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Disinfection of biological agents in the field using a mobile advanced oxidation process

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Disinfection of biological agents in the field using a mobile advanced oxidation process

U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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Disclaimer

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Acronyms and Abbreviations

ANOVA	Analysis of Variance
AOP	advanced oxidation process
COD	chemical oxygen demand
EPA	Environmental Protection Agency
gpm	gallons per minute
H_2O_2	hydrogen peroxide
HO_2^-	hydroperoxide ion
LR	log reduction
MPN	most probable number
mS/cm	microsiemens per square cemtimeter
O_2	oxygen
O_3	ozone
•OH	hydroxyl radical
ORD	Office of Research and Development
ppm	parts per million
PSA	pressure swing adsorption
scfh	standard cubic feet per hour
TDS	total dissolved solids
TSS	total suspended solids
UV	ultraviolet

Executive Summary

The Army's Net Zero Initiative is an energy-conservation program that focuses on energy as well as water and waste usage procedures. All Net Zero projects are geared toward helping the military installation or community become more sustainable and resilient, with an emphasis on taking a systems approach. Net Zero projects must advance the state of the science and are focused on three general topic areas: water, energy, and waste, and the nexuses among them. This project examined the inactivation and/or removal of biological contaminants in dirty wash water using a portable ozone-UV AOP process. The strain of *E. coli* used in these experiments is not a biological warfare agent, but acts as a surrogate for certain of the vegetative biological agents such as the enterohemorrhagic strain designated *E. coli* 0157:H7.

When operated at lower flow rates (4 gpm [15.1 L/min] or less), inactivation of *E. coli* by AOP treatment ranged from 4 to 6 log. Increasing flow rate to 6 gpm reduced inactivation to 1 to 2 log. Decreased flow rates resulted in a longer periods of exposure to the AOP treatment, and which may have permitted more *E. coli* contact with the disinfection process. The decrease in flow rates from 6 gpm (22.7 L/min) to 4 gpm (15.1 L/min) appears small, but the results demonstrate a significant change in the rate of *E. coli* inactivation. The data suggests that increasing contact time via recirculation may be necessary for flow rates above 6 gpm (22.7 L/min).

Statistical analyses conducted using Analysis of Variance (ANOVA) suggests no significant relationship between the level of *E. coli* inactivation and the amount of suspended solids in the source water. However, the ANOVA results do suggest a significant relationship between inactivation and flow rate, which can be influenced by disinfectant contact time. Although *E. coli* inactivation was not complete at either flowrate, the once-through flow system could be adjusted to recirculate water for additional treatment. If the AOP was utilized in the field to disinfect and reuse wash water for vehicle washing, adequate contact time would be needed to ensure personnel are not contaminated. Due to time constraints, additional testing was not possible, but the benefits could be examined in future research. Finally, the equipment used in this study is scalable. Treated volumes were 100 to 150 gallons in this study, but the UV and ozone units could be sized to handle more flow if larger volumes were generated during washing activities.

1.0 Introduction

1.1 Project Background and Objectives

The Army's Net Zero Initiative is an energy-conservation program that focuses on energy as well as water and waste usage procedures. All Net Zero projects are geared toward helping the military installation or community become more sustainable and resilient, with an emphasis on taking a systems approach. Net Zero projects must advance the state of the science and are focused on three general topic areas: water, energy, and waste, and the nexuses among them.

Net Zero seeks to reduce water consumption and improve water reuse on military installations throughout the world. The U.S. Army and the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD) are currently partnering to promote and demonstrate innovative water reduction and reuse technologies on Army installations in support of the Army's Net Zero Initiative. In 2011, Fort Riley, the source of the waste water used in this study, was selected to participate in the Army's Net Zero Initiative as one of six Net Zero Water Pilot Installations. A Net Zero Water Installation limits the consumption of freshwater resources and returns water back to the same watershed so as not to deplete the groundwater and surface water resources of that region in quantity and quality.

One area of interest is treating and potentially reusing the large volumes of wash water used to clean military vehicles. Wash racks, or areas where military vehicles are washed with large volumes of potable water, generate waste water contaminated with oil, grease, some metals and mixtures of suspended solids (dirt and mud). Reusing wash rack water can be difficult due to the contamination, but reuse would become even more difficult if disinfection of biological contaminants were needed. Biological contamination could come from sources such as untreated sewage if, for example, a military vehicle were near an open sewer during combat or exercises. It could also come from deliberate contamination with a biological warfare agent while in theater. Access to the wash rack water provides a unique opportunity to evaluate disinfection of biological agents in the field with waste water that could hinder the disinfection process.

The use of a mobile disinfection system can support community water conservation goals and is key to the military for locations, domestically and in theater, where copious volumes of fresh water are not readily available. In theater, immediate access to chemical disinfectants such as chlorine bleach or chloride dioxide may not be available. Furthermore, transport of large amounts of such chemicals could prove impractical or hazardous. In situations where chemical disinfection is impracticable, disinfection technologies such as ultraviolet light and ozone are

favorable since they do not need chemicals or reagent, and power can be supplied by a mobile generator. Ozone and UV light are powerful disinfectants on their own, but when ozone is exposed to UV light, hydrogen peroxide and hydroxyl radicals are formed, both of which are also potent disinfectants. The in situ production of a potent oxidizer such as the hydroxyl radical at concentrations strong enough to disinfect water is known as an advanced oxidative process (AOP).

This project will examine the inactivation and/or removal of biological contaminants in wash water using a portable ozone-UV AOP process. The AOP equipment is mounted on a trailer that can be towed with a pick-up truck. Wash water will come from the wash racks at Ft. Riley, Kansas, and *Escherichia coli* will act as the biological contaminant. The strain of *E. coli* used in these experiments is not a biological warfare agent, but acts as a surrogate for certain of the vegetative biological agents such as the enterohemorrhagic strain designated *E. coli* 0157:H7. Data from these experiments will help decision makers in the Army determine if vegetative biological agents are disinfected to a degree that the wash rack water could be reused for further vehicle washing or some other use. In addition, the wash rack water is representative of water washed from cars or structures after an outdoor contamination event. Therefore, this data may be applicable to a scenario where a wide area biological contamination event occurs, and dirty water must be disinfected before being disposed of in a sewer.

2.0 Methods & Procedures

2.1 AOP System Design

The AOP trailer system consists of a 1-inch stainless steel pipe loop system, a variable speed recirculation pump, a medium pressure ultraviolet (UV) lamp and a low pressure UV lamp, an oxygen (O_2) concentrator, an ozone (O_3) generator, an ozone injection system, and an O_3 destructor (Figure 1). Influent *E. coli* samples were taken from the blend tank used to feed the AOP unit. Water quality samples for determining TSS, pH, etc. were taken just after initial entrance to the unit. Effluent samples for bacteria and water quality were taken from the sampling port immediately in front of the location where the treated water was discharged. Water with biological agents was exposed to the ozone, UV light and products generated from the UV-ozone reaction as it passed through the AOP unit.

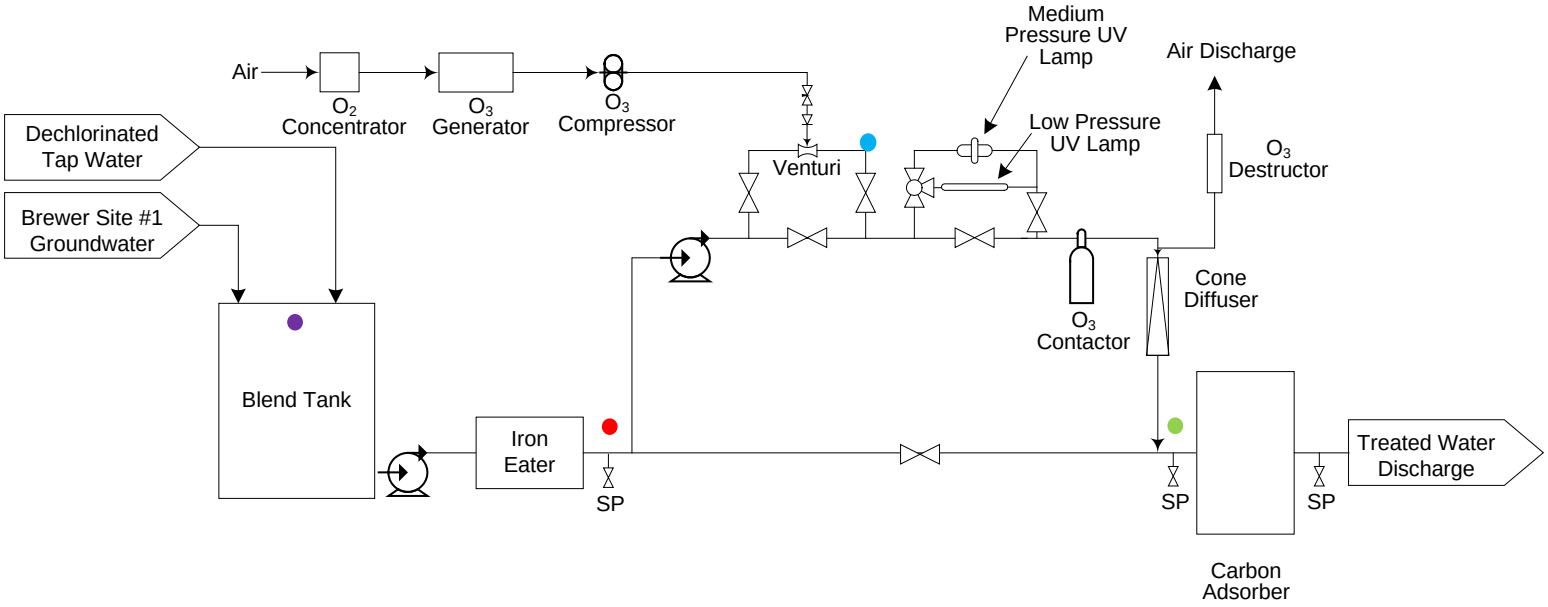


Figure 1: Schematic diagram of the pilot-scale AOP system with sampling ports.

UV radiation was provided by the medium pressure UV reactor (Aquionics InLine 20 UV System, Aquionics, Inc., Erlanger, KY) in Figure 2 (the low pressure lamp in Figure 1 was not used in this study). Ozone was generated using an O_2 concentrator and an O_3 generator. The O_2 concentrator separates O_2 from compressed air through a pressure swing adsorption process. The pressure swing adsorption process uses a molecular sieve (a synthetic zeolite), which adsorbs nitrogen and other impurities from the air at high pressure and desorbs them at low pressure. The O_2 concentrator is designed for a maximum airflow rate of 6.6 standard cubic feet per hour

(scfh) (187 L/hr). The O_2 is then fed into the O_3 generator. In the reaction chamber of the O_3 generator, the feed gas is exposed to multiple high-voltage electrical discharges, producing O_3. The O_3 is injected into the system through a venturi-type, differential pressure injector (Mazzei® ¾-inch (1.9 cm) MNPT Model 684, Mazzei Injector Company, LLC., Bakersfield, CA) located on the discharge side of the system recirculation pump (¾-horsepower (0.56 kW) G&L Pump NPE/NPE-F, Xylem Inc., Rye Brook, NY). When the contaminated water enters the injector inlet, it is constricted towards the injection chamber and emerges as a high-velocity jet stream. The increase in velocity through the injection chamber results in a decrease in pressure, thereby enabling O_3 to be drawn through the suction port and entrained into the motive stream. The venturi is assisted by an ozone compressor (Dia-Vac® pump, Air Dimensions, Inc., Deerfield Beach, FL) to allow the system to operate at lower differential pressures while maintaining a high ozone concentration in the system. The ozone concentrations are further increased by the use of an ozone cone diffuser shown in Figure 3. Excess O_3 is converted back to O_2 using an O_3 destruct unit before it is vented into the atmosphere. The recirculation pump is connected to a variable-speed controller (1AB2 AquaBoost® II Controller, Goulds Pumps, Seneca Falls, NY), which enables the flow rate in the loop to be set to any desired value. Maximum flow through the system is 12 gpm.

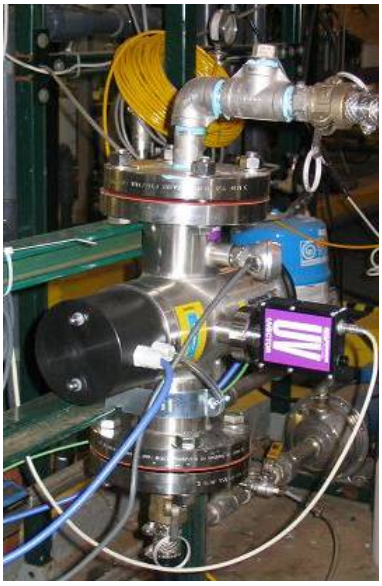


Figure 2: Medium pressure UV lamp system.

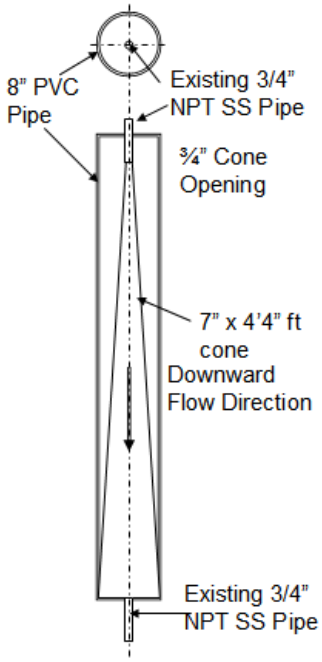


Figure 3: Cone diffuser used for ozone concentration with national pipe threat taper (NPT) stainless steel (SS) pipe.

2.2 AOP Treatment Process

The medium pressure mercury vapor UV lamp installed in the AOP system provided UV-C radiation at an emission spectrum between 200 nm and 300 nm with a power requirement of 0.9 kW and a UV dose >10 mJ/cm^2. The UV unit had one setting, so UV conditions were constant for all experiments in the study. Preliminary tests were performed by running carbon-filtered tap water and ozone through the AOP system to test the capacity of the ozone generator and to determine the ozone concentration in the AOP system. The setting of the ozone generator was adjusted during the preliminary tests to achieve the target ozone concentration of approximately 4 to 6 mg/L in the AOP system. The levels of ozone in effluent samples were never definitively established due to the high reactivity of O_3 and the presence of turbidity and organic constituents in the samples. Settings for the ozone generator were left at the highest values possible and the presence of ozone in the effluent was recorded throughout testing.

The AOP disinfection technology is UV irradiation combined with O_3. Due to the high molar extinction coefficient of ozone, UV radiation can be applied to ozonated water to form highly

reactive hydroxyl radical (•OH). Because photolysis of O_3 generates H_2O_2, the UV/O_3 process involves the disinfection mechanisms present in O_3/H_2O_2 and UV/H_2O_2 AOPs. For instance, H_2O_2 in conjunction with O_3 can enhance the formation of •OH. H_2O_2 is a weak acid that partially dissociates into hydro-peroxide ion (HO_2^-) in water. The HO_2^- ion can rapidly react with O_3 to form •OH. Meanwhile, hydroxyl radicals are produced from the photolytic dissociation of H_2O_2 in water by UV radiation. Disinfection can occur either by direct photolysis or by reactions with •OH.

2.3 Experimental Procedure

The investigation consisted of the treatment of *E. coli* in clean tap water, dirty water from Fort Riley wash racks, and naturally sourced water from a local runoff collection pond using the mobile AOP trailer. All tests were conducted on the Kansas State University campus in the Biological and Agricultural Engineering Department workshop. Water from the wash racks was used directly without dilution to establish whether turbidity interfered with disinfection. Carboys of water from the wash racks at Ft. Riley were collected as needed along with water from a local runoff pond. For reference, Ft. Riley is approximately 15 miles from the Kansas State University campus.

Preparing and running the AOP trailer for an individual test required approximately 2 hours per run with 24 hours of preparation between tests for bacteria propagation and final enumeration. Before experiments began, the AOP system was flushed for 20 minutes with tap water. Experiments were conducted by filling the feed tank with *E. coli* at an initial microbial density of $1x10^6$ most probable number (mpn)/ml. Water was fed to the AOP unit at two different rates, 6 gallons per minute (gpm) (22.7 L/min) or 4 gpm (15.1 gpm). During experiments where flow was maintained at 4 gpm (15.1 L/min), flow periodically dipped to between 3 and 3.5 gpm (11.4 to 13.2 L/min). Therefore, results at the 4 gpm (15.1 L/min) flowrate are reported as 4 gpm (15.1 L/min) or less.

One sample was taken from the feed tank to determine the initial *E. coli* concentration (T_i). Water exiting the AOP unit (effluent samples) were sampled at the last sampling point before the water left the AOP unit (C_e). Samples were taken at 1, 5, 10, 15, and 20 minutes after the contaminated water feed to the AOP unit had started. Disinfection was assessed by examining the log reduction (LR) of samples taken at 1, 5, 10, 15, and 20 minutes compared to the initial microbial density in the feed tank using the following equation:

$$LR = -log\frac{C_e}{T_i}$$

Disinfection was also assessed by calculating percent reduction of *E. coli* in water through the following equation:

$$\%Reduction = \frac{T_i - C_e}{T_i} \times 100$$

Table 1 summarizes the primary experimental design parameters for AOP disinfection of *E. coli*.

Table 1: Experimental Design Parameters

Parameters	Designed Values
Source water	Pond/Lagoon water, Dechlorinated tap water
Dilution water	Pond/Lagoon water, Dechlorinated tap water
Target contaminant	*Escherichia coli*
Concentration of contaminant	10^6 to 10^7mpn/ml
AOP method	UV irradiation/O_3
Type of UV lamp	Medium-pressure UV lamp
UV intensity	preset level kept constant
Ozone concentration in the AOP unit	4 to 6 mg/L
Temperature range	20 to 23°C
Flow rates	4 gpm (15.1 L/min) (or less) and 6 gpm (22.7 L/min)
Recirculation ratio	None (once-through flow)
Test Duration	20 minutes

gpm = gallons per minute; mpn = most probable number

2.4 Evaluation objectives

Critical measurements (those key to the study), sampling location, reporting units, and sampling frequency are summarized in Table 2.

Table 2: Summary of Critical Experimental Parameters

Measurement	Reporting Unit	Sampling Location	Measurement Purpose
E. coli	mpn/ml	One sample from the blend tank (T); AOP System Effluent (E) at 0, 1, 5, 10, 15, and 20 minutes after the start of a test run.	Primary microbial contaminant for study
Ozone	mg/L	Outlet sampling port, 2 grab sampling events per test run (at the beginning and end of the test run); ozone sample port	Disinfectant concentration

E = effluent; mpn = most probably number; T = blend tank

The information in Table 2 highlights critical parameters for experiment. The initial concentration of *E. coli* was compared to concentrations in the effluent to evaluate how much inactivation took place. The presence of ozone, while difficult to measure precisely in the effluent, was also measured from a sampling port in the AOP system when clean tap water was flowing. This verified that ozone was being produced at 5.8 mg/L in the AOP unit, which was within the expected range of 4 to 6 mg/L.

Table 3 summarizes the AOP system operating parameters, reporting units, sampling type, sample locations, and sample frequencies.

Table 3: AOP System Operating Parameters

Inline AOP Measurements	Reporting Unit	Sample Type	Sampling Location	Sampling Frequency
Temperature	ºC	Analog gauge reading	On-line gauge	2 readings per test run (at the beginning and end of the test run)
Flow rate	gpm	Digital flow meter reading	On-line meter	2 readings per test run (at the beginning and end of the test run)
Water pressure	psi	Analog gauge reading	On-line gauge	2 readings per test run (at the beginning and end of the test run)
Air flow into the ozone generator	scfh	Flow meter	On-line meter	2 readings per test run involving ozone (at the beginning and end of the test run)

C = AOP influent; E = AOP effluent; gpm = gallons per minute; psi = pounds per square inch; scfh = standard cubic feet per hour, T = Blend Tank

Table 3 lists AOP system operation parameters that were monitored during test run. Temperature was influenced by the daily ambient conditions, but was stable between 20 and 23 °C. Flowrate, water pressure, and air flow were determined by inline sensors on the AOP system. Maintaining consistent pressure, temperature and ozone generator air flow between each experiment provided uniformity by which to compare results.

The measurements in Table 4 are indicative of the influent water quality used during each test (tap water, pond water or wash rack water). Correlations of these measurements with inactivation were used to compare the impact of water quality on inactivation.

Table 4: Water Quality Parameters

Measurement	Reporting Unit	Sample Type	Sampling Location	Sampling Frequency
*TDS	mg/L	Sample from supply tank	Mixing Tank	1 sample per test run
*Conductivity	m S/cm	Sample from supply tank	Mixing Tank	1 sample per test run
*Total N	ppm	Sample from supply	Mixing Tank	1 sample per test run

		tank		
*Total P	ppm	Sample from supply tank	Mixing Tank	1 sample per test run
COD	mg/L	Sample from supply tank	Mixing Tank	1 sample per test run
pH	Standard unit	Sample from supply tank	Mixing Tank	1 sample per test run
TSS	mg/L	Sample from blend tank, influent and effluent	Mixing Tank	10 sampling events per test run (T, C0, C5, C10, C15, C20, E5, E10, E15, E20)

C = AOP influent; COD = chemical oxygen demand; E = AOP effluent; m S/cm = micro Siemens per centimeter; T=Blend Tank; TDS = total dissolved solids; TSS = total suspended solids

*Conducted by Kansas State University's Soil Testing Lab

3.0 Sampling and Measurement Procedures

3.1 Sampling Containers, Holding Times and Preservation

Sampling containers, preservation techniques, and holding times for grab sample measurement are presented in Table 5. Aliquots of each sample were deposited into the proper containers and the appropriate preservation technique were applied in accordance with the guidelines in Table 5.

Table 5: Sample Containers, Preservation Methods, and Holding Times for Grab Samples

Parameter	Sample Container	Preservation Method	Holding Time
E. coli	Sterile 200-ml glass sample bottle	Cool to 4 ± 2 °C	24 hours from collection
Ozone	200-ml glass bottle	None	Samples analyzed immediately in the field
pH	200-ml glass bottle	Cool to 4 ± 2 °C	Samples analyzed immediately, or held for no more than 4 hours
TSS, TDS, Total N, Total P, Conductivity	200-ml glass sampling bottle	Cool to 4 ± 2 °C	Samples analyzed immediately, or held for no more than 48 hours

TDS = total dissolved solids; TSS = total suspended solids

3.2 Preservation Procedure for Microbial Samples

Microbial samples from the supply tank and AOP unit influent/effluent were collected in 200-ml glass sampling bottles. Once the bottles were full the samples were immediately analyzed or placed in a refrigerator at 4 ± 2 °C until analysis within 24 hours (see table 5).

3.3 Analytical Laboratories

All analyses and measurements listed in Tables 2 and 3 were conducted at the Kansas State

University Soil Testing Lab. *E. coli* strain K-12 was obtained from EPA. The stock culture obtained from EPA was stored at 4 °C, and sub-cultured in tryptic soy broth before experiments.

3.4 Sampling and Analytical Procedures

Analytical procedures are summarized in Table 6. When collecting a grab sample, the sample tap was opened and water allowed to flow for approximately 10 seconds to flush the sampling port.

Table 6: Analytical Methods Used to Analyze Grab Samples

Parameter	Units	Method	Citation
E. coli	mpn/ml	9221 B, C	Rice EW, Baird RB, Eaton AD, Clesceri LS (editors). Standard Methods for Examination of Water and Wastewater, 22nd Edition. Washington DC: APHA, AWWA, WEF.
Ozone	mg/L	4500-O_3-B	Standard Methods for Examination of Water and Wastewater, 22nd Edition
pH	pH units	150.1	U.S. Environmental Protection Agency (EPA), Methods for the Chemical Analysis of Water and Waste, March 1983. Cincinnati, OH: EPA. EPA/600/4-79-020
TSS	mg/L	SM 2540 D	Standard Methods for Examination of Water and Wastewater, 22nd Edition
*TDS	mg/L	SM 2540C	Standard Methods for Examination of Water and Wastewater, 22nd Edition
COD	mg/L	SM 5200D/Hach 8000	Standard Methods for Examination of Water and Wastewater, 22nd Edition
*Conductivity	µS/cm	SM 2510	Standard Methods for Examination of Water and Wastewater, 22nd Edition
*Total N	mg/L	USGS WRIR 03-4174	Patton CJ, Kryskalla JR. Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. Denver: USGS. USGS WRIR 03-4174
*Total P	mg/L	USGS WRIR 03-4174/EPA 365.2	USGS WRIR 03-4174

COD = chemical oxygen demand; mpn = most probable number

* Conducted at the Kansas State University Soil Testing Lab (http://www.agronomy.k-state.edu/services/soiltesting/)

Samples were labeled in accordance with the following identification scheme: date, sample location, sample time, and experiment number. Temperature, flow and pressure readings were recorded 2 times per test run (at the beginning and the end of the test run). The information in Table 7 lists the source for each test batch of water, its characteristic properties, and flowrate maintained during treatment.

Table 7: Summary of Experiments

Test Run	Source Water	Flowrate (gpm)	TSS (mg/L)	Supply Volume (gal)	Run Time
TW2	Tap	6	0	100	10
LW1	Lagoon	4	197	100	20
LW2	Lagoon	4	121	100	20
LW3	Lagoon	3.5-4	70	100	20
PW10	Pond	6	52	150	20
PW11	Pond	6	110	150	20
PW12	Pond	6	70	150	20
PW2	Pond	6	49	100	10
PW3	Pond	5.5-6	65	150	20
PW5	Pond	6	682	150	20
PW6	Pond	3	155	150	20
PW7	Pond	6	50	150	20
PW8	Pond	3	278	100	20
PW9	Pond	3	176	100	20
TW1	Tap	4	67	100	20
TW3	Tap	4	210	100	20

gpm = gallons per minute; LW = lagoon water (wash rack), PW = pond water, TSS = total suspended solids; TW = tap water

4.0 Results and Discussion

4.1 Water Quality and E. coli Disinfection Results

Table 8 shows the water quality data for each sample reported by the Kanas State University Soil Testing Lab. Note that the lagoon water samples from the Ft. Riley wash racks were on average lower in TDS, TSS, Total N and Total P. On average, TSS and TDS were 3-4 times higher in pond water than lagoon water, and total N and P were 8-12 times higher. Before experiments began, it was assumed that the parameters in Table 8 could contribute to disinfectant demand and inhibit disinfection. Since the wash rack water was more dilute in these constituent (TDS, TSS, Total N, Total P) than the pond water, more pond water experiments were conducted so that the impact of more concentrated samples could be evaluated.

Table 8: Water Quality Sample Results for Tap Water, Pond Water, and Lagoon Water

Test	TSS (mg/L)	TDS (mg/L)	Conductivity (m S/cm)	Total N (ppm)	Total P (ppm)	COD (mg/L)	pH
TW2	0	0	NA	NA	NA	NA	7
PW2	38	NA	NA	NA	NA	NA	-
PW3	65	648	0.93	11.0	0.9	123	8
PW5	682	569	0.81	15.9	1.66	150	8
PW6	155	616	0.88	13.5	1.22	142	8
PW7	52	571	0.82	15.7	1.17	143	8
PW8	278	591	0.84	17.4	1.36	150	7
PW9	176	601	0.86	16.0	1.23	150	8
LW1	197	356	0.51	4.12	0.33	47	8
LW2	121	368	0.53	4.41	0.34	60	8
LW3	70	365	0.52	4.00	0.29	37	8
PW10	52	573	0.82	10.0	1.01	145	8
PW11	110	591	0.84	12.7	1.46	150	8
PW12	70	604	0.86	12.2	1.31	155	8
TW1	67	NA	NA	NA	NA	NA	NA
TW3	120	NA	NA	NA	NA	NA	NA

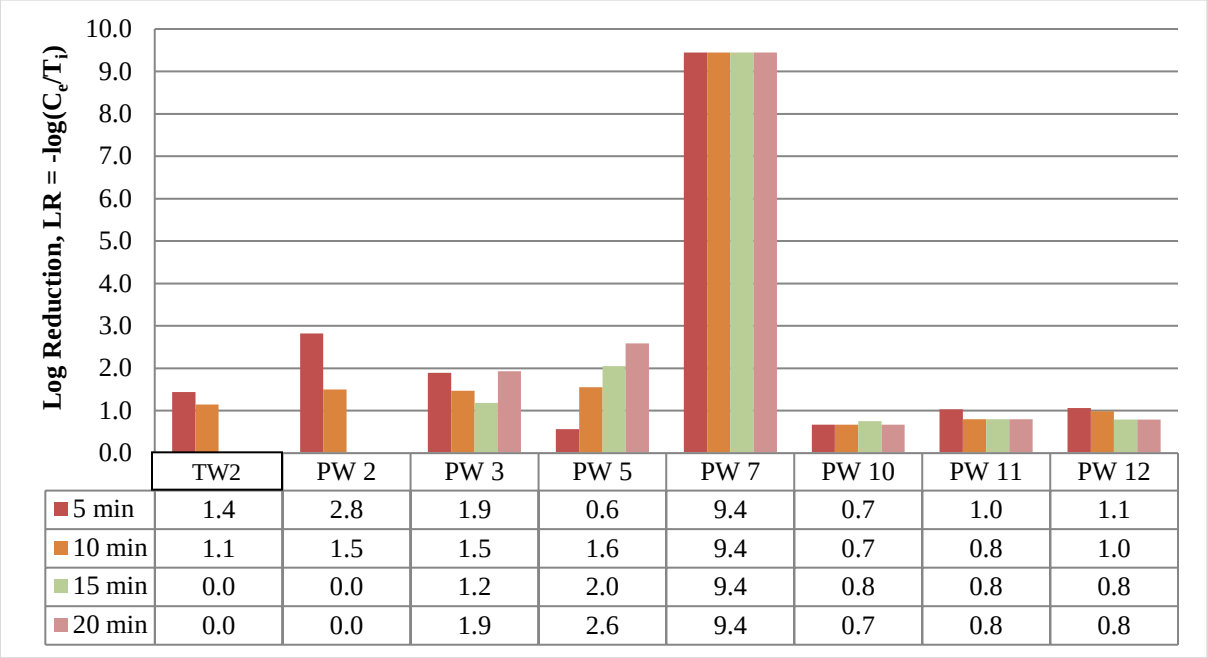
COD = chemical oxygen demand; LW = lagoon water (from the wash racks); PW = pond water; TDS = total

dissolved solids; TSS = total suspended solids; TW = tap water; NA = Not Analyzed

Figures 4 and 5 show the amount of *E. coli* inactivation achieved in the AOP system at high (6 gpm [22.7 L/min]) and low (4 gpm [15.1 L/min] or less) flowrates. Note that these figures do not include the *E. coli* inactivation sample taken from the AOP system at 1 minute. In 11 out of the 16 experiments, it was noticed that 4 to 5 log reductions of *E. coli* was observed in the volume of water that had been treated at the 1 minute time point, with consistently lower log removal occurring in the samples from the remaining treated water. It was eventually determined that flushing the AOP system with tap water before the experiment resulted in residual chloraminated water lingering in the AOP system. Chloramine levels in the local tap water were typically 2 to 2.5 mg/L. *E. coli* samples taken at 1 min had been in contact with this water before it was flushed out, and the high log reductions observed resulted from disinfection with monochloramine, not the AOP process. Samples after 1 min reflect *E. coli* disinfected by the AOP process only.

The data in Figure 4 shows the disinfection performance of the AOP process for flow rates at 6 gpm (22.7 L/min). Except for the PW7 experiment, *E. coli* inactivation ranged from 1 to 2 log over the course of the 20 minute experiment. Figure 5 shows inactivation of *E. coli* at a flowrate of 4 gpm (15.1 L/min) or less. *E. coli* inactivation ranged from 4 to 6 log over the course of the 20 minute experiment. The lower flowrates may have permitted more *E. coli* contact with UV, ozone and hydroxy radicals in the AOP system.

In Figures 4 and 5, the experiments using PW 6 and 7 resulted in log reductions of approximately 9.5 log, which was higher than the other studies. These samples has TSS levels of 155 and 50 mg/L respectively, which are the first highest and third lowest TSS value tested. It is possible that mixing in the AOP unit was more efficient during these tests or that the ozone generator produced more ozone in the piping that in other studies. It should also be noted that calculation of 9+ log removal was possible due to the nature of the Colitert test used to detect coliforms. The test requires 100 ml of water, in tests including these two, the initial *E. coli* density in the feed tank was between 10^7 to 10^8 MPN/ml, which is slightly higher than the desired target concentration. When analyzing these 100 ml volume with 10^8 MPN/ml, the total sample has 10^{10} MPN. The Colitert test can detect down to 1 MPN in a 100 ml volume, which allowed calculation of 9-log removal in the PW 6 and 7 tests.



	TW2	PW 2	PW 3	PW 5	PW 7	PW 10	PW 11	PW 12
5 min	1.4	2.8	1.9	0.6	9.4	0.7	1.0	1.1
10 min	1.1	1.5	1.5	1.6	9.4	0.7	0.8	1.0
15 min	0.0	0.0	1.2	2.0	9.4	0.8	0.8	0.8
20 min	0.0	0.0	1.9	2.6	9.4	0.7	0.8	0.8

Figure 4: Log inactivation of *E. coli* at high flowrate (6 gpm) for tap water and pond water (refer to Table 7 for definition of the samples).

	PW 6	PW 8	PW 9	LW 1	LW 2	LW 3	TW 1	TW 3
5 min	9.7	5.1	5.8	5.8	4.2	5.8	2.7	3.2
10 min	9.7	5.1	6.3	6.3	6.0	5.6	3.8	2.8
15 min	9.7	5.3	5.1	6.2	5.5	5.7	3.4	3.2
20 min	9.7	5.0	5.0	6.3	5.2	5.5	2.7	2.8

Figure 5: Log inactivation of *E. coli* at low flowrate (4 gpm or less) for tap water, pond water, and lagoon water (refer to Table 7 for definition of the samples).

4.2 Comparison of data grouped by solids and flow rate

Figure 6 shows the percent inactivation for water samples grouped by TSS. The various groups represent 3-5 tests with each grouping containing experiments at both flow rate ranges. For 0-60 mg/L the reduction averaged 93.2% with similar rates for 60-100 mg/L and 100-160 mg/L at 96.0% and 95.2%, respectively. The highest TSS levels or >160 mg/L experienced 98.4% inactivation. The error bars represent standard deviation within the data groups. Reduction values were based on the average inactivation for all associated effluent sampling times. TSS values ranged from 0 mg/L to 682 mg/L among the 16 water samples used in this study. The overlapping standard deviation for log reduction within each grouping of TSS levels suggests that suspended solids were not a significant factor that influenced inactivation.

Figures 7 shows *E. coli* inactivation experiments grouped by the flow rate. By reducing the flowrate to 4 gpm (15.1 L/min) (or less) the level of inactivation was increased by 2 to 3 logs. The two groupings, 6 gpm (22.7 L/min) and 4 gpm (15.1 L/min), each consist of 8 individual tests and their average reduction. The error bars represent the standard deviation of inactivation within the two groups. The standard deviation error bars do not overlap, which suggests that there is a significant difference between the two groups.

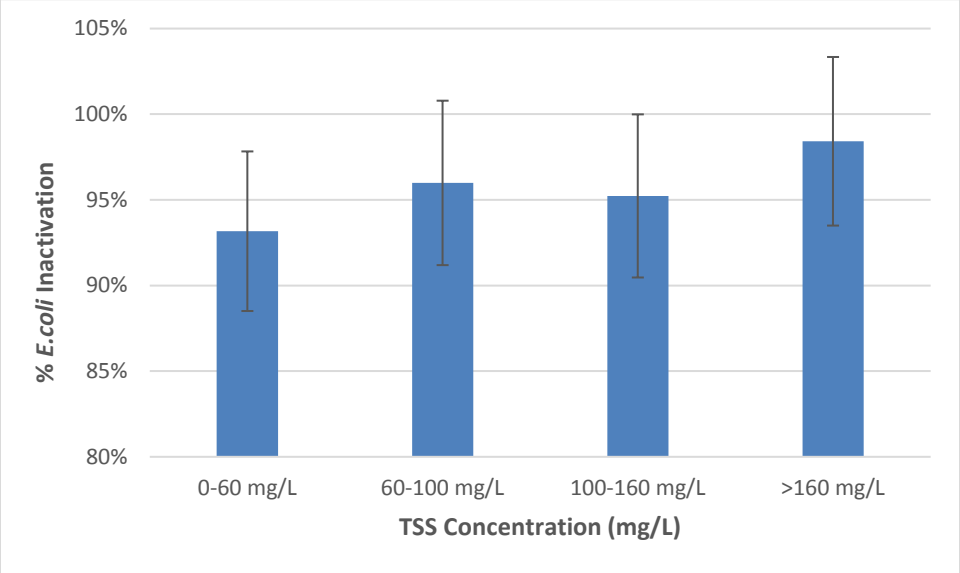


Figure 6: Percent inactivation of *E. coli* at 0-60, 60-100, 100-160 and >160 mg/L total suspended solids (TSS).

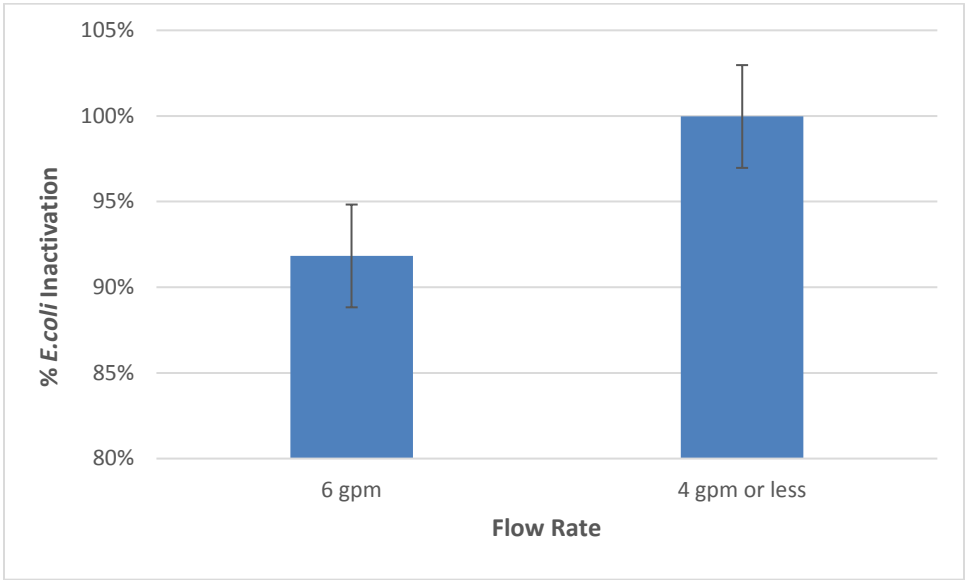


Figure 7: Percent inactivation of *E. coli* at high (6 gpm) and low (4 gpm or less) flow rates.

4.3 Statistical Analysis using ANOVA

In Table 9, flowrate (independent variable) was compared against the level of inactivation (response variable) to determine if inactivation is dependent on flow rate using single factor Analysis of Variance (ANOVA). The high F value indicates greater variation between the two groups rather than within the sample groups indicating flowrate is a significant contributor to inactivation. Flowrate impacts contact time, or the time of exposure to the AOP treatment process. The connection between increased disinfectant contact time and higher inactivation is a well-established principle. By reducing the flowrate, even marginally by 2 gpm (7.6 L/min), the rate of inactivation increased substantially. In a system requiring additional contact time, the alternative to reducing flowrate would be re-treating a batch of water, or recirculating water through the treatment system.

Table 9: Single Factor ANOVA Results for Flowrate

SUMMARY

Groups	*Count)*	*Sum*	*Average*	*Variance*
Low (4 gpm or less)	8	37.42194	4.677743	1.360293
High (6 gpm)	8	12.54557	1.568197	1.484671

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	38.67711	1	38.67711	27.18988	0.000131	4.60011
Within Groups	19.91475	14	1.422482			
Total	58.59186	15				

Count = # of experiments tested; Df = degrees of freedom; F = F statistic; F crit = critical F value MS = mean square; P-value = probability; SS = sum of squares

Variation between groups is lower than variation within groups of high and low TSS (Table 10). The low F statistic illustrates this relationship indicating that TSS does not have a significant influence on inactivation. The variation within the data shows that whether or not TSS is elevated does not influence effectiveness of the AOP process to inactivate *E. coli*. This is interesting to note since increased turbidity in the water should block UV light to some extent. The data suggests that the UV light was still able to react with ozone to create sufficient amount of hydroxyl radical, or that ozone alone is the dominant disinfectant in the AOP process and was unaffected by the increased turbidity.

Table 10: Single Factor ANOVA Results for Total Suspended Solids

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Low (0-70 mg/L, Avg: 52 mg/L)	8	19.15929	2.394912	3.220706
High (110-682 mg/L, Avg: 230 mg/L	8	30.80822	3.851028	3.937974

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.481098	1	8.481098	2.369459	0.146022	4.60011
Within Groups	50.11076	14	3.57934			
Total	58.59186	15				

Count = # of experiments tested; df = degrees of freedom; F = F statistic; F crit = critical F value; MS = mean square; P-value = probability; SS = sum of squares

5.0 Conclusions

When operated at lower flow rates (4 gpm [15.1 L/min] or less), inactivation of *E. coli* by AOP treatment ranged from 4 to 6 log. Increasing flow rate to 6 gpm reduced inactivation to 1 to 2 log. Decreased flow rates resulted in a longer periods of *E. coli* exposure to the AOP treatment, and increased the time available for •OH to form. The decrease in flow rates from 6 gpm (22.7 L/min) to 4 gpm (15.1 L/min) irrespective of the TSS appears small, but the results demonstrate a significant change in the rate of *E. coli* inactivation. The data suggests that increasing contact time via recirculation may be necessary for flow rates above 6 gpm (22.7 L/min).

Statistical analyses conducted using ANOVA suggests no significant relationship between the level of *E. coli* inactivation and the amount of suspended solids in the source water. However, the ANOVA results do suggest a significant relationship between inactivation and flow rate, which can be influenced by disinfectant contact time. Although *E. coli* inactivation was not complete at either flowrate, the once-through flow system could be adjusted to recirculate water for additional treatment. If the AOP was utilized in the field to disinfect and reuse wash water for vehicle washing, adequate contact time would be needed to ensure personnel are not contaminated. Due to time constraints, additional testing was not possible, but the benefits increased contact time could be examined in future research. Furthermore, the equipment used in this study is scalable. Treated volumes were 100 to 150 gallons in this study, but the UV and ozone units could be sized to handle more flow if larger volumes were generated during washing activities.

The original objective of this study was to help decision makers in the Army determine if vegetative biological agents are disinfected to a degree that wash rack or other wash water could be reused for further vehicle washing or some other use. Since the wash rack water is representative of water washed from cars or structures after an outdoor contamination event, this data may be applicable a wide area biological contamination event, where dirty water must be disinfected before being disposed of in a sewer. For both scenarios, the data in this study shows that 4 to 6 log reduction of *E. coli* is possible if the flow rate used results in appropriate contact time (4 gpm in this case). Therefore, if *E. coli* contamination is 4 log or less, the AOP technology used in this study should be considered a tool that could be used for reuse and/or disposal of wash rack or other dirty water.

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