Two Stage Decontamination of Agricultural Equipment Using Power Washing Followed by Disinfectant Treatments

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Abstract: Foreign agricultural pests can become problematic to the environment, economy, animal, plant, and human health if widely transported on contaminated equipment or vehicles. Two equipment decontamination studies were conducted using a mobile power washer and disinfectant treatments. The first study factors were: steel and fabric surfaces, power washing conditions, disinfectants, and disinfectant adjuvants. The second study factors were: relative humidity conditions, disinfectant type, disinfectant additive, and number of repeat disinfectant applications. Efficacy for the power washing and disinfectant treatments was based on log10 reduction of *Bacillus subtilis* spores attached to the two surface types. Power washing increased log10 reduction of spores by 3 to 4 log, when washing was followed by disinfectant treatments. The optimal decontamination treatment in the first study was power washing for 30 seconds at a distance of 10 cm, using a commercial chlorine dioxide formulation (Electro-Biocide) with Reign (1%) that resulted in a 4.7 log10 reduction of *B. subtilis* spores on steel washers. In the second study the optimal treatment was power washing for 10 seconds at a nozzle distance of 20 cm with a commercial disinfectant (Easy Decon DF-200) mixed with 20% glycerol resulting in a 5.1 log10 reduction of *B. subtilis* spores on wool fabric samples.

Keywords: Decontamination, Power washing, Disinfectants.

1. INTRODUCTION

Farm to farm transport of agricultural equipment and interstate transport of agricultural products and animals are pathways for pathogens and insect pests to be widely distributed in the USA [1]. Therefore the decontamination of field equipment, vehicles, and storage facilities is extremely vital in stopping the spread of exotic pathogens or pests. Typically the decontamination process involves either physical or chemical methods, or a combination of the two, such as power washing followed by a liquid disinfectant treatment [2]. Physical decontamination often involves steam power cleaning, 160° C dry heat for at least two hours, or ultraviolet light [3]. Power washing is used in order to remove and dislodge high levels of grime and "dried liquid" spills from contaminated surfaces [4]. Generally, power washing is the first stage of equipment decontamination, which is followed by a disinfectant application in order to inactivate any pathogens remaining on the equipment [5]. Two stage decontamination methods involving power washing and disinfectants provide a higher level of sanitation that is needed for high risk, foreign pests, which threaten both agricultural crops and animals in the United States.

In order to ensure worker safety and reduce bio-contamination issues, a non-pathogenic, surrogate species *Bacillus subtilis* (*B. subtilis*) was selected for both field studies. *B. subtilis* is a gram-positive, rod shaped bacterium that is commonly found in the soil, air, and within plant compost [6]. It is an endospore forming bacteria that creates a dormant spore in order to survive harsh conditions [7-9]. Also, *B. subtilis* spores are considered excellent surrogates for a variety of efficacy studies due to the fact that they are extremely resistant to heat, radiation, and a wide range of chemicals.

The goal of these two studies was to evaluate the ability of a two stage decontamination process, involving power washing followed by disinfectant treatments, to inactivate *B. subtilis* spore inoculated on samples that were placed on field equipment. The two studies were designed evaluate the basic parameters of power washing and disinfectant treatments, in order to improve the overall performance and success of a two stage decontamination process. The first study

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evaluated the effects of sample type (steel washer and wool/nylon fabric), power washing (time and nozzle distance), disinfectant adjuvant type (glycerol or Reign), and glycerol oil coated on sample surface (yes or no) on *B. subtilis* spore inactivation. The second study evaluated the effects of relative humidity conditions (ambient or high RH), disinfectant type (Accel; Ogena Solutions, Stoney Creek, ON, Canada, Easy Decon DF-200; Intelegard, Lafayette, CO, Electro Biocide; Strategic Resource Optimization LLC, Denver, CO, and Virkon-S; Lanxess, Cologne GER), glycerol concentration (0, 10, and 20%), and number of repeat disinfectant applications (1, 2, and 3) on *B. subtilis* spore inactivation.

2. GENERAL MATERIALS AND METHODS

Both field studies were conducted at the Colorado State University Agricultural Research Development and Education Center (ARDEC) near Fort Collins, CO in June, 2015. Both studies were factorial designs with multiple study factors for testing power washing and disinfectant effects on inactivating *B. subtilis* spores.

In the first study the steel washers and wool/nylon fabric samples were prepared prior to the start of the experiment and inoculated with *B. subtilis* spores by a private microbiology laboratory. The steel washer samples represent hard surfaces and wool/nylon samples represent the porous surfaces of agricultural equipment and vehicles. After media culturing the spores were suspended in water and treated with isopropanol to kill any vegetative cells. Steel washers (5 cm) were inoculated with 300 µl of spores with an initial count of 10^6 Colony Forming Units/ml (CFU/ml). Wool/nylon fabric strips (4 x 15 cm) were also inoculated with 300 µl of spores an initial spore count of 10^8 CFU/ml. The initial spore density was different between the washers and the wool samples because the spore suspension was prepared as two separate batches for the two sample types. Washers and fabric strips were shipped in insulated boxes with ice packs, and stored at 4°C until the start of the study. During the study, samples were stored in portable coolers with ice packs in order to maintain cold temperatures. Samples were refrigerated, then shipped to the private laboratory for sample assays. Samples were assayed by culturing *B. subtilis* spores on semi-selective media in order to enumerate the viable spore counts.

Inoculated steel washers were attached to the frame of a chisel plow using neodymium magnets (K & J Magnetics, Pipersville, PA) approximately 25 cm apart in order to reduce any potential disinfectant drift between samples. The inoculated fabric strips were attached to a wood stud clamped to the back of the chisel plow. A mobile power washing trailer (S-K Environmental LLC, Okanogan, WA) was used in both studies for the first stage of the decontamination process. This power washer had a water reclaim mat and sump pump to recycle the waste water, a two stage, fabric “sock” water filtration system, a diesel power generator and air compressor. The power washer had an operational capacity of 1, 893 liters with a nozzle pressure of 13,789 kPA. The mobile washer had a 1,703- liter supply tank, 11 liter chemical/final rinse tank, a 94 liter collection tank, and a 3,411 liter sludge tank.

After power washing occurred and samples appeared to be dry, four commercial disinfectants were applied with a hand spray bottle (Double Mist Trigger Sprayer, Kwazar, West Midland, UK) using four trigger pulls (approx. 4 ml per sample). Once the appropriate exposure time was complete, the disinfectants were neutralized in order to prevent continued spore inactivation. Electro-Biocide (EB), a chlorine dioxide formulation was neutralized with sodium thiosulfate (2.5%). Accel was neutralized with sodium bicarbonate (10%), while Easy Decon DF-200 and Virkon-S were both neutralized with a 50-50 mixture of sodium thiosulfate and sodium bicarbonate. All neutralizers were applied with identical hand sprayer bottles using six trigger pulls (approx. 6 ml) per sample. Samples were then placed in individual Whirl-Paks and allowed to dry for 15 to 30 minutes.

The pH and Oxidative Reduction Potential (ORP) for each of the disinfectants and adjuvant combinations were measured prior to the start of each study, using an Orion 3 Star pH/ORP Multi-Meter (Thermo Scientific, Waltham, MA).

All study designs were created with the SAS-JMP Design of Experiment (DOE) program in order to reduce the number of samples and cost for each study. Analysis of each study was limited to two-way interactions for all model interaction terms. Statistical significance was set at α=0.05. The average spore counts for the storage and transit controls were found to be 10^7 and 10^6 CFU/sample for steel washers and fabric samples respectively. The storage and transit control and treated *B. subtilis* spore counts were transformed into log10 reduction data. Transformation of the data ensures that the final treated spore counts are related back to the spore counts that could be

recovered from the storage and transit control samples. The log10 reduction formula is as follows: A was the number of viable spores recovered from the control treatment surfaces, and B was the number of actual sores recovered from the treated surfaces:

$$Log\ 10\ Reduction\ of\ Viable\ \ B.\ subtilis\ spores = Log\ \ (A/B)$$

The SAS-JMP Least Squares program was used to analyze the log10 reduction data for each of the studies.

3. STUDY 1: POWER WASHING COMBINED WITH ELECTRO-BIOCIDE TREATMENTS

3.1. Materials and Methods

The objective for this study was to determine the effects of EB mixed with two disinfectant adjuvants and their concentrations, power washing time and nozzle distance, and the addition of glycerol to the metal washer and fabric strip samples on viable *B. subtilis* spore reduction. This field study had four study factors: 1) treatment surfaces, 2) the combination of power washing time and nozzle distance, 3) EB and adjuvants at four concentrations, and 4) pre-treatment of glycerol on treatment surfaces. The treatment surfaces were either steel washers or wool nylon fabric strips inoculated with *B. subtilis* spores as previously described. The second set of study factors were a combination of power washing time of 0, 15, or 30 seconds and a nozzle distance of either 10 or 20 cm. EB was used alone or mixed with one of two adjuvants: glycerol or Reign mixed at either 0, 1, 10, or 20%. The pretreatment of washers and fabric strips with or without glycerol was used in order to stimulate an organic challenge for each of the decontamination treatments. EB is an EPA registered disinfectant consisting of a proprietary formulation of chlorine dioxide (200 ppm of ClO_2), a sarcosinate surfactant, and a pH-buffering agent to maintain the pH near 7.

3.2. Results

EB, when mixed with either of the two adjuvants at 20%, had the lowest pH values (Table **1**). Reign was mixed with EB had a lower pH in comparison to glycerol mixed with Reign at the same (20%). EB without any adjuvants had the highest ORP value of 837 mV (Table **1**). In general, Reign had less impact on ORP values in comparison to glycerol, when compared at equal concentrations.

Data analysis showed that sample type, power washing time, and nozzle distance, EB adjuvant, and adjuvant concentrations all significantly increased log10 reduction of *B. subtilis* spores. Pre-treating washers and fabric strips with glycerol was not significant, therefore the data were pooled. Two, two-way interaction terms were found to significantly increase log10 reduction of the spores (Table **2**), *i.e*. sample type interacted with the EB adjuvant and concentration, and sample type interacted with the pressure washing time and distance. The SAS-JMP test for variable importance showed that power washing increased the log10 reduction values of viable spores the most (Probability=0.46) when compared to the importance of sample type (Probability=0.026) of EB adjuvant and concentration (Probability=0.012). This analysis was competed using the 'Variable of Importance' test where factors were presented in an independent model and calculations were completed

Table 1: Average pH and Oxidation Reduction Potential (ORP) Values for Glycerol and Reign at the Four Concentrations (%), when Mixed with the Disinfectant Electro-Biocide

Adjuvant	Concentration of Adjuvant (%)	pH	ORP (mV)
Glycerol	0	6.1	827
Glycerol	1	6.1	828
Glycerol	10	6.0	819
Glycerol	20	5.8	808
Reign	0	6.0	837
Reign	1	5.6	833
Reign	10	5.0	825
Reign	20	4.7	810

Table 2: Least Square Fit Model Results for the Three Study Factors, Electro-Biocide (EB), Adjuvant Concentration, Pressure Washing Time and Distance, and Two Way, Two-Way Interactions. Pre-Treatment of Treatment Surfaces with Glycerol was not Significant and therefore was not Included in the Final Model

Source	Prob>F
Sample Type	<0.0001
EB Adjuvant/Concentration	<0.0001
Pressure Washing Time and Distance	<0.0001
Treatment Surface * EB Adjuvant/Concentration	0.0006
Treatment Surface * Pressure Washing Time and Distance	0.0135

using predicted values in order to rank values based on their importance for each factor [10, 11]. For steel washers, the optimum log10 reduction was 4.73, and the treatment parameters were: EB mixed with Reign at 1%, and power washing for 30 seconds at a nozzle distance of 10 cm (Table **3**). For the fabric strips, the optimum log10 reduction was 4.85, and the treatment parameters were: EB mixed with Reign at 20% and power washing for 30 seconds at a nozzle distance of 20 cm.

When EB was applied alone, without power washing, log10 reduction of spores was 0.85 and 0 for steel washers and fabric strips, respectively. In contrast, combining power washing with a disinfectant treatment increased log10 reduction of spores by 3 to 4 log in comparison to applying disinfectants alone. By doubling the nozzle distance from 10 to 20 cm, the log10 reduction of viable spores was reduced by approximately 9% when applied to steel washers. However, when applied to fabric strips, doubling the nozzle distance did not reduce log10 reduction of *B. subtilis* spores. When EB was applied without power washing, all EB and adjuvant combinations had equivalent log10 reduction of spore counts for the steel washers. However, when Reign was added to EB at 1, 10, and 20% there was an increase in log10 reduction of spores on fabric strips, across all power washing treatments.

4. STUDY 2: EVALUATION OF DECONTAMINATION CONDITIONS AND METHODS FOR IMPROVING SPORE EFFICACY

4.1. Materials and Methods

The objective of this study was to reduce disinfectant evaporation rates after being applied to a surface, in order to extend exposure time and thereby increase the effectiveness of the disinfectant. Three techniques were used in this second study to reduce disinfectant evaporation rates; 1) increasing the relative humidity by enclosing the equipment and treatment surfaces inside a shelter with a water fogging sprayer, 2) adding the adjuvant glycerol to the tested disinfectants, and 3) repeating applications of the disinfectant in order to keep samples continuously wet with the disinfectant.

Ambient relative humidity was measured under open-air conditions. A water fogging manifold was used in an enclosed chamber to raise the relative humidity in order to test the effects of high humidity and disinfectant treatments on log10 reduction of *B. subtilis* spores. All of the relative humidity data was collected with a temperature/relative humidity sensor (VP-3) and data logger (EM50) (Meter Environmental, Pullman, WA).

This study included four disinfectants: Accel, EasyDecon DF-200, Virkon-S, and Electro-Biocide. Each of these disinfectants were mixed with the

Table 3: Predicted Log10 Reduction of *B. Subtilis* Spores using the Least Squares Fit Model, for Steel Washers and Fabric Treatment Surfaces, Pressure Washing time, Pressure Washing Distance, and the Adjuvants Glycerol and Reign, for the First Study

Treatment Surface	Pressure Washing Time (Seconds)	Pressure Washing Distance (cm)	EB Alone	EB + 1% Glycerol	EB + 10% Glycerol	EB + 20% Glycerol	EB + 1% Reign	EB + 10% Reign	EB + 20% Reign
			Log 10 Reduction						
Steel	0		0.85	0.96	0.63	0.84	0.96	1.00	0.73
Steel	15	10	4.25	4.36	4.03	4.24	3.36	4.40	4.13
Steel	15	20	3.81	3.92	3.59	3.81	3.93	3.96	3.70
Steel	30	10	4.57	4.69	4.36	4.57	4.69	4.73	4.46
Fabric	30	20	4.22	4.33	4.00	4.21	4.33	4.37	4.10
Fabric	0		0.00	0.00	0.00	0.00	0.47	0.55	0.81
Fabric	15	10	3.26	3.26	2.98	3.10	3.78	3.85	4.12
Fabric	15	20	3.12	3.12	2.84	2.96	3.64	3.71	3.98
Fabric	30	10	3.74	0.74	3.46	3.58	4.26	4.33	4.60
Fabric	30	20	3.99	3.99	3.71	3.83	4.51	4.59	4.85

adjuvant glycerol at concentrations of 0, 10, or 20%. In the second study, only wool/nylon fabric strips were used, and power washing was set at 10 seconds with a nozzle distance of 20 cm for all treatments. The fabric strips were sprayed with 1, 2, or 3 applications per disinfectant treatment, with a two minute time interval between each application. After power washing, the fabric strips were allowed to dry before being treated with the appropriate disinfectant and then neutralized as previously described.

4.2. Results

The average relative humidity and temperature averaged 50% (± 0.5) and 27° C during the power washing and disinfectant treatments conducted under ambient environmental conditions. Inside the enclosed shelter, with the water fogger, the relative humidity and temperature averaged 100% and 33°C for the power washing and disinfectant treatments conducted under high relative humidity conditions (data not presented).

Accel had the lowest pH at 1.9, and Easy Decon DF-200 had the highest pH at 9.9, regardless of the glycerol concentration. Virkon-S had the highest measured ORP value of 973 mV, while DF-200 had the lowest at 133 mV (Table **4**). The average pH in this study was 2.0, 2.6, and 3.4 for Accel, Virkon-S, and EB, respectively. Correlation analysis revealed that log10 reduction and pH of the disinfectants had a positive correlation (p-value<0.0001) across all of the treatments.

Data analysis using the Least Squares model revealed that both relative humidity and disinfectant type were significant factors (Table **5**). Also, there were three, two-way interaction terms, which included all three study factors, there were included in the final log10 reduction model. Repeat disinfectant applications didn't affect log10 reduction, therefore the term was deleted from the model in order to pool the data across this study factor. The significant interactions were: relative humidity x disinfectant type, relative humidity x adjuvant concentration, and disinfectant x adjuvant concentration. There were 24 decontamination treatments that either positively or negatively affected log10 reduction of *B. subtilis* spores within this study. The JMP test for variable importance predicted that disinfectant type had the most influence on the log10 reduction of viable spore values (Probability=0.41) when compared to the importance of relative humidity conditions (Probability=0.06) or glycerol concentration (0.03).

Table 5: The Least Squares Fit Model Results with Fixed Effects for the Three Study Factors: Relative Humidity, Adjuvant Concentration, and Disinfectants for the Second Study

Source	Prob>F
Relative Humidity	0.0441
Adjuvant Concentration (%)	0.4876
Disinfectant	<0.0001
Relative Humidity * Disinfectants	0.0080
Relative Humidity * Adjuvant Concentration (%)	0.0046
Disinfectant * Adjuvant Concentration (%)	0.0303

Table 4: Average pH and Oxidation Reduction Potential (ORP) Values for Electro-Biocide (EB), Accel, Virkon-S, and DF-200, Based on their Final Concentration and the Three Glycerol Concentrations

Disinfectant	Disinfectant Rate (ppm)	Glycerol Concentration (%)	pH	ORP (mV)
Electro-Biocide	200	0	3.8	837
Electro-Biocide	200	10	3.3	878
Electro-Biocide	200	20	3.2	863
Accel	2,600	0	2.0	539
Accel	2,600	10	2.0	542
Accel	2,600	20	1.9	541
Virkon-S	10,000	0	2.8	973
Virkon-S	10,000	10	2.5	911
Virkon-S	10,000	20	2.6	908
DF-200	54,782	0	9.9	124
DF-200	54,782	10	9.8	133
DF-200	54,782	20	9.7	137

The optimal log10 reduction for this study was 5.1, and the treatment parameters were: Easy Decon DF-200 at high relative humidity conditions and DF-200 mixed with glycerol at 20% (Table **6**). Without the addition of glycerol, Accel, DF-200, and EB had equivalent log10 reduction values, while Virkon-S had a lower spore reduction estimate at high relative humidity conditions. In contrast, at ambient relative humidity conditions and without the addition of glycerol, DF-200, EB, and Virkon-S were determined to have equivalent spore efficacy, while Accel had a lower log10 reduction estimate. EB had decreased effectiveness as glycerol concentrations increased under ambient relative humidity conditions. Under high relative humidity conditions, it was determined that adding glycerol to either DF-200 or Virkon-S increased the log10 reduction estimates.

DISCUSSION

Unfortunately there are few peer-reviewed, decontamination papers published that report the effectiveness of power washing in combination with disinfectant treatments. There is also a lack of research that evaluates the length of time for power washing, power washing distance, or number of disinfectant applications for microbial efficacy. Previous research involving disinfectants conducted under controlled laboratory conditions achieved efficacy results as high as 5 or 6 log10 reduction values [12]. However, disinfectant efficacy results in a controlled environment do not always translate to real world conditions. Decontamination field studies conducted under real-world conditions seldom achieve efficacy results comparable to laboratory studies.

Oxidation reduction potential measures the electrochemical potential to acquire electrons and indirectly measures the strength of oxidant disinfectants [13]. In general, the greater the ORP values the greater the disinfectant efficacy. Virkon-S had the highest ORP (973 mV) while DF-200 had an ORP of 131 mV because it is formulated with two active ingredients and is not a 100% oxidant disinfectant. The ORP of disinfectants containing non-oxidant, active ingredients is not a reliable predictor of their effectiveness as can be seen in the results for the DF-200 disinfectant. DF-200 had the lowest ORP value, but the highest log10 reduction, which is due to the concentration of active ingredients in the formulation, and the inherent toxicity of the combined active ingredients.

The first study demonstrated that power washing was the most effective decontamination method for increasing log10 reduction of *B. subtilis* spores. However, power washing only dislodges spores from a surface, therefore allowing viable spores to be transported into wastewater [14]. Power washing systems should be designed to capture the wastewater, filter, and sanitize the recycled water before it can be discharged or reused for subsequent power washing treatments. High risk pathogens should not be released into the ground water or soil, but should be contained and inactivated in order to avoid any further risks to plant, animal, or humans.

The average decrease in log10 reduction was approximately 9% when the power washing nozzle distance was increased from 10 to 20 cm. In contrast, the average increase in log10 reduction was approximately 13% when the power washing time was

Table 6: Predicted log10 Reduction of *B. Subtilis* Spores using the Least Squares Fit Model for Ambient and High Relative Humidity Conditions, Disinfectant Types, and Glycerol Concentrations for the Second study

Relative Humidity	Disinfectant	0%[a]	10%	20%
		Log10 reduction		
Ambient	Accel	3.14	2.94	2.75
Ambient	DF-200	4.03	4.2	4.38
Ambient	Electro-Biocide	4.07	3.6	3.13
Ambient	Virkon-S	3.85	3.67	3.5
High	Accel	3.61	3.85	4.09
High	DF-200	3.83	4.44	5.06
High	Electro-Biocide	3.84	3.81	3.78
High	Virkon-S	3.1	3.37	3.63

increased from 15 to 30 seconds per treatment surface. These results show that by holding the power washing nozzle at 10 cm from the surface resulted in a similar log10 reduction where power washing time was increased from 15 to 30 seconds. Improving power washing efficacy can best be achieved by reducing the nozzle distance to the surface instead of increasing the spray time per surface area.

The EB adjuvant test in the first study shows that mixing either adjuvant with EB did not improve the efficacy of the disinfectant to kill spores, with the exception of EB and Reign when applied to the fabric strips. When comparing the power washing parameters to disinfectant properties, much larger gains in log10 reduction could be achieved by focusing on refining the power washing parameters.

In the second study, the relative humidity treatments resulted in mixed log10 reduction effects that were dependent on the disinfectant type. By increasing the relative humidity, the log10 reduction for Accel increased, but had no effect on EB efficacy, and had a negative effect on Virkon-S mixed with glycerol at 0%. In theory, a higher relative humidity should have resulted in lowering the droplet evaporation rates since the air is already saturated with moisture [15, 16]. Therefore a higher relative humidity should have allowed disinfectant applications to not dry out too quickly which would translate into increased inactivation of *B. subtilis* spores. However, this was only observed for the Accel treatments.

Repeat disinfectant applications was also hypothesized as a strategy to extend disinfectant contact time thereby increasing log10 reduction of *B. subtilis* spores. The results show that the number of repeat applications had no effect on log10 reduction of spores, which suggests that the disinfectant application methods may have been flawed while conducting this study.

CONCLUSIONS

These studies demonstrate that power washing is an important step in equipment decontamination. However, under real world conditions it is not realistic to power wash a single point on the equipment for 30 seconds at a nozzle distance of 10 cm. In addition, power washing only transfers bio-contaminates into wastewater, which must then be captured and sanitized. This would necessitate that current power washers be retrofitted with a water sanitation system to avoid further spread of pests and pathogens. The three methods that were evaluated for extending the disinfectant contact time increased sporicidal efficacy under specific conditions, but they should only be considered after making improvements in power washing techniques. The overall results of these studies suggest caution, or at least lowered expectations, that decontamination of field equipment could result in a 5 or 6 log10 reduction of pathogens when applied under real world conditions.

Further future research is needed in order to determine the scalability of decontamination from equipment that is designed for small farm equipment to large scale decontamination of multiple vehicles, or sea containers at ports and border stations. Additional research is needed to evaluate wastewater decontamination systems which reclaim wastewater from power washing systems for recycling and disposal. Other research is needed to evaluate automated decontamination systems where power washing and disinfectant systems are programmed, much like automated car washes.

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REFERENCES

[1] Beck GK, Zimmerman JD, Schardt J, Stone RR, Lukens S, Richard J et al. Invasive Species Defined in a Policy Context: Recommendations from the Federal Invasive Species Advisory Committee. Invasive Plant Sci Manag 2006; 1(4): 414-421. https://doi.org/10.1614/IPSM-08-089.1

[2] OSHA: United States Department of Labor. Hazardous Waste: Decontamination.https://www.osha.gov/SLTC/hazardouswaste/training/decon.html

[3] Block SS. Disinfection, Sterilization, and Preservation. 5th Addition. Lippincott Williams and Wilkins; Philadelphia PA. 2000.

[4] Alphin RL, Ciaverelli CD, Hougentogler DP, Johnson KJ, Rankin MK, Benson E. Postoutbreak disinfection of mobile equipment. Avian Dis 2010; 54(1): 772-776. https://doi.org/10.1637/8763-033109-ResNote.1

[5] Guan J, Chan M, Brooks BW, Rohonczy E, Miller LP. Vehicle and Equipment Decontamination during Outbreaks of Notifiable Animal Diseases in Cold Weather. J ABSA Int

2017; 22(3): 114-122. https://doi.org/10.1177/1535676017719846

[6] Kirk E. "Bacillus subtilis." Missouri S&T Microbiology. Web 2009.

[7] Utkhede RS, Smith EM. Promotion of apple tree growth and fruit production by the EBW-4 strain Bacillus subtilis in apple replant disease soil. Can. J Microbiol 1992; 38:1270-1273. https://doi.org/10.1139/m92-209

[8] Asaka O, Shoda M. Biocontrol of Rhizoctonia solani damping off of Tomato with Bacillus subtilis. Appl Environ Microbiol 1996; 62:4081-4085.

[9] Emmert EB, Handelsman J. Biocontrol of plant disease: A Gram Positive Persepctive. FEMS Microbiol Lett 1999; 171: 1-9. https://doi.org/10.1111/j.1574-6968.1999.tb13405.x

[10] JMP. Assess Variable Importance. Statistical Discovery from SAS. https://www.jmp.com/support/help/14/assess-variable-importance.shtml. (2018)

[11] Steinberg B. What is the Variable Importance Measure? Dan Steinberg's Blog for Salford Systems. https://www.salford-systems.com/blog/dan-steinberg/what-is-the-variable-importance-measure. (2018)

[12] Maillard JY. Innate resistance to sporicides and potential failure to decontaminate. J Hosp Inf 2011; 77:204-207. https://doi.org/10.1016/j.jhin.2010.06.028

[13] James CN, Copeland RC, Lytle DA. Relationships between Oxidation-Reduction Potential, Oxidant, and pH in Drinking Water. American Water Works Association. (2004)

[14] Ramirez A. Animal Health: Four steps to effective cleaning and disinfecting. National Hog Farmer. http://www.nationalhogfarmer.com/health-diseases/1015-effective-cleaning-disinfecting-steps. (2009)

[15] Gupta A. How Relative Humidity Affects Evaporation. Environmental Science: Weather and Climate Change. Bright Hub. http://www.brighthub.com/environment/science-environmental/articles/104601.aspx. (2011)

[16] Hovis J. Sustainability: What Causes Humidity? Scientific American. https://www.scientificamerican.com/ article/what-causes-humidity/. (2017)

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