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Testing the Efficacy of a Combination of Microwave and Steam Heat for Log Reduction of the Microbial Load Following a Simulated Poultry Mass Mortality Event

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Abstract

In the event of a natural disaster or other unexpected devastation, the need arises for a means of waste treatment and removal. In the case of agricultural waste, specifically poultry, safe and effective disposal is paramount to avoid the serious side effects of disease and contamination of the surrounding environment and populations. In this trial, microwave radiation, coupled with steam heat, was used to treat organic waste (1,136 kg of culled turkey carcasses), designed to simulate a small-scale poultry mortality event. A total of 40 inoculated samples consisting of 20 Bacillus atrophaeus *spore samples and 20* Salmonella enterica *samples provided the criteria for testing the decontamination of poultry waste. The samples were inserted in the microwave unit with the organic waste at 5-minute intervals, post-grinding. Average transit time through the unit was 75 minutes. Subsequent bacterial colony enumeration was conducted using standard FDA-approved protocols and provided quantitative results for analysis. The system generated an approximate seven-log reduction in the microbial load of Salmonella and a five-log reduction in Bacillus spores. These results illustrate the potential effectiveness of using microwave radiation and steam heat technology for management of agriculture-based mass mortality events.*

Introduction

Several methods for disposing of animal carcasses in the livestock industry are now in use. Some of these are rendering, incineration, alkaline hydrolysis and onsite burial or composting (Sander et al., 2002; Casper, 1993; Glanville & Trampel, 1997). All of these procedures have some drawbacks and may not decontaminate the waste, so alternative methods are currently being explored which could be used in case of a large- scale natural or bioterrorist event. Using microwave radiation in combination with steam heat has been shown both in the United States and abroad to be an effective method for reducing the microbial loads associated with medical waste (Diaz et al., 2005, Edlich et al, 2006). Microwave-based sterilization technology has several advantages over more traditional sterilization methodologies. Microwave-based sterilization systems do not have the chemical by-products of chemical-based sterilization methods, nor do they require the set up of an incinerator or produce potential pollutants created during incineration, which would necessitate scrubbing or secondary combustion to eliminate. Furthermore, they do not require a large volume of steam for sterilization as is used in an autoclave-based sterilization system (Diaz et al., 2005). While the microwave system does require a small amount of steam to help transmit the heat generated from the microwave units, the volume of steam required is significantly less than that needed for entirely steam-based sterilization systems.

These microwave sterilization systems may be self-contained mobile units that could be transported to remote locations for bulk sterilization onsite, an important consideration in preventing the spread of infectious agents (McQuiston et al., 2005). These microwave systems have been shown to work effectively on medical waste streams, but their ability to handle bulk organic material has not been documented. This study examines the capability of a portable microwave unit (Sanitec Industries) to provide multiple logarithm reductions in both vegetative bacterial cell counts and bacterial spore counts in laboratory-inoculated samples of an organic waste stream. The waste stream used in this study was designed to simulate a poultry mass mortality event.

A total of 40 samples, 20 *Bacillus atrophaeus* spore samples and 20 *Salmonella enterica* vegetative cell samples, were used to test the efficacy of microwave radiation with steam heat to treat simulated organic waste. Culled turkey carcasses (1136 kg: 2,500 pounds) mixed with wood chips in a 65%/35% (w/w) ratio served as the simulated poultry mortality event. The test samples were inserted in the entry port and processed through the Sanitec waste treatment unit. Following standard U.S. Food and Drug Administration (FDA) protocol 2400 (www.fda.gov/opacom/morechoices/fdaforms/FDA-2400a.pdf), treated samples were enumerated to provide the initial quantitative data for this new technology. The goal of this study was to demonstrate that a combination of steam heat and microwave radiation would be effective in reducing vegetative cell and spore counts from culled turkey carcasses to levels of at least 6-logs and 4-logs respectively. These levels of load reduction are considered to be Level III reduction, the standard for regulated medical waste sterilization (Holliday et al., 2000; Technical Assistance Manual, STAATT, EPRI, 1998).

Material and Methods

Sanitec Microwave Technology Process Description

The Sanitec microwave technology is a stand-alone medical waste treatment device that incorporates a loading system, feed hopper, treatment chamber, and discharge shoot. The technology is housed in an all-weather steel enclosure (Figure 1). A mobile unit (trailer mounted) was utilized for this test. The units are designed to treat in excess of 250 kg/per hour of waste. Systems are installed either inside or outdoors, and require only a standard 480 electric line and standard water hookups for the onboard steam generator. The technology has been in use for over 15 years in the U.S. and abroad.

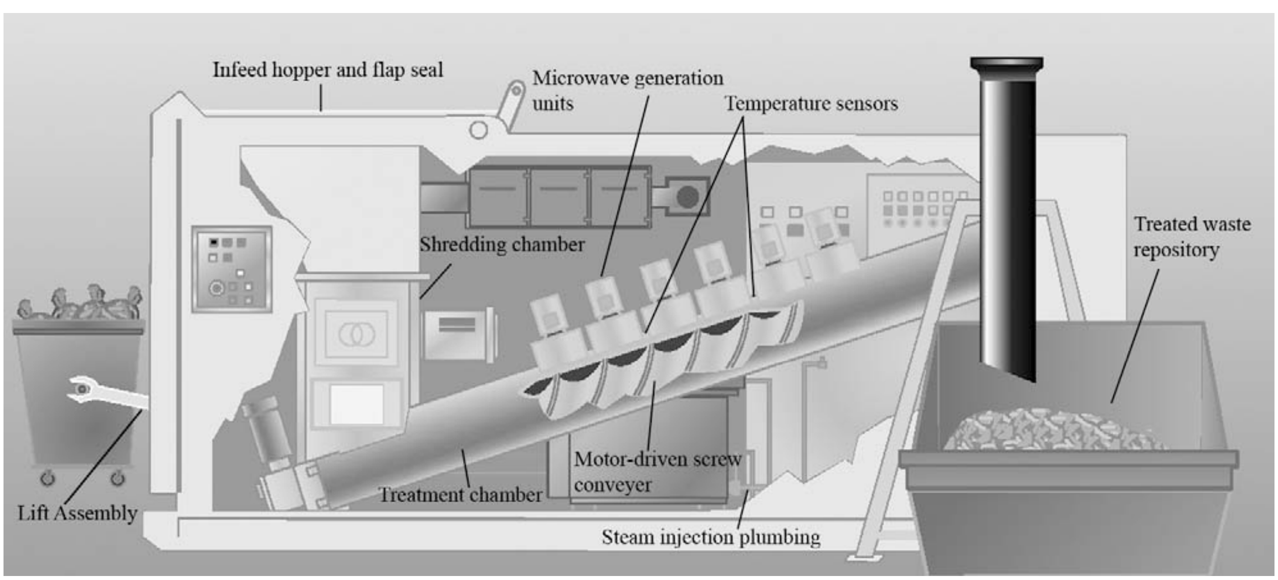
The treatment process is computer controlled. A hydraulic lift mechanism hoists and dumps waste containers into a hopper on top of the unit (Figure 1). The hopper, which has a sealed lid, opens and closes automatically. Once waste has been introduced, the hopper lid closes and material is directed towards a shredding device by a feed arm. The feed arm assists in forcing material into the shredder. Sensors monitor the amount of material falling into a transfer chamber below the shredder. This chamber contains a motor-driven screw. As the screw conveys the waste into the treatment chamber, 150ºC (300ºF) steam is injected at four locations. The purpose of the steam is to uniformly moisten waste and to assist in the treatment process. A series of microwave generators input energy to maintain uniform heating of the waste at a minimum temperature of 95ºC (203ºF). The action of the screw also provides additional mixing to ensure uniform heating. The waste is transported along the screw so that the final exposure time and temperature profile is a minimum of 30 minutes at 95ºC (203ºF). The final waste product then falls into a waste transport container that may be used to transport the treated waste to a repository.

Bacterial Strains

A commercial *Bacillus atrophaeus* (ATCC 9372) spore suspension (approximately 2 x 10^{10} spores/ml) was purchased from BIoCI Systems, Inc., Raleigh, NC. Two 250 ml *Salmonella enterica* subsp. *enterica* serovar Anatum (ATCC 9270) cultures were grown aerobically overnight in Tryptic Soy Broth (TSB, Difco, Franklin Lakes, NJ) in a Barnstead Lab-Line shaker incubator (Barnstead International, Dubuque IA) at 37°C, 250 RPM. *Salmonella* cells were concentrated by pelleting the overnight cultures using a Sorvall RC-5B floor centrifuge (5,000 RPM in a GS-3 rotor, for 15 minutes at 4°C). The cell pellets were

Figure 1

Schematic drawing of the Sanitec Industries Microwave Technology Unit.



then resuspended in 25 ml of TSB to give a final concentration of approximately 10^{10} cells/ml (as determined previously via direct microscopic count compared to A_{600} of the culture) for inoculation into the chicken liver samples.

Sample Preparation and Inoculation

Whole chicken livers were purchased from a local supermarket in Raleigh, North Carolina and were stored at 4°C at the North Carolina Department of Agriculture and Consumer Services (NCDA & CS) Food and Drug Protection Division, Food Microbiology Laboratories in Raleigh, North Carolina. The day prior to inoculation, 10 g samples of liver were excised from the whole livers, washed in 70% ethanol and stored individually in covered sterile plastic Petri dishes at 4°C. On the day of the experiment, the liver samples were inoculated with 500 µl of either concentrated *Salmonella* cells or *Bacillus* spores. The inoculated chicken liver samples were placed in glassine bags (Tidi Brand self-seal autoclave bags, Banta Healthcare, Neenah, WI), sealed and then placed in recovery bags and subsequently stored in a chilled cooler until they were loaded into the Sanitec Mobile Unit at the test site. Field controls remained in the cooler and were enumerated after all treated samples were recovered.

Simulated Mortality Event

The Emergency Programs division, NCDA & CS provided 1136 kg (2,500 pounds) of culled turkey carcasses to simulate a mass mortality event. The carcasses were mixed with short lumber pieces, approximately 10.16 cm (4 inches) wide by 2.54 cm (1 inch) deep by 45.7 cm (18 inches) long, at a ratio of 65%/35% w/w carcass to wood, and the mixture was placed in the Sanitec microwave unit. Loads of approximately 227 kilograms (500 lb) were placed into the feed hopper to ensure consistent operation of the unit. The wood pieces were used to prevent the culled carcasses from binding the internal screw system of the Sanitec Mobile Unit. Alternating single samples from each group (*Salmonella* or *Bacillus*) were added at 5 minute intervals to the ground turkey/wood mixture via a sample port located distal to the grinding head but proximal to the microwave units. The treated samples remained in the waste stream for approximately 75 minutes and were retrieved from the ejected waste and transported back to the NCDA & CS labs for immediate plating and enumeration.

Bacterial Enumeration

Liver samples were macerated in a stomacher for 2 minutes in 90 ml of 1x PBS in Nasco Whirlpack™ filter bags. The untreated control and treated samples were enumerated via a pour plate method using Tryptic Soy Agar (TSA, Difco, Franklin Lakes, NJ) according to FDA 2400 protocols (Standard Plate Count, Coliform counts, and Simplified Count Methods, www.fda.gov/opacom/morechoices/fdaforms/FDA-2400a.pdf). All *Bacillus* and *Salmonella* isolates were verified on selective media, TSA supplemented with 3.5% NaCl and XLT4 Agar (USDA Microbiology Laboratory Guidelines; Difco, Franklin Lakes, NJ), respectively. Plates were incubated aerobically at 35°C for 6 days. Preliminary counts were made after 2-3 days of incubation, and at day 6, the counts were repeated.

Results and Discussion

To test the efficacy of using Sanitec's mobile microwave unit for decontamination of an agriculture-based organic waste-stream, a poultry mortality event was simulated using a mixture of culled turkey carcasses and wood chips. This material was ground in the Sanitec unit and 10 gram-bacteria (*Salmonella* or *Bacillus*) inoculated liver samples entered the waste stream between the grinding heads and the microwave units after the unit was brought to operating temperature (150ºC; 300ºF).

Bacterial plating was performed in duplicate for each sample following FDA 2400 plating protocols, and the plates were incubated for 6 days at 35ºC. After 2-3 days of incubation, initial plate counts were conducted following established protocols for the NCDA&CS Labs. At the end of the 6-day incubation, counts were conducted again with no change in counts observed. A second series of counts was conducted using selective media specific for *Salmonella* and *Bacillus* (see Materials and Methods). This second enumeration was conducted to overcome the confounding variable of environmental contaminants detected at lower dilutions of treated samples on TSA plates. The control (untreated sample) plates were used as guides for colony selection for plating on the selective media and these numbers were recorded as colony forming units per gram (CFU/g) counts (Tables 1 and 2).

The pre-treatment microbial load for *Salmonella* (field control) was 7.5 +/- 3.3 x 10^8 CFU/g and the pre-treatment microbial load for *Bacillus* spores was 6.7 +/- 0.5 x 10^7 CFU/g (Table 1). Following treatment using the Sanitec Mobile Unit, *Salmonella* colony counts were less than 10^1 CFU/g. *Bacillus* was recovered at 8.1 +/- 9.7 x 10^2 CFU/gm (Table 2). One of the 40 samples loaded into the Sanitec Mobile Unit was discarded due to the failure of the glassine bag. As illustrated in Figure 2, the exponential reduction after treatment for *Salmonella* was greater than a 7.5-log reduction, while the *Bacillus* spores had a greater than 5.5-log reduction. This substantial reduction in viable bacteria indicates that the Sanitec unit is effective in decontaminating an organic waste stream. The reduction in viable vegetative cells was within the guidelines established for regulated medical waste sterilization. This decontamination method demonstrated appropriate reduction of viable spores and, as such, can be considered an effective means of decontaminating agriculture-based waste streams.

Table 1

Enumeration of field control samples from the Mobile Microwave unit test. The field control samples were kept in a cooler onsite during the length of the test. Dilution plating was carried out using FDA 2400 protocols.

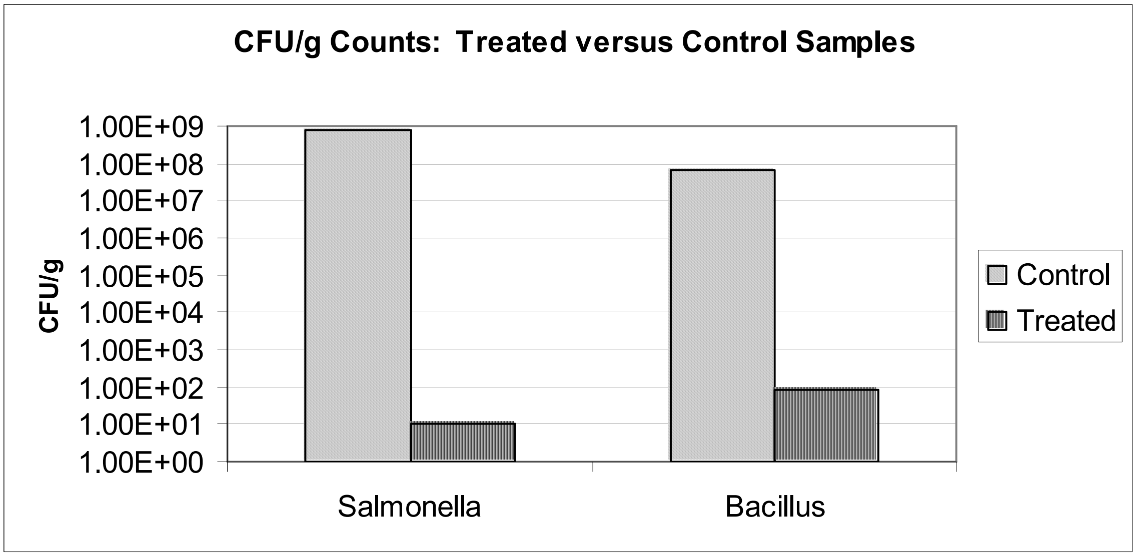
Salmonella enterica							
Field Control	E6	E6	E7	E7	E8	E8	
S41	TNTC	TNTC	41	34	2	7	3.75E+08
S42	TNTC	TNTC	94	101	8	17	9.75E+08
S43	TNTC	TNTC	91	89	15	7	9.00E+08
							7.5 +/- 3.3E8
Bacillus atrophaeus							
Field Control	E6	E6	E7	E7	E8	E8	
B44	76	50	22	6	3	0	6.30E+07
B45	85	61	7	9	0	0	7.30E+07
B46	65	64	4	7	0	0	6.50E+07
							6.7 +/- 0.5E7

Table 2

CFU/gm recovery of treated *Salmonella* and *Bacillus* samples from the Sanitec test. (Sample S11 was punctured during treatment and was eliminated from the study.)

Salmonella	**E1**		***Bacillus***	**E1**	**E2**
S1	0		B21	25	0
S2	0		B22	0	0
S3	0		B23	0	0
S4	0		B24	9	0
S5	0		B25	22	19
S6	0		B26	1	0
S7	0		B27	0	0
S8	0		B28	8	0
S9	0		B29	5	0
S10	0		B30	5	0
S12	0		B31	1	0
S13	0		B32	6	0
S14	0		B33	10	0
S15	0		B34	0	0
S16	0		B35	23	0
S17	0		B36	1	0
S18	0		B37	1	0
S19	0		B38	14	0
S20	0		B39	30	0
			B40	0	0

Figure 2
Graphical representation of the log reductions generated by the Sanitec mobile microwave unit. Growth was reported at 10^1 for *Salmonella* since that was the limit of detection for enumeration.



Conclusions

The Sanitec mobile microwave unit is capable of effecting multiple logarithm reductions in bacterial counts within an organic waste stream consisting of whole turkey carcasses and wood material which was spiked with chicken liver samples inoculated with *Salmonella enterica* vegetative cells or *Bacillus atrophaeus* spores. While the Sanitec unit, in its current configuration, can handle poultry carcasses, other types of livestock mortality events will need to be studied in the future to determine this method's utility in decontamination of other agriculture-based waste-streams. The primary limiting factor of the current unit for use in decontamination of agriculture waste streams is the design of the grinding head used to break down the carcasses. It is not suitable for grinding the bones of larger animals such as hogs or cattle. From this study it is concluded that this microwave-based decontamination system is a safe and effective method for disposal of carcasses from a mass poultry mortality event.

Acknowledgements

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References

Casper, J. (1993). The Maryland plan for disaster recovery: Disposal of dead animals. *Journal of the American Veterinary Association, 203*, 997-999.

Diaz, L. F., Savage, G. M., & Eggerth, L. L. (2005). Alternatives for the treatment and disposal of healthcare wastes in developing countries. *Waste Management, 25*, 626-637.

Edlich, R. F., Borel, L., Jensen, H. G., Winters, K. L., Long, W. B., Gubler, K. D., Buschbacher, R. M., Becker, D. G., Chang, D. E., Korngold, J., Chitwood, W. R., Lin, K. Y., Nichter, L. S., Berenson, S., Britt, L. D., & Tafel, J. A. (2006). Revolutionary advances in medical waste management. The Sanitec system. *Journal of Long-Term Effects of Medical Implants, 16*, 9-18.

Glanville, T. D., & Trampel, D. W. (1997). Composting alternative for animal carcass disposal. *Journal of the American Veterinary Medical Association, 210*, 1116-1120.

Holliday, M. G., Ford, M., Burrell, P., & Gould, F. K. (2000). Heat disinfection of clinical waste: microbiological assessment and monitoring of effectiveness. *British Journal of Biomedical Science, 57,* 107-113.

Isolation and Identification of Salmonella from Meat, Poultry and Egg Products. 2004 United States Department of Agriculture Food Safety and Inspection Service. Microbiology Laboratory Guidebook. www.fsis.usda.gov/PDF/MLG_4_03.pdf

McQuiston, J. H., Garber, L. P., Porter-Spalding, B. A., Hahn, J. W., Pierson, F. W., Wainwright, S. H., Senne, D. A., Brignole, T. J., Akey, B. L., & Holt, T. J. (2005). Evaluation of risk factors for the spread of low pathogenecity H7N2 avian influenza virus among commercial poultry farms. *Journal of the American Veterinary Medical Association, 226,* 767-772.

Sander, J. E., Warbington, M. C., & Myers, L. M. (2002). Selected methods of animal carcass disposal. *Journal of the American Veterinary Association, 220,* 1003-1005.

Standard Plate Count, Coliform Counts, and Simplified Count Methods. Department of Health and Human Services, Public Health Service. Food and Drug Administration. www.fda.gov/opacom/morechoices/fdaforms/FDA-2400a.pdf

Technical Assistance Manual: State Regulatory Oversight of Medical Waste Treatment Technologies: A Report of the State and Territorial Association on Alternative Treatment Technologies (*STAATT*). (1998). EPRI Palo Alto, CA: TR-112222.

CDC/NIH Recommended Precautions for Laboratory Work with HIV

Facility	Practices & Procedures	Activities Involving
BSL-2	BSL-2	clinical specimens body fluids human/animal tissues infected with HIV
BSL-2	BSL-3	growing HIV at research lab-scale growing HIV-producing cell lines working with conc. HIV preparations droplet/aerosol production
BSL-3	BSL-3	HIV at industrial-scale levels, large-volume, or high-concentration production and manipulation

Important Biosafety Reference Available on the Web

International Society for Analytical Cytology (ISAC) Biosafety Standard for Sorting of Unfixed Cells

Cytometry Part A: 71A:414-437 (2007). Published in 2007 by Wiley-Liss, Inc. "Standard Safety Practices for Sorting of Unfixed Cells," UNIT 3.6 of *Current Protocols in Cytometry* is available free online at: www.isac-net.org