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5 **Disinfection of Swine Wastewater Using Chlorine, Ultraviolet Light and Ozone**

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1 **Abstract**

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

20 **Keywords:** Disinfection; Antibiotic resistance; Swine wastewater; Chlorine; Ultraviolet light,
21 Ozone

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 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 11200 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 ground water 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 (24%) and beta-lactams (21%), and *Aeromonas* to tetracycline (28%) and co-trimoxazole (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; Krapac et al., 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 chlorotetracycline (CTC), lincomycin (LIN), sulfamethazine (SMN) and tetracycline (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, 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 (1 L), 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 bacteria 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_2PO_4, 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 Lagoon A and Lagoon B were measured to be 7.85 and 7.42, respectively (Table 1). The typical physical-chemical properties of the centrifuged samples from Lagoon A and Lagoon B are described in Table 1.

2.2. Reagents

Potassium phosphate monobasic (HPLC grade, 99.6%), certified ACS grade hydrochloric acid (37.6%) and sodium hydroxide (98.5%) were purchased from Fisher Scientific (Fairlawn, NJ). NaOH and HCl solutions were prepared in a series of appropriate concentrations and sterilized for pH adjustment. Sodium hypochlorite (> 4% by weight), obtained from Aldrich (Milwaukee, WI), was used as the source of free chlorine. Its real concentration was determined to be 44,400 mg/L as Cl_2. Chlorotetracycline (CTC), sulfamethazine (SMN) and tetracycline (TET) were purchased from Sigma (St. Louis, MO), and lincomycin (LIN) was purchased from ICN Biomedicals Inc. (Aurora, OH). Millipore water with a resistivity of > 18.2 MΩ·cm was

produced by a Millipore Simplicity 185 water purification system (Millipore Co., Bedford, MA) from distilled water.

2.3. Analysis

Sample pH was measured with a digital Corning pH meter (Model 320) coupled with a combination pH probe (Corning Inc., Corning, NY). Dissolved organic carbon (DOC) was analyzed by using a Total Organic Carbon Analyzer (Model TOC-5000A, Shimadzu Co., Kyoto, Japan) after appropriate sample dilution. Soluble chemical oxidation demand (SCOD), free ammonia, total alkalinity were determined by Hach methods 8000 (dichromate reactor digestion), 10045 (AccuVac Ampuls), and 8203 (digital titration with H_2SO_4 solution) with a DR/2010 portable spectrophotometer (Hach Co., Loveland, CO). A digital conductivity meter coupled with a platinum probe from Fisher Scientific (Fairlawn, NJ) was used to measure sample conductivity and total dissolved solids (TDS) concentration. The concentrations of acetate, chloride, bromide, phosphate and sulfate were determined by using ion chromatography (Model DX-120, Dionex Co., Sunnyvale, CA) with a Dionex IonPac AS9-HC column (4 × 250 mm) for ion separation and 9 mM Na_2CO_3 solution as mobile phase running isocratically at a flow rate of 1.0 mL/min. A Varian spectrophotometer (Model Cary 50 Conc., Varian Australia PTY Ltd., Australia) was used to determine the ultraviolet absorbance of lagoon wastewater at 254 nm and the concentration of aqueous ozone at 260 nm. Free chlorine and total chlorine concentrations were analyzed by using Hach DPD methods 8021 and 8167, respectively, after appropriate sample dilution.

2.4. Disinfection Procedures

In the chlorination experiments, sodium hypochlorite was used as the source of free chlorine. Five mL aliquots of sample were distributed to a series of 25-mL sterile conical vials.

A desired amount of chlorine was spiked into these vials to achieve chlorine doses of 5, 10, 30, 50, 100, 250, and 500 mg/L. After addition of chlorine, the samples were immediately vortexed and allowed to react for 2.5 hr before performing enumeration tests. The disinfection reactions proceeded on ice (about 3 –4°C) to simulate unfavorable winter temperature conditions. In warm seasons, higher disinfection efficiencies are anticipated because disinfection reactions will proceed more quickly. Sodium hypochlorite was added to a new sample every 15 min. This time interval was required to complete plating of each sample in the subsequent bacterial enumeration tests.

For the chlorination tests and subsequent antibiotic exposures, the samples from each lagoon were first centrifuged, and a desired amount of chlorine was added thereafter with a dose of 0, 50 and 500 mg/L. After the disinfection was allowed to proceed for 2.5 hr on ice, the samples were plated onto brain heart infusion (BHI) medium amended with individual antibiotics. The concentration of LIN, CTC and TET in the BHI medium was prepared at 32 mg/L, while a high concentration of 256 mg/L was used for SMN due to its lower antibiotic effectiveness (Salmon et al., 1995).

During the ultraviolet light experiments, a 200-mL graduated glass cylinder (I.D. 3.7 cm) was used as the reactor with aluminum foil wrapped around the outside to enhance radiation efficiency. A low pressure mercury vapor 254 nm lamp (Pen Ray, Model 90-0004-01) was situated along the central line of the reactor. The light intensity of the lamp at 254 nm was 5.4 mW/cm^2 at 1.9-cm radius as provided the manufacturer (UVP Inc., Upland, CA). The effective dose rates of the UV lamp were calculated to be 0.366 and 1.282 mW/cm^2 for Lagoon A and Lagoon B samples, respectively, based on the wastewater absorbance and reactor geometry using the Point Source Summation Method [White, 1992]. This apparatus was allowed to warm up for

10 minutes before initiating the experiments. The lagoon sample (180 mL) was added into the reactor and mixed gently with a magnetic stir bar. An aliquot of 5 mL sample was periodically withdrawn midway down the reactor through a Teflon tube and glass syringe at pre-selected times to perform bacteria enumeration tests.

For the ozonation experiments, gaseous ozone was produced from compressed oxygen by corona discharge in an ozone generator (Model GLS-1, PCI-WEDECO Environmental Technologies, West Caldwell, NJ). An ozone gas stream was bubbled through a stone diffuser into an ozone receiving solution (Millipore water buffered with 10 mM KH_2PO_4 and pH adjusted to 7.7). Ozone was saturated in the aqueous phase within 5 min. The ozone-saturated solution was spiked into a series of 25-mL sterile conical vials, each containing 5 mL of lagoon sample, to reach an ozone dose of 10, 20, 40, 100, 150, and 200 mg/L. Other procedures were exactly the same as those used in the chlorination experiments. Ozone residual was not monitored during the reaction because ozone decay was expected to be fast in the lagoon wastewaters. Preliminary experiments indicated that the half-life of ozone decay was about 3 min in pH 7.0 Millipore water buffered with 10 mM KH_2PO_4. The lagoon wastewaters contained a significant amount of organic materials, thus all the ozone would be depleted within the 2.5 hr reaction time. The effect of sample dilution due to batch addition of the ozone saturated solution was corrected for during bacteria enumeration.

All the chlorination, UV irradiation, and ozonation of lagoon bacteria were conducted in two parallel experiments (i.e., sample duplicates) for statistical data analysis.

2.5. Bacteria Enumeration

Both standard most probable number (MPN) analysis and plate count technique were used for enumerating bacteria. Brain heart infusion medium (37 g/L; Beckton-Dickinson) was used for both analyses.

Most probable number analysis was performed by preparing 10-fold serial dilutions of the disinfectant treated sample to 10^{-10} in triplicate. The serially diluted tubes were incubated at 37 °C for 3 days before analyzing. Tubes exhibiting increased turbidity after incubation were considered as positive. Final enumeration of bacteria was done by comparing the distribution pattern of positive tubes with a standard MPN table (Atlas et al., 1984).

The plate count analysis was performed first by a single 10-fold serial dilution of the disinfectant treated sample to 10^{-10}. Next, approximately 20–25 sterile glass beads of 3-mm diameter were dispensed to the BHI medium plates. An aliquot of 100 uL of sample was withdrawn from each dilution tube and plated onto the center of three plates (in triplicate). The plates were shaken in a side to side motion for approximately 10 seconds to evenly distribute the sample. After removing the glass beads, the plates were incubated in an inverted position at 37°C for 3 days before analyzing. After incubation, the plate colonies were counted by using a Darkfield Colony Counter (Reichert Scientific Instruments, Buffalo, NY).

2.6. DNA Extraction and Sequencing

DNA was extracted from cultures grown overnight by using the UltraClean Soil DNA Kit (MO BIO Laboratories, Inc., Solana Beach, CA). The 16S rDNA of bacteria resistant to high levels of chlorine was amplified by using the universal bacterial primers 27F and 1492R synthesized by MWG Biotech (High Point, NC). The sequences of the 27F and 1492R primers are 5' – AGA GTT TGA TC(AC) TGG CTC A – 3' and 5' – TAC GG(CT) TAC CTT GTT

ACG ACT T – 3’, respectively. The PCR mixture (20 µL) consisted of 30 pmol of each primer, 1 U Taq DNA polymerase, 2 µL 10X PCR buffer, 1 µL 25 mM Mg $(OAc)_2$, 0.2 µL 200 uM dNTP, and 1 µL of the DNA extraction from each isolate. A touchdown PCR program was used to amplify the target of interest. Amplification products were confirmed by running 10 µL of aliquots of each PCR reaction on a 0.7% agarose gel stained with ethidium bromide. Restriction fragment length profiles (RFLPs) were conducted as a fast screen of chlorine resistant isolates to determine differences in these bacteria by nucleotide sequence. Briefly, colonies from the highest chlorine dosed (i.e., 500 mg/L Cl_2) samples were isolated. DNA was extracted and purified as detailed above. A general RFLP double digest was performed: 7 µL water, 2 µL buffer C (final assay concentrations - 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, and 50 mM NaCl), 1 µL bovine serum albumin, 0.5 µL enzyme Rsa I, 0.5 µL enzyme Hae III, and 10.5 µL PCR product. Reaction mixtures were incubated at 37°C for 3 hr. All RFLP reactions were analyzed with electrophoresis by using an ethidium bromide stained 2.0% agarose gel.

Amplicons of the 16S rDNA were purified by using the QIAGEN QIAquick PCR Purification Kit, and subsequently sent to MWG Biotech for Comfort Read® sequencing reactions and analysis. The 16S rDNA sequences of the above isolates were edited and BLAST searched in GenBank by using CHROMAS PRO to determine the closest potential relative.

3. Results

3.1. Disinfection with Chlorine

The effect of chlorine dose on bacteria inactivation is shown in Figure 1. Results indicate that for all lagoon samples, the bacteria were effectively inactivated as the chlorine dose was increased from 0 to 30 mg/L. However, the inactivation curve leveled off as the chlorine dose

was further increased from 50 to 500 mg/L. For example, the disinfection efficiency was 3.1 log for Lagoon A non-centrifuged sample at the chlorine dose of 30 mg/L. At the chlorine dose of 500 mg/L, the disinfection efficiency only increased to 3.6 log. A complete inactivation of bacteria could not be achieved even at a chlorine concentration as high as 500 mg/L. This implies that a small portion of bacteria were highly resistant to chlorine disinfection in both non-centrifuged (raw) and centrifuged lagoon samples.

Results in Figure 1 also show that a higher efficiency of bacteria inactivation was achieved in Lagoon A than in Lagoon B. This may be due to the difference in the starting bacteria populations and the microbial diversity as well. For example, at the chlorine dose of 30 mg/L, the disinfection efficiency was 2.2 log for the non-centrifuged sample of Lagoon B as compared to 3.1 log for that of Lagoon A. For the same lagoon, bacteria inactivation was slightly more effective in the centrifuged sample than in the raw sample. This indicates that the suspended solids did inhibit bacteria inactivation, but the inhibition was not significant.

The decay of total chlorine was monitored as the disinfection proceeded for the chlorine doses of 5, 30, and 500 mg/L, as shown in Figure 2. Results indicate that for the same lagoon, the raw sample consumed total chlorine more rapidly than the centrifuged sample, as affected by the different amounts of suspended solids present. Furthermore, less residual total chlorine was detected in the samples of Lagoon A than those in Lagoon B. This probably resulted in more bacteria removal in Lagoon A samples as observed in our experiments. At the chlorine dose of 5 mg/L, almost all total chlorine was consumed after 10 min. At the chlorine dose of 30 mg/L, the concentration of residual total chlorine was 12.6 mg/L for the centrifuged Lagoon B sample, and ranged from 4.2 to 5.4 mg/L for the other three samples after 3 hr. When the chlorine dose increased to 500 mg/L, a significant amount of residual total chlorine (150–215 mg/L) was

detected in all samples even after 6 hr. Although the residual total chlorine persisted throughout the course of disinfection at a significantly high concentration, approximately 1000–5000 cfu/mL bacteria survived the disinfection process, exhibiting a high resistance to chlorine disinfection.

It should be pointed out that the breakpoint of chlorination had never been reached even at the highest chlorine dose of 500 mg/L. A Cl_2:N ratio of 7.6:1 is required to reach the breakpoint of chlorination where the residual combined chlorine level is reduced to a minimum. After the breakpoint, free chlorine starts to predominate instead of combined chlorines. The typical ammonia concentrations were determined to be 420 and 279 mg/L (as NH_3-N) in the samples of Lagoon A and Lagoon B, respectively (Table 1). To reach the breakpoint, a chlorine dose of 3192 and 2120 mg/L would be required for the samples of Lagoon A and B, respectively. The second-order rate constant of monochloramine formation reaction was reported to be as high as 3.07×10^6 ($M^{-1} \cdot s^{-1}$) at 25°C (Qiang and Adams, 2004). Therefore, upon the addition of chlorine, ammonia would rapidly consume the majority of chlorine to primarily form monochloramine. A small portion of chlorine may also be directly consumed by bacteria and dissolved natural organic materials present in the lagoon samples, depending on respective reaction rate constants. As a result, the total chlorine monitored mainly consisted of monochloramine, while the concentration of free chlorine was negligible. The primary disinfectant was actually monochloramine, instead of free chlorine, during the course of bacteria inactivation.

3.2. Chlorine Resistant Isolates

As stated above, the disinfection curves (Figure 1) leveled off in the chlorine dose range of 50–500 mg/L for all lagoon samples, suggesting the presence of chlorine resistant bacteria.

These chlorine resistant bacteria were isolated and identified. Results indicate that two unique colony-types dominated the culture plates at a chlorine dose of 30 mg/L. Above this chlorine dose, only two colony-types were observed to survive the chlorine disinfection in each lagoon sample. Five colonies, possessing either unique morphology, from each lagoon were isolated and restriction fragment length profiling (RFLP) double digests were performed on each. The RFLP double digests yielded exactly the same patterns for all isolates. However, these colonies were distinguishable by their morphologies. The first isolate, denoted C1, formed colonies approximately 7 mm in diameter with volcanic morphology in appearance. The second isolate, denoted C2, formed colonies approximately 25 mm in diameter, were irregular and with globular lobes. A candidate of each isolate was selected for sequencing analysis of their 16S rDNA. The BLAST results indicate that the two isolates, C1 and C2, were most closely related to *Bacillus subtilis* and *Bacillus licheniformis,* respectively, both with 99% similarity to their prospective relatives. Our confirming experiments show that the two isolates demonstrated their ability to grow overnight on BHI media amended with an initial concentration of 500 mg/L chlorine. Although it was observed that total chlorine continuously decayed due to the reaction between chlorine and the BHI medium (e.g., 180 mg/L after 1 min, 90 mg/L after 1 hr, and 10 mg/L after 15 hr), it clearly indicated on a qualitative basis that the isolates were resistant to chlorine.

3.3. Effect of Chlorine on Antibiotic Resistant Bacteria

In the swine facility where Lagoon B was located, only bacitracin was applied for the treatment of swine disease. In contrast, in the swine facility where Lagoon A was located, a large number of antibiotics were applied to swine including amikacin, amoxicillin, ampicillin, cephalexin, chlortetracycline, lincomycin, oxytetracycline, procaine penicillin, sulfadimethoxine, sulfamethoxazole, tiamulin, tilmicosin, and trimethoprim. Our analysis of lagoon samples with

LC/MS indicated that no antibiotics were detected in Lagoon B. However, four antibiotics were detected in Lagoon A: lincomycin (1.47 mg/L), oxytetracycline (0.11 mg/L), isochlorotetracycline (a major degradation product of chlorotetracycline, 0.4 mg/L), and sulfamethazine (1.24 mg/L).

Based on the above information, LIN, CTC, SMN and TET were selected as model antibiotics in this study. The effect of chlorine on antibiotic resistant bacteria is shown in Figure 3. Results indicate that without chlorine treatment (i.e., 0 mg/L chlorine dose), a significant portion of bacteria could survive antibiotic-amended BHI medium. The percentages of culturable bacteria, as compared to antibiotic-free controls, were 83, 46, 79 and 22% in Lagoon A samples, and 23, 100, 29 and 4% in Lagoon B samples, corresponding to CTC, LIN, SMN and TET amended media, respectively. This clearly shows that the lagoon bacteria have reduced susceptibility to selected antibiotics. When bacteria were challenged with both chlorine, at 50 and 500 mg/L, and antibiotics, a statistically notable significance of bacteria inactivation was only observed for TET-amended cultures from Lagoon A, and LIN- and TET-amended cultures from Lagoon B, as compared to respective antibiotic-free control cultures. The tetracycline-resistant bacteria in Lagoon B were completely inactivated with exposure to 50 or 500 mg/L chlorine. In general, the bacteria from Lagoon A exhibited a weaker susceptibility to antibiotics than those from Lagoon B. This is consistent with the historical use of many antibiotics including the ones analyzed in the swine facility associated with Lagoon A. As mentioned above, four antibiotics were detected in Lagoon A with a concentration ranging from 0.11 to 1.47 mg/L. The presence of these antibiotics may provide a selection pressure on the bacteria of Lagoon A to develop and maintain antibiotic resistance. However, tetracycline-resistance did not confer a protection against chlorine-inactivation. It appears that bacteria that were exposed

to tetracycline were more susceptible to chlorine. One mechanism of chlorine damage is the disruption of cell membranes (Venkobachar, et al., 1997). On the other hand, one of the mechanisms of tetracycline resistance is the production of efflux membrane pumps that transport protons into the cell while pumping tetracycline out (Walsh, 2003). These efflux pumps might allow more free chlorine to interact with bacterial cell membranes and lead to their disruption.

3.4. Disinfection with UV Light

The effect of UV dose on bacteria inactivation is shown in Figure 4. Results indicate that UV is effective in disinfecting all lagoon samples, although a slightly higher efficiency was observed for the centrifuged samples. At the irradiation time of 10 min, which corresponds to an effective UV dose of 220 mJ/cm^2 for lagoon A samples and 770 mJ/cm^2 for lagoon B samples, a bacteria inactivation efficiency of 3.4–4.2 log could be achieved that reduced the number of bacteria to less than 1,000 cfu/mL in all samples. Further increasing the irradiation time to 30 min, which corresponds to an effective UV dose of 660 mJ/cm^2 for lagoon A samples and 2300 mJ/cm^2 for lagoon B samples, could essentially inactivate all bacteria in lagoon samples (except the non-centrifuged Lagoon B sample that had only about 100 cfu/mL bacteria left). It is seen that the UV irradiation is unlikely to be economically feasible due to its high energy consumption. The commonly applied UV dose for disinfecting wastewater is generally less than 100 mJ/cm^2 (Bourrouet et al., 2001; Jolis et al., 2001). Occasionally, a UV dose of 170–300 mJ/cm^2 has been applied to achieve a higher efficiency of bacteria inactivation (Thompson et al., 2003; Lazarova and Savoys, 2004).

3.5. Disinfection with Ozone

The effect of ozone dose on bacteria inactivation in centrifuged lagoon samples is shown in Figure 5. It was observed that there exists an initial lag phase on the disinfection curves. The

bacteria inactivation was ineffective up to an ozone dose of 20 and 10 mg/L for Lagoon A and Lagoon B samples, respectively. It implies that at a low ozone dose, the majority of ozone was preferentially consumed by natural organic materials (non-bacterial) present in lagoon samples. Since Lagoon A contained more natural organic materials than Lagoon B, as reflected by the values of COD and DOC listed in Table 1, more ozone was required to pass this lag phase for the Lagoon A sample. After the lag phase, bacteria could be effectively inactivated. At the ozone dose of 100 mg/L, the efficiency of bacteria inactivation could reach 3.3 and 3.9 log for Lagoon A and Lagoon B samples, respectively. Further increasing the ozone dose did not significantly enhance the bacteria removal efficiency, probably due to accelerated self-decomposition of ozone at a high concentration.

4. Discussion

As described above, chlorine is relatively effective in inactivating lagoon bacteria. At a moderate dose of 30 mg/L chlorine, a bacteria inactivation efficiency of 2.2–3.4 log could be readily achieved. The real disinfectant, however, was monochloramine instead of free chlorine due to the presence of a large amount of dissolved ammonia. If ammonia is removed from the lagoon wastewater prior to chlorination, the disinfection efficiency may be greatly improved because free chlorine has a much stronger disinfection potential than monochloramine. One potential mechanism of ammonia removal is the precipitation of struvite (magnesium ammonium phosphate, $MgNH_4PO_4 \cdot 6H_2O$), a promising technology for both N and P removal from anaerobic swine lagoon effluent (Nelson et al., 2003). In addition, the retention time of the swine wastewater is a long as several months due to the small flow rate of the wastes into the anaerobic lagoons, as informed by the CAFOs operators. Therefore, an extended reaction time may be applied to improve the disinfection efficiency.

In this study, the chlorination experiments were conducted at pH 7.7 where free chlorine consisted of approximately 50% hypochlorous acid (HOCl) and 50% hypochlorite (OCl^-) (pK_a = 7.63 at 15 °C, Morris, 1966). The pH value of the lagoon sample, which controls the speciation of HOCl, is critical to disinfection effectiveness because the relative disinfection efficiency of HOCl is about 40–80 times that of OCl^- (Tchobanoglous and Burton, 1991). It is thus reasonably expected that after ammonia removal, lowering the sample pH (i.e., increasing the fraction of HOCl) will significantly enhance the disinfection efficiency. However, without ammonia removal, changing pH does not seem to affect the disinfection activity notably because the primary disinfectant is monochloramine. The pK_a value of monochloramine was reported to be about -1.45 (Gray et al., 1978), so the speciation of monochloramine is negligible except under an extremely acidic pH condition.

UV light has become widely accepted for wastewater disinfection. There are now over 2,000 wastewater treatment plants using either low- or medium-pressure UV technology worldwide (Kalisvaart, 2004). UV irradiation could effectively inactivate bacteria in lagoon samples. However, this technology is limited by its high energy consumption due to the strong absorbance of UV light by lagoon wastewater. The UV transmittances at 254 nm are only 0.10% and 2.19% at 1-cm light path length for the centrifuged Lagoon A and Lagoon B samples, respectively (Table 1). The suspended solids in lagoon samples also inhibit the penetration of UV to bacteria. Therefore, the UV irradiation technology seems inapplicable to swine lagoon bacteria.

The disinfection curves of ozonation showed a similar shape to those of chlorination that leveled off above a certain chemical dose. As mentioned above, this is most probably due to the accelerated self-decomposition of ozone at a high concentration. It was reported that a

transferred ozone dose of 30–50 mg/L achieved a 2 log reduction of fecal coliform in the effluent of a wastewater treatment plant (Gehr et al., 2003). Our results show that an ozone dose of 100 mg/L could achieve a 3.3–3.9 log reduction of total bacteria in lagoon samples, but about 1.1–1.8x10^4 cfu/mL bacteria still survived the ozone treatment. To suppress the self-decomposition of ozone at a high concentration, pulse dosing of ozone at a reduced concentration (e.g., 30–50 mg/L) may be considered. Furthermore, due to the small flow rate of the swine lagoons, a total ozone dose as high as 200-300 mg/L may still be economically affordable.

5. Conclusions

This study investigated the disinfection potential of chlorine, ultraviolet light and ozone against swine lagoon bacteria to prevent the release of antibiotic resistant bacteria into other environments. It was observed that a significant fraction of lagoon bacteria are resistant to the antibiotics investigated: chlorotetracycline, lincomycin, sulfamethazine and tetracycline. Chlorine could achieve a 2.2–3.4 log bacteria reduction at a dose of 30 mg/L. However, two chlorine resistant bacteria were isolated and identified as *Bacillus subtilis* and *Bacillus licheniformis* with 99% similarity to known species. UV irradiation was able to essentially inactivate almost all bacteria, but high energy consumption makes this technology infeasible due to the low UV transmittance in swine wastewater. Ozone could achieve a 3.3–3.9 log bacteria reduction at a dose of 100 mg/L.

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Figure 2. Decay of total chlorine as a function of disinfection time. Experimental conditions: 10 mM KH_2PO_4, pH 7.7, on ice.

Figure 3. Effect of chlorine dose on antibiotic resistant bacteria inactivation. Data are from 3-plate count assays that were run twice (mean ± standard deviation). Experimental conditions: 10 mM KH_2PO_4, pH 7.7, 2.5 hr chlorination time (on ice); 32 mg/L CTC, LIN and TET, and 256 mg/L SMN in BHI media.

Figure 4. Effect of UV dose on bacteria inactivation. Data are from 3-tube MPN assays that were run twice (mean of MPN ± standard deviation). Experimental conditions: 10 mM KH_2PO_4, pH 7.7, room temperature (23 ± 1 ^oC).

Figure 5. Effect of ozone dose on bacteria inactivation. Data are from 3-tube MPN assays that were run twice (mean of MPN ± standard deviation). Experimental conditions: 10 mM KH_2PO_4, pH 7.7, 2.5 hr disinfection time (on ice).

Table 1
Click here to download Table: WR4069 Table 1.pdf

Table 1. Typical physical-chemical properties of centrifuged lagoon samples

Properties	Lagoon A	Lagoon B
pH	7.85	7.42
DOC (mg/L)	425.2	222.7
SCOD (mg/L)	1215	839
NH_3-N (mg/L)	420	279
Total alkalinity (mg/L)	1853	1235
Conductivity (uS/cm)	5070	3300
TDS (mg/L)	3380	2200
Acetate (mg//L)	441.6	278.8
Chloride (mg/L)	215.2	95.8
Bromide (mg/L)	4.4	2.5
Phosphate (mg/L)	61.1	46.4
Sulfate (mg/L)	4.1	68.5
$UVA_{254\ nm}$ (cm^{-1})	2.98	1.66
$UVT_{254\ nm}$ (% @ 1 cm)	0.10	2.19
SUVA (L/mg-m)	0.70	0.75

Figure 1

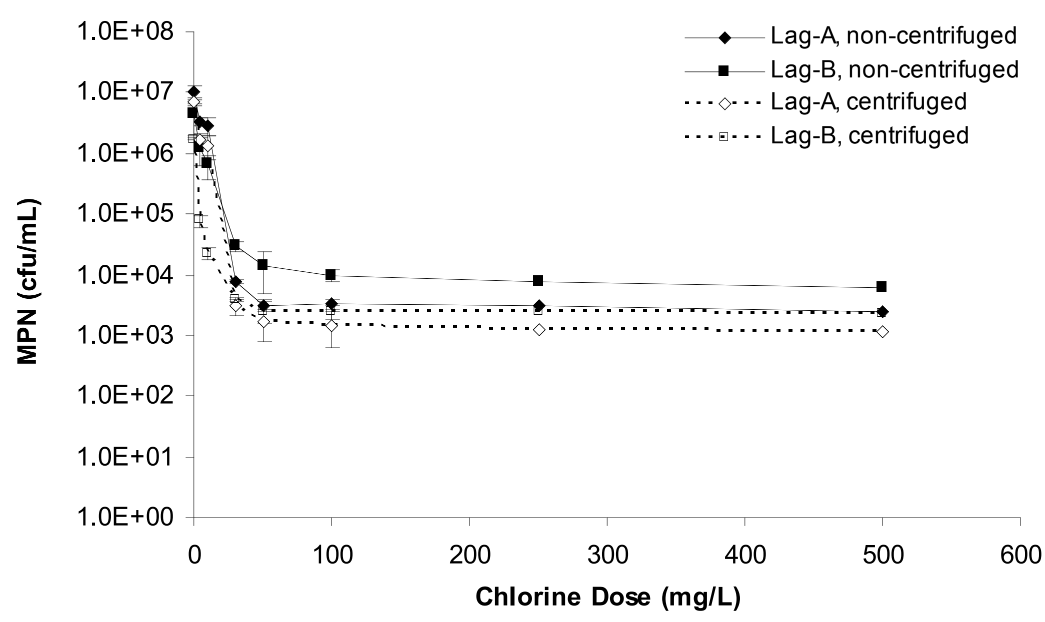

Figure 2

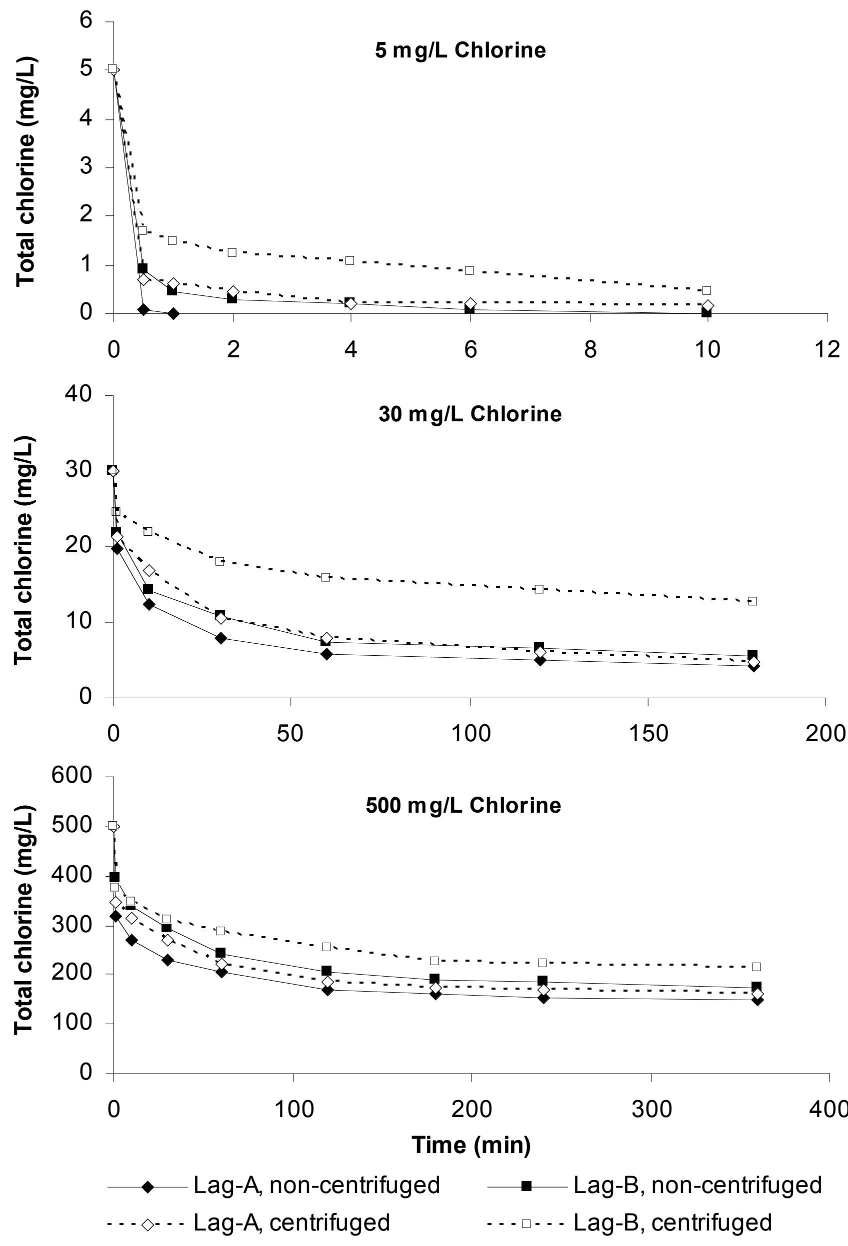

Figure 3

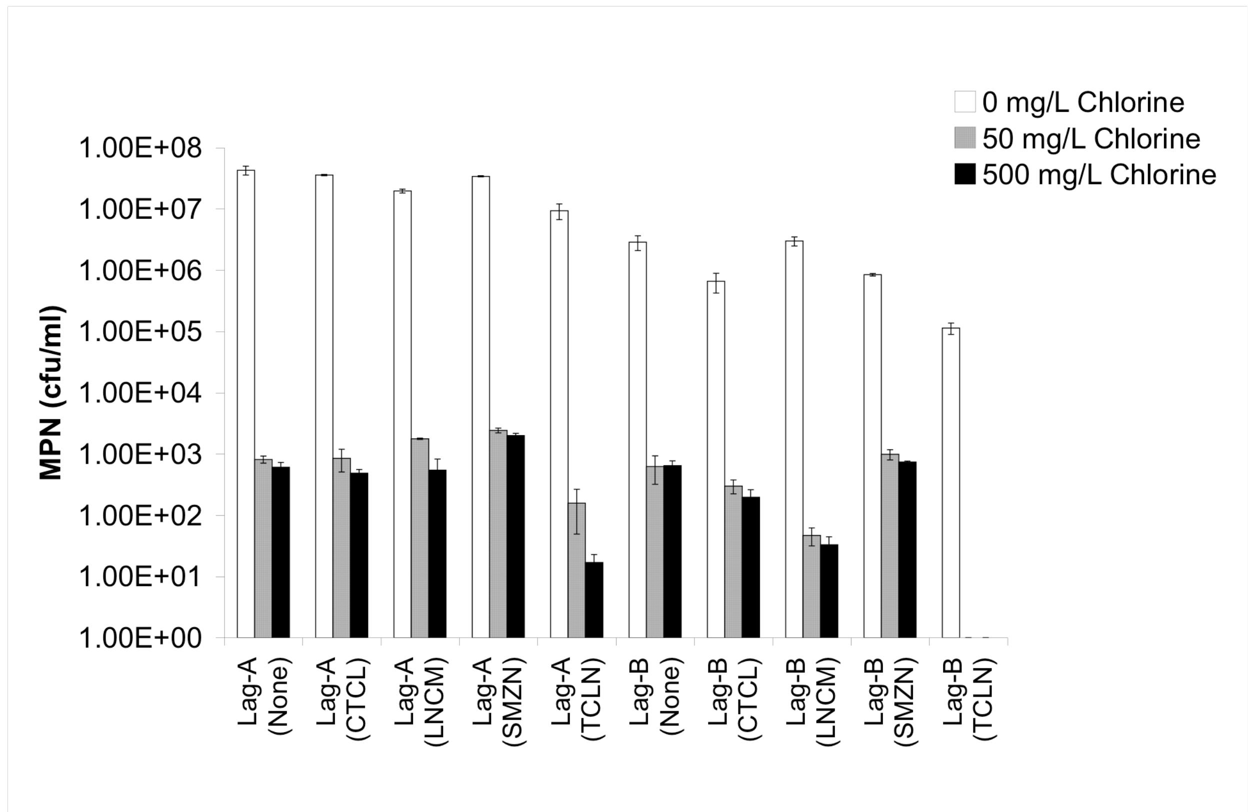

Figure 4

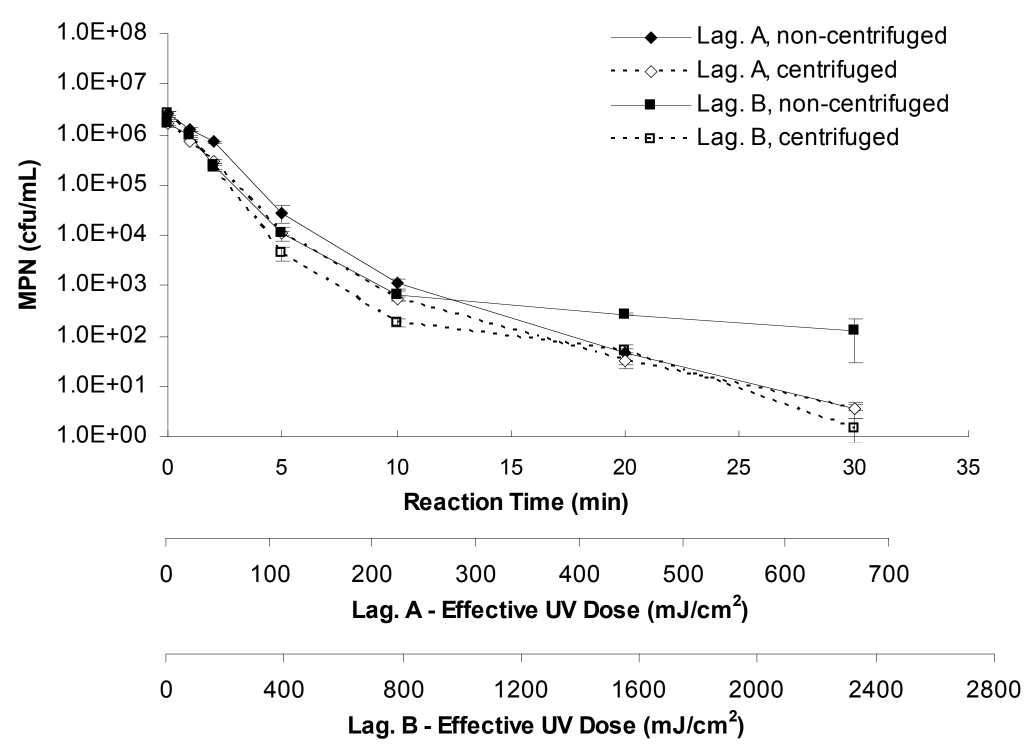

Figure 5