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HOG WASTE TREATMENT TO CONTROL MICROBIAL CONTAMINATION

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ABSTRACT

The presence and control of human pathogens in waste from commercial swine farms has become a public health and policy issue for swine waste treatment and management practices. This research quantified enteric microbe reductions in anaerobic swine waste lagoons and alternative treatment systems (anaerobic mesophilic digestion, constructed wetlands, aerobic biofiltration, aerated solids recovery biological treatment, gravel media filtration, and overland flow). Swine waste samples were analyzed for the bacterial pathogen *Salmonella* and a suite of 6 microbial indicators: fecal coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens* spores, somatic coliphages and male-specific (F-specific) coliphages. In untreated swine waste lagoon influent, average *Salmonella* spp. concentrations were 3800 MPN/100 mL. *Salmonella* reductions were 96% and 97% in primary and secondary lagoons, respectively. Fecal coliforms, *E. coli* and enterococci reductions were only slightly higher than those of *Salmonella* (97-98%) in each lagoon cell. *C. perfringens* spores were less effectively reduced in lagoons than were the other enteric bacteria studied: 84% in primary lagoons and another 92% in secondary lagoons. Somatic and male- (F-) specific coliphages were reduced similarly to fecal coliforms, *E. coli* and enterococci (97% in primary lagoons and another 96% in secondary lagoons). In a surface flow constructed wetland treating lagoon liquid at a swine nursery, fecal coliforms, *E. coli* and enterococci were reduced by 98, 99 and 87%, respectively. *Salmonella* spp. were reduced by 96% and *C. perfringens* spores, somatic coliphages and F-specific coliphages by 97, 99 and 98%, respectively. In a field pilot scale gravel media filter and an overland flow system, reductions of enteric microbial indicators were far less than achieved in the constructed wetland. In an aerobic biofilter field system, reductions of fecal coliforms, *E. coli* and enterococci were 97, 97 and 96%, respectively. *Salmonella* spp. were reduced by 94% and *C. perfringens* spores, somatic coliphages and F-specific coliphages by 84, 95 and 97%, respectively. In aerated solids recovery biological treatment systems on two farms, reductions of fecal coliforms, *E. coli* and enterococci were 99.3, 99.3, and 99.5%, respectively, in the closed-loop portion of the treatment system. Reductions were 99.4% for *Salmonella* and for *C. perfringens* spores, somatic coliphages and F-specific coliphages they were 81, 99.5 and 99.8%, respectively. In the "temporary storage areas" of waste liquids (i.e., former waste lagoons retrofitted to hold excess water from the aerated solids recovery biological treatment systems), microbial indicators (except *C. perfringens* spores) and *Salmonella* generally were >99.99% lower than in the untreated, flushed swine waste. Hence, replacing lagoons with alternative swine waste treatment systems can provide additional reductions of enteric microbes in liquid subsequently applied to sprayfields. The results of this study show that high levels of *Salmonella* and other enteric microbes are present in flushed swine waste and swine waste lagoons. Alternative treatment techniques were found to be as effective or more effective than lagoons for reducing enteric microbes in flushed swine waste. Despite these biological treatments, swine waste effluents still contained measurable levels of *Salmonella* pathogens and the levels of fecal indicator bacteria were higher than allowed for land application of domestic or municipal wastewater. Therefore, greater efforts are needed to insure that land-applied swine waste effluents do not result in enteric microbial contamination of ground water, surface water or edible crops.

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SUMMARY AND CONCLUSIONS

- Enteric microbes, including the human pathogens *Salmonella* spp., were found at high concentrations in untreated, flushed swine waste.
- With the exception of *C. perfringens* spores (potential treatment indicators for parasite pathogens like *Cryptosporidium parvum* and *Ascaris* spp.), enteric microbe concentrations were appreciably reduced in anaerobic swine waste lagoons and a mesophilic digester. There were appreciable further reductions of microbes in a second lagoon used in series with a primary lagoon or digester, resulting in greater overall reductions .
- Relatively high concentrations of enteric microbes, including *Salmonella*, remained in anaerobic swine lagoon or digester liquids.
- Alternative or additional waste treatment techniques, including constructed wetlands, aerobic biofiltration and aerobic solids recovery biological treatment, were found to be as effective for reducing *Salmonella* and enteric microbial indicators in swine waste as were anaerobic lagoons and a mesophilic digester.
- A marl-gravel media filter and overland flow system developed for swine waste nitrification were found to achieve lower enteric microbe reductions relative to the other treatment systems studied.
- Constructed wetlands were found to achieve appreciable reductions of *Salmonella* and enteric microbial indicators in anaerobic swine lagoon liquid. *Salmonella*, fecal coliforms and *E. coli* were reduced by 96-99% in this system. Enterococci were less effectively reduced (~87%) and appeared to be inadequate indicators of *Salmonella* reductions in the wetland treatment system. The viral indicators, somatic and F-specific coliphages, were reduced to a similar extent as were fecal coliforms and *E. coli* (98-99%). The constructed wetland system was the only treatment system studied that achieved similar removal of *C. perfringens* spores (97%) as was observed for other enteric microbes (e.g., fecal coliforms, coliphages).
- An aerobic biofilter system achieved substantial reductions of *Salmonella* and enteric microbial indicators (an average of 94-97%; with the exception of *C. perfringens* spores, which were reduced an average of 84%). The hydraulic residence time of <24 hours was much lower than typical hydraulic residence times of 6-12 months in anaerobic lagoons.
- Enteric microbe reductions in the closed-loop portion of an aerated solids recovery biological treatment system were also high (99.4-99.8%), except for *C. perfringens* spores, which were reduced an average 81%. In the "temporary storage areas" (TSA) used to store excess wastewater from the closed-loop system, concentrations of enteric microbes were about 99%

lower than in the last treatment reactor (Bioreactor 2) of the closed-loop aerated solids recovery biological treatment systems.

- The results of this study show that alternative treatment systems can be effective for reducing *Salmonella* and other enteric microbes in swine waste to an extent similar to, or greater than, reductions achieved in anaerobic swine waste lagoon systems, and at much lower hydraulic residence times and treatment system volumes.
- The TSA data show that lagoons replaced by alternative treatment systems can be utilized as storage areas for effluent from alternative systems. Furthermore, the resultant enteric microbe concentrations in TSA water subsequently applied to land can be 99% lower than was measured in primary swine waste lagoons, which is another order of magnitude lower than concentrations measured in secondary swine waste lagoons.

RECOMMENDATIONS

Human enteric pathogens (e.g., *Salmonella* bacteria) are present at high concentrations in swine waste, and in some cases the concentrations exceed those found in human, domestic or municipal raw sewage. Therefore, reductions of these microbes should be an essential component of swine waste treatment system performance evaluations in order to ensure protection of public health, livestock health and the environment.

Because enteric microbes were incrementally reduced substantially (90-99% per treatment unit) in anaerobic lagoon and mesophilic digestion systems, the use of a second lagoon in series with a primary lagoon or digester is recommended when greater pathogen reductions are needed or desired. Based on existing knowledge of microbial dynamics and related lagoon treatment performance from the literature, it is likely that even further reduction in enteric microbe could be achieved by using additional lagoon cells in series (e.g., three lagoons in-series, etc.). The use of such multiple-cell treatment systems is recommended if even greater reductions in pathogens are needed or desired. It should be noted, that the microbial reductions measured in lagoon systems were for those built and operated according to NRCS standards, which have hydraulic residence times on the order of 6 months or more (and associated large reactor volumes).

The effective alternative waste treatment techniques studied (i.e., constructed wetlands, aerobic biofilter, aerated solids recovery biological treatment) were shown to be capable of achieving enteric microbe reductions of similar or greater magnitude than those achieved in primary lagoons. Because their enteric microbe reductions were comparable to two lagoons in-series systems, but at much shorter hydraulic residence times (and smaller reactor volumes), further evaluation and consideration of these systems is recommended. Perhaps relatively less risk of microbial contamination of the environment would be associated with these alternative treatment systems if there were treatment system failures or catastrophic events like flooding. This is because these alternative treatment systems require far less time and reactor volume to achieve comparable microbial reductions to those of anaerobic lagoons.

The investigation of former lagoons used as temporary storage areas (TSAs) for wastewater effluent from an aerated solids recovery biological treatment system showed that lagoons replaced by alternative waste treatment systems can be effectively utilized for storage of effluent from the alternative systems. The use of former lagoons as temporary storage areas prior to land application is recommended as a beneficial and cost-effective way to further utilize these existing structures. This is because the liquid from these former lagoons can have enteric microbial concentrations that are more than 1 to 2 orders of magnitude lower than in the waste lagoons investigated in this study. These lower microbial concentrations decrease the risks that water applied to sprayfields will result in microbial contamination of nearby streams, ground water aquifers or other environmental receptors.

Effluents from swine waste lagoons and some other swine waste treatment systems can still contain measurable levels of human pathogens such as *Salmonella* bacteria as well as concentrations of fecal indicator bacteria well above the allowed limits for land application of municipal wastewater. Therefore, greater efforts to determine the levels of human pathogens and fecal indicator bacteria in land applied swine wastewater effluents and their fate in the environment are recommended. In particular, the potential for enteric microbes from land applied swine water effluents to contaminate ground water, surface water and edible food crops needs to be determined. If such contamination is found, it may indicate the need for improved treatment and other control measures in swine waste treatment and management systems to further reduce the potential health risks from enteric microbe contamination of environmental media.

INTRODUCTION

Background

Current practice of commercial swine agriculture in North Carolina and elsewhere is to raise animals indoors in secure barns having sub-floor pits or other collectors for the manure (feces and urine) produced by the animals. Typically, the resulting swine waste is periodically flushed or otherwise removed from the barns for subsequent treatment and disposal. Lagoons are the most common method of storing and treating flushed swine waste in North Carolina, as well as throughout much of the United States. In the 1990s, the U.S. swine production industry experienced substantial growth and consolidation, with a concomitant increase in both the number and size of waste lagoons. Public concern has been growing regarding the public health and environmental threats posed by current swine lagoon management practices. Government agencies have responded at the local level in North Carolina by establishing a moratorium on new swine lagoon construction. In addition, the major swine producers in North Carolina negotiated and agreed to a lagoon phase-out (State of North Carolina, Office of the Attorney General, 2000). At the federal level, new regulations have been proposed for improved management of animal waste produced by concentrated animal feeding operations (CAFOs) (USEPA, 2001).

Pathogens of swine are a concern for swine waste management because many of them are also infectious to humans, including bacteria like *Salmonella*, *Yersinia*, and *Leptospira*, protozoan parasites like *Cryptosporidium parvum* and *Giardia lamblia*, and viruses like swine hepatitis E virus (Cole et al. 1999). Enteric microbes are present in flushed swine waste at high concentrations (Hill and Sobsey 1998; 2001) and when herds are infected with pathogens of the enteric or reproductive systems these infectious microbes will be present in the farm's waste management system. When waste is applied to land, these pathogens may persist and be transported to groundwater, surface water, or other aquatic or food resources that on-farm or neighboring individuals may ingest or contact. *Salmonella* spp. are commonly found on swine farms in the United States and are highly prevalent in swine herds (Davies et al. 1997a; Davies et al. 1997b). Thus, *Salmonella* are bacterial pathogens with high potential for transport off swine farms to nearby water resources and food commodities.

The alternative swine waste treatment systems investigated in this research received attention by university and non-university researchers because of their potential for improving swine waste treatment and management in North Carolina. Some of these systems, like overland flow (Carlson et al. 1974; Hunt and Lee 1976) have been available for many years for municipal and animal waste treatment. Others, like constructed wetlands (Hammer et al. 1993; Cronk 1996), aerobic biofiltration (Kantardjieff et al. 1996) and gravel media filtration (Szögi et al. 1997) also have been used for municipal and domestic waste water treatment and have generated recent research interest for swine waste treatment.

Study Purpose and Design

In this study, wastewater samples from anaerobic swine waste lagoon systems and alternative treatment systems were analyzed for *Salmonella* and a suite of six microbial indicators of fecal contamination, namely fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, somatic coliphages, and male-specific (F-specific) coliphages, in order to evaluate enteric microbe reductions in alternative treatment systems. Microbial indicators are commonly used as models or surrogates for the presence and persistence of enteric pathogens in waste treatment systems and the environment. Fecal coliforms, Gram-negative, rod-shaped bacteria fermenting lactose (possessing Beta-galactosidase activity) at 44.5°C, are traditional bacterial indicators of enteric bacterial pathogens like *Salmonella*. *E. coli*, the archetypical fecal coliform, is a more specific bacterial indicator of fecal contamination than fecal coliforms because the fecal coliform test detects some non-fecal bacterial species (e.g., *Klebsiella* spp.). Enterococci (especially *E. faecalis* and *E. faecium*), the Gram-positive, spherical to oval bacteria growing in chains, resistant to bile salts and sodium chloride and commonly inhabiting the human and animal gut, are possibly better representatives of the survival of more environmentally-resistant bacterial pathogens. *C. perfringens* is a Gram-positive, rod-shaped, spore-forming, anaerobic bacterium inhabiting the human and animal gut that has been suggested as a possible indicator for the persistence of environmentally-stable helminth ova (e.g., *Ascaris* spp) and protozoan parasite cysts and oocysts (*Giardia lamblia* and *Cryptosporidium parvum*). Somatic and F-specific coliphages (viruses infecting *E. coli* and perhaps some other coliform bacteria) are potential indicators for human and animal enteric viruses. These two groups of coliphages are differentiated by how they infect their host bacteria, either by attaching directly to the bacterial outer cell membrane or cell wall (somatic coliphages) or to hair-like appendages ("male-specific" or F-specific pili or fimbriae) protruding from the bacterial cell wall.

In this study the reductions of *Salmonella* bacterial pathogens and six microbial fecal indicators- namely fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, somatic coliphages and F-specific coliphages- were determined in a variety of alternative swine wastewater treatment processes and systems. These treatment processes included anaerobic lagoons, which are the current treatment designated as Best Management Practice in North Carolina (followed by land application of the stored lagoon liquid using spray irrigation). Other treatment processes investigated in this study were (1) mesophilic anaerobic digestion in an in-ground, covered reactor, followed by storage in a lagoon, (2) surface flow constructed wetlands, (3) an aerobic, two-stage, fixed-film, biofiltration system (the Ekokan process), (4) an aerated, two-stage, biological treatment system with biosolids recovery (Bion system), (5) a gravel media filtration system for nitrification, and (6) an overland flow treatment system. All of these alternative treatment processes were available for study at either full-scale (the covered, mesophilic, anaerobic digester and secondary lagoon treatment system) or at pilot-scale [constructed wetland, aerobic biofilter (Ekokan[®]) system, aerated solids recovery (Bion[®]) system, gravel media nitrification filter and overland flow system]. Treatment systems were studied by repeated sampling and microbial analyses of influents and effluents of the treatment processes

and systems for time periods ranging from several months to more than one year. The reductions of the various microbial pathogens and indicators and in some cases other analytes (e.g., nutrients such as forms of nitrogen) were determined for treatment processes and systems.

REVIEW OF THE LITERATURE

Introduction

Fecal wastes and other wastes (e.g., respiratory secretions, urine and sloughed feathers, fur or skin) of various agricultural (livestock) and feral animals often contain high concentrations of human pathogens (disease-causing microorganisms) (Strauch and Ballarini, 1994).

Concentrations of some pathogens occur at levels of millions to billions per gram of wet weight feces. Per capita fecal production by agricultural animals such as cattle and swine far exceeds that of humans. Furthermore, the trend for production facilities to harbor thousands to tens of thousands of animals in relatively small spaces results in the generation of very large quantities of concentrated fecal wastes that must be effectively managed to minimize environmental and public health risks. As shown in Table 1, animal pathogens posing potential risks to human health include a variety of viruses (such as swine hepatitis E virus) (Halbur et al., 2001; Meng et al., 1997), bacteria (such *Salmonella* species) (Davies et al., 1997), and parasites (such as *Cryptosporidium parvum*) (Pell, 1997; Sischo et al., 2000; Slifko et al., 2000).

Table 1. Some Human Pathogens Potentially Present in Animal Wastes

Microbe Category	Some Important Microbes of Human Health Concern
Viruses/Groups	Hepatitis E virus (swine), Reoviruses, Rotaviruses, Adenoviruses*, Caliciviruses*, Influenza viruses (Orthomyxoviruses)*
Bacterium/Group	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Escherichia coli</i> ***, <i>Aeromonas hydrophila</i> **, <i>Yersinia enterocolitica</i> , <i>Vibrio</i> spp., <i>Leptospira</i> spp., <i>Listeria</i> spp.
Parasites (Helminths and Protozoans)	<i>Ascaris</i> spp. (helminth); <i>Cryptosporidium parvum</i> , <i>Giardia lamblia</i> , and <i>Balantidium coli</i> (protozoans)

*Humans and animals (including swine) often have unique strains of these viruses, but not always.

**Some strains of these bacteria are non-pathogenic and others are pathogenic; occurrence varies.

Some pathogens, such as *Salmonella* spp. and hepatitis E virus in swine, are endemic in commercial livestock and difficult to eradicate from both the animals and their production facilities. Because these pathogens are so widely prevalent in animals, they are often present in fresh animal manure and other animal wastes. Therefore, the pathogens in animal manure and other wastes pose potential risks to human and animal health both on and off animal agriculture production facilities, especially if the wastes are not adequately treated and contained (Crane et al., 1983; Graczyk et al., 2000). Manure and other animal waste management technologies must be capable of reducing or containing these pathogens in order to prevent or minimize human and animal exposures to them that would pose health risks (Cole et al., 1999; 2000; Darwin and

Yukifumi, 1998). Of importance to the understanding of pathogens in animal wastes and their potential environmental and public health impacts are the following topics: (1) the types of pathogens potentially present in the manure of swine and other agricultural animals, (2) the levels of some important microbial pathogens and indicators for them that have been detected in swine and other animal wastes, (3) the potential for off-farm release or movement of pathogens present in manure and other wastes under current or proposed management practices, and (4) the extent to which these pathogens are reduced by currently used and candidate manure treatment and management technologies.

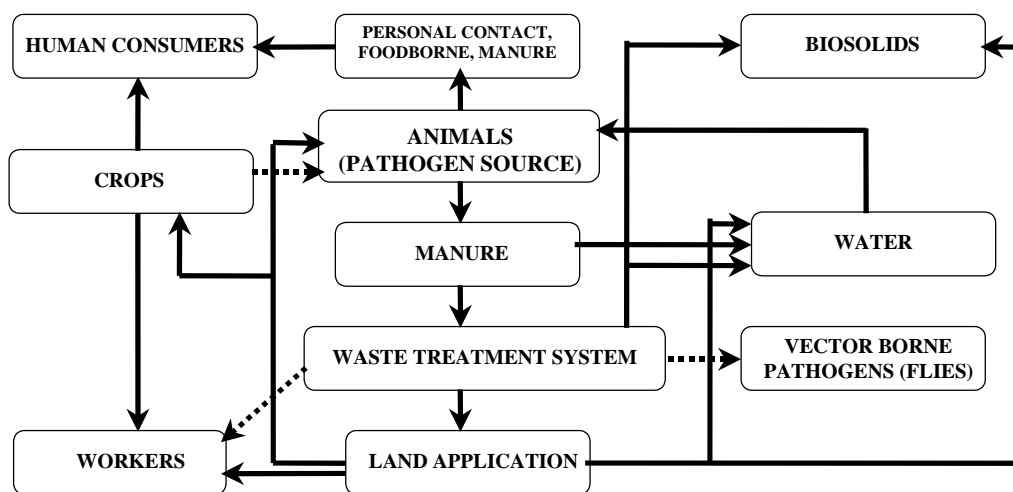
In addition to the ability of many animal pathogens to pose health risks to exposed humans and animals, there are also growing concerns about the presence of high concentrations of antibiotics and antibiotic-resistant bacteria in agricultural animal manures (Witte, 1998; Meyer et al., 2000). Antimicrobials are widely used therapeutically and subtherapeutically in animal production for disease prevention and growth promotion, respectively. Subtherapeutic antimicrobial use is associated with increased antibiotic resistance (AR) and multiple AR in enteric bacteria in swine and other animals (Langlois et al., 1978; Levy, 1978; Gellin et al., 1989; Mathew et al., 1998; 1999; Moore et al., 1996) and other livestock (Khachatourians 1998; Davis et al., 1999). Furthermore, *E. coli* have been implicated in AR gene transfer to other enteric bacteria (Abdul and Venable, 1986). Enteric bacteria with AR genes can spread from farm animals to other animals and to farm workers (Marshall et al., 1990; Nijsten et al., 1994; 1996; Ozanne et al., 1987; Saida et al., 1981). Research and outbreak data have shown that AR *Salmonella* have lower infective doses and cause increased incidence of human salmonellosis (Cohen and Tauxe, 1986). Therefore, the presence of antibiotic resistant bacteria in animal manures is another potential health risk of concern from both on-farm exposure and off-farm contamination.

Potential Pathways for Pathogen Movement on and off Farms

Pathogens from animal manures and other wastes have the potential to contaminate water, land and air if containment and treatment do not adequately manage the wastes. The range of potential pathways by which pathogens can move on and off of farms are shown in Figure 1.

Pathogen presence, persistence and transport. Available data suggest that pathogens are capable of persisting for days to weeks to months, depending on the pathogen, the medium and the environmental conditions. Even pathogens that are considered relatively non-persistent in various environmental media, such as vegetative, non-sporeforming bacteria, can persist for relatively long periods in animal manures and in media such as water. For example *Escherichia coli* O157:H7, an important water- and food-borne bacterial pathogen has been shown to survive in dairy cattle drinking water for at least 16 days at both 5 and 15 °C (Rice and Johnson, 2000). The protozoan parasite *Cryptosporidium parvum* has been shown to survive in fecally contaminated water for weeks to months, depending on temperature and water quality (Fayer and Trout, 1998).

Figure 1. Pathways for Pathogen Transport and Exposure on and off Farms.



The presence of pathogens in animal manures is well documented. However, pathogen levels in untreated, treated and recycled manures and the extent of environmental contamination by such pathogens is uncertain and is likely to vary with manure management practices. For example, typical concentrations of *E. coli* (a microbial indicator of fecal contamination of which some strains are true pathogens) in fresh barn flush or anaerobic lagoon influent from swine are about 10,000,000 per 100 ml and those of *Salmonella* spp. are about 10,000-100,000 per 100 ml (Hill and Sobsey, 1998). Typical concentrations of these microbes in anaerobic lagoon liquid are lower by about 1-2 orders of magnitude: 10,000-100,000 per 100 ml for *E. coli* and 100-1,000 per 100 ml for *Salmonella* spp. Dry manure solids also may contain high concentrations of pathogens and management systems for this type of animal manure also may pose risks of pathogen exposure to human and animals, depending on pathogen reductions during storage or treatment. Enteric microbe concentrations often decline with time in stored manure stockpiles (Forshell and Ekesbo, 1993). However, pathogen recontamination by animals and other vectors (e.g., birds, mice, and flies) also is a concern (Iwasa et al., 1999).

Pathogen exposures have been shown to vary with manure management systems. For example, dairy cattle herds maintained on farms on which alleyways were flushed with water to remove manure were 8 times more likely to have samples test positive for verotoxin-producing *E. coli* O157 than were herds maintained on farms cleaned by use of other methods of manure removal (Garber et al., 1999). As another example, the risks of pathogens to animals in relation to animal husbandry and management practices has been studied for Salmonellosis in cattle (Warnick et al., 2001). Due to observed increased clinical salmonellosis in cattle herds, a case-control study was done to identify potential risk factors. The potential impact of exposure to poultry-related factors was of particular interest because of the close proximity of poultry and cattle operations in the study area. *Salmonella* was isolated from 4.7% of 531 fecal, feed, water, and environmental samples collected, with *Salmonella typhimurium* the most frequent serotype in clinical cases in participating herds and from other samples. Significant risk factors for

salmonellosis were: (1) the number of mature cows in the herd, (2) the percent change in the number of mature cows during the study year, (3) having calves usually born in a building rather than outdoors, (4) poultry manure spreading on bordering property, (5) signs of rodents in cattle-housing or feed-storage areas and (6) contact of wild geese with cattle or feed.

Current treatment and management requirements for animal manure often are based on the principle of no discharge. That is, the manure constituents are to be recycled and remain on the farm. In this context, there are no specific requirements for the microbial/pathogen quality of liquid or solid manure residues assumed to remain on the farm. If animal waste management practices do not adequately destroy or contain pathogens, however, there is the potential for both on-farm contamination and off-farm movement and contamination. Despite the assumptions and stated policies of zero-discharge or no off-farm movement of animal manure and its pathogens, there are many potential pathways for manure-associated pathogen movement off farms. For all of these pathways, such movement has been documented. Off-farm movement or transport of the animals and their associated wastes to other locations has occurred and is an infectious disease concern within the animal industry. Pathogen contamination of farm workers is also possible, especially contamination with feces, respiratory secretions and combined solid and liquid barn wastes on hands and other body parts or on clothing. Infection of farm workers can lead to further transmission of pathogens to family members and other contacts, as well as to other animals.

Groundwater contamination. Groundwater contamination by pathogens from manure treatment systems and land application of liquid manure on fields is possible and has been documented. This is especially possible with unlined or poorly lined basins (such as unlined anaerobic lagoons) and in more pervious soils with seasonally high water tables (such as the porous, sands, limestone and shale soils). For example, it has been shown that land application of dairy manure significantly increased fecal bacteria in manure leachate compared with unmanured treatments (Conboy and Goss, 1999). After manure application, leachates collected from lysimeters 90 cm below the soil surface contained high concentrations (between 3,000-60,000 fecal coliforms/100 ml) of fecal bacteria. Fecal bacteria in leachate declined to nondetectable levels within 60 days of manure application. Fecal bacteria moved below the crop root zone whenever there was rainfall of sufficient duration or intensity to cause flow after manure application. The potential for groundwater contamination depended on soil structure and water flow more than on fecal bacteria survival at the soil surface. Pathogen impacts on ground water quality from both human fecal wastes and animal manure sources also have been documented by reported infectious disease outbreaks. For example, waterborne outbreaks of *E. coli* O157:H7 illness in humans have occurred in rural Ontario, Canada, New York state and Scotland. These outbreaks were attributed to contamination of groundwater sources of drinking water by cattle or other animal manures (Licence et al, 2001; Jackson et al., 1998; MMWR, 1999).

Surface water contamination. Runoff to surface water from land application of manure on fields and from feedlots or pastures also is possible (Baxter-Potter and Gilliland, 1988). Such contamination of surface water can impact the beneficial uses of the water, such as a drinking

water source, primary contact recreation (bathing, water skiing, etc.), irrigation and fishing. Enteric microbe/pathogen concentrations in surface waters adjacent to farms and other human and animal land uses where fecal waste are present become greatly elevated during storm events. As documented by wet weather sampling and analyses of surface waters adjacent to animal agricultural production facilities, biosolids application sites and urbanized areas, it is typical for the levels of pathogens to increase by factors of 100- to 1000-fold during precipitation events (Cole et al., 2001). Vegetative buffer strips have been suggested as a method to minimize the impacts of microbes of fecal origin on ambient water quality, and studies support the benefits of this management practice. For example, Larsen et al. (1994) conducted a series of runoff and infiltration studies with bovine feces placed various distances from a collection point to assess effectiveness of vegetative fiber strips. Effectiveness was based on the ability of the separation distance to reduce the number of fecal coliform (FC) bacteria being transported from the manure to the edge of the plots under conditions of variable distance, soil permeability, and rainfall intensity. The FC bacteria were 83% lower at the edge of the manure than in the manure and levels were further reduced as the separation increased.

Airborne contamination. Airborne transmission of bioaerosols from animal house exhaust and during land application of manure by spray irrigation also is possible. Atmospheric emissions from animal production units can include solid particles harboring microorganisms and their products (endotoxins and exotoxins) (Marquis and Marchal, 1998). According to Hartung (1995) the concentration of airborne microorganisms in livestock housing is between 100 and several 1000 cells per liter of air. The average concentrations of dust in animal barns vary between 0.5 and 20 Mg/m³. The dust contains high amounts of protein and carries endotoxins, both of which have an allergenic potential. The microbial content of the outdoor air in areas with high animal densities also appears to be higher than in non-livestock regions, raising that concern that these emissions may affect human health. It was recommended that reduction measures begin with the housing and manure removal systems and with feeding and management practices. Spray irrigation systems for animal manure also are a concern for bioaerosol transmission because enteric microbes have been isolated from such aerosols more than 400 ft from spray irrigation equipment (Bausum et al., 1982). Produce irrigated with liquid manure containing pathogens also may become contaminated, especially if the manure is sprayed onto the edible portions of the plants as opposed to application by drip or subsurface irrigation.

Pathogen and Microbial Indicator Reductions by Manure Treatment and Management Practices

Pathogen persistence in animal wastes depends on the type of pathogen, the type of waste and the waste storage or treatment system(s). The extent of pathogen reduction in animal manures

has been studied to some extent and the available data suggest that some systems are capable of extensive pathogen reduction. Treatment processes include physical, chemical and biological methods. Estimated pathogen reductions in animal manures are summarized in Table 2. Many of the processes shown in Table 2 have been extensively studied for pathogen and indicator microbe reductions in municipal wastewaters and biosolids. By comparison, considerably less information is available on the reduction of the same or similar pathogens in by these same or similar processes in animal waste treatment systems. Consequently, the estimated pathogen reductions shown in Table 2 are based on data from both municipal and animal wastes.

Storage. Storage of animal wastes is considered a management approach capable of achieving pathogen reductions. However, there is considerable variability in the extent of pathogen reduction by storage, depending on the pathogen types, the composition and consistency of the manure and the storage conditions. For example, the persistence of five animal viruses, representing picorna-, rota-, parvo-, adeno-, and herpesviruses, and the coliphage f2 was determined in the field by exposing the viruses to different animal wastes and storage conditions of ambient temperature and pH (Pesaro et al., 1995). The time, in days, required for a 90% reduction of virus titer varied widely, ranging from less than 1 week for herpesvirus to more than 6 months for rotavirus. Virus inactivation progressed substantially faster in liquid cattle manure, a mixture of urine and water (pH >8.0), than in semiliquid waste that consisted of mixtures of feces, urine, water, and bedding materials (pH <8.0). Overall, it was shown that some enteric viruses in manures may persist for prolonged periods of time under non-aerated conditions.

Mesophilic biological processes. Most mesophilic biological treatment processes reduce pathogens by about 90-99% or 1-2 log₁₀. For example, anaerobic lagoon treatment, a current BMP for swine manure in some states, produces microbial indicator (bacteria and viruses) and pathogenic bacteria (*Salmonella* spp.) reductions of about 90-99% (Hill and Sobsey, 1998) in a single-stage lagoon. However, spores of the anaerobic bacterium *Clostridium perfringens* are less extensively reduced (about 80%). Furthermore, fecal indicator microbes in lagoon effluent are still at levels of tens of thousands to hundreds of thousands per 100 ml. These levels of fecal indicator bacteria are well above state standards and federal guidelines for maximum allowable fecal coliform levels in municipal wastewater effluent or sludge (biosolids) applied to land or wastewater effluent discharged to water. *Salmonella* levels in swine anaerobic lagoon effluents are typically hundreds to thousands per 100 ml. In two-stage lagoon systems pathogen reductions of 99-99.9% are possible, including for *Salmonella* (Hill, 2001). Additional pathogen reductions can be expected with additional lagoons in series. Pathogen reductions in swine lagoons show some seasonal effects, with enteric microbe reductions being higher during warmer months than during colder months. This is probably because pathogen die-off and the activities of biological processes are greater at higher temperatures. Other mesophilic biological manure treatment and storage methods have also been studied for pathogen reductions. Constructed wetlands have been shown to reduce enteric microbes in anaerobic lagoon effluent by 90-99% (Hill and Sobsey, 1998; 2001). Activated sludge treatment of swine manure has been reported to achieve ~99% reduction of enteric bacteria (Bicudo and Svoboda, 1995). Aerobic biofiltration

Table 2. Summary of Animal Waste Treatment Processes and Estimated Pathogen Reductions

Treatment Process	Est. Pathogen Reduction (log ₁₀)	Factors Influencing Performance
Physical		
Heat, mesophilic (<50°C)	Typically, 1-2	Temperature, pathogen, contact time, pH, etc.
Heat, Thermophilic (>50°C)	Typically, >4	Temperature, pathogen, contact time, pH, etc.
Freezing	Variable	Pathogen, waste composition and conditions, temperature, etc.
Drying or desiccation	Typically >4 at <1% moisture; Typically <1 at >5% moisture	Pathogen, contact time, pH, etc.
Gamma Irradiation	Typically >3	Pathogen, dose, waste, etc.
Chemical		
High pH (>11)	Inactivation at high pH, e.g., alkaline/lime stabilization; >3-4	Pathogen, contact time, pH, etc.
Low pH (<2 to <5)	Inactivation at low pH; acidification: typically, <2	Pathogen, contact time, pH, etc.
Ammonia	Inactivation at higher pH where NH ₃ predominates	Pathogen, contact time, pH, other waste constituents
Biological Processes		
Aerobic, mesophilic	Typically 1-2	Pathogen, solids separation, contact time, reactor design, temp.
Aerobic, thermophilic (composting)	Typically >4	Pathogen, solids separation, contact time, reactor design, mixing methods, temperature
Anaerobic, mesophilic	Typically 1-2	Pathogen, contact time, reactor design, solids separation, temperature
Anaerobic, thermophilic	Typically >4	Pathogen, contact time, reactor design, solids separation, temperature
Ensiling, mesophilic	Variable	Ensiling conditions and pathogen
Land application	Highly variable and largely unknown; potentially high	Pathogen and site-specific factors: temperature, precipitation, vadose zone, loading, sunlight, riparian buffers, etc.

has been found to reduce microbial indicators by about 90-99% (Hill et al., 2002). Anaerobic mesophilic digestion also reduces pathogens in animal manures. Mesophilic digestion achieves reductions of about 90-99.99% ($2 \log_{10}$) for enteric bacteria (Duarte et al., 1992). However, overland flow achieves only 60-75% reduction of enteric bacteria and viruses (Hill and Sobsey, 1998).

Thermophilic biological processes. Thermophilic digestion and other thermophilic biological processes, such as composting are capable of achieving considerably greater pathogen reductions than mesophilic digesters and other mesophilic biological processes. The higher temperatures in thermophilic digestion and other thermophilic biological processes, such as composting, are highly microbiocidal. Thermophilic digestion of manures and biosolids achieves 3-6 \log_{10} (99.99-99.9999%) reductions of fecal coliforms, $>3 \log_{10}$ reductions of viruses (Berg and Berman, 1980), and $>3 \log_{10}$ reductions of *Cryptosporidium parvum* (Barbee, 1998).

Chemical and physical disinfection processes. Agricultural animal wastes usually are not treated by chemical or physical disinfection processes. However, such processes have the potential to achieve substantial inactivation of enteric pathogens, as has been demonstrated in municipal wastewater and biosolids (Martins et al., 1986). UV irradiation has been studied as a disinfection process for inactivation of enteric microbes in biologically treated swine lagoon liquid, and microbial reductions of 99-99.9% have been achieved for enteric bacteria and viruses (Hill et al., 2002). As noted above and in Table 2, thermal processes such as thermophilic digestion and composting have the potential to achieve substantial reductions of pathogens. This is because the high temperatures of these processes (typically 50-65°C) are capable of inactivating many pathogens. Pathogen reductions of $>99.99\%$ have been reported for typical composting treatment systems applied to swine waste (Bhamidimarri, Rao and Pandey, 1996).

Land application. Land application methods and rates for manures also influence enteric microbe survival in soil (Dazzo et al., 1973) and well as movement. This has not been extensively studied for pathogens in land-applied manures. Stored manure also can attract vectors, and these vectors can either introduce or spread pathogens. Recent studies demonstrate that improved manure management strategies can reduce enteric microbial impacts on water quality. For example, implementation of best management practices (BMP) in a 5.3 km² experimental watershed was done to assess the effect of the BMPs on water quality (Gallichand et al., 1998). It was found that 90% of the point source pollution came from leaking liquid manure tanks and from manure piles stored directly on the ground. Corrective measures consisted of the construction of animal waste tanks and changes in the timing and amount of manure application. The most problematic water quality parameters were phosphorus, fecal coliforms, and fecal streptococci. Time series analyses showed a highly significant decrease in total phosphorus concentration and a significant decrease in fecal streptococci. Overall, water quality improvements were detected in the watershed following the implementation of BMPs over a period of 2.6 years. Hence, the results of this and other studies indicate that changes in BMPs for animal manures can reduce enteric microbial contamination impacts on surface and ground waters in the vicinity of animal production facilities.

Summary, Conclusions and Recommendations

A variety of different kinds of pathogens are likely to be present in animal manures and other wastes. Some of the important pathogens potentially present in animal manures are not present in the United States, but there are growing concerns that non-endemic pathogens may be introduced either accidentally or deliberately into United States animal populations. New pathogens continue to be discovered in agricultural animal populations and the host ranges of these pathogens are uncertain. There are concerns that some of these recently discovered animal pathogens might be able to infect humans or that they have the potential to recombine with human pathogens and produce new strains capable of infecting humans. Particular pathogens of concern in this regard are hepatitis E virus and orthomyxoviruses (influenza viruses).

The reductions of pathogens by animal waste treatment processes and animal waste management systems have not been extensively studied. Therefore, there are considerable uncertainties about the extent to which pathogens survive waste treatment processes, are released into the environment and are available to be transported off farms. Off farm contamination can potentially occur inadvertently, such as in unplanned and uncontrolled releases by runoff, or aerosolization or infiltration into soils and ground water. It can also occur purposefully when biosolids and other manure residuals are transported off of farms to be land applied, marketed or for other beneficial uses. The extent to which pathogens are removed and inactivated in various waste treatment processes and management systems is uncertain and needs further investigation.

The reductions of some pathogens by some animal waste treatment processes have been determined in laboratory and pilot scale field studies. In general, thermophilic processes, such as pasteurization, thermophilic digestion and composting are capable of producing extensive ($>4 \log_{10}$) pathogen inactivation, and therefore, resulting treated residuals are likely to contain only low pathogen concentrations. Further studies are recommended to better characterize pathogen inactivation in thermophilic processes for manure treatment and to define the optimum conditions to achieve extensive pathogen reductions.

Drying of some animal manures is a widely practiced management approach in some places. However, little is known about the extent to which pathogens are inactivated in manure drying processes or during dry storage because there have been few if any studies to document their effectiveness. Desiccation or drying to very low moisture levels ($<1\%$) has been shown to result in extensive ($>4 \log_{10}$) inactivation of pathogens in municipal biosolids and in soils. Therefore, studies are recommended to determine the rate and extent of pathogen inactivation in drying and desiccation processes for animal manures.

Most mesophilic biological treatment processes for animal manures are not likely to reduce pathogen levels by more than $1-2 \log_{10}$ or 90-99%, unless several treatment reactors or processes are used in series. Therefore, treated manures, effluents or biosolids from such processes may still contain high concentrations of pathogens. The fate of these pathogens in subsequent

management operations, such as land application or prolonged storage is uncertain and has not been adequately determined. Therefore, further studies on effectiveness of mesophilic treatment processes in reducing pathogens and on the fate of pathogens in these post-treatment management processes are recommended.

Chemical treatments of animal manures are typically by lime or other alkaline treatment. Such treatment is widely practiced for municipal biosolids but less so for animal wastes. Alkaline stabilization for pathogen inactivation has been highly effective in municipal biosolids and promising results have been obtained when it has been applied to animal biosolids. Therefore, further studies are recommended to better characterize pathogen inactivation by alkaline treatments of animal biosolids with respect to solids composition, pH and storage and handling conditions.

The ultimate fate of pathogens in animal manure management systems remains uncertain, especially for large scale, multi-stage systems involving treatment or storage followed by land application at production facilities with large numbers of animals and minimum acreage (confined animal feeding operations). Because of the magnitude of the quantities of animal wastes generated by these facilities and the potentially high pathogen loadings that can result if the treated manure residuals still contain high pathogen concentrations, further investigation of the fate of pathogens in these systems and their surrounding environments is recommended.

Definitive or reference methods to recover and detect many of the pathogens in animal manures and their treated residual solids and liquids have not been reported, especially for emerging pathogens, such as hepatitis E virus, bacteria such as *E. coli* O157:H7 and *Yersinia enterocolitica*, and parasites such as *Giardia lamblia* and *Cryptosporidium parvum*. Therefore, the extent to which these pathogens are removed or inactivated in animal waste treatment processes or management systems remains uncertain due to limitations associated with the pathogen recovery and detection methods. The development, evaluation and application of reliable, sensitive and affordable methods to recover and detect pathogens in animal manures and their treated residual solids and liquids is recommended.

In principle, methods are available to recover and detect some indicator microbes in animal manures and their treated residual solids and liquids. However, the methods for some indicators, such as bacterial viruses (coliphages) and spores of *Clostridium perfringens* have not been adequately verified and collaboratively tested in these types of samples. Such verification and performance characterization studies are recommended. Also recommended are comparative studies on the removal, inactivation and fate of indicator microbes and animal pathogens in manure treatment processes and management systems. If such studies show that the indicator microbes reliably reflect or predict the responses of the animal pathogens in manure treatment processes and management systems, it then becomes possible to use them in practical, rapid and affordable monitoring and surveillance activities to assess treatment processes and system performance and the pathogen quality of the treated residuals.

METHODS AND MATERIALS

Anaerobic Lagoon

Lagoon samples were collected manually on an approximately monthly basis at four farms in North Carolina: (1) a 2,600-head commercial swine nursery having a single-stage anaerobic lagoon system, (2) a commercial farrow-to-wean farm housing 4,000 sows and having a two-stage treatment system (with primary treatment performed in an in-ground anaerobic digester and secondary treatment in an open-air lagoon), (3) a 300-sow farrow-to-feeder, 500-head farrow-to-finish educational farm operated by NCSU and having a two-lagoon system (both open to the atmosphere), and (4) a 12,000-head, 1,200-sow commercial farrow-finish farm having a single anaerobic lagoon. To the best of the author's knowledge, the lagoon systems were built and operated in accordance with Natural Resources Conservation Service (NRCS) standards (e.g., Conservation Practice Standard 359 for Waste Treatment Lagoons).

Anaerobic Digester

The anaerobic digester was located at a commercial farrow-to-wean farm housing 4,000 sows. The unheated digester was constructed as a 26,000-m³ (7-million-gallon) in-ground reactor, with a surface area of 6,500 m² (1.6 acres) covered by a 40-mil-thick high density polyethylene (HDPE) material. Average volatile solids (VS) loading to the digester was approximately 2.2 lbs VS/1000 ft³/d (35 mg VS/L/d) (Cheng et al., 1999). The estimated hydraulic residence time (HRT) in the digester was 4 months. The temperature of liquid in the digester was similar to, and varied seasonally with, ambient air temperatures. Samples were collected manually from the influent pipe to the digester (representing pit plug flush water from the storage pits beneath the swine houses) and the continuously flowing effluent discharge from the digester before it entered a secondary storage/treatment lagoon. Thirteen pairs of flushed waste and digester effluent samples were collected between December 1998 and May 2000.

Constructed Wetlands

The constructed wetland system has been operating at the 2,600-head swine nursery since 1992 (Hunt et al. 1998). The surface flow (SF) system contains two cells (each 3.6 m x 33.5 m) in series, planted with bur-reed (*Sparganium americanum*) and cattails (*Typha angustifolia* and *Typha latifolia*), as well as volunteer plant species that have since colonized the system. Wastewater from the anaerobic lagoon at the farm was diluted 1:1 with water (groundwater or water from a storage pond) and pumped through the system at a total nitrogen loading rate of 25 kg/ha/d. The HLR of lagoon liquid to the field system averaged 2 cm/d. The nominal HRT in the wetland system was about 10 days. A total of 19 sets of samples (influent and effluent from each wetland cell) were collected from the system between March 1997 and May 2000.

Gravel Media Filter

The media filter was installed at the 2,600-head swine nursery by researchers at North Carolina State University (NCSU) to investigate its effectiveness for nitrifying anaerobic lagoon liquid. The “marl-gravel” media filter received anaerobic lagoon liquid in parallel with two other treatment systems at the site (the surface flow constructed wetland and the overland flow cell). It was operated at an areal hydraulic loading rate (HLR) of 322 L/m²/hr and had a HRT of 6 hrs due to recirculation. Between March 1997 and June 1998, eight sets of influent and effluent samples were collected from the media filter system.

Overland Flow Cell

As with the gravel media filter, the overland flow cell was installed at the 2,600-head swine nursery by NCSU researchers to study its nitrification performance. The overland flow cell had dimensions of 4 m by 20 m and a slope of 2.5%. Anaerobic lagoon liquid was applied to the overland flow cell at an average areal HLR of 3 cm/d. Between March and October 1997, six sets of influent and effluent samples were collected from the overland flow system.

Aerobic Biofilter

The aerobic biofilter was installed by Ekokan LLC in May 1997 at NCSU’s educational farm on Lake Wheeler Road, Raleigh, NC. Swine waste was automatically flushed every 3 hours from the 300 sow, farrow-to-feeder, 500-head finishing facility at the farm. Solids were separated from the settled swine waste using a rotary screen (100 mesh, 150 micron size) and the screened wastewater was pumped through the two-stage biofilter system at a flow rate of 5450 L/d (1440 gpd). Each biofilter tower had a height of 3 m, a cross-sectional area of 0.5 m² and a hydraulic residence time (HRT) of 9 hours (for an overall system HRT of 18-24 hours). The medium in each biofilter was a perforated plastic material designed for high suspended solids loadings. Air was injected at the bottom of each biofilter by two blowers at a rate of 0.2 m³/min at 400 mbar pressure. The first biofilter tower (Biotower #1) was designed and operated to enhance organics removal, while the second biofilter (Biotower #2) functioned primarily as a nitrification reactor. Biotower #1 was backwashed four times each day and Biotower #2 once every two days. Eleven sets of samples (system influent, Biotower #1 effluent and Biotower #2 effluent) were collected between June 1998 and January 2000 and analyzed for enteric microbes.

Aerated Solids Recovery Biological Treatment

The aerated solids recovery biological treatment system (commercial name: Bion NMS™), designed and operated by Bion Technologies Inc., was investigated at two commercial swine farms in North Carolina. One was an 8000-head nursery and the other a 10 000-head finishing farm. This patent-pending wastewater treatment process consists of a “solids ecoreactor,” three “bioreactors,” a liquid storage area (referred to as a “temporary storage area,” or TSA), and a

sprayfield. The system components at the nursery were comprised of earthen basins lined with clay to meet North Carolina state permeability requirements. The same components at the finishing farm were lined with an impermeable synthetic liner. The Bion system is designed as follows. Flushed waste from the swine houses first enters a bioreactor (Bioreactor 1) where an aerator both aerates and mixes the wastewater in the reactor. The wastewater then enters a solids ecoreactor, configured such that biosolids are deposited on the floor of the cell and water flows through to an effluent point leading to Bioreactor 2. The treatment system is designed with two of these ecoreactors in parallel, one “online” through which wastewater flows and solids settle, and another “offline” in which solids from a previous collection cycle are allowed to biologically stabilize until they are harvested for dewatering and processing into a commercial end-product. In Bioreactor 2, effluent from the online ecoreactor is mixed and aerated again. Water from Bioreactor 2 is used to flush waste from the swine houses and thus represents the last point in the closed-loop portion of the treatment system. Excess water from Bioreactor 2 is piped to Bioreactor 3, a settling cell designed to remove residual solids and provide additional biological treatment. At the nursery, water from Bioreactor 3 was periodically pumped into a 4 million-gallon TSA (the former waste lagoon used by the farm prior to installation of the Bion system). Water from the TSA was then sprayed onto a 9-acre sprayfield. At the finishing farm, a new TSA was constructed with a sufficient volume to retain accumulated water from Bioreactor 3 prior to application to a 43-acre sprayfield. Hydraulic residence times in the treatment systems was about 10 days. Solids residence times in the solids ecoreactors were 4-6 months. An aerial photograph of the Bion system on one of the North Carolina swine farms studied is shown in Figure 2.

Microbiological Analyses

Wastewater samples were collected and analyzed within 24 hours for the following enteric microbial indicators: fecal coliforms, *Escherichia coli*, enterococci, *C. perfringens* spores, somatic coliphages, and F-specific coliphages. Wastewater samples were collected aseptically as grab samples in sterile polypropylene bottles. Samples were kept chilled during transport to the laboratory, and they were stored at 4°C until analyzed. Analysis of samples commenced within 24 hours of sample collection.

Bacterial indicators were enumerated by filtering diluted samples through 47-mm, 0.45- μ m pore-size cellulose filters in standard, sterile membrane filtration apparatuses and incubating the membrane filters on appropriate agar media: fecal coliforms by incubating membranes on mFC agar media for 2 hours at 37°C and then 44.5°C for another 20 \pm 2 hours; *E. coli* by transferring membranes from mFC plates having countable colonies to plates containing nutrient agar and 4-methylumbelliferyl- β -D-glucuronide (MUG), incubating the plates for 3-4 hours at 37°C, and observing colonies under long-wavelength UV light for blue fluorescence; enterococci by incubating membranes on modified mE agar for 48 hours at 41°C; and *C. perfringens* spores by heat treating samples at 60-65°C for 20 minutes, incubating filter membranes on mCP agar in an anaerobic jar for 18-24 hours at 41°C, and exposing the plates to ammonium hydroxide fumes.

Figure 2. Bion system of aerated biological treatment with solids recovery



Swine waste is flushed from the animal houses into an aerated Bioreactor (**B**) where bio-conversion processes begin. **B** effluent flows into a Solids Ecoreactor (**SE**) where biomass settles to the bottom for further bioconversion. The liquid fraction flows into another Bioreactor (**B**) before being returned back to the animal houses as flush water. The settled biomass is periodically harvested and formulated into horticultural products.

Viral indicators were enumerated by standard plaque assays using single- or double-agar layer, pour plate plaque techniques (Adams 1959; Grabow and Coubrough 1986). Assay media were tryptic soy agars containing either nalidixic acid or ampicillin and streptomycin for host bacteria *E. coli* CN-13 (somatic host) or *E. coli* Famp (F-specific host), respectively. Samples were incubated overnight at 37°C for plaque formation.

Salmonella were enumerated using the most probable number (MPN) technique as follows: pre-enrichment for 20-24 hours at 37° C in buffered peptone water (Difco) (Edel and Kampelmacher 1973); enrichment for 24 hours at 43° C in Rappaport-Vassiliadis R10 broth (Difco) (Vassiliadis 1983); parallel isolation on Salmonella-Shigella agar (Difco) and Rambach® agar (CHROMagar Microbiology); and biochemical testing of a subset of presumptive positive colonies using BBL® Enterotube™ II media (Becton Dickinson).

All microbiological methods used in this study are standard procedures and detailed descriptions of the protocols can be found in the following standard handbooks and protocol documents: Standard Methods for the Examination of Water and Wastewater (APHA, 2000), Bacteriological Analytical Manual (US Food and Drug Administration, 2001), and Method 1602: Male-specific

(F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (US Environmental Protection Agency, 2001).

Physical and chemical parameters, including total suspended solids (TSS), chemical oxygen demand (COD), and pH were measured according the methods described in Standard Methods for the Examination of Water and Wastewater (APHA, 2000).

RESULTS AND DISCUSSION

Anaerobic Lagoons

Average hydraulic residence time (HRT), total suspended solids (TSS), chemical oxygen demand (COD) and pH values in the three primary lagoons and two secondary lagoons studied are shown in Table 3. Average HRTs differed to some extent among the lagoons, with the primary lagoons having estimated average HRTs of 8-15 months and the secondary lagoons having somewhat shorter estimated average HRTs of 2-6 months. Levels of TSS and COD were similar for each type of lagoon. COD concentrations in the primary anaerobic lagoons were similar to levels reported for other swine lagoons (Cheng et al., 2000; CDC, 1998). Average COD concentrations were on the order of 1000 mg/L in the primary lagoons, a level similar to “strong” untreated domestic wastewater, and were approximately 500 mg/L in the secondary lagoons, a level similar to “medium-strength,” untreated domestic wastewater (Metcalf & Eddy, 1991). TSS concentrations in flushed waste influent to the primary lagoons were 4200, 1500, and 11 000 mg/L at Farms 1, 3 and 4, respectively, indicating that the primary swine lagoons removed 71 to 97% of suspended solids. The extent of TSS reduction depended largely on the concentration of solids in the flushed waste influent to the lagoons. TSS removal in the secondary lagoons was lower: 24% at Farm 2 (for an average digester effluent TSS concentration of 330 mg/L) and 32% at Farm 3, further illustrating that TSS removal performance in lagoons is strongly influenced by influent TSS concentration.

Table 3. Average physico-chemical and hydraulic characteristics of swine waste lagoons*

Lagoon	HRT (months)	TSS (mg/L)	COD (mg/L)	pH (range)
Farm #1	8	330	1100	7.6-7.9
Farm #2				
• Secondary	6	250	550	8.1-8.7
Farm #3				
• Primary	9-15	430	1000	7.8-8.1
• Secondary	2-4	290	540	8.0-9.2
Farm #4	7-9	310	1200	7.9-8.1

* TSS concentrations in flushed waste influent to the primary lagoons were 4200, 1500, and 11 000 mg/L at Farms 1, 3 and 4, respectively.

The pH levels of the primary lagoons varied over a narrow range (7.6 to 8.1). The pH levels for the secondary lagoons (8.0 to 9.2) were for samples collected during mid-afternoon and likely reflect the activity of algae that were typically visible in the lagoons during warm-weather

months. Based on the somewhat elevated pH in these secondary lagoons and the visible presence of photosynthetic green algae, these lagoons were likely facultative, at least during warm-weather months.

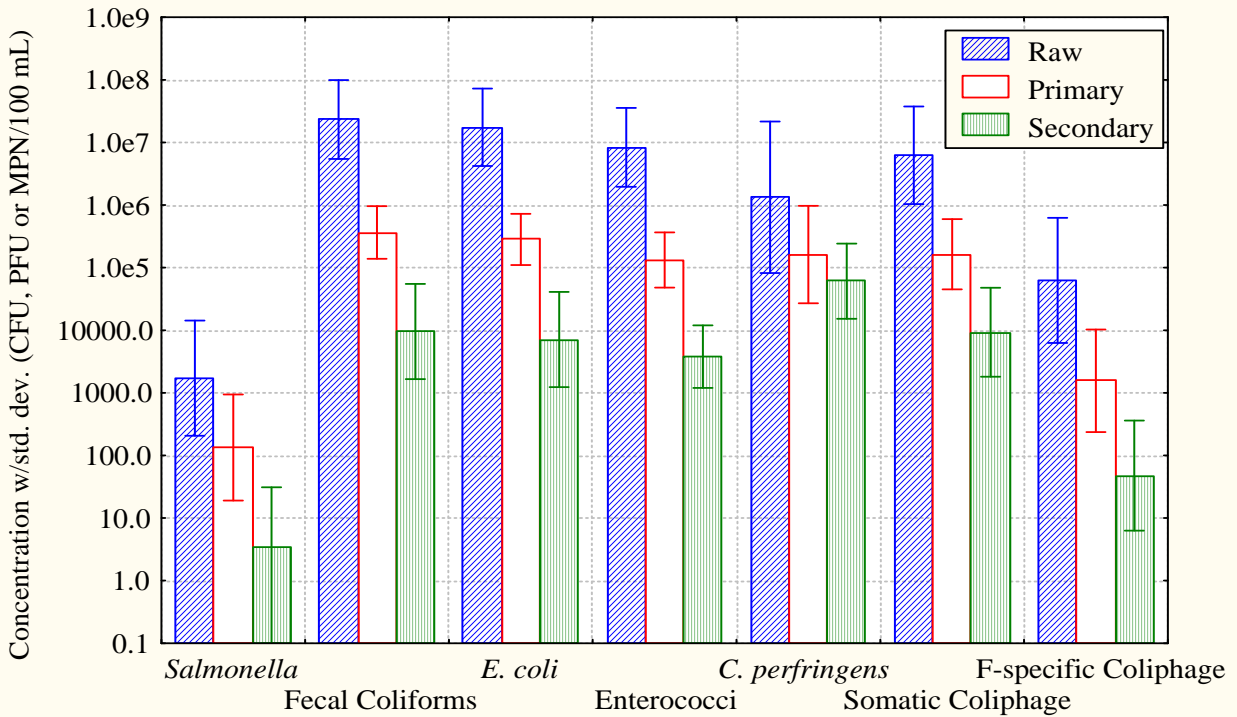
The geometric mean concentrations of *Salmonella* and enteric microbial indicators in flushed (“Raw”) swine waste, primary anaerobic lagoons and secondary anaerobic lagoons are shown in Figure 3 as an average of the four lagoon systems studied. The average *Salmonella* concentration in flushed swine waste at the four farms studied was 3800 MPN/100 mL. *Salmonella* spp. were reduced by approximately 96% in primary lagoons and by a further 97% when a secondary lagoon was used. Concentrations of *Salmonella* were, in general, on the order of 1-10 MPN/100 mL in the secondary lagoons. Fecal coliforms, *E. coli*, and enterococci were reduced to a similar, but slightly greater, extent than *Salmonella*. Fecal coliforms and *E. coli*, measured at average concentrations of 2.7×10^7 and 2.0×10^7 colony forming units (CFU) per 100 mL of flushed swine waste, respectively, were both reduced by 98% in primary lagoons and secondary lagoons. Enterococci, which were present at a slightly lower average concentrations in flushed swine waste (9.2×10^6 CFU/100 mL), were reduced by 98% in primary lagoons and by a further 97% in secondary lagoons.

C. perfringens spores were less efficiently reduced in lagoons than the other enteric bacteria studied. Geometric mean *C. perfringens* spore concentrations in flushed swine waste averaged 1.3×10^7 CFU/100 mL at the four farms studied, but varied between 130 000 CFU/100 mL at the nursery to 5.1×10^7 CFU/100 mL at the farrow-wean farm. Average reductions of *C. perfringens* spores were 84% in primary lagoons and 92% in secondary lagoons, suggesting that lagoon systems using multiple lagoons in-series may be needed to achieve substantial reductions of parasitic pathogens. *C. perfringens* spore concentrations in the secondary lagoons were 98-99.7% lower than in untreated swine waste influent (fresh barn flush) to the systems.

In lagoon influent (fresh barn flush), geometric mean somatic coliphage concentrations were similar to those of vegetative bacteria at about 10^7 per ml, but F-specific coliphage concentrations were appreciably lower at about 10^5 per ml. Both somatic coliphages and F-specific coliphages were reduced to a similar extent as measured for fecal coliforms, *E. coli* and enterococci. Somatic and F-specific coliphages were reduced by 97% in primary lagoons and a further 96% in secondary lagoons. Overall, somatic and F-specific coliphage concentrations in secondary lagoons were 99.7-99.9% lower than in flushed swine waste influent to the treatment systems.

The results from the lagoon studies show that frank human pathogens, like *Salmonella* spp., are present in swine waste. *Salmonella* concentrations were appreciably reduced in anaerobic swine waste lagoons, with substantial additional reductions achieved during secondary lagoon treatment. Fecal coliforms, *E. coli* and enterococci appeared to be reliable indicators for the reduction of *Salmonella* in anaerobic lagoon systems, though their overall

Figure 3. Geometric mean concentrations of *Salmonella* and microbial indicators in flushed swine waste and lagoon liquid



Raw = flushed swine waste from barns; primary = primary lagoons; secondary = secondary lagoons

relative reductions were slightly greater than those observed for *Salmonella*. The results for the coliphages show that these viral indicator microbes are reduced to a similar, though possibly slightly lower, extent than fecal coliforms, *E. coli* and enterococci. The results for *C. perfringens* spores show that these microbes were the least effectively reduced enteric microbes in anaerobic swine lagoons. These results were not unexpected, as the environmentally stable spores of *C. perfringens* are likely primarily removed during lagoon treatment by sedimentation, as opposed to inactivation and degradation mediated by the microbiocidal processes that might affect viruses and vegetative bacteria. Similarly, helminth ova and parasitic cysts and oocysts may be removed less efficiently in swine lagoons than are bacterial or viral pathogens and more like *C. perfringens* spores due to their relatively high resistance to environmental processes.

Mesophilic Covered Anaerobic Digester

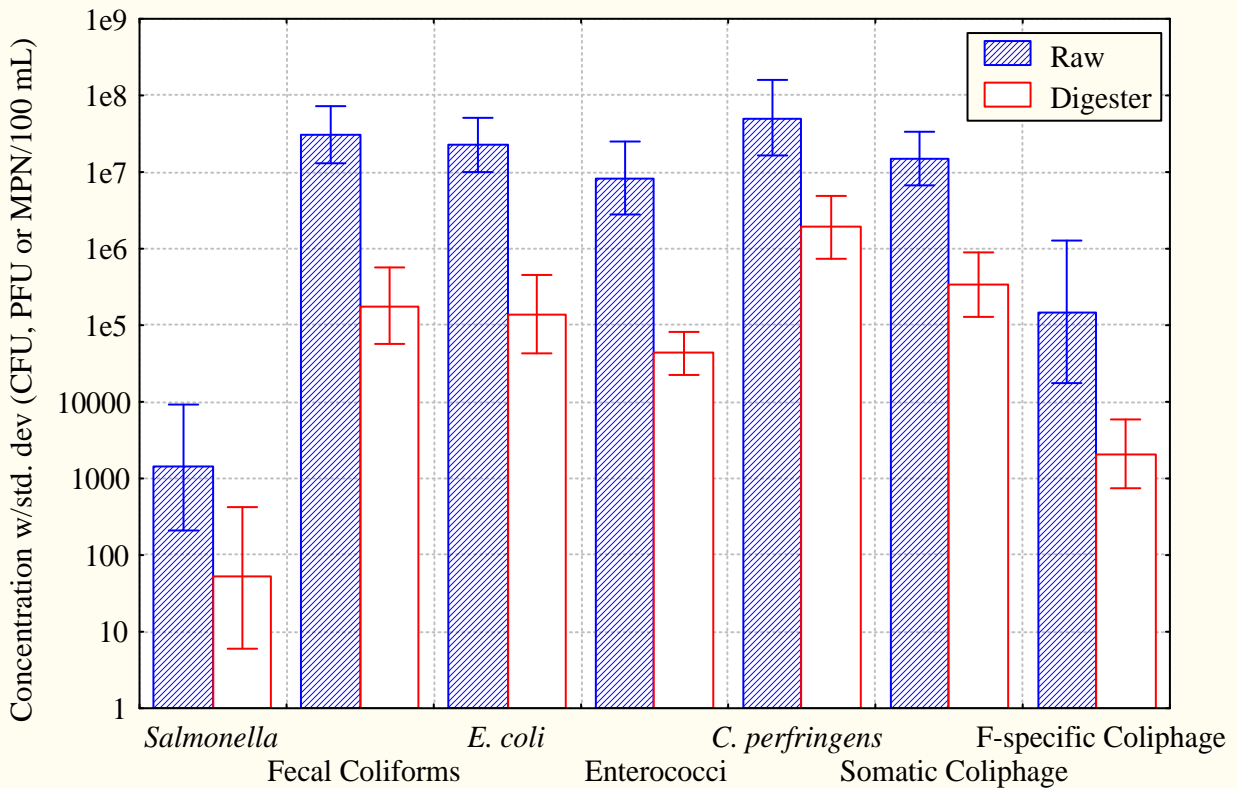
The mesophilic covered digester receiving swine waste as fresh barn flush typically reduced COD and TSS by 94% and 76% during the period of this study (Cheng et al., 1999). Concentrations of *Salmonella* spp. in flushed swine waste entering the mesophilic covered anaerobic digester varied from 23 to 16 000 MPN/100 mL, with a geometric mean of 1400 MPN/100 mL (Figure 4). *Salmonella* and *C. perfringens* spores were appreciably reduced (96% for both), but these reductions were lower than those for the other enteric microbes tested. Geometric mean reductions of the non-spore-forming bacterial indicators (fecal coliforms, *E. coli* and enterococci) were similar (99.4-99.5%), and significantly higher than for *Salmonella*. Fecal coliforms and *E. coli* were reduced from influent concentrations of 3.1×10^7 and 2.3×10^7 , respectively, to concentrations of 180 000 and 140 000, respectively, in digester effluent. The viral indicators, somatic and F-specific coliphages, were also substantially reduced in the anaerobic digester, with geometric mean reductions for both being 98%. Somatic coliphages were detected at concentrations approximately 100 times higher than F-specific coliphages in flushed swine waste influent to the digester. *C. perfringens* spore removals in the anaerobic digester (96%) were significantly lower than for fecal coliforms, *E. coli*, enterococci, and F-specific coliphages.

When enteric microbial indicator reductions in the anaerobic digester are compared with those for the primary lagoons studied at other commercial farms, the results show that the digester achieved significantly greater reductions of enterococci and *C. perfringens* spores (95% confidence limits of 1.7-1.9 log₁₀ and 0.5-0.7 log₁₀, respectively). Differences for reductions of other microbial indicators were not significant between the two types of treatment systems. Overall, the covered mesophilic digester was as effective or more effective than anaerobic lagoons in reducing *Salmonella* pathogens and enteric microbial indicators. Because the covered anaerobic mesophilic digester takes up less surface area, is covered to collect off gases, including odorants, and has a much lower hydraulic retention time than anaerobic lagoons (about 2 months versus 6-15 months), this treatment appears to be an effective alternative technology for swine waste treatment.

Constructed Wetlands

The pilot scale constructed wetlands operated at a Nitrogen (N) loading of 25 kg/ha/year and achieved average nitrogen mass removal efficiencies of 84% (cattail-burreed) and 90% (rush-bulrush). (Mass removal was defined as % mass reduction of NH₃-N + NO₃-N in the effluent with respect to the nutrient mass inflow for 270 application days per year) (Rice et al., 2000).

Figure 4. Geometric mean concentrations of *Salmonella* and microbial indicators in anaerobic digester influent and effluent

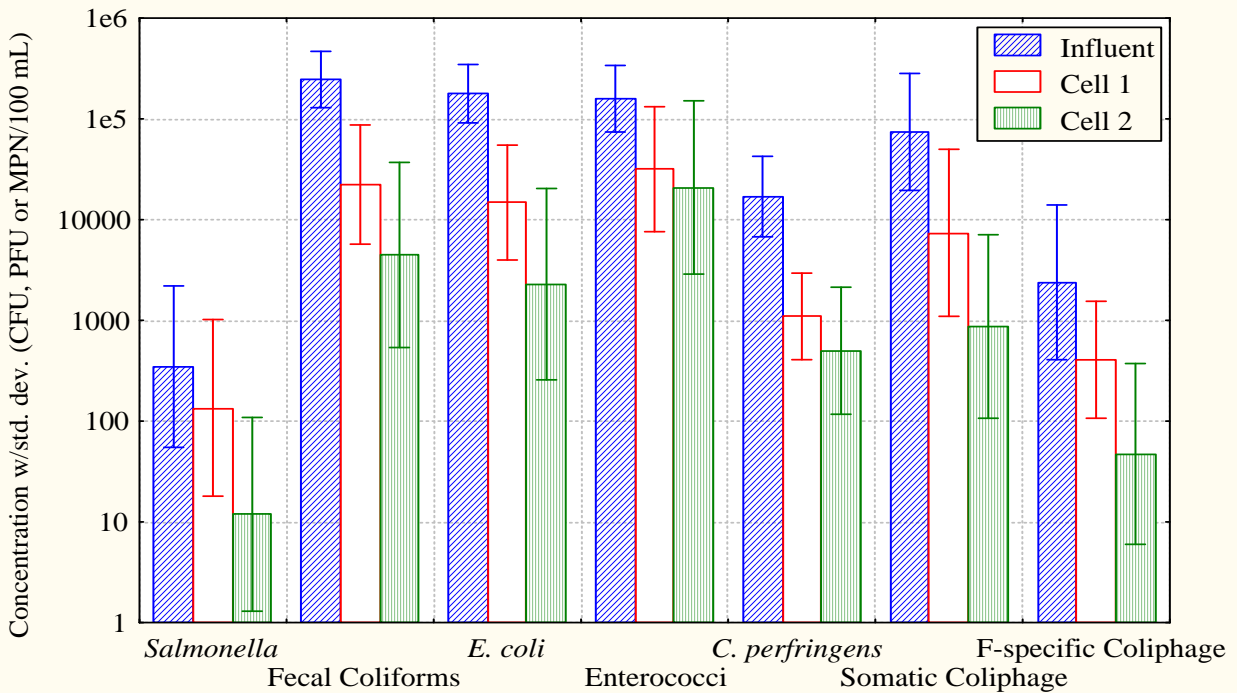


Raw = flushed swine waste from barns;
 Digester = effluent from covered mesophilic digester

Concentrations of *Salmonella* and enteric microbial indicators in wetland influent and in each wetland cell are shown in Figure 5 as geometric mean values with standard deviations.

As shown, the influent to the surface flow constructed wetland had geometric mean concentrations of fecal coliforms and *E. coli* of 240 000 and 180 000 colony forming units (CFU) per 100 mL, respectively. These bacterial indicators were reduced by 1.0 log₁₀ (91%) and 1.1 log₁₀ (92%), respectively, in Cell 1 of the wetland system, and overall by 1.7 log₁₀ (98%) and 1.9 log₁₀ (99%), respectively, for the two wetland cells in series. Enterococci were less effectively reduced than were the fecal coliforms and *E. coli* in the field-scale constructed wetland system: 0.7 log₁₀ (80%) in Cell

Figure 5. Geometric mean concentrations of *Salmonella* and enteric microbial indicators in a surface flow constructed wetland



Influent = swine lagoon effluent diluted 1:1 with water
 Cell 1 = effluent from the first cell of the constructed wetland
 Cell 2 = effluent from the second cell of the constructed wetland

1 and by 0.9 log₁₀ (87%) overall. Enterococci (as well as other fecal streptococci) are generally thought to be more resistant to environmental degradation than fecal coliforms, including *E. coli*. These data suggest that enterococci may be good indicators for more environmentally stable bacterial pathogens. It is also possible, however, that the low reductions of enterococci during some sampling rounds may reflect the reproduction of these organisms in the wetland system (Clausen et al., 1977; Anderson et al., 1997). *Salmonella* were measured at far lower concentrations than the indicator bacteria in influent to the system, as expected. *Salmonella* were reduced from an influent geometric mean of 350 MPN/100 mL to a mean of 130 MPN/100 mL in Cell 1 (a 0.4 log₁₀, or 63% reduction) and a mean of 12 MPN/100 mL in effluent from the system (a 1.5 log₁₀, or 96% reduction through the 2-cell system).

C. perfringens spore concentrations were reduced by 1.2 log₁₀ (93%) in Cell 1 effluent and by 1.5 log₁₀ (97%) in system effluent (Figure 5), suggesting that environmentally-stable enteric microbes (e.g., *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts and helminth ova) would be substantially removed from wastewater by a similarly designed and operated SF constructed wetland system. Because bacterial spores and parasites are stable in the

environment, it is still possible that release of these microbes may occur periodically or during system perturbations (e.g., precipitation events). Somatic and F-specific coliphages were reduced to a similar extent in each cell of the SF constructed wetlands system: $1.0 \log_{10}$ (90%) and $0.8 \log_{10}$ (83%), respectively, in Cell 1 effluent and $1.9 \log_{10}$ (99%) and $1.8 \log_{10}$ (98%), respectively, in system effluent.

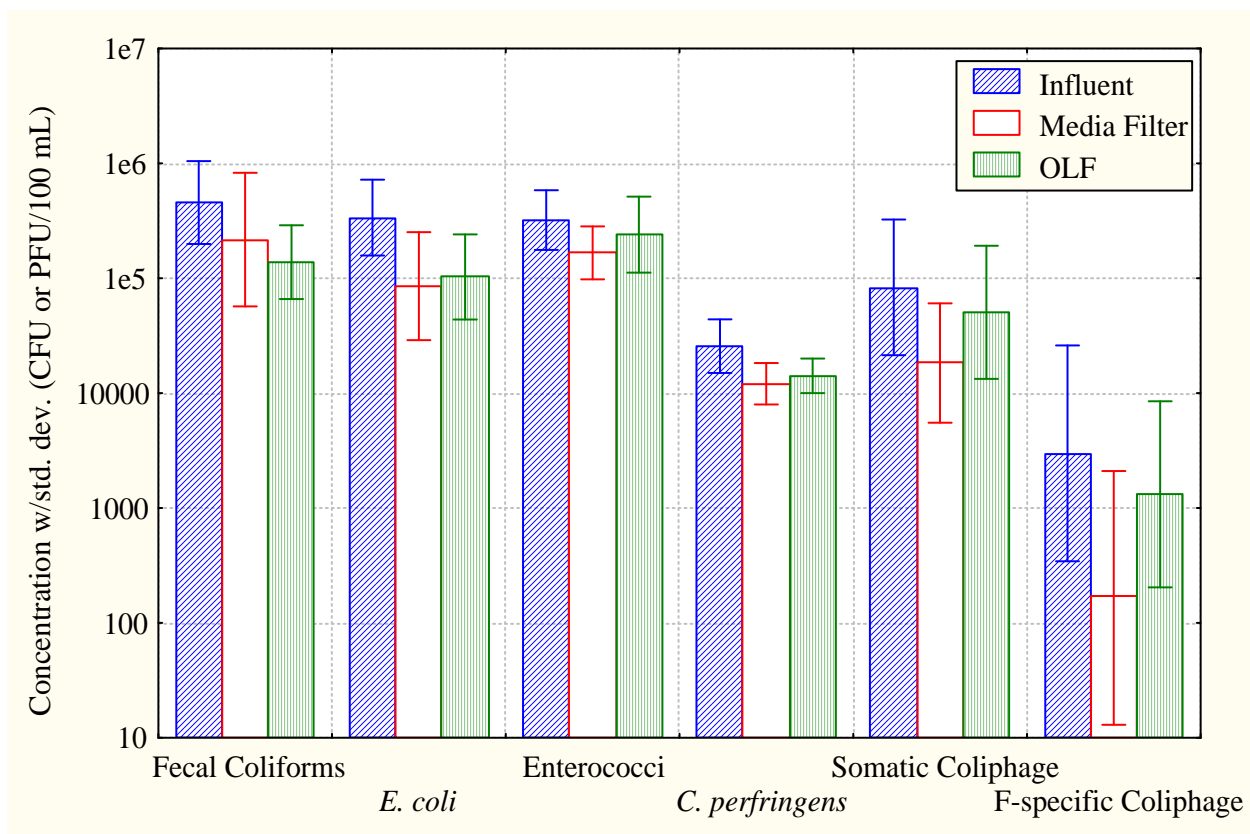
Overall, the results of these studies indicate that surface flow constructed wetlands have the potential to achieve appreciable reductions of *Salmonella* bacteria and fecal indicator microbes as well as high rates of N mass removal. Reductions of *Salmonella* and fecal indicator bacteria were similar to those in anaerobic lagoons in a single wetland cell and superior to those in anaerobic lagoons using two wetland cells in series. Therefore, such constructed wetland treatment systems appear to provide a promising alternative to anaerobic lagoons that can achieve high levels of N and pathogen reductions.

Gravel Media Filter and Overland Flow

Two nitrification treatment alternatives, overland flow and a marl-gravel media filter with encapsulated nitrifiers were investigated for enteric pathogen and indicator reductions. Typically, the lagoon liquid that served as the wastewater influent to the marl-gravel filter treatment system contained 365 mg/L of total Kjeldahl nitrogen (TKN), mostly (> 95%) as ammonia-N, 93 mg/L of total phosphorus, 740 mg/L, chemical oxygen (COD), and a pH of 8.2. The operating conditions of the media nitrification filter used in this study were as follows: hydraulic residence time = 6 hours, total N loading rate = 926 mg/l (Total N = TKN + $\text{NO}_3\text{-N}$; inflow nitrate concentration = 0). At these operating conditions nitrification efficiency [$\text{NO}_3\text{-N}$ conc. outflow/TN conc. inflow) x 1005] was 54% (Vanotti et al., 1999). In the overland flow treatment system lagoon liquid was applied five days a week with a hydraulic rates of 3.0 cm/day. Total N application rate was 99 Kg/ha/day. Because the sandy soil of the system was highly permeable, obtaining a functional surface flow was achieved by applications during four hours each day. Hydraulic losses were similar to the expected evapotranspiration losses (0.5-0.8 cm/day). On a mass basis, average total N removal efficiency was 42%, which is equivalent to 41.6 kg N/ha/day, respectively. Low nitrate recovery value of 7% was observed after treatment, suggesting that simultaneous denitrification occurred in the saturated soil layer, a typical feature of overland flow systems (Vanotti et al., 1999).

Concentrations of enteric microbial indicators in swine lagoon liquid treated by the gravel media filter and overland flow cell are shown in Figure 6 as geometric means with standard deviations.

Figure 6. Geometric mean concentrations of microbial indicators in lagoon liquid influent and effluent from media filter and overland flow systems for nitrification



Influent = anaerobic lagoon swine waste effluent
 Cell 1 = effluent from the media filter
 Cell 2 = effluent from the overland flow system

In general, the enteric microbe reductions measured in these systems were less than were measured in secondary lagoon systems at other farms. Enteric microbe reductions in the medium filter were greater than in the overland flow cell except for fecal coliforms, which were reduced by 58% in the medium filter and 66% in the overland flow cell. Reductions of *E. coli*, enterococci, *C. perfringens*, somatic coliphages and F-specific coliphages in the media filter were 80, 54, 52, 83 and 97%, respectively. Reductions of these microbes in the overland flow cell were 70, 37, 30, 51, and 84%, respectively.

It should be reiterated that these treatment systems were being investigated as nitrification systems by other researchers, so loading rates were selected for nitrification efficiency, not pathogen reduction. HRTs in these systems (6 hours in the medium filter and approximately 24 hours in the overland flow cell) were far shorter than in lagoons operated per NRCS guidelines, for which HRTs can be greater than 6-12 months. Nevertheless, these results indicate that swine treatment systems designed and operated for nutrient reductions and

conversions do not necessarily achieve appreciable pathogen reductions under some of their proposed operating conditions.

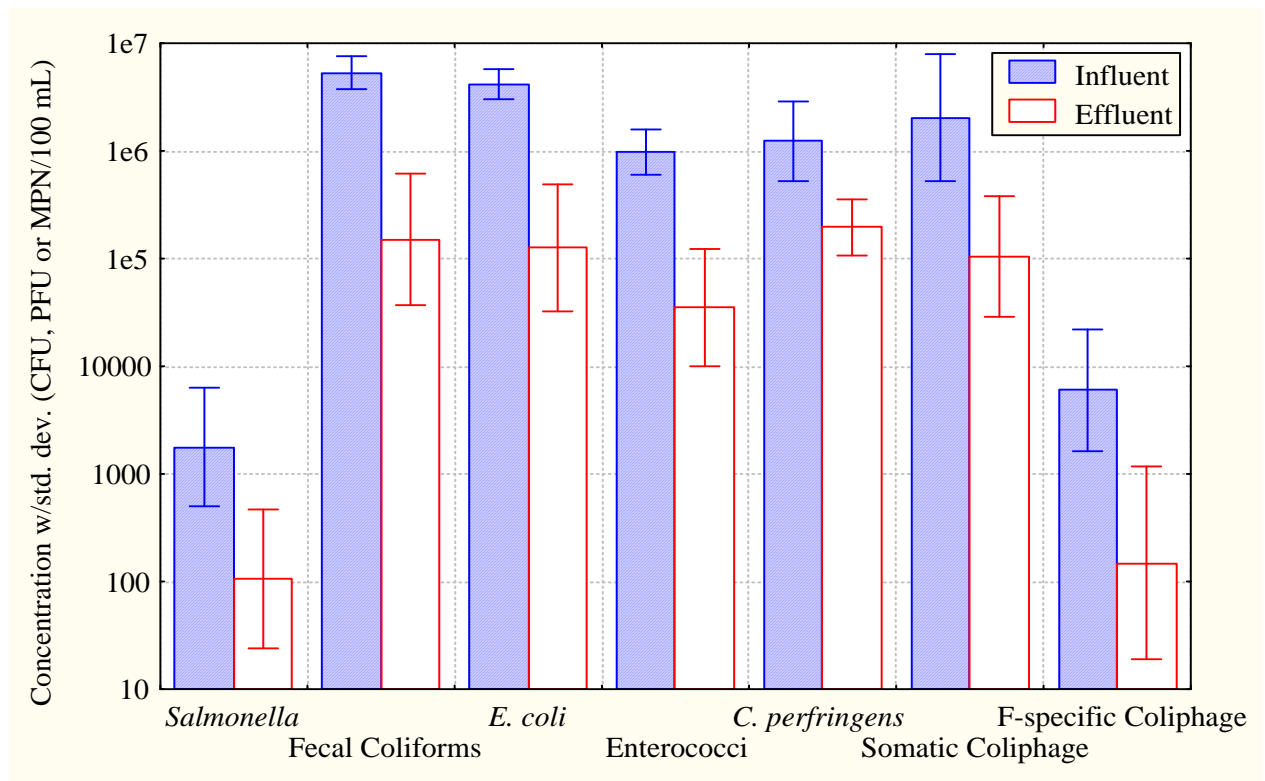
Aerobic Biofilter

The pilot plant treatment system was composed of two upflow, aerated biofilters connected in series, and treatment performance for parameters such as solids, organics and nutrients have been previously described (Westerman et al., 2000). In studies on the performance of the pilot scale aerobic biofilter at hydraulic flow rates to the first biofilter of 4 to 5 m³/day and an average loading of 6.6 kg COD day per m³ of media, the reductions in concentrations from influent to effluent were: 72% for COD, 57% for volatile solids, 76% for SS, 72% for TKN, 82% for NH₃-N, 49% for total N, and 26% for total P. Temperature affected the reductions, with higher reductions at higher temperatures. Most of the reduction in concentrations occurred in the first biofilter, but the second biofilter still had significant reductions as a percentage of influent concentrations to the second biofilter. During low temperatures, the first biofilter had very little nitrification, demonstrating some advantage for having the two biofilters in series for nitrification at low temperature. Under warm weather conditions (average temperature of 27° C), the treatment system removed about 88% of biochemical oxygen demand (BOD), 75% of chemical oxygen demand (COD), and 82% of total suspended solids (SS) with loading of 5.7 kg COD/m³/day of biofilter media. The total Kjeldahl nitrogen (TKN), total ammonia nitrogen (NH₃-N), and total nitrogen (Total-N) reductions averaged 84%, 94% and 61%, respectively, during warm weather, with a significant portion of the NH₃-N being converted to nitrite plus nitrate nitrogen (NO₂+NO₃-N). At higher organic loading (over 9 kg COD/m³/day), the biofilters had only slightly lower percentage removal rates. Operation at lower temperatures (average of 10° C) resulted in lower performances. The COD, TKN, NH₃-N, and Total-N removal averaged 56%, 49%, 52%, and 29%, respectively. The COD mass removal rate was linear with loading rate over the range of approximately 2-12 kg COD/m³/day of filter. A mass balance average for a 12- month operating period indicated that about 30% of the influent volume, 35% of Total-N and 60% of total phosphorus (Total-P) were removed with the biofilter backwash. The unaccounted-for nitrogen was about 24% and could have been lost as ammonia volatilization or possibly through denitrification within the biofilm.

Geometric mean concentrations of enteric microbes in the aerobic biofilter system influent, effluent from the first biofilter tower (BF1) and effluent from the second biofilter tower (BF2, or system effluent) are shown in Figure 7. Geometric mean concentrations of enteric microbes in influent to the biofilter system were: 1800 MPN/100 mL for *Salmonella* spp., 5 200 000 CFU/100 mL for fecal coliforms, 4 200 000 CFU/100 mL for *E. coli*, 980 000 CFU/100 mL for enterococci, 1200 000 CFU/100 mL for *C. perfringens* spores, 2 000 000 plaque forming units (PFU)/100 mL for somatic coliphages, and 6100 PFU/100 mL for F-specific coliphages.

Figure 7. Geometric mean concentrations of *Salmonella* and microbial indicators in an aerobic

biofilter treatment system for swine wastewater



Influent = swine wastewater (barn flush liquid with solids separated)
 Effluent = effluent from the aerobic biofilter treatment system

Salmonella spp. were reduced by 1.2 log₁₀ (94%) in the aerobic biofilter system. Reductions of fecal coliforms and *E. coli* were slightly higher, with both removed by 1.5 log₁₀ (97%). The biofilter system reduced enterococci to a similar extent as measured for fecal coliforms and *E. coli*, with enterococci being reduced by 1.4 log₁₀ (96%). As anticipated, *C. perfringens* spores were the least efficiently reduced of the microbes studied in the biofilter system. These bacterial spores were reduced by 0.8 log₁₀ (84%). Somatic and F-specific coliphages were reduced by 1.3 log₁₀ (95%) and 1.6 log₁₀ (97%), respectively in the biofilter system, a level similar to the reductions measured for the bacterial indicator microbes in the biofilter system.

As with other alternative treatment systems to anaerobic swine waste lagoons, the aerobic biofilter system is designed to remove nutrients and other waste constituents through solids removal. In the case of the biofilter system, this is done through backflushing the biofilters. Table 4 shows the results of four rounds of backwash sampling during the Spring and Summer of 1998 and 1999. In general, concentrations of microbial indicators in backwash from Biofilter #1,

with the exception of F-specific coliphages, were similar in magnitude to the geometric mean concentrations of these microbes in swine wastewater influent to the system. The geometric mean concentration of F-specific coliphages in Biofilter #1 backwash was approximately an order of magnitude lower than measured in system influent wastewater. These results show that the backwash from the biofilter system contains a high concentration of enteric microbes, likely including pathogens, and should be further handled appropriately to minimize potential environmental contamination and human health risks.

Table 4. Geometric mean enteric microbe concentrations in backwash from aerobic biofilter system (CFU or PFU/100 mL)

Sample	Fecal Coliforms	<i>E. coli</i>	Enterococci	<i>C. perfringens</i> Spores	Somatic Coliphages	F-specific Coliphages
Biofilter #1	2 200 000	1 800 000	440 000	1 000 000	830 000	200
Backwash Biofilter #2	180 000	180 000	70 000	860 000	140 000	48
Backwash						

Overall, the concentrations of enteric microbes in effluent from the aerobic biofilter system were generally lower than were measured in single-stage swine waste lagoons. The aerobic biofilter system also achieved these enteric microbe reductions in considerably less time than lagoons require. Swine waste lagoons are typically designed to have HRTs of over 6 months compared to the 18- to 24-hour HRT in the aerobic biofilter system. Based on the results of these pathogen and microbial indicator reduction studies and related performance studies for reductions of solids, organic and nutrients, the aerobic, fixed film biological treatment system for swine waste water appears to have considerable potential for effective treatment of swine wastewater to reduce pathogens and other key waste constituents.

Aerated Solids Recovery Biological Treatment System

In this treatment system swine waste is first treated aerobically in a bioreactor, then in a long, shallow earthen reactor called an ecoreactor. The ecoreactor serves as a holding cell, where solids are converted to biosolids, which can be harvested for use as fertilizer or a soil amendment. Liquid undergoes further treatment in a secondary aerated reactor and is then recycled to the swine barns for pit recharge. Excess wastewater and rainfall is treated in a third aerated bioreactor. Performance of the aerated solids recovery system for waste constituents such as solids, organics and nutrients has been described by Classen (1999). Mass inputs and

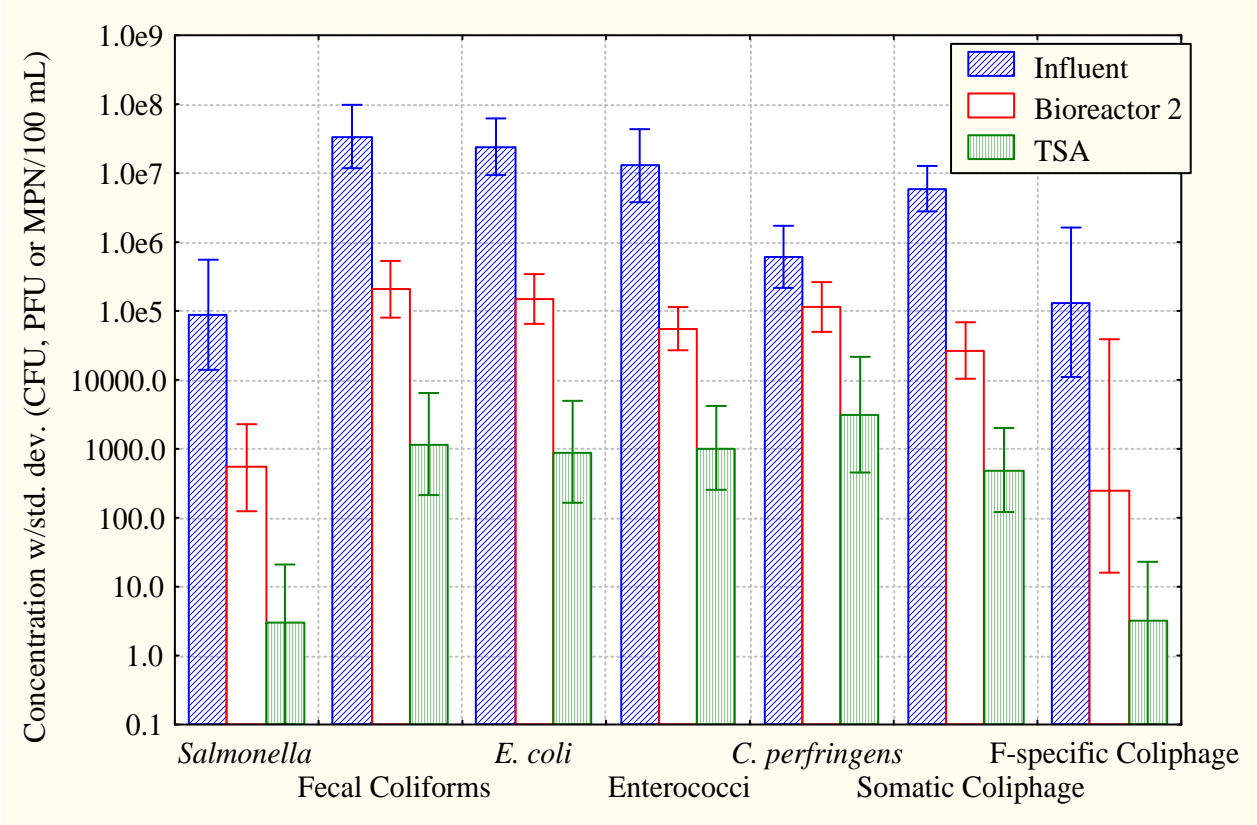
removal efficiencies were calculated using estimated flow rates based on pump capacities and time of operation. System removal efficiencies for chemical oxygen demand (COD) and total suspended solids (TSS) were 95% and 93%, respectively, not including the storage pond. The largest removals of COD and TSS occurred in the solids ecoreactor, although substantial removal also occurred in the first and second bioreactors. Nutrient removals were 53% for ammonia nitrogen (N), 69% for total Kjeldahl N, and 87% for total P. Initial data suggests that little N, but substantial P is removed from the liquid effluent in the solids ecoreactor.

Figure 8 shows the combined geometric mean reductions of *Salmonella* and enteric microbial indicators for the two aerated solids recovery biological treatment systems from which samples were collected.

Geometric mean concentrations of enteric microbes in influent to the biosolids recovery/aeration treatment systems were: 88 000 MPN/100 mL for *Salmonella* spp., 3.4×10^7 CFU/100 mL for fecal coliforms, 2.4×10^7 CFU/100 mL for *E. coli*, 1.3×10^7 CFU/100 mL for enterococci, 610 000 CFU/100 mL for *C. perfringens* spores, 5.9×10^6 PFU/100 mL for somatic coliphages, and 130 000 PFU/100 mL for F-specific coliphages. Reductions of *Salmonella*, fecal coliforms, *E. coli*, and enterococci were similar within the closed-loop portion of the system (from influent through Bioreactor 2 effluent): 99.4, 99.4, 99.4 and 99.5%, respectively. Somatic and F-specific coliphage reductions in the closed-loop portion of the two systems averaged approximately 99.5 and 99.8%, respectively. *C. perfringens* spores were the least efficiently reduced enteric microbe studied, being reduced by an average of 81%, within the closed-loop portion of the aerated solids recovery biological treatment systems (a level similar to the reduction achieved in the aerobic biofilter system discussed previously).

Excess wastewater from the closed-loop systems was stored in the former primary waste lagoon at each farm, referred to as “Temporary Storage Areas” (TSAs) by the system designers/operators. Water from the TSAs was applied to fodder crop land. Average concentrations of *Salmonella*, fecal coliforms and *E. coli* in the TSAs were 3 MPN/100mL, 1200 CFU/100 mL and 900 CFU/100 mL, respectively, each approximately 99.996% lower in the TSA than in influent to the aerated solids recovery biological treatment system. Similarly, concentrations of enterococci, somatic coliphages and F-specific coliphages were 99.992, 99.992 and 99.998% lower in the TSAs than in barn-flush wastewater entering the two treatment systems. *C. perfringens* spores were the least-reduced of the microbes studied in the TSAs, with average concentrations in the TSAs being 99.5% lower than in the barn-flush influent to the treatment systems, consistent with the research results for the other swine waste treatment systems investigated as part of this study (except for the laboratory-scale subsurface flow reactors).

Figure 8. Geometric mean concentrations of *Salmonella* and enteric microbial indicators in the aerated solids recovery biological treatment system*



Influent = swine wastewater (barn flush)
 Bioreactor 2 = effluent from the second bioreactor; closed loop effluent
 TSA - liquid in the temporary storage area

Overall, the magnitudes of the enteric microbe reductions in the aerated solids recovery system were similar or greater than the reductions achieved by the primary anaerobic swine lagoons investigated as part of this study. In addition, concentrations of enteric microbes such as *Salmonella*, fecal coliforms, *C. perfringens* spores and somatic coliphages in TSA water were substantially lower than were measured in primary anaerobic lagoon liquid (approximately 98, 99.8, 97 and 99.8% lower, respectively). The additional reductions that were achieved during storage of excess wastewater in the TSAs (generally, 1.5-2.0 log₁₀ for all microbes studied) further support the potential for this treatment approach to be an effective alternative to lagoon storage of flushed swine waste. Further studies are needed to determine if the final biosolids from the treatment system are both low in pathogens and suitable as either soil amendment or

fertilizer. Preliminary studies of this final biosolids material suggest that it may meet the US EPA microbial requirements for class A biosolids as applied by municipal waste treatment systems, but further studies are needed to determine if this biosolids quality can be consistently achieved. Overall this aerated solids recovery biological treatment system appears to have considerable potential to appreciably reduce pathogens in swine waste and, therefore, deserves consideration as a candidate alternative technology to anaerobic lagoons.

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