RESEARCH REPORT
ENVIRONMENT

Title: Reduction of *Salmonella* and Other Fecal Microbes in Swine Waste Treatment Systems - **NPB #99-112**

Investigator: Mark D. Sobsey

Institution: University of North Carolina at Chapel Hill

Co-Investigator: Vincent R. Hill

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I. ABSTRACT

The presence and control of human pathogens in waste from commercial swine farms has emerged as a public health and policy issue that impacts management practices for swine waste treatment. This project focused on quantifying the reductions of fecal microbes in commercial swine waste lagoons and constructed wetlands. Constructed wetlands are a promising alternative or additional treatment technique for flushed swine waste. Swine waste samples were analyzed for a suite of six microbial indicators (fecal coliforms, *Eschericia coli*, enterococci, *Clostridium perfringens* spores, somatic coliphages and male-specific coliphages) and *Salmonella* spp., a group of pathogenic bacteria. In untreated swine waste from flushed and pit-plug systems at four swine farms, the average concentration of *Salmonella* was measured to be 3800 MPN/100 mL. *Salmonella* were reduced by approximately 96% in primary anaerobic lagoons at these farms and by a further 97% in the secondary lagoons used at two of the farms. In general, fecal coliforms, *E. coli* and enterococci were reduced to a similar, but slightly greater, extent than *Salmonella* (≈ 97-98%) in each lagoon cell provided. *C. perfringens* spores, investigated as a potential model for the removal of helminth ova and protozoan parasite cysts and oocysts in swine waste, were less efficiently reduced in lagoons than the other enteric bacteria studied, being reduced by an average of 84% in primary lagoons and another 92% in secondary lagoons. Somatic coliphages and F+ coliphages, investigated as potential models for the removal of enteric viruses pathogenic to humans, were reduced to a similar extent as measured for fecal coliforms, *E. coli* and enterococci (≈ 97% in primary lagoons and a further 96% in secondary lagoons). In a field-scale surface flow (SF) constructed wetland operated as a secondary treatment system receiving anaerobic lagoon liquid, fecal coliforms, *E. coli* and enterococci were reduced by 98, 99 and 87%, respectively. *Salmonella* were reduced by 96% in this constructed wetlands system, and *C. perfringens* spores, somatic coliphages and male-specific coliphages by 97, 99, and 98%, respectively. In laboratory-scale SF and subsurface flow (SSF) wetland reactors, temperature and

loading rate were shown to be significant variables affecting the performance of the reactors for reducing concentrations of enteric microbes and nutrients in swine lagoon liquid. At temperatures of 10, 20 and 30°C and total Kjeldahl nitrogen loading rates of 10, 25 and 40 kg/ha/d, the vegetated SSF reactor generally achieved higher microbial and nutrient reductions than either the vegetated SF reactor or the unvegetated SSF control reactor. The results of this study show that significant reductions of fecal microbes can occur in anerobic swine waste lagoons, but that high concentrations of these microbes remain in lagoon liquid. Alternative, or additional, treatment using constructed wetlands can achieve significant removal of pathogens and nutrients from swine waste if important design variables such as temperature and mass/hydraulic loading rates are considered.

II. INTRODUCTION

Numerous microbes found in the fecal waste of swine are known to cause disease in human hosts. Bacteria such as *Salmonella typhimurium*, *Eschericia coli*, *Yersinia enterocolitica*, and protozoa such as *Cryptosporidium parvum* can be found in swine wastes as normal enteric microflora or as pathogens (Cole et al., 1999). Understanding the presence and persistence of these and other potentially zoonotic pathogens in swine waste is important to protect the health of farm workers, the surrounding community and natural resources.

Current waste management methods on swine farms often rely on lagoons for waste storage and treatment prior to land application. Research on anaerobic lagoons receiving swine waste indicates that the liquid from these treatment systems can have high concentrations of enteric microbes, including fecal coliform levels of over 300,000 organisms/100 mL (Hill and Sobsey, 1998), and can contain pathogens such as *Salmonella*. Development of alternative treatment systems for swine waste is ongoing and much research has been focused on the use of low-cost techniques such as constructed wetlands to provide additional treatment of lagoon wastewater (Cronk, 1996; Hunt et al., 1998). Currently, little data is available regarding the levels of fecal microbes in swine waste. A better understanding of the microbial quality of swine waste is important for issues such as worker safety, environmental quality and public health protection, and livestock health protection, especially if livestock ingest fecal pathogens, for example, through ingestion of contaminated ground water. It is likely that alternatives to the lagoon-sprayfield system will be required for swine operations, so it is prudent for the pork industry to investigate cost-effective treatment methods like constructed wetlands.

III. OBJECTIVES

This study was designed to provide insights into the following questions: (1) what concentrations of *Salmonella* and other fecal microbes can be found in untreated and lagoon-treated swine waste?, (2) what reductions in *Salmonella* and other fecal microbe concentrations can be achieved using constructed wetland treatment of lagoon liquid?, (3) are the observed reductions of these fecal microbes in a laboratory-scale SF wetland reactor significantly different than in a SSF wetland reactor?, (4) does the presence of wetland plants in a SSF wetland system have a significant effect on fecal

microbe and nutrient removal?, and (5) what variables (e.g., microbial indicator reductions, hydraulic residence time (HRT), temperature, total suspended solids removal, etc.) contribute significantly to the prediction of the reduction of the pathogen *S. typhimurium* in the waste treatment lagoons and constructed wetlands systems studied?

IV. PROCEDURES

Microbiological analyses: Wastewater samples were collected and analyzed within 24 hours for the following enteric microbial indicators: fecal coliforms, *Eschericia coli*, enterococci, *C. perfringens* spores, somatic coliphages, and F+ coliphages. Bacterial indicators were enumerated by filtering diluted samples through 47-mm, 0.45-mm 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 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 then exposing the plates to ammonium hydroxide fumes. Viral indicators were enumerated using single- or double-agar layer, pour plate plaque techniques (Adams, 1959; Grabow and Coubrough, 1986), with somatic and F+ coliphages detected on host bacteria *E. coli* CN-13 and *E. coli* Famp, respectively. *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).

Anaerboic lagoon sampling: Lagoon samples were collected manually on an approximately monthly basis at four farms: 1) a 2,600-head commerical swine nursery in Duplin County, NC having a single-stage anaerobic lagoon system, 2) a commercial farrow-to-wean farm housing 4,000 sows and having a two-lagoon treatment system (the primary lagoon being a covered anaerobic "digester" and the secondary lagoon being open to the atmosphere), 3) a 300-sow farrow-to-feeder, 500-head farrow-to-finish educational farm operated by North Carolina State University 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.

Operation and sampling of constructed wetland systems: The field-scale constructed wetland system has been operating at a 2,600-head swine nursery in Duplin County, North Carolina since 1992 (Hunt et al., 1998) The SF system contains two cells (each 3.6 m x 33.5 m) in series, planted with bur-reed (*Sparganium americanuum*) 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 hydraulic loading rate (HLR) of lagoon liquid to the field system averaged 2.0 cm/d.

Polyethylene laboratory-scale reactors (76 cm x 30 cm x 61 cm) were installed in a walk-in incubator in October 1998: a SF reactor with soft-stem bulrush (*Scirpus tabermontanii*) planted in sandy loam (30 cm deep), a SSF reactor with soft-stem bulrush planted in 30-cm-deep PermaTill® (Carolina Stalite Co.; Salisbury, North Carolina), an expanded-slate gravel (9.5-mm average diameter), and an unplanted SSF control. Full spectrum ® "Sunshine" plant grow-lights (General Electric) were suspended above each reactor and operated on a 12-hour on/off cycle. Beginning in February 1999, lagoon liquid diluted 1:1 with tap water was pumped semi-continuously from a central influent distribution tank (completely mixed and refrigerated at 2-6□C) into each reactor using peristaltic pumps controlled by on/off timers. Between September 1999 and January 2001, the reactors were studied at total Kjeldahl nitrogen (TKN) loading rates of 40 kg/ha/d (3.8 cm/d HLR), 25 kg/ha/d (2.3 cm/d HLR), and 10 kg/ha/d (1.1 cm/d HLR) at constant ambient temperatures of 10, 20 and 30□C. Tracer tests were conducted at each loading rate using sodium fluoride to measure the hydraulic residence time (HRT) in each reactor. *Salmonella typhimurium*, isolated from the primary lagoon at the 4,000-sow farrow-wean farm, was spiked into the influent tank to maintain an approximate influent concentration of 1,000,000 MPN per 100 mL.

V. RESULTS

Anaerobic Lagoons

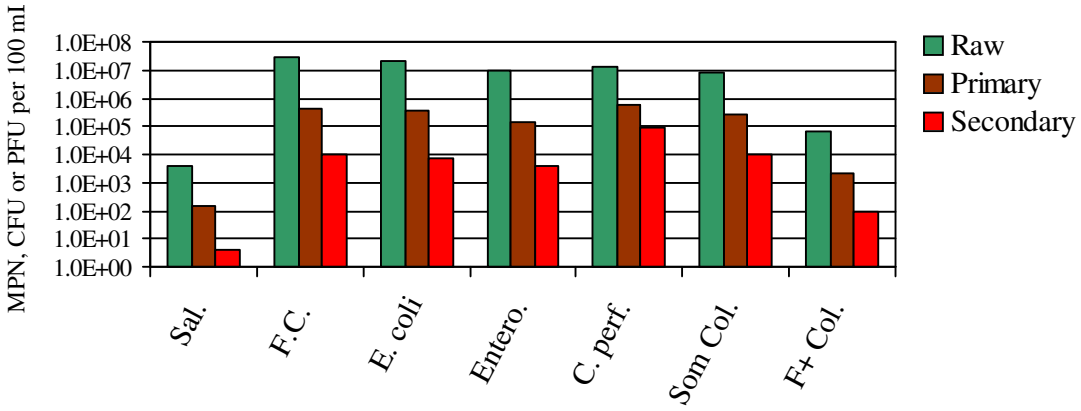
The average *Salmonella* concentration in flushed swine waste at the four farms studied was 3800 Most Probable Number (MPN)/100 mL (Figure 1). *Salmonella* were reduced by approximately 96% in primary lagoons and by a further 97% when a secondary lagoon was used. Fecal coliforms, *E. coli* and enterococci were investigated as possible models for the reduction of *Salmonella* in the lagoon systems. In general, these microbes 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, present at a slightly lower average concentration in flushed swine waste (9.2×10^6 CFU/100 mL), were reduced by 98% in primary lagoons and by a further 97% at farms having a second lagoon.

C. perfringens spores, investigated as a potential model for the removal of helminth ova and protozoan parasite cysts and oocysts in swine waste, 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.

Somatic coliphages and F+ coliphages were reduced to a similar extent as measured for fecal coliforms, *E. coli* and enterococci. Both somatic and F+ coliphages were reduced by 97% in primary lagoons and a further 96% in secondary lagoons.

The results of the swine waste lagoon study show that fecal microbes, including *Salmonella* spp., are significantly reduced in anaerobic lagoon systems, but such high concentrations of these microbes remain that additional or alternative management of flushed swine waste may be needed to protect public health and environmental resources.

Figure 1. Concentrations of fecal microbes in anaerobic swine waste lagoon systems



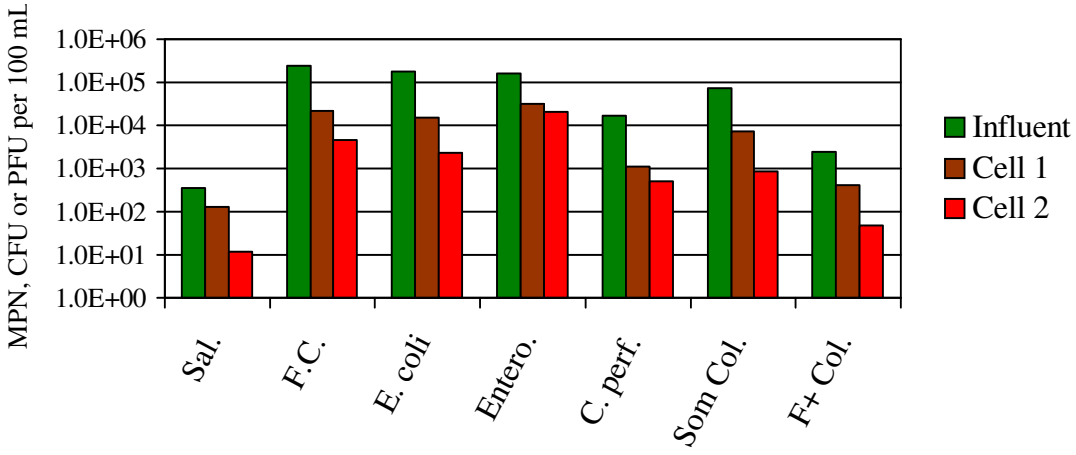
Constructed Wetlands: Field-Scale Surface Flow

The influent to the field-scale 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 (Figure 2). These bacterial indicators were reduced by about 1.0 $\log_{10}$ (91%) and 1.1 $\log_{10}$ (92%), respectively, in Cell 1 of the wetland system, and overall by 1.7 $\log_{10}$ (98%) and 1.9 $\log_{10}$ (~99%), respectively. Enterococci were less effectively reduced than were the fecal coliforms and *E. coli* in the field-scale constructed wetland system: 0.7 $\log_{10}$ (80%) in Cell 1 and 0.9 $\log_{10}$ (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. As expected, *Salmonella* were measured at far lower concentrations than the fecal indicator bacteria in influent to the field wetland system. *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 $\log_{10}$, or 63% reduction) and to a mean of 12 MPN/100 mL in effluent from the system (a 1.5 $\log_{10}$, or 96% overall reduction through the 2-cell system).

C. perfringens spore concentrations were reduced by 1.2 $\log_{10}$ (93%) in Cell 1 effluent and by 1.5 $\log_{10}$ (97%) overall in system effluent (Figure 2), suggesting that environmentally-stable enteric microbes (e.g., *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts and helminth ova) would be similarly removed from wastewater by a SF constructed wetland system with this type of design and operation. Because bacterial spores and parasites are relatively stable in the environment, however, it is still possible that release of these microbes may occur periodically or during system perturbations (e.g., precipitation events). Somatic and F+ 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.

Figure 2. Reduction of fecal microbes in surface flow constructed wetland treating swine lagoon liquid



The results of the constructed wetlands field study show that fecal microbes, including *Salmonella* spp., in swine lagoon liquid can be significantly reduced in a surface flow constructed wetland system. However, reductions are not 100% and pathogens such as *Salmonella* bacteria, as well as fecal indicator bacteria, bacterial spores and viruses remain in the treated wastewater. Therefore, adequate containment and overall management of constructed wetland effluents is needed to prevent migration or movement of these microbes off of farms.

Constructed Wetlands: Laboratory-Scale Study

The results in Figures 3, 4 and 5 show that, in general, TKN loading rate (as well as hydraulic loading rate) significantly affected the reductions of fecal microbes in the SF and SSF reactors (with improved reactor performance observed as loading rates decreased). At the various TKN loading rates studied, reductions of fecal coliforms, *S. typhimurium* and somatic coliphages were in general significantly greater in the SSF wetland reactor than in either the SSF control (no vegetation) or the SF wetland reactor. These results are quantitative evidence supporting the hypothesis that vegetation has a positive effect on SSF reactor performance for reducing enteric microbes in wastewater. In addition, the results show that design of a wetland reactor for subsurface flow can greatly improve fecal microbe removal from wastewater.

Results from the laboratory study also show that temperature can significantly affect enteric microbial reductions in wetland reactors (Figures 6, 7 and 8), with generally greater microbial reductions at higher temperatures. However, it is unclear why the fecal coliform and *S. typhimurium* reductions were highest at 10°C in the SF wetland reactor. The expected temperature trends in this reactor were observed for somatic coliphages (Figure 10).

In general, reductions of fecal coliforms in the SSF wetland reactor ranged from moderate [1.1 $\log_{10}$ (92%)] to very high [4.4 $\log_{10}$ (99.996%)] depending on temperature and loading rate. Reductions of *S. typhimurium* in the SSF reactor also ranged from

low [0.7 $\log_{10}$ (82%)] to very high [4 $\log_{10}$ (99.99%)]. Reductions of these microbes were comparatively lower in the SF wetland reactor: 0.1 to 1.5 $\log_{10}$ (15 to 97%) and 0 to 1.5 $\log_{10}$ (0 to 97%), respectively.

The results of this study show that enteric microbes (including the bacterial pathogen *Salmonella*) are present at high concentrations in flushed swine waste. Anaerobic lagoons can substantially reduce the concentrations of these microbes, especially if more than one lagoon in-series is used, but high concentrations of *Salmonella* and indicator microbes remain in lagoon liquid. The results of both field and laboratory studies indicate that constructed wetlands can be effective for further reducing fecal microbe concentrations in swine lagoon liquid and may, with appropriate pretreatment, be feasible as alternatives to lagoon treatment systems. The laboratory study showed that effective designs of constructed wetland systems for maximum pathogen removal must consider the effects of hydraulic/mass loading rates and seasonal temperature changes on constructed wetland performance.

Figure 3. Reductions of fecal coliforms in laboratory wetland reactors at 20 C and variable TKN loading rates

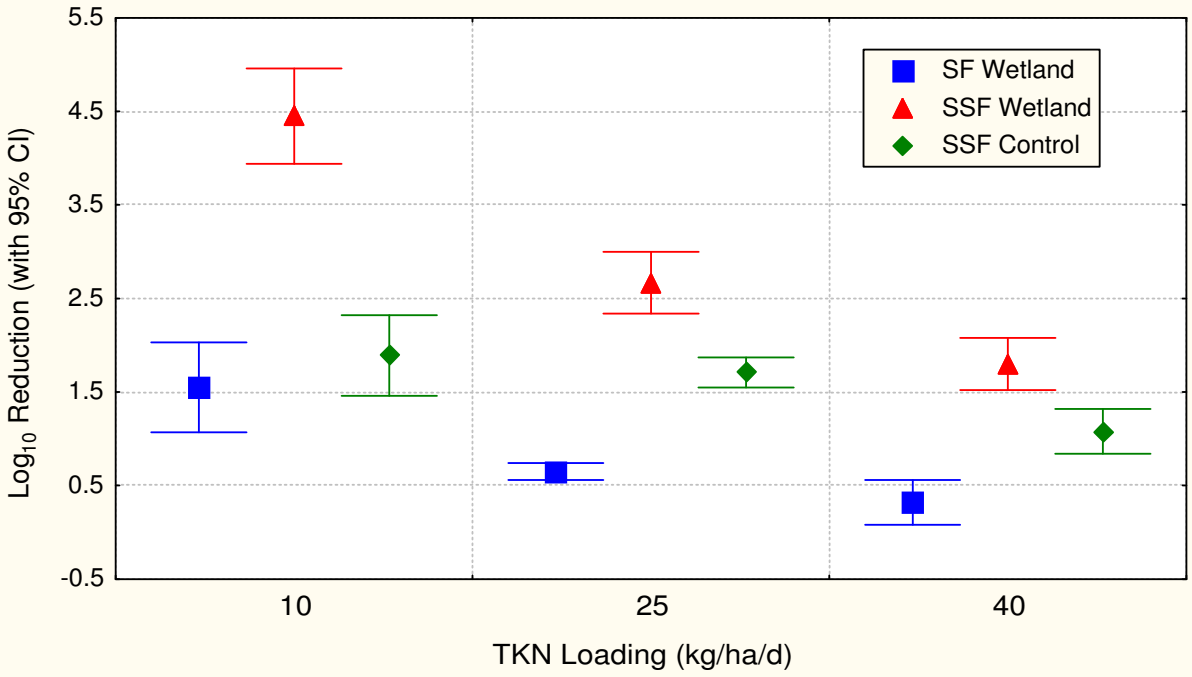


Figure 4. Reductions of Salmonella in laboratory wetland reactors at 20 C and variable TKN loading rates

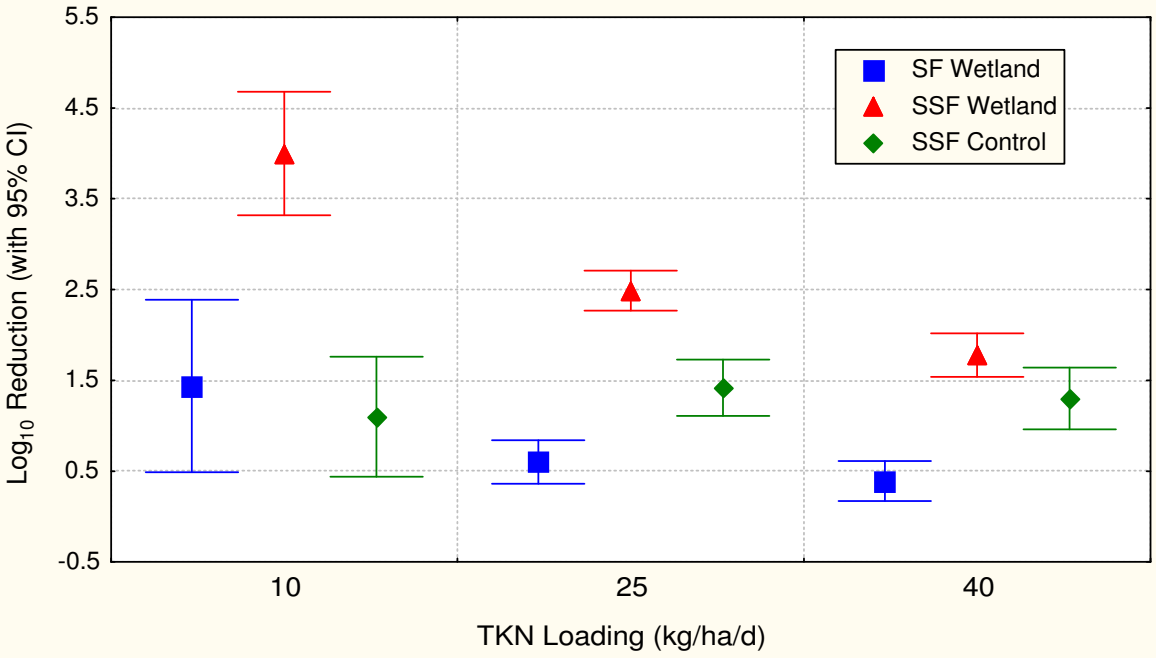


Figure 5. Reductions of somatic coliphages in laboratory wetland reactors at 20 C and variable TKN loading rates

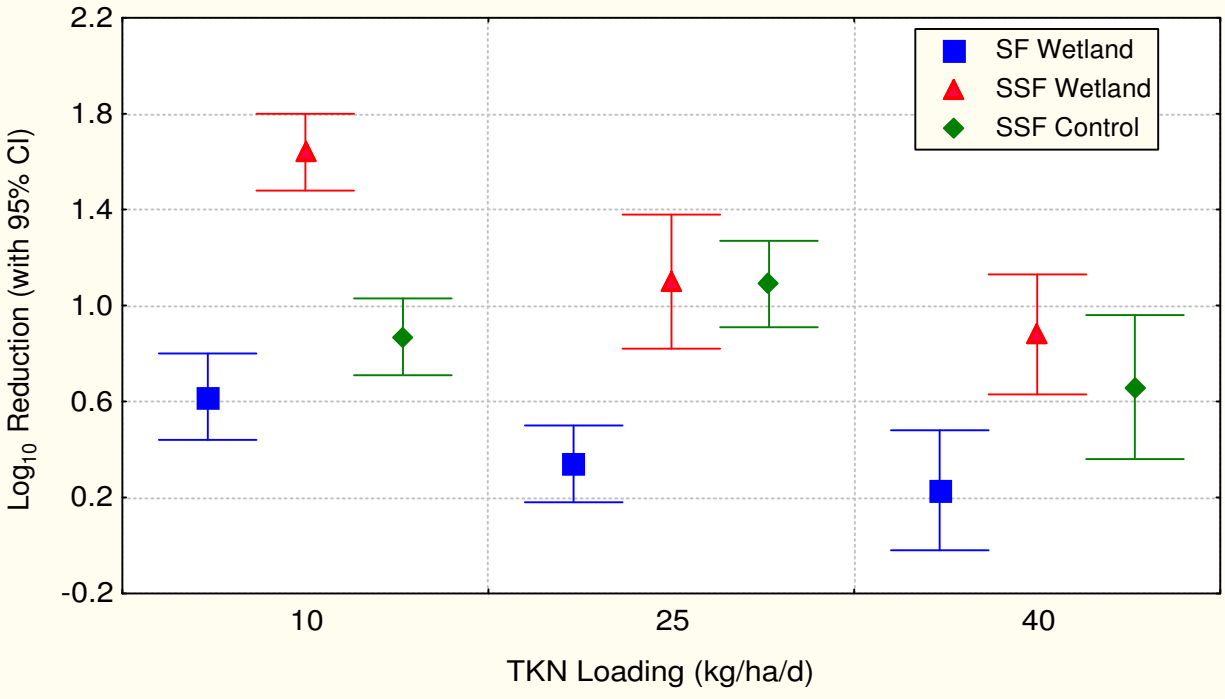


Figure 6. Effect of temperature on reduction of Salmonella in laboratory wetland reactors at TKN loading rate of 25 kg/ha/d

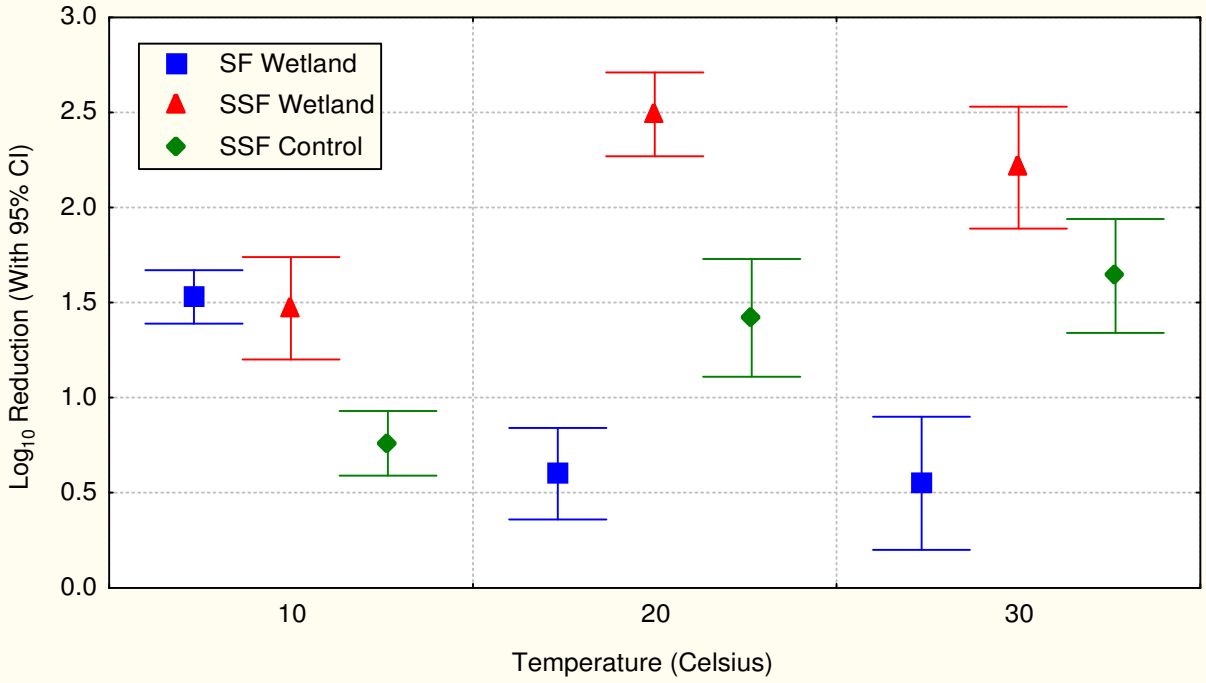


Figure 7. Effect of temperature on reductions of fecal coliforms in laboratory wetland reactors at TKN loading rate of 25 kg/ha/d

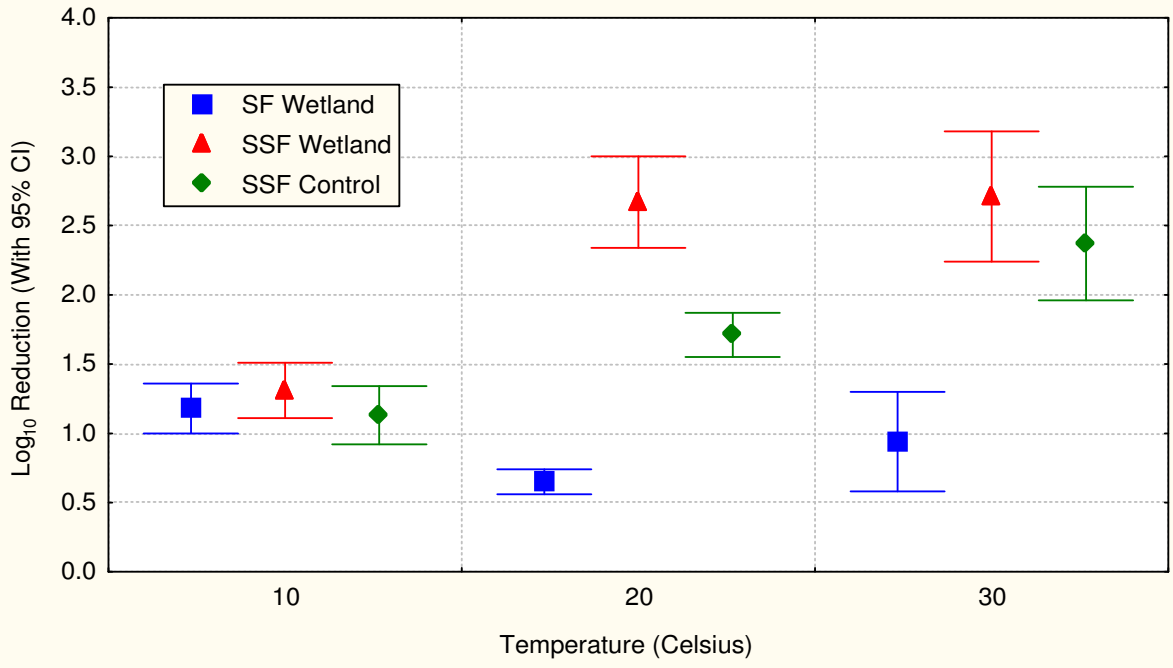
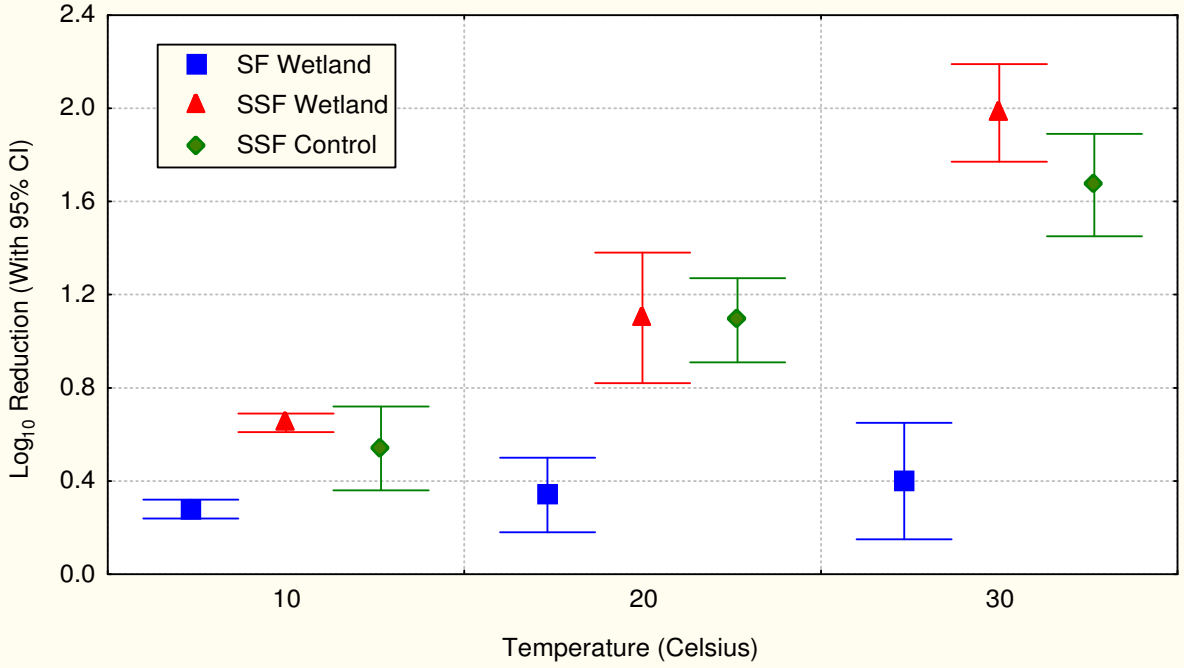


Figure 8. Effect of temperature on reductions of somatic coliphages in laboratory wetland reactors at TKN loading rate of 25 kg/ha/d



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