and fecal coliforms, and enterococci as a result of each step in the treatment system (Table 2). In general, the lowest concentrations of salmonellae and microbial indicators of fecal contamination occurred after the nitrification/denitrification and phosphorus removal steps. Solid–liquid separation decreased 0.5–1 $\log_{10}$ the concentration of *Salmonella* and microbial indicators. This is lower than the 98% TSS reduction obtained in the same treatment step and suggests that flocculation treatment is less effective to capture suspended microbes compared to colloidal manure particles.

Subsequent biological N removal conditions destroyed most of the microbes in the liquid with total concentration reductions of 4.5 $\log_{10}$ for total coliforms, 4.4 $\log_{10}$ for fecal coliforms, 4.1 $\log_{10}$ for enterococci, and 2.4 $\log_{10}$ for *Salmonella*. Daily mean water temperature varied from 33.1 to 14.1 °C during the July–October period when microbial testing was conducted. During treatment, the liquid was passed through a succession of oxic and anoxic tanks that affected microbe survival. Average process ORP_h (standard hydrogen oxygen reduction potential) was −8.7, +91.1, and +200.9 mV in influent, denitrification tank and nitrification tank, respectively. Corresponding average dissolved oxygen concentration was 0, 1.35 and 3.90 mg/l. Hydraulic retention time (HRT) was 31.2 h in the denitrification tank and 13.2 h in the nitrification tank. Exposure of microbes to these contrasting conditions

Table 1
Average physico-chemical characteristics of liquid manure effluent before treatment and at each step of the treatment system[a]

Sampling point[b]	TSS (g/l)	COD (g/l)	TKN (mg/l)	TP (mg/l)	pH
Lagoon wash water	0.26 (0.03)	0.64 (0.05)	193 (39)	89 (16)	7.89 (0.05)
Homogenization tank	10.59 (1.28)	8.27 (1.07)	688 (49)	480 (76)	7.01 (0.06)
Post-separation	0.19 (0.02)	1.32 (0.10)	232 (13)	112 (25)	7.29 (0.03)
Post-nitrification/denitrification	0.22 (0.12)	0.29 (0.03)	11 (5)	75 (9)	7.62 (0.06)
Post-phosphorus removal	0.09 (0.01)	0.18 (0.02)	2 (2)	7 (2)	10.3 (0.07)

[a] Values are mean (standard error) for five sampling dates.
[b] Lagoon supernatant liquid (1) was used to flush manure from barns. Flushed raw manure was collected and mixed in homogenization tank (2) and passed through the multi-step treatment system consisting of solid–liquid separation (3) biological N removal (4) and phosphorus removal (5).

Table 2
Microbiological analyses of liquid manure effluent before treatment and at each step of the treatment system[a]

Sampling point[b]	Total coliforms (log_{10}/ml)	Fecal coliforms (log_{10}/ml)	Enterococci (log_{10}/ml)	Confirmed *Salmonellae*[c] (log_{10}/ml)
Lagoon wash water	3.40 (0.58)	4.18 (0.40)	3.46 (0.27)	2.61 (1.06)
Homogenization tank	6.79 (0.45)	6.23 (0.24)	5.73 (0.44)	3.89 (0.87)
Post-separation	6.07 (0.50)	5.75 (0.08)	4.78 (0.73)	3.27 (1.01)
Post-nitrification/denitrification	2.25 (0.49)	1.88 (0.35)	1.63 (0.34)	1.54 (0.87)
Post-phosphorus removal	<0.30	<0.30	<0.30	<0.30

[a] Values are mean (standard deviation) of log_{10} colony forming units (cfu) per ml for duplicate samples for five runs of the system; < indicates there were no colonies to count, thus only the upper threshold limit value can be calculated. To compare means in a column, $LSD_{0.05} = 0.42$, 0.31, 0.40, and 0.88 log_{10}/ml for total coliforms, fecal coliforms, Enterococci and Salmonellae, respectively.

[b] Lagoon supernatant liquid (1) was used to flush manure from barns. Flushed raw manure was collected and mixed in homogenization tank (2) and passed through the multi-step treatment system consisting of solid–liquid separation (3), biological N removal (4) and phosphorus removal (5).

[c] Presumptively positive salmonellae were confirmed by serological test.

was intensified due to the internal recycle (2.5 Q) between nitrification and denitrification used in the process. Our data suggest that these conditions were extremely effective for pathogen destruction.

Alkali treatment is well known to destroy pathogens (Sobsey et al., 2001; Hrazdira et al., 2002; Callis and Gregg, 1986), but its application to liquid manure is difficult due to ammonia loss and large chemical requirement at high pH. In wastewater containing a high ammonia concentration, the lime dose required to elevate the pH is increased due to the ammonium-ammonia equilibrium that neutralizes the hydroxyl ions. This means that the alkali is used to convert ammonia into gas form before effective increase of pH above 9 is achieved. Ammonia volatilization from animal facilities is an environmental problem in and of itself. However, this problem is solved using a biological N removal step preceding the alkali treatment. Since ammonia N has been eliminated (Table 1), increased pH does not result in significant gaseous N loss.

Salmonella and pathogen indicators were eliminated with the phosphorus treatment in the sequence due to elevated pH used to precipitate phosphorus; there were no colonies to count at the upper threshold limit value of <2 cfu/ml. Total reductions obtained were >6.5 log_{10} for total coliforms, >5.9 log_{10} for fecal coliform, >5.4 log_{10} for enterococci, and >3.6 log_{10} for *Salmonella*. These reductions were significantly higher than reductions of 3.4, 2.1, 1.8, and 1.3 log_{10}, respectively, obtained by lagoon treatment in the same farm (Lagoon wash, Table 2). Thus, use of treated water after N or P treatment to flush manure from the houses may contribute to additional improvements in animal and environmental hygiene.

4. Conclusions

An important part of manure and byproduct management is treatment before land application because it can reduce pathogens, manure volume, odor potential, and stabilize nutrients while improving the handling and spreading characteristics. Thus, effective treatments can contribute significantly to pollution prevention and hazard reduction. Reduction of *Salmonella* ssp. and microbial indicators of fecal contamination was demonstrated in a multi-step treatment system consisting of solid–liquid separation, nitrification–denitrification, and phosphorus extraction with lime at high pH. Elimination of COD and TKN and alternating oxic and anoxic conditions during the nitrification–denitrification treatment were effective conditions to decrease *Salmonella* and indicator microbes in liquid swine manure. Subsequent alkali treatment during the P removal process produced a sanitized effluent which may be important for biosecurity reasons; there were no colonies to count at the upper threshold limit value of <2 cfu/ml. Total reductions obtained by treatment were >6.5 log_{10} for total coliforms, >5.9 log_{10} for fecal coliform, >5.4 log_{10} for enterococci, and >3.6 log_{10} for Salmonella.

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