DESTRUCTION OF PATHOGENS IN LIQUID SWINE MANURE BY BIOLOGICAL NITROGEN REMOVAL AND PHOSPHORUS TREATMENT

VANOTTI M.B., MILLNER P.D.**, HUNT P.G.*, ELLISON A.Q.**

** United States Department of Agriculture, ARS, Coastal Plains Research Center, 2611 W. Lucas St, Florence, SC 29501, USA; vanotti@florence.ars.usda.gov*
*** United States Department of Agriculture, ARS, Animal Waste Pathogens Laboratory, 10300 Baltimore Ave., Bldg. 001, Rm 140, Beltsville, MD 20705, USA.*

ABSTRACT

Concern has greatly increased about the potential for contamination of water, food, and air by pathogens present in manure, byproducts, and bioaerosols. We evaluated pathogen reduction in a multistage system where first the solids and liquid in swine manure are separated with polymer, followed by biological N removal using nitrification and denitrification and then P extraction through lime precipitation. Each step of the treatment system was evaluated for its effectiveness in reducing pathogens by counting total and fecal coliforms, enterococci, and salmonellae on selective and differential nutrient media. Before treatment, concentrations of total and fecal coliforms were 1.10 x 10^7colony forming units (cfu)/mL and 1.28 x 10^6 cfu/mL, respectively. Enterococci concentration was 9.52 x 105 cfu/mL and salmonella concentration was 2.3 x 10^4 cfu/mL. Results showed a consistent trend in reduction of pathogens as a result of each step in the treatment system. Solids separation reduced 67% to 83% of the pathogens compared with 98% removal of volatile suspended solids in the same process step. Biological N removal with alternating anoxic and oxic conditions destroyed a large amount of the microbes with total reductions of 5 logs (99.997%) for total coliforms, 4 logs for fecal coliforms and enterococci, and 2 logs for salmonellae. Salmonellae and pathogen indicators were eliminated with the phosphorus treatment in the sequence due to elevated pH (10.3); there were no colonies to count at the upper threshold limit value of < 3 x 10^1 cfu/mL. Our results indicate that nitrification/denitrification treatment is very effective in reducing pathogens in liquid swine manure and that the phosphorus removal step via calcium precipitation produces a sanitized effluent which may be important for biosecurity reasons.

INTRODUCTION

The usefulness of composting, heat drying, and alkaline stabilization processes to destroy infectious microorganisms contained in solid manure and biosolids in general is well known. However, little is known about rates of pathogen reduction in new treatments developed for liquid manure and wastewater effluents. We developed an alternative system for treatment of liquid swine manure to replace anaerobic lagoon technology commonly used in the USA to treat swine waste. In this multistage system, solids and liquid are first separated with polyacrylamide (PAM) polymer, followed by biological N removal using nitrification and denitrification and then by P extraction using a lime precipitation process. The pilot system was successfully tested for two years at the Swine Unit at NC State University Lake Wheeler Field Rd. Laboratory (Vanotti et al., 2001). During a three-month period in the second year of the study (July-Oct. 2001), we evaluated how pathogens were reduced by each of the process units comprising the total system. A full-scale system based on these studies is being constructed in Duplin Co., NC for demonstration and verification of Environmental Superior Technology under the Smithfield Foods/PSF & NC Attorney General Agreement program.

SOLIDS-LIQUID SEPARATION STEP

The usefulness of polymers to flocculate and increase separation of solids from liquid swine manure has been demonstrated (Vanotti and Hunt, 1999). Along with the solids there is a significant capture of organic nutrients associated with the clumping of the small particles that usually escape screens or clog sand filters. For solids separation, we used an in-line PAM injector and mixer to flocculate the solids in the flush and two sand filter beds (20 x 16 ft) for dewatering (Vanotti et al., 2001). Flushed manure was first mixed in a homogenization tank and pumped at 500 L/min to the polymer injection unit before application to the sand filter bed. The polymer used was Magnifloc c-1596, Cytec Industries, Inc . The beds received 30-cm depth of flocculated liquid during each pour. Separation efficiencies of 98% for total suspended solids (TSS) and volatile suspended solids (VSS), 93% BOD_5, 77% total phosphorus (TP) and 66% total kjeldahl nitrogen (TKN) were obtained on the average (Table 1). Organic N and P were efficiently separated (88 and 84%, respectively) but not the soluble fractions.

Table 1. Performance of solids-liquid separation in the multistage treatment process.[a]

	Homogenization tank (mg/L)	Post-Polymer (mg/L)	Separation Efficiency (%)
TSS	10591	190	98
VSS	8563	145	98
BOD_5	2932	217	93
TKN	688	232	66
Total P	480	112	77

[a] Polymer flocculation and sand filtration

BIOLOGICAL NITROGEN REMOVAL STEP

Soluble ammonia and phosphorus levels, which may constitute 35-45% of total N and 15-25% of total P, are mostly unaffected by polymer separation and need further treatment when land application is not an option. A Biogreen system that uses nitrifying pellets (Vanotti et al., 2000) in an aerated tank and denitrifying sludge in anoxic tanks was used to treat the effluent after solids separation with PAM (see figure). The pilot unit was designed to treat 1 m^3/day of liquid at 10°C water temperature. It contained a 1.3 m^3 anoxic denitrification tank (1) with ~ 3 g/L MLVSS to remove soluble carbon and NO_3-N, a 0.55 m^3 nitrification tank containing 100 L of

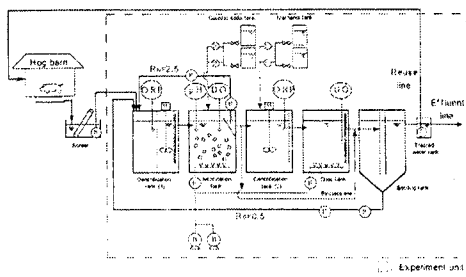

Table 2. Performance of the biological nitrogen removal step in the multistage treatment system.[a]

	Post-Polymer (mg/L)	Post-N Removal (mg/L)	Removal Efficiency (%)
Alkalinity	1381	338	76
BOD_5	217	56	74
TKN	232	11	95
NH_3-N	173	0	100

[a] Nitrification/denitrification after solids separation

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

polyethylene glycol (PEG) 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 to enhance settling, and a 0.63 m^3 tank for settling and recycling of suspended solids. Pellets were provided by the Hitachi Plant Construction & Engineering Co. of Japan. Nitrification activity after 60 days of initial acclimation was 790 g N/100-L pellets/day. Nitrified water was recirculated (R_N) to the denitrification tank (1) at a rate of 5 m^3/day and settled sludge was recycled (R_S) at 0.5 m^3/day. The system performed to expectations removing 95% of TKN and all the ammonia in the wastewater (Table 3). The natural alkalinity in wastewater (1380 mg/L) was sufficient for a complete nitrification without needs for alkali supplements.

PHOSPHORUS EXTRACTION STEP

Table 3. Performance of phosphorus removal step in multistage treatment system.[a]

	Post-N Removal	Post-P Removal	% Efficiency
pH	7.6	10.3	--
Alkalinity	338	364	--
BOD	56	4	93
Total P	75	7	91
PO_4-P	54	1	98

[a] Except pH, values are concentration in mg/L

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). Hydrated lime (2% $Ca(OH)_2$) was added and mixed with the effluent after biological N removal in a stirred tank and subsequently settled in a conic tank where the precipitate was removed from the bottom of the tank. Chemical was injected to reach a set point pH of 10.5 in order to remove soluble P (Table 3) and kill pathogens (Table 4). The amount of calcium applied averaged 2.1 mol per 1 mol of P contained in the wastewater.

MICROBIOLOGICAL ANALYSES

Each step of the treatment system was evaluated for its effectiveness in reducing pathogens, by counting total and fecal coliforms, enterococci, and salmonellae on selective and differential nutrient media using the standard protocols for pathogens and indicator microbes for the examination of compost and water. 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. Samples were collected five times approximately 3 weeks apart during a three month period (July-Oct., 2001) from 1) the homogenization tank receiving liquid manure from the barns, 2) the effluent of the sand filter after liquid-solids separation (post-polymer), 3) the effluent

after the nitrification/denitrification step (post-N removal), and 4) the effluent after the phosphorus extraction step (post-P removal). One set of duplicate samples was overnight shipped with coldpacks to the ARS Animal Waste Pathogens Laboratory in Beltsville, MD for microbial analysis (Table 4). Another set of duplicate samples was transported on ice to the ARS Coastal Plains Research Center for water quality analyses (reported in Tables 1,2 and 3). Before microbial analysis, the pH of the post-P removal samples was brought down from 10.3 to 8.0 using 6N H_2SO_4.

Table 4. Microbiological analyses of effluents at each step in the treatment system.[a]

Treatment Point	Total Coliforms (cfu/mL)	Fecal Coliforms (cfu/mL)	Enterococci (cfu/mL)	Confirmed Salmonellae[b] (cfu/mL)
Homogenization Tank	1.10×10^7	1.28×10^6	9.52×10^5	2.32×10^4
Post-Polymer	2.30×10^6	4.26×10^5	1.59×10^5	7.21×10^3
Post-Nitrification/ Denitrification	3.05×10^2	6.80×10^1	4.10×10^1	3.10×10^2
Post-Phosphorus Removal	$<3.5 \times 10^1$	$<1.8 \times 10^1$	$<2.1 \times 10^1$	$<2.1 \times 10^1$

[a] *Values are mean of 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.*
[b] *Presumptively positive salmonellae were confirmed by serological test.*

Results showed a consistent trend in reduction of salmonellae, total and fecal coliforms, and enterococci as a result of each step in the treatment system (Table 4). In general, the lowest concentrations of salmonellae and pathogen indicators occurred after nitrification/ denitrification and phosphorus removal steps. Solids-liquid separation with cationic polymer reduced 68% to 83% of the pathogens. This is lower than reductions of 98% total and volatile suspended solids in the same treatment step (Table 1). Biological N removal conditions destroyed most of the microbes in the liquid with total reductions of 5 logs (99.997%) for total coliforms, 4 logs for fecal coliforms and enterococci, and 2 logs for salmonellae. The N removal process recycles the liquid several times between oxic and anoxic tanks and also promote use of endogenous carbon in denitrification. Our data suggest that these conditions are extremely effective for pathogen destruction. Salmonellae and pathogen indicators were eliminated with the phosphorus treatment in the sequence due to elevated pH (10.3) used to precipitate phosphorus; there were no colonies to count at the upper threshold limit value of $< 3 \times 10^1$ cfu/mL. Our results indicate that nitrification/ denitrification treatment is very effective in reducing pathogens in liquid swine manure and that the phosphorus removal step via calcium precipitation produces a sanitized effluent which may be important for biosecurity reasons. Such is the case with controlling the introduction and spread of Foot and Mouth Disease virus as an aerosol during liquid manure spreading since the virus is particularly susceptible to pH above 9.

REFERENCES

Vanotti, M. B. and P.G. Hunt.1999. Solids and nutrient removal from flushed swine manure using polyacrylamides. *Transactions of the ASAE* 42(6):1833-1840.

Vanotti, M.B., P.G. Hunt, J.M. Rice and F.J. Humenik. 2000. Nitrifying high-strength wastewater. *Industrial Wastewater*, Sept./Oct.: pp. 30-36. Water Environment Federation, Alexandria, VA, USA.

Vanotti, M.B., A.A. Szogi and P.G. Hunt. 2001. Wastewater treatment system. Patent Application Serial No. 09/903,620. U.S. Patent & Trademark Office, Washington, DC, USA.

Vanotti, M.B., J.M. Rice, P.G. Hunt, F.J. Humenik, A.Q. Ellison, C.A. Baird, P. Millner and A.A. Szogi. 2001. Evaluation of polymer solids separation, nitrification denitrification and soluble phosphorus removal system for treating swine manure. Proc. Int. Symposium Addressing Animal Production and Environmental Issues. North Carolina State Univ., Oct. 3-5, 2001. Sheraton Imperial, Research Triangle Park, NC, USA.