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# Disinfection of swine wastewater using chlorine, ultraviolet light and ozone

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## ABSTRACT

Veterinary antibiotics are widely used at concentrated animal feeding operations (CAFOs) to prevent disease and promote growth of livestock. However, the majority of antibiotics are excreted from animals in urine, feces, and manure. Consequently, the lagoons used to store these wastes can act as reservoirs of antibiotics and antibiotic-resistant bacteria. There is currently no regulation or control of these systems to prevent the spread of these bacteria and their genes for antibiotic resistance into other environments. This study was conducted to determine the disinfection potential of chlorine, ultraviolet light and ozone against swine lagoon bacteria. Results indicate that a chlorine dose of 30 mg/L could achieve a 2.2–3.4 log bacteria reduction in lagoon samples. However, increasing the dose of chlorine did not significantly enhance the disinfection activity due to the presence of chlorine-resistant bacteria. The chlorine resistant bacteria were identified to be closely related to *Bacillus subtilis* and *Bacillus licheniformis*. A significant percentage of lagoon bacteria were not susceptible to the four selected antibiotics: chlortetracycline, lincomycin, sulfamethazine and tetracycline (TET). However, the presence of both chlorine and TET could inactivate all bacteria in one lagoon sample. The disinfection potential of UV irradiation and ozone was also examined. Ultraviolet light was an effective bacterial disinfectant, but was unlikely to be economically viable due to its high energy requirements. At an ozone dose of 100 mg/L, the bacteria inactivation efficiency could reach 3.3–3.9 log.

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## 1. Introduction

The number and size of concentrated animal feeding operations (CAFOs) are on the rise, and with them comes a rise in the amount of waste produced. In the United States, there are

approximately 1.3 million livestock farms, with about 257,000 of these farms regarded as animal feeding operations (AFOs) (US EPA, 2003). Animal agriculture commonly uses anaerobic lagoons and pit systems for waste disposal (Sweeten, 1980). These lagoons depend on both microbial activity and

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management practices such as solids separation prior to treatment, periodic solids removal, and suitable organic loadings to help maintain functionality (Barker and Drigger, 1985; Miner et al., 2000). Factors that can adversely affect microbial activity include organic overloading, temperature and pH fluctuations, salt buildup, ammonia accumulation, and the use of disinfectants and antibiotics (Hilpert et al., 1984; Poels et al., 1984; Hansen et al., 1998; Zahn et al., 2001; Do et al., 2003).

Veterinary antibiotics are widely used as additives in food or water at CAFOs to prevent and treat animal disease outbreaks due to their prophylactic and therapeutic qualities as well as to promote animal growth (Carlson and Fangman, 2000). It is estimated that more than 3000 tons of veterinary antibiotics are used in the European Union, and from 8500 to 11,200 tons in the United States each year (Dell, 2003). However, these antibiotics pass through animal bodies and are commonly excreted in urine, feces and manure as parent compounds, conjugates, or oxidation and hydrolysis byproducts (Tolls, 2001). The animal wastes are discharged to anaerobic lagoons for biological treatment and temporary storage. However, many antibiotics are not amenable to biodegradation (Daughton and Ternes, 1999) and accumulate in the lagoons. As a result, the lagoons can act as reservoirs of various antibiotics and subsequently, a portion of lagoon bacteria may develop strong resistance to these antibiotics. Seepage and runoff of the lagoon wastewater and farm application of the lagoon sediments as fertilizer may lead to the contamination of both surface and groundwater with antibiotics and antibiotic-resistant bacteria, thus posing a severe threat to public health (Chee-Sanford et al., 2001). In fact, a variety of antibiotics were detected at relevant concentrations in U.S. streams in a recent national reconnaissance (Kolpin et al., 2002). Goni-Urriza et al. (2000) evaluated the impact of an urban effluent on antibiotic resistance of freshwater bacterial populations and reported that 72% of *Aeromonas* strains and 20% of *Enterobacteriaceae* strains were resistant to nalidixic acid. *Enterobacteriaceae* also exhibited resistance to tetracycline (TET) (24%) and beta-lactams (21%), and *Aeromonas* to TET (28%) and cotrimoxazole (27%). Recent studies have also shown that the potential contamination of groundwater with bacteria and antibiotic-resistant genes was found up to 100 m downstream of swine lagoons (Chee-Sanford et al., 2001; Krapac et al., 1998, 2000).

There has been little research on disinfection of the bacteria associated with animal wastes generated at CAFOs. Chlorine, UV light and ozone are commonly used as disinfectants in water and wastewater treatment facilities. The major objective of this study was to examine the potential disinfection efficiency of chlorine, UV light and ozone on swine lagoon bacteria. The susceptibility of lagoon bacteria to selected antibiotics, including chlortetracycline (CTC), lincomycin (LIN), sulfamethazine (SMN) and TET, was also tested. It was reported that microorganisms associated with cell debris, fecal material, or wastewater solids were more protected from disinfection (Berman et al., 1988). Therefore, the effect of suspended solids on disinfection efficiency was assessed in this work.

## 2. Materials and methods

### 2.1. Lagoon samples

Wastewater samples were obtained from lagoons at two different swine production facilities located in central Missouri. One is classified as a functional lagoon while the other is classified as non-functional. The functional lagoon (Lagoon A) is a recharge pit system with semi-annual solids removal. The lagoon receives swine wastes from two barns that can hold 2000 weaner pigs and are collected in a pit before being flushed and washed down. The water recycled from the lagoon is used to wash down the wastes from the pit. The lagoon size is 65.5 m (L) × 58.8 m (W) with the depth ranging from 2.4 to 5.5 m. The aqueous phase of this lagoon turns purple in the warm weather. The purple color indicates the probable presence of photosynthetic purple bacteria that can consume odoriferous compounds such as hydrogen sulfide, ammonia, and excess volatile fatty acids (Kobayashi et al., 1983; Do et al., 2003). The non-functional study lagoon (Lagoon B) is the initial stage of a two-stage system without solid separation, recycle and solid removal. The first-stage lagoon receives wastes from three barns that contain approximately 375 hogs ranging in age from farrowing with sows to finishing. This farm uses groundwater to flush the swine wastes into this lagoon. This first stage is a primary treatment lagoon for the swine wastes where solids accumulate, while the second stage receives overflow from the initial stage lagoon. The treatment lagoon size is 54 m (L) × 21.6–36.6 m (W) with the depth ranging from 0.3 to 2.7 m. The sludge depth varies from 0.3 to 1.2 m. This lagoon has a grayish or black color and possesses a high sludge accumulation. The black color is indicative of organic overloading in the lagoon (USDA, Natural Resources Conservation Service, 1999).

Samples were taken at a depth of 0.3 m below the surface at the middle of each lagoon by using a Van Dorn style water sampler (Cole Parmer, Vernon Hills, IL). After being dispensed into sterile Nalgene® polyethylene bottles (1L), the samples were immediately stored on ice, transported to the lab, and maintained at 4 °C until used for experiments.

To assess the effect of suspended solids on the disinfection potential, a portion of lagoon samples was centrifuged at 1000g for 5 min at 4 °C by using an IEC B-22M Programmable centrifuge (International Equipment Company, Needham Heights, MA). The supernatant was thereafter stored on ice until use to suppress bacterial growth. A low speed and a short time for centrifugation were adopted here in an attempt to only remove large suspended particles but retain most of the bacteria in the supernatant.

Both centrifuged and non-centrifuged samples were buffered with 10 mM KH<sub>2</sub>PO<sub>4</sub>, and adjusted to pH 7.7 for experiments. This pH value was selected because it closely represented the natural pH conditions of both study lagoons. The pH values of Lagoons A and B were measured to be 7.85 and 7.42, respectively (Table 1). The typical physical-chemical properties of the centrifuged samples from Lagoons A and B are described in Table 1.

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