Decontamination using Chlorine Dioxide Disinfectant with Adjuvants Verses Hydrogen-Peroxide and Pentapotassium Disinfectants on Farm Equipment

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Abstract: Agricultural machinery and farm equipment are potential sources of infectious material that can lead to the contamination and spread of diseases if proper action isn't taken. Two stage decontamination methods, involving power washing followed by disinfectant applications, are needed to clean farm equipment, agricultural transport vehicles, and storage units. The field experiments confirm that pressure washing surfaces is an extremely important step in enhancing spore efficacy. Log10 reduction values were 5.45 and 2.90 for disinfectant applications with and without power washing, respectively. Both experiments show that the commercial chlorine dioxide disinfectant Electro-Biocide was an effective disinfectant alone and when mixed with adjuvants. Increasing the concentration of some tested adjuvants resulted in more spores being removed or killed, however this was not true for all adjuvants tested in these two experiments.

Keywords: Decontamination, Disinfectants, Power Washing, Adjuvants.

1. INTRODUCTION

Invasive species are simply defined as non-native species or microorganisms whose introduction can severely affect economic, ecological, or human health [1]. Invasive species can be introduced deliberately as means of stocking, or to control other species, or accidently, through live food trade, contaminated fishing gear or movement of equipment [2]. If introduced invasive species become established in a new environment they can be difficult and expensive to remove or control [3]. Invasive species are one of the top threats to native wildlife species, and nearly half of threatened or endangered species are at risk world wild [3].

Within the United States a wide variety of species that may not already occur in a particular area, including plant seeds and spores can be transported and introduced vast distances from their original place of origin and can establish a new community where the new ecosystem cannot exist without being compromised [4]. These new species can upset the already existing natural balance of wildlife and plant life and potentially endanger other resources or species. These species are often transported via soil and mud that is picked up from off road vehicles and agricultural equipment [4]. Control of agricultural disease and pest outbreaks relies on a variety of strategies including quarantine, disposal, and decontamination [5, 6]. Quarantine is the separation of people, animals, or plants to reduce or prevent the spread of disease or pests [7]. Containment or quarantine is the first priority to stabilize and prevent potential outbreaks [6]. Quarantine is typically the precursor to disposal or decontamination [6]. Disposal control strategies involve permanently eradicating the contaminated materials; typically, by either complete incineration or deep burial [6]. There are currently two general decontamination procedures used to control biological agents: physical decontamination or chemical disinfectants [8]. Physical decontamination typically involves dry heat up to 160°C for two hours, power washing, or steam treatments often within an autoclave [9, 10]. UV-C light can also be used for surface sterilization, but it can be difficult to use in practice due to its poor penetrating power, required long contact periods, and limited research as an effective control method [9, 10]. Chemical disinfection requires the physical cleaning of a surface followed by the application of a disinfectant using EPA registered disinfectants for specific uses [8]. Chemical disinfection often includes hypochlorite solutions that are either liquid or liquid-like foam/gel disinfectants for both surface and water purification [11].

To decontaminate field equipment used in agricultural operations many methods have been

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tested, but these methods need to be validated for efficacy. They must be scaled to both small and large pieces of machinery and sensitive equipment, all while being cost effective. Currently pressure washing is used to disinfect contaminated field equipment and vehicle surfaces followed by the application of a disinfectant [5]. However, this and other methods are not always plausible due to the vast variety and number of machinery and equipment that may need to be treated. This is even more challenging during peak planting, growing, and harvesting seasons at agricultural operations.

The evaluation of decontamination equipment and disinfectants under field conditions using pest control efficacy as the performance measurement requires non-pathogenic surrogates to be selected as a test organism. The spore forming bacterium *Bacillus subtilis* (*B. subtilis*) was used for these studies. It is a Gram-positive rod shaped bacterium that has an excellent survival rate for studies with long-term storage requirements, it is non-pathogenic, and readily cultured and assayed inexpensively [12]. It is common in the soil, air and plant compost [12], and is an spore forming bacterium that creates dormant endospores for surviving in harsh conditions [13-15, 12]. *B. subtilis* is extremely resistant to variable temperatures, and is an important test organism since the spores are resistant to heat, radiation, and various chemicals [12].

The goal of these two studies was to evaluate the ability of a mobile pressure washer combined with disinfectant treatments to decontaminate farm equipment using samples that inoculated with *B. subtilis*.

2. MATERIALS AND METHODS

Two field studies were conducted at the Colorado State University Agricultural Research Development and Education Center (ARDEC) near Fort Collins, CO in May and June of 2014.

B. subtilis spore samples for the studies were prepared by MicroChem Laboratory (Round Rock, TX), prior to the start of each experiment. The spore/vegetative cell suspensions were treated with isopropanol to kill vegetative cells, so that only spores remained within the suspensions. Three hundred µl of the dormant spores were prepared at a concentration of 10^6 Colony Forming Units per ml (CFU/ml) for the studies and were pipetted onto 5.08 cm steel washer surfaces (Grainger Inc, Lake Forest, IL), and then dried to bind the spores to the surfaces. All samples were shipped in insulated boxes with ice packs to the study site and stored at 4°C until the day of the study. Control samples were inoculated and assayed at the MicroChem lab, while a second set of transit control samples were shipped and stored along with the treatment samples. During the two-day study periods, samples were stored in the field in portable coolers with ice packs. Upon completion of the experiments, treated samples were returned to 4°C cold storage until they were returned to MicroChem where they were assayed for viable spores on semi-selective media to enumerate the CFU count per sample.

The steel washers were labeled according to their respective treatments and attached to a chisel plow frame with neodymium magnets (0.6 x 2.5 x 5.1 cm) (K & J Magnetics, Pipersville, PA). The washers were placed with their inoculated surface facing out on the horizontal surface and organized by power washing time. The magnets and corresponding washers were placed 25 to 50 cm apart to reduce disinfectant drift onto other samples.

Both studies used a 13.8 MPa mobile power washer (S-K Environmental LLC, Okanogan, WA). This particular mobile power washer had an operational capacity of 1,891L, was fitted with a 1,730 L supply tank, a 11.41 L chemical/final rinse tank, a 94 L collection tank, and a 3,411 L sludge tank. The power-washing nozzle was positioned 10 cm to 15 cm from the washer surfaces. Once the washers appeared to be dry, disinfectants were applied using a hand spray bottle (Double Mist Trigger Sprayer, Kwazar) with four trigger pulls (1 ml per trigger pull).

Samples that were disinfected with Electro-Biocide [EB (Strategic Resource Optimization LLC, Denver, CO]were neutralized with sodium thiosulfate (Fisher Scientific, Pittsburgh, PA), mixed at 2.5% or 25g/1,000 ml of water. Samples disinfected with Accel (Ogena Solutions, Stoney Creek, ON, Canada)or Virkon-S (DuPont, Wilmington, DE) were neutralized with sodium bicarbonate (Arm & Hammer, Church & Dwight Co., Inc. Princeton, NJ) mixed at 10% to neutralize the disinfectants and not affect the final bacterial count. Neutralizers were applied with an identical hand sprayer bottle with six trigger pulls (1 ml per trigger pull) to the sample surface. After the neutralizer application, the sample surfaces were collected after air dried for 15 to 30 minutes. The samples were then placed in pre-sterilized Twirl Pak bags, labeled, and placed into isolated coolers with ice packs for 15 to 30 minutes until they were transferred to 4°C, until they could be returned to MicroChem for assaying for spore counts.

2.1. Statistical Analysis

Both study designs were facilitated by a consulting statistician using Design of Experiments Software (JMP Software, SAS Institute Inc., Clary, NC) to reduce the number of samples. Both factorial studies were analyzed for only two way interactions (α = 0.05; with α = 0.07 considered as marginally significant).

The raw spore data (viable spore counts per sample [CFU/sample]) were analyzed by two different methods: log10 reduction and probability of viable spores remaining after a treatment. Log10 spore reductions were calculated where A was the median number of viable spores recovered from control samples and B was the number of variable spores recovered from the treated samples using the equation:

$$Log10\ Reduction = Log\ (^{A}/_{B})$$

The probability of viable spores recovered from treated samples was calculated as:

$$Probability\ Viable\ Spores = \frac{Treated\ CFU\ per\ sample}{Control\ CFU\ per\ sample}$$

Both spore count transformations were analyzed in this study to determine if either data transformation affected the final results. Log10 reduction was analyzed using both the Least Squares model and the Generalized Linear Model (GLM). The GLM model uses data generated from a binomial distribution (logistic regression model) which is analyzed to determine the probability of viable spores remaining after each decontamination treatment. Both the Least Squares and GLM models included all significant factors and two-way interaction terms to predict the log10 reduction and probability of viable spores for each treatment.

2.2. Experiment 1: Decontamination with a Chlorine Dioxide Disinfectant and Five Adjuvants

This field study had four factors: 1) three power washing times, 2) six chlorine dioxide formulations, 3) three chlorine dioxide contact times, and 4) two organic challenges (with and without grease added to the steel washers prior to the application of the disinfectants). The three power washing times were 0, 5, and 10 seconds per sample. The six chlorine dioxide disinfectant treatments included a commercial formulation known as Electro-Biocide (EB) and five solutions of EB mixed with commercial adjuvants. The adjuvants were Attach, Bond Max, Reign, Tactic (Loveland Products, Fort Collins, CO), and glycerol (Fisher Scientific, Pittsburgh, PA). Each EB and adjuvant combination was applied once to a sample, then timed for 5, 10, or 15 minutes before spraying the sample with a neutralizer. All of the adjuvants were mixed at 1% v/v (volume/volume percent) of final EB volume for a rate of 1:100. The pH and oxidative reduction potential (ORP) values were measured for each disinfectant treatment at the conclusion of the experiment with a pH/ORP multi-meter (Orion 3 Star, Thermo Scientific, Waltham, MA).

The washers assigned to an organic challenge treatment were lightly coated with a white grease (White Axle Grease, B'laster Cooperation, Cleveland, OH), which was applied immediately before power washing. All of the washers assigned to a treatment were then power washed for either 0, 5, or 10 seconds. When the washers appeared to be visually dry following pressure washing, the washers were sprayed with the EB solutions (with and without the five adjuvants), then timed and neutralized. The washers for that treatment were then removed from the magnets, dried, labeled, and placed in individual pre-sterilized Whirl-Paks (Nasco, Fort Atkinson, WI) bags for storage in a cooler with ice packs.

2.3. Experiment 2: Decontamination of Chlorine Dioxide Disinfectants in Combination with Two Adjuvants Verses Non-Chlorine Dioxide Disinfectants

The study factors for this experiment were 1) two EB formulations with adjuvants verses two non-chlorine dioxide disinfectants, 2) a power washing time of 10 seconds, 3) a single exposure time of 10 minutes, and 4) glycerol added: yes or no. The two formulations of EB that were tested included two adjuvants: Reign (Loveland Products, Fort Collins, CO) and glycerol (Fisher Scientific, Pittsburgh, PA). The two non-chlorine dioxide disinfectants tested were Accel) and Virkon-S. The two adjuvants were mixed at a higher target rate of 10% v/v for a ratio of 10:100. Accel was mixed at 125 ml per 1,875 ml of water to achieve a 1:16 v/v. Accel is based on an accelerated hydrogen peroxide formulation that includes an accelerant adjuvant for an approximate 2,600 ppm H_2O_2. Virkon-S was mixed at 8 tablets per 3,785 ml of water to achieve a 1:100 v/v solution. Virkon-S tablets react with water to form a hypochlorous acid solution of 10,000 ppm or 1% v/v HOCl. The resulting concentrations of EB, Accel, and Virkon-S were 200, 2,600, and 10,000 ppm, respectively for their active ingredients.

Power washing was set for 10 seconds per sample for all treatments. When samples were visually dry they

were treated with the appropriate disinfectant solution and left exposed for 10 minutes. Once the disinfectant exposure time was complete, samples were neutralized and then collected 15 to 30 minutes later and placed into pre-sterilized Whirl-Pak bags for storage. The pH and ORP values were also measured as previously described.

3. RESULTS

3.1. Experiment 1

During the two-day time period for the first study weather data was collected hourly at the Colorado State University ARDEC farm. The average temperature, relative humidity, and wind speed with gusts for both days are listed in Table **1**. Weather data shows that low humidity conditions were present and therefore evaporation rates were high which decreased the disinfectant contact time below the ten minute recommended time listed on the labels. In this experiment, EB with no adjuvants had the highest measured ORP, while EB with Attach had the lowest ORP (Table **2**). EB with Tactic and Reign had the lowest and highest pH values, respectively.

The data analysis for this experiment involved transforming the raw data, the first method involved log10 reduction analysis using the Least Squares Fit model, and it revealed that all four study factors were significant, along with four two way interactions (Table **3**). Multiple interaction terms within the model incorporated the study factors thus confounding most generalizations about the effects of the study factors on spore efficacy. Power washing was found to be the most effective for increasing the log10 reduction of *B. subtilis* spores when using this transformation method. Log10 reduction of spores increased from 2.6 to 5.1 when power washing time was increased from 0 to 10 seconds, with EB alone, a 10 minute exposure time, and no grease applied (Table **4**). The decontamination treatment with the highest log10 reduction value was 5.5 and was seen in the treatment that had power washing for 10 seconds, EB in combination with glycerol at a 10 minute exposure time, and no grease applied to the sample (Table **4**). Control samples with no pressure washing or disinfectant treatment had a *B. subtilis* spore count of 7.03 log CFU per sample. A 4 and 5 log10 reduction value is equivalent to a 99.99 and 99.999% spore reduction respectively. The four two-way interactions were determined to have a mix of

Table 1: Weather Data Collected Hourly over the Two Day Time Period for the First Study

Date and Time	Temp (°C)	RH (%)	Wind Speed (mph)	Wind Gust (mph)
05/06/14 09:00	16.17	46.8	5	11.6
05/06/14 10:00	16.61	55.2	7	11.0
05/06/14 11:00	17.56	50.6	7	10.9
05/06/14 12:00	19.28	41.6	5	11.2
05/06/14 13:00	22.56	21.4	8	14.2
05/06/14 14:00	23.5	18.3	10	19.2
05/06/14 15:00	23.5	20.9	11	21.2
05/06/14 16:00	22.72	20.7	9	16.7
05/06/14 17:00	21.67	21.4	7	17.0
05/07/14 09:00	11.22	80.2	5	8.9
05/07/14 10:00	12.61	74.3	6	10.5
05/07/14 11:00	13.39	68.4	6	10.4
05/07/14 12:00	14.39	62.3	8	14.2
05/07/14 13:00	12.5	70.0	8	17.6
05/07/14 14:00	9.5	80.2	11	17.5
05/07/14 15:00	7.33	82.9	7	14.7
05/07/14 16:00	10.72	76.2	11	18.0
05/07/14 17:00	11.11	72.2	NA	NA

both positive and negative effects on spore efficacy; therefore no general trends could be discerned between the study factors.

Table 2: Disinfectant pH and ORP Measured Values for EB and Adjuvants for the First Study. EB is the Disinfectant Electro-Biocide

Disinfectant/Adjuvant	pH	ORP (mV)
EB with Attach	5.34	588
EB with Bond Max	5.69	708
Electro-Biocide (EB)	5.88	726
EB with glycerol	5.79	715
EB with Reign	5.95	720
EB with Tactic	5.29	694

Table 3: Least Squares Fit Model Results for log10 Reduction Estimates with Fixed Effects for All Four Study Factors and Significant Two Way Interactions. EB is the Disinfectant Electro-Biocide

Factor	Prob>ChiSq
EB Formulation	0.0019
EB Exposure Time (min)	0.0002
Power Washing Time (sec)	<0.001
Grease Application (yes or no)	0.0055
EB Formulation *EB Exposure Time (min)	<0.001
EB Formulation *Power Washing Time (sec)	0.0068
EB Exposure Time (min)*Grease Application (yes or no)	0.0287
Power Washing Time (sec)*Grease Application (yes or no)	<0.001

The second data transformation method involved the analysis of the probability of viable spores with the use of the GLM model. The model showed that only two of the four study factors were significant, along with six two way interactions (Table **5**). These six interaction terms contained all four of the study factors. The decontamination treatment with the lowest probability of viable spores after treatment was determined to be power washing for 5 seconds with EB applied alone at 5 minutes of exposure time without the presence of grease (data not shown) for a probability value of 0.0000087, which is equivalent to a 99.999% spore inactivation rate; or 5 log10 reduction. When comparing between the two model results, numerous interactions are shown amongst the study factors for both analysis types. Therefore, spore efficacy can be deemed as dependant on the total number of interactions amongst the study factors, thus once again confounding any generalized statements about the results beyond the generalization that both test models showed comparable log10 reduction values.

3.2. Experiment 2

In the second study the highest and lowest ORP values were measured for Virkon-S and Accel, respectively. The highest and lowest pH values were measured for EB + Reign and Accel, respectively (Table **6**).

This study unlike experiment 1 was analyzed only with the Least Squares Fit model for log10 reduction since it was a single factor study. The disinfectant treatment with the highest log10 reduction was EB mixed with glycerol (10%) for a log10 reduction value of 4.8 (Table **6**). Accel was determined to be the second best disinfectant treatment with a log10 reduction value of 4.4.

4. DISCUSSION AND CONCLUSIONS

The overall goal of these two studies was to evaluate the ability of a mobile pressure washing unit combined with disinfectant treatments to decontaminate agricultural equipment using samples that were inoculated with the spore forming bacterium *B. subtilis*. The bio-activity of oxidant disinfectants like those tested in this study is highly influenced by a number of factors such as the pH of the formulation, concentration, contact time, and surface type [16]. Preliminary tests evaluated the chemical reactivity between the Electro-Biocide formulation and five adjuvants (data not shown). The test involved measuring any changes in pH and ORP values over 45 hours. When Tactic and Bond Max were mixed with Electro-Biocide, both demonstrated steady drops in both pH and ORP during the test period. In contrast, both Reign and glycerol had stable pH and ORP values and showed excellent stability with Electro-Biocide. When EB was mixed with Attach there appeared to be stability of both pH and ORP values, but only for a short time. This indicates that Attach would work best when applied shortly after it was mixed with Electro-Biocide.

The ORP values for Electro-Biocide mixed with the five adjuvants in the first study ranged from 588 to 726 mV. Bacteria such as *E. coli* and *Salmonella* are generally killed within a few seconds when an oxidant

Table 4: Log10 Reduction of *B. Subtilis* Spores Predicted by the Least Squares Fit Model for the Four Study Factors Tested in the First Study with the Standard Error (SEM) Values in Parenthesis under the log10 Reduction Values. EB is the Disinfectant Electro-Biocide

Disinfectant/ Adjuvant	Grease Application	Power Washing at 0 Seconds			Power Washing at 5 Seconds			Power Washing at 10 Seconds		
		Exposure Time (Min)			Exposure Time (Min)			Exposure Time (Min)		
		5	10	15	5	10	15	5	10	15
EB+Attach	No	2.7	2.4	2.2	4.9	4.5	4.4	5.1	4.7	4.6
		(0.024)	(0.022)	(0.026)	(0.023)	(0.020)	(0.025)	(0.022)	(0.019)	(0.034)
EB+BondMax	No	2.0	2.9	3.3	3.9	4.8	5.2	3.7	4.7	5.1
		(0.029)	(0.025)	(0.034)	(0.027)	(0.019)	(0.024)	(0.027)	(0.031)	(0.018)
EB+glycerol	No	2.8	2.9	2.7	5	5.1	4.9	5.3	5.5	5.2
		(0.023)	(0.027)	(0.025)	(0.025)	(0.026)	(0.019)	(0.031)	(0.024)	(0.021)
EB	No	2.1	2.6	2.4	3.9	4.4	4.2	4.6	5.1	4.9
		(0.030)	(0.027)	(0.028)	(0.026)	(0.034)	(0.022)	(0.023)	(0.027)	(0.015)
EB+Reign	No	2.8	2.5	3.0	4.7	4.3	4.8	5.1	4.7	5.1
		(0.035)	(0.017)	(0.024)	(0.029)	(0.026)	(0.017)	(0.020)	(0.027)	(0.024)
EB+Tactic	No	3.1	2.3	2.9	4.1	3.3	3.8	4.5	3.7	4.2
		(0.030)	(0.021)	(0.025)	(0.020)	(0.019)	(0.019)	(0.019)	(0.020)	(0.029)
EB+Attach	Yes	3.2	3.3.	3.2	4.5	4.6	4.6	4.4	4.5	4.5
		(0.022)	(0.020)	(0.028)	(0.017)	(0.018)	(0.022)	(0.023)	(0.018)	(0.031)
EB+BondMax	Yes	2.5	3.8	4.3	3.6	4.9	5.4	3.2	4.5	5.0
		(0.027)	(0.023)	(0.029)	(0.03)	(0.019)	(0.025)	(0.028)	(0.027)	(0.022)
EB+glycerol	Yes	3.3	3.8	3.7	4.7	5.2	5.0	4.8	5.3	5.1
		(0.022)	(0.029)	(0.020)	(0.027)	(0.023)	(0.017)	(0.025)	(0.020)	(0.018)
EB	Yes	2.6	3.6	3.4	3.5	4.5	4.4	4.0	5.0	4.8
		(0.031)	(0.025)	(0.029)	(0.024)	(0.033)	(0.022)	(0.022)	(0.024)	(0.016)
EB+Reign	Yes	3.3	3.4	3.9	4.4	4.4	5.0	4.4	4.5	5.0
		(0.029)	(0.021)	(0.021)	(0.023)	(0.023)	(0.016)	(0.019)	(0.028)	(0.022)
EB+Tactic	Yes	3.6	3.3	3.9	3.8	3.4	4.0	3.9	3.5	4.2
		(0.029)	(0.022)	(0.019)	(0.022)	(0.017)	(0.022)	(0.021)	(0.017)	(0.028)

disinfectant has an ORP value of 650-700 mV [17]. In 1972, the World Health Organization (WHO) adopted an ORP standard for drinking water disinfection at 650 mV. However, the WHO standards are based on continuous exposure time to water with an ORP at 650 mV [18]. Bacterial endospores are some of the most resilient life forms to biocidal processes such as disinfection, making them ideal surrogates for decontamination and sterilization field experiments [16].

The concentration of Electro-Biocide, Accel, and Virkon-S was 200, 2,600, and 10,000 ppm, respectively, and the ORP values were 715, 520, and 1,020 mV, respectively. Comparisons of these oxidant disinfectant properties revealed there is little correlation between the concentration and ORP values across all three tested disinfectants in study two. There is also little correlation between the log10 reduction estimates and the ORP values for each disinfectant (Table **5**). The poor correlation between disinfectant ORP or concentration and spore efficacy suggests that other factors besides concentration or ORP have a significant effect on the overall performance of the disinfectants.

The first study tested a power washing variable in combination with three disinfectant variables where it

Table 5: The Generalized Linear Model (Logistic Regression) Results with Fixed Effects for All Study Factors and Two Way Interactions in the First Study. EB is the Disinfectant Electro-Biocide

Factor	Prob>ChiSq
EB Formulation	<0.001
EB Exposure Time (min)	0.0573
Power Washing Time (sec)	<0.001
Grease Application (yes or no)	0.6724
EB Formulation *EB Exposure Time (min)	<0.001
EB Formulation *Power Washing Time (sec)	<0.001
EB Formulation *Grease Application (yes or no)	<0.001
EB Exposure Time (min)*Power Washing Time (sec)	<0.001
EB Exposure Time (min)*Grease Application (yes or no)	<0.001
Power Washing Time (sec)*Grease Application (yes or no)	<0.001

Table 6: The Average pH and ORP Values of the Disinfectants in Experiment 2 with the log10 Reduction of *B. Subtilis* Spores Predicted by the Least Squares Fit Model for the Four Tested Disinfectant Formulations at a Power Washing Time of 10 Seconds and Disinfectant Exposure Time of 10 Minutes. EB is the Disinfectant Electro-Biocide

Disinfectant	pH	ORP (mV)	Log10 Reduction
EB+Reign	5.95	720	3.8
EB+glycerol	5.79	715	4.8
Accel	2	520	4.4
Virkon-S	3	1023	4.2

was hypothesized that increasing power washing time, the addition of adjuvants, increasing disinfecting time, and grease free surfaces would increase *B. subtilis* spore efficacy. It was also hypothesized that the study factors would interact with one another in an unpredictable, but positive manner that would increase the spore efficacy and result in a log10 reduction value higher than 4. Both models in the first study showed log10 reduction values greater than or equal to a value of 5, which is equivalent to a 99.999% reduction rate. The second study also combined power washing with disinfectant treatments as a single factor study. For this experiment it was hypothesized that by adding adjuvants that have the potential to increase droplet contact time to Electro-Biocide the authors would see an improved spore efficacy over two other common disinfectant formulations. This hypothesis was found to be partially correct in that one Electro-Biocide adjuvant combination did improve spore efficacy when compared to the regularly used non-chlorine dioxide disinfectants.

In the first study four of the five adjuvants were found to increase the log10 reduction when mixed with Electro-Biocide in comparison to Electro-Biocide alone; given that the other study factors were set at: no power washing, 5 minutes of exposure time, and no application of grease. The addition of either glycerol or Reign increased the log10 reduction by 33 and 35% respectively, compared to Electro-Biocide applied alone when the other study factors were set at, no power washing, 5 minutes of exposure time, and no application of grease. Adjuvants are typically mixed with disinfectant formulations to increase the overall biological efficacy, and are often registered with the EPA as a single formulation. In addition, there are after-market adjuvants that can be combined with disinfectants to improve their overall performance. A well-known method for enhancing the performance of sodium hypochlorite (bleach), for example, involves the addition of an after-market acidifier to lower the pH of the solution [11]. The rationale for combining Electro-Biocide with the five tested adjuvants in the first study was to extend the droplet contact time by adding polymers or oils to the solution. Extending the contact time for disinfectants becomes crucial for decontamination of field equipment during low humidity conditions that occur in the summer months of some regions in the United States. Under low humidity conditions, disinfectant contact times could drop to 30-60 seconds, which is much lower than the listed contact time of 10 minutes on most disinfectant labels. The weather conditions during the two days of the first study in May of 2014 had a temperature range of 9.5º C to 23.5 ºC, and a relative humidity range of 18 to 83%. Under these conditions the sample surfaces appeared visually dry 2 or 3 minutes after the spray application took place.

Based on the JMP significance of factor test completed, power washing was determined to be the most effective study factor for increasing the log10 reduction of the tested *B. subtilis* spores (total effect = 0.38). The significance of factor test is a statistical test that is used in order to distinguish deviations from sampling errors due to any possible deviations signifying any real difference between hypothesis and observations that are common in factorial studies [19].

When comparing the model results, power washing for 10 seconds (compared to 0 seconds) increased the log10 reduction by 96% when Electro-Biocide was applied with a contact time of 10 minutes and no grease applied. Guan *et al*. [20] in 2017 evaluated power washing with a disinfectant application for decontamination of field equipment and found that power washing increased the log10 reduction of *Geobacillus stearothermophilis* spores by 95% in comparison to no power washing. The amount of material removed from a treated surface depends on the design of the power washing system; power washing units like the S-K Environmental mobile washer were found to remove almost 90% of soil in a U.S. Army Corps of Engineering study [4]. Power washing has been found to dislodge spores from treated surfaces depending on water pressure [21]. Both studies presented in this paper show that power washing is an effective method for dislodging spores from sample surfaces. In the first study, disinfectant results were variable and inconsistent when samples were not power washed prior to the application of the disinfectant (data not shown). However, power washing alone does not inactivate spores or other pathogens, instead it transfers the microbes into waste water that must then be sanitized.

In the first experiment, grease was added as an organic challenge to stimulate machinery grease, which may be present on equipment being treated, resulting in a possible efficacy reduction of Electro-Biocide and/or the adjuvants. When grease was pre-applied to the samples, the log10 reduction of *B. subtilis* spores increased with increasing power washing time and EB contact time when it was applied alone without adjuvants (Tables **4**, **5**, and **6**). In the field experiment completed by Guan *et al*. in 2017 [20], they found that pre-applying dirt to spore samples dramatically reduced the spore efficacy based on the log10 reduction model estimates. The decision to use grease instead of a dirt mixture in the first experiment resulted in a counter-intuitive decontamination effect on spore efficacy. In general, spore efficacy increased with treatments where grease was present, which contradicts the grease challenge hypothesis in the original study design. The addition of grease to samples was expected to partially inactivate the oxidant disinfectants; however the general increase in the log10 reduction results suggests that the grease may be inherently toxic to the *B. subtilis* spore structures. Another possible explanation of the increased efficacy is that the grease treatment improved the rate of dried spore detachment from the sample surfaces. Guan *et al*.'s 2017 [20] results show that the choice of materials used to add dirt/grim to the sample could have unpredictable effects on the final efficacy results.

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