Supporting Information

Review of decontamination techniques for the inactivation of *Bacillus anthracis* and other spore-forming bacteria associated with building or outdoor materials

Joseph P. Wood,[†,*] Alden Charles Adrion[†,‡]

[†]United States Environmental Protection Agency, Office of Research and Development, National Homeland Security Research Center, Research Triangle Park, North Carolina, USA

[‡]Oak Ridge Institute for Science and Education Postdoctoral Fellow

*Corresponding author.

E-mail address: wood.joe@epa.gov

Other Liquid-Based Sporicidal Chemistries of Note

Calcium hypochlorite. At very high concentrations, calcium hypochlorite (50,000 ppm free available chlorine, or FAC) was effective against *B. anthracis* in suspension,[1] but more information is needed on efficacy at lower concentrations and to determine an effective concentration for use as a spray-applied liquid. One study reported no viable *B. subtilis* spores on many porous and nonporous building surfaces after treatment with spray-applied detergent solutions containing acidified calcium hypochlorite or supertropical bleach (calcium hypochlorite with calcium oxide [lime]) at 2,000-ppm FAC, but confirmation of efficacy greater than 2-3 LR was limited by the method detection limit.[2] Subsequent tests of acidified calcium hypochlorite (5,000 ppm FAC) showed low to moderate efficacy against spores of *B. atrophaeus* on porous and nonporous surfaces.[3]

Ozonated water. Ozone is a strong oxidant, typically generated in the gas phase from air or pure oxygen using a corona discharge system.[4] Ozonated water is produced by bubbling a mixture of ozone and air through water, allowing the ozone to dissolve. A recent trade publication article suggests that aqueous solutions of ozone see niche use as a sanitizer of surfaces in seafood processing, but the lack of data on the frequency of its use in general suggests it is largely experimental or pilot-scale.[4-6] Potable commercial ozonated-water generators capable of producing the sporicidal concentrations described below (~10 mg/L) are available.[7, 8]

Ozonation has been used to treat surfaces by bubbling ozone (continuous ozonation) through the solution containing the surface or by transferring the surface to a previously ozonated solution, which has a half-life of 20-30 min.[9] A qualitative carrier test found that 4-log CFU of *B. subtilis* OSU494 dried on porcelain was completely inactivated after 10 min immersed in a solution ~10 mg/L aqueous ozone.[10] Immersion for up to 40 min, however, was ineffective against

B. subtilis OSU494 dried on porous silk-suture loops with an organic burden; all replicates tested positive for growth.[10] Despite its short half-life in solution, continuous ozonation during immersion treatment may not be necessary. Immersion in a solution with an initial ozone concentration of 8-14 mg/L for one min was effective (8 LR) against *B. subtilis* OSU494 on nonporous surfaces (stainless steel and laminated paperboard).[11] Immersion in 10-mg/L ozone (initial estimated concentration) for 30 min, however, was ineffective (2 LR) against *B. atrophaeus* on diamond dental burs.[12]

Glutaraldehyde. Glutaraldehyde, which is most effective in an alkaline solution, acts faster than does formaldehyde against *B. anthracis* spores (unspecified avirulent strain) in suspension[13] and, unlike formaldehyde, is still recommended by the CDC for use as a sterilant in healthcare facilities.[14] Despite its faster inactivation kinetics, immersion of nonporous surfaces in solutions of 2-3 wt% glutaraldehyde required CTs of several h to be effective against *B. subtilis*, although *B. anthracis* may be in inactivated faster since it was inactivated faster in suspension than was *B. subtilis*.[15, 16] Lastly, along with formaldehyde, glutaraldehyde was evaluated in preliminary trials for the decontamination of Gruinard Island. Solutions of 5 wt% glutaraldehyde applied liberally to lightly contaminated soil (< 400 CFU of an unspecified *B. anthracis* strain/g-soil) often resulted in no detectable spores in locations sampled 10 days after the application.[17]

Emerging decontamination technologies

While there has been an extensive amount of R&D published over the past 15-20 years on relatively novel approaches for the inactivation of bacterial spores, the majority of the techniques discussed below currently have limited commercial availability and/or would not be applicable as decontamination techniques for *B. anthracis* spores at full scale or for other reasons. Some of these technologies may have some niche uses (e.g., for medical device or food decontamination), but

their availability may be hindered by their small-scale use, high cost, lack of technological readiness, or sub-optimal efficacy. Many of these are physical-based, e.g., irradiative, techniques. Because of the abundance of literature on these emerging approaches, we briefly discuss here a few of these technologies, and provide a summary and references for other emerging techniques.

One of the more popular emerging technologies that has received extensive attention and funding the past decade has been the use of cold plasmas for antimicrobial purposes; see, for example, Hertwig et al.,[18] who provide a review of this technology. According to Roth et al.,[19] plasma sterilization methods use electric fields to excite gas mixtures, resulting in production UV photons, free radicals, ions, and free electrons. One common facet and drawback of this technology is that the plasma source is usually relatively small and must be in close proximity (on the order of mm to cm) of the surface being decontaminated.

The use of photo-based techniques for antimicrobial purposes is another popular emerging sterilization technique. While UVC light generated via low pressure mercury vapor lamps (i.e., at 254 nm wavelength) is a well-established sporicidal technology and was discussed in more detail in this review, many other photo-based approaches are still at a low readiness level. Examples of this class of technologies would include those producing UVC light (200-280 nm) via light emitting diodes;[20, 21] Kim et al. [22] demonstrated that UV-C light produced via LEDs at 266 nm was more biocidal than UVC-254. The use of blue light, i.e., 405 nm wavelength has also been evaluated.[23] [24] The use of various light sources and radiative wavelengths coupled with chemical additives to act as catalysts [25, 26] or photosensitizers [24] has been reported in the literature as well. Vimont et al. [27] provide a review of pulsed light technology, which is characterized by short but high intensity bursts of light generated from various sources at various wavelengths. For example, the use of xenon-based lamps as a pulsed light source is gaining prominence in the literature; see

Chaine et al. [28] and Vimont et al.[27] Lastly, exposure of *B. anthracis* spores to simulated sunlight (UVA/B) was shown to attenuate populations up to several LR, depending on the material.[29]

In addition to the plasma and photo-based techniques, Table S1 summarizes some of the more frequently published yet emerging germicidal technologies reported in the literature since 2002.

Applicability of *B. anthracis* spore inactivation techniques to *Clostridium difficile*

While the primary purpose of the critical review is to aid in the selection of sporicidal techniques that could be employed to decontaminate buildings or outdoor areas contaminated with *B. anthracis* spores, the technologies reviewed may also be considered as potential decontamination options for other virulent spore-producing bacteria, such as *Clostridium difficile*, *Bacillus cereus*, and others. *C. difficile* is a major public health concern and a source of hospital acquired infections.[30] The US Centers for Disease Control and Prevention estimates that 29,000 died in the U.S. from infection of *C. difficile* in 2011.[31] Environmental contamination is a primary source of transmission of *C. difficile,*[32] and as such, patients can become infected with *C. difficile* from poorly decontaminated surfaces.[33] Similar to *B. anthracis* spore inactivation techniques, there is a fair amount of literature related to the evaluation of techniques for inactivation of *C. difficile* spores. A thorough review of this literature is needed (see for example recent publications related to the evaluation of biocides for *C. difficile* [34-36]), but beyond the scope of this review.

Nevertheless, understanding the relative resistance of *B. anthracis* and *C. difficile* spores to decontaminants would enable prediction of sporicide performance against *B. anthracis* spores based upon the decontamination data generated for *C. difficile*, and vice versa.[37] There are only a few direct side-by-side laboratory efficacy studies that have been conducted to compare the two

species: Oie et al. [38] showed that in suspension tests with sodium hypochlorite, *C. difficile* was less resistant than *B. anthracis* in terms of the time required to inactivate a 10^6 population. In another study [37] which compared sporicidal efficacy against spores of *C. difficile* and *B. anthracis* using chlorine dioxide gas, hydrogen peroxide vapor, or pAB, inactivation results for *C. difficile* were either comparable to *B. anthracis* or showed greater resistance than *B. anthracis*, depending on the material and the chemistry.

Table S1. Emerging Sterilization and Decontamination Technologies

Technology	Principle	Limitations for use	Example references showing use for inactivation of spores
Cold plasmas	Use gas mixtures excited via electrical field to produce reactive chemical species and other potentially germicidal components	Small-scale use only	18, 19, 39-42
Photo-based	Use of novel sources of light (e.g., xenon, LED), unique wavelengths, with and without the use of photo-catalysts or photosensitizers; or pulsed light (short bursts of high intensity)	Limited efficacy data reported for spores on building materials; shadowing	20, 21, 23-25, 43
Ultrasound	Mechanical effects and sonochemical reactions produced by acoustic cavitation	For liquids only	44
Supercritical carbon dioxide (CO_2) and other high pressure transmitting fluids	High pressure CO_2, operated at temperature of approximately 31 °C and pressures of 7.4 MPa.	Small-scale chamber use	45-48
Microwave irradiation	Non-ionizing radiation typically using a frequency of 2450 MHz	Small-scale chamber use	49-52
Pulsed electric field	Pulses of high voltage (typically 20 - 80 kV/cm) to foods placed between 2 electrodes, usually 1 sec bursts	Used for food preservation	53-55
Bacteriophages	Use of naturally occurring viruses to attack and inactivate *B. anthracis* Sterne spores	Literature reported primarily for their use against non-spore forming agents	56, 57
Germinants	Accelerate germination of spores with enzymes, nutrients, or other techniques (heat, pressure) to form vegetative bacteria, which are more sensitive to antimicrobial agents	Expense, insufficient germination efficacy	48, 58, 59

SI References

1. Rogers, J. V.; Ducatte, G. R.; Choi, Y. W.; Early, P. C., A preliminary assessment of Bacillus anthracis spore inactivation using an electrochemically activated solution (ECASOL (TM)). *Lett. Appl. Microbiol.* **2006,** *43*, (5), 482-488.
2. Fielding, G.; Dimmick, R.; Neihof, R.; Echols, W. *Disinfection with Hypochlorite: Application to Clothed Men, Construction Materials, and Electronic and Electrical Items*; NRL 7067; DTIC Document: Naval Research Laboratory, Washington, D.C., 1970.
3. OConnor, L. E.; Harper, B.; Larsen, L. *A comparison of decontamination technologies for biological agent on selected commercial surface materials*; NA; Army Soldier and Biological Chemical Command: U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD, 2001.
4. Guzel-Seydim, Z. B.; Greene, A. K.; Seydim, A. C., Use of ozone in the food industry. *LWT - Food Science and Technology* **2004,** *37*, (4), 453-460.
5. Cullen, P. J.; Norton, T., Ozone Sanitisation in the Food Industry. In *Ozone in Food Processing*, Wiley-Blackwell: 2012; pp 163-176.
6. Higgins, K. T., Is Ozone The Next Sanitation Superstar? *Food Process.* January, 2014.
7. McClain Ozone 2015 Mobile Sanitation System Specifications. http://mcclainozone.com/wp-content/uploads/2013/10/2015-Sanitation-System-Specification-Sheet-7-1-15NP1.pdf (October 2, 2017),
8. Ozone Solutions Mobilezone. https://www.ozonesolutions.com/industrial-ozone-generators/mobilezone (October 2, 2017),
9. Khadre, M. A.; Yousef, A. E.; Kim, J. G., Microbiological aspects of ozone applications in food: A review. *J. Food Sci.* **2001,** *66*, (9), 1242-1252.
10. Rickloff, J. R., An evaluation of the sporicidal activity of ozone. *Appl. Environ. Microbiol.* **1987,** *53*, (4), 683-686.
11. Khadre, M. A.; Yousef, A. E., Decontamination of a multilaminated aseptic food packaging material and stainless steel by ozone. *J. Food Saf.* **2001,** *21*, (1), 1-13.
12. César, J.; Sumita, T. C.; Junqueira, J. C.; Jorge, A. O. C.; do Rego, M. A., Antimicrobial effects of ozonated water on the sanitization of dental instruments contaminated with E. coli, S. aureus, C. albicans, or the spores of B. atrophaeus. *Journal of Infection and Public Health* **2012,** *5*, (4), 269-274.
13. Rubbo, S. D.; Gardner, J. F.; Webb, R. L., Biocidal activities of glutaraldehyde and related compounds. *J. Appl. Bacteriol.* **1967,** *30*, (1), 78-87.
14. Rutala, W. A.; Webber, D. J.; Healthcare Infection Control Practices Advisory Committee Guideline for disinfection and sterilization in healthcare facilities, 2008. https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf (October 19, 2017),
15. Tomasino, S. F.; Pines, R. M.; Cottrill, M. P.; Hamilton, M. A., Determining the efficacy of liquid sporicides against spores of Bacillus subtilis on a hard nonporous surface using the quantitative three step method: collaborative study. *J. AOAC Int.* **2008,** *91*, (4), 833-852.
16. March, J. K.; Pratt, M. D.; Lowe, C. W.; Cohen, M. N.; Satterfield, B. A.; Schaalje, B.; O'Neill, K. L.; Robison, R. A., The differential effects of heat-shocking on the viability of spores from Bacillus anthracis, Bacillus subtilis, and Clostridium sporogenes after treatment with peracetic acid- and glutaraldehyde-based disinfectants. *Microbiologyopen* **2015,** *4*, (5), 764-73.

17. Manchee, R. J.; Broster, M. G.; Anderson, I. S.; Henstridge, R. M.; Melling, J., Decontamination of Bacillus anthracis on Gruinard Island? *Nature* **1983,** *303*, (5914), 239-40.
18. Hertwig, C.; Steins, V.; Reineke, K.; Rademacher, A.; Klocke, M.; Rauh, C.; Schluter, O., Impact of surface structure and feed gas composition on Bacillus subtilis endospore inactivation during direct plasma treatment. *Front. Microbiol.* **2015,** *6*, 774.
19. Roth, S.; Feichtinger, J.; Hertel, C., Characterization of Bacillus subtilis spore inactivation in low-pressure, low-temperature gas plasma sterilization processes. *J. Appl. Microbiol.* **2010,** *108*, (2), 521-31.
20. Tran, T.; Racz, L.; Grimaila, M. R.; Miller, M.; Harper, W. F., Jr., Comparison of continuous versus pulsed ultraviolet light emitting diode use for the inactivation of Bacillus globigii spores. *Water Sci. Technol.* **2014,** *70*, (9), 1473-80.
21. Würtele, M. A.; Kolbe, T.; Lipsz, M.; Külberg, A.; Weyers, M.; Kneissl, M.; Jekel, M., Application of GaN-based ultraviolet-C light emitting diodes – UV LEDs – for water disinfection. *Water Res.* **2011,** *45*, (3), 1481-1489.
22. Kim, S.-J.; Kim, D.-K.; Kang, D.-H., Using UVC light-emitting diodes at wavelengths of 266 to 279 nanometers to inactivate foodborne pathogens and pasteurize sliced cheese. *Appl. Environ. Microbiol.* **2016,** *82*, (1), 11-17.
23. Maclean, M.; Murdoch, L. E.; MacGregor, S. J.; Anderson, J. G., Sporicidal effects of high-intensity 405 nm visible light on endospore-forming bacteria. *Photochem. Photobiol.* **2013,** *89*, (1), 120-6.
24. Eichner, A.; Gollmer, A.; Spath, A.; Baumler, W.; Regensburger, J.; Konig, B.; Maisch, T., Fast and effective inactivation of Bacillus atrophaeus endospores using light-activated derivatives of vitamin B2. *Photochem Photobiol Sci* **2015,** *14*, (2), 387-96.
25. Zacarías, S. M.; Satuf, M. L.; Vaccari, M. C.; Alfano, O. M., Photocatalytic inactivation of bacterial spores using TiO2 films with silver deposits. *Chem. Eng. J.* **2015,** *266*, 133-140.
26. Prasad, G. K.; Ramacharyulu, P. V. R. K.; Merwyn, S.; Agarwal, G. S.; Srivastava, A. R.; Singh, B.; Rai, G. P.; Vijayaraghavan, R., Photocatalytic inactivation of spores of Bacillus anthracis using titania nanomaterials. *J. Hazard. Mater.* **2011,** *185*, (2–3), 977-982.
27. Vimont, A.; Fliss, I.; Jean, J., Efficacy and Mechanisms of Murine Norovirus Inhibition by Pulsed-Light Technology. *Appl. Environ. Microbiol.* **2015,** *81*, (8), 2950-2957.
28. Chaine, A.; Levy, C.; Lacour, B.; Riedel, C.; Carlin, F., Decontamination of sugar syrup by pulsed light. *J. Food Prot.* **2012,** *75*, (5), 913-7.
29. Wood, J. P.; Meyer, K. M.; Kelly, T. J.; Choi, Y. W.; Rogers, J. V.; Riggs, K. B.; Willenberg, Z. J., Environmental persistence of Bacillus anthracis and Bacillus subtilis spores. *PLoS One* **2015,** *10*, (9), e0138083.
30. Lawley, T. D.; Clare, S.; Deakin, L. J.; Goulding, D.; Yen, J. L.; Raisen, C.; Brandt, C.; Lovell, J.; Cooke, F.; Clark, T. G., Use of purified Clostridium difficile spores to facilitate evaluation of health care disinfection regimens. *Appl. Environ. Microbiol.* **2010,** *76*, (20), 6895-6900.
31. U.S. Centers for Disease Control and Prevention Clostridium difficile infection https://www.cdc.gov/hai/organisms/cdiff/cdiff_infect.html (February 13, 2018),
32. Wilcox, M.; Fraise, A.; Bradley, C.; Walker, J.; Finch, R., Sporicides for Clostridium difficile: the devil is in the detail. *J. Hosp. Infect.* **2011,** *77*, (3), 187-188.
33. Anderson, D. J.; Chen, L. F.; Weber, D. J.; Moehring, R. W.; Lewis, S. S.; Triplett, P. F.; Blocker, M.; Becherer, P.; Schwab, J. C.; Knelson, L. P., Enhanced terminal room disinfection and acquisition and infection caused by multidrug-resistant organisms and Clostridium difficile (the

Benefits of Enhanced Terminal Room Disinfection study): a cluster-randomised, multicentre, crossover study. *The Lancet* **2017,** *389*, (10071), 805-814.
34. Rutala, W. A.; Gergen, M. F.; Weber, D. J., Efficacy of different cleaning and disinfection methods against Clostridium difficile spores: importance of physical removal versus sporicidal inactivation. *Infect. Control Hosp. Epidemiol.* **2012,** *33*, (12), 1255-1258.
35. Joshi, L. T.; Phillips, D. S.; Williams, C. F.; Alyousef, A.; Baillie, L., Contribution of spores to the ability of Clostridium difficile to adhere to surfaces. *Appl. Environ. Microbiol.* **2012,** *78*, (21), 7671-7679.
36. Barbut, F., How to eradicate Clostridium difficile from the environment. *J. Hosp. Infect.* **2015,** *89*, (4), 287-295.
37. U.S. Environmental Protection Agency *Comparative efficacy of sporicidal technologies for the decontamination of Bacillus anthracis, B. atrophaeus, and Clostridium difficile spores on building materials*; EPA 600/R-14/405; U.S. Environmental Protection Agency, Washington, DC, 2015.
38. Oie, S.; Obayashi, A.; Yamasaki, H.; Furukawa, H.; Kenri, T.; Takahashi, M.; Kawamoto, K.; Makino, S., Disinfection methods for spores of Bacillus atrophaeus, B. anthracis, Clostridium tetani, C. botulinum and C. difficile. *Biol. Pharm. Bull.* **2011,** *34*, (8), 1325-9.
39. Stapelmann, K.; Fiebrandt, M.; Raguse, M.; Awakowicz, P.; Reitz, G.; Moeller, R., Utilization of low-pressure plasma to inactivate bacterial spores on stainless steel screws. *Astrobiology* **2013,** *13*, (7), 597-606.
40. Dobrynin, D.; Fridman, G.; Mukhin, Y. V.; Wynosky-Dolfi, M. A.; Rieger, J.; Rest, R. F.; Gutsol, A. F.; Fridman, A., Cold Plasma Inactivation of *Bacillus cereus* and *Bacillus anthracis* (Anthrax) Spores. *Ieee Transactions on Plasma Science* **2010,** *38*, (8), 1878-1884.
41. Wang, S.; Doona, C. J.; Setlow, P.; Li, Y. Q., Characterization of cold atmospheric plasma inactivation of individual bacterial spores using Raman spectroscopy and phase contrast microscopy. *Appl. Environ. Microbiol.* **2016**.
42. Sharma, A.; Pruden, A.; Yu, Z.; Collins, G. J., Bacterial Inactivation in Open Air by the Afterglow Plume Emitted from a Grounded Hollow Slot Electrode. *Environ. Sci. Technol.* **2005,** *39*, (1), 339-344.
43. Kozlova, E.; Safatov, A.; Kiselev, S.; Marchenko, V. Y.; Sergeev, A.; Skarnovich, M.; Emelyanova, E.; Smetannikova, M.; Buryak, G.; Vorontsov, A., Inactivation and mineralization of aerosol deposited model pathogenic microorganisms over TiO2 and Pt/TiO2. *Environ. Sci. Technol.* **2010,** *44*, (13), 5121-5126.
44. Gao, S.; Hemar, Y.; Ashokkumar, M.; Paturel, S.; Lewis, G. D., Inactivation of bacteria and yeast using high-frequency ultrasound treatment. *Water Res.* **2014,** *60*, 93-104.
45. Howell, J.; Niu, F.; McCabe, S. E.; Zhou, W.; Decedue, C. J., Solvent removal and spore inactivation directly in dispensing vials with supercritical carbon dioxide and sterilant. *AAPS PharmSciTech* **2012,** *13*, (2), 582-9.
46. Hossain, M. S.; Nik Ab Rahman, N. N.; Balakrishnan, V.; Alkarkhi, A. F.; Ahmad Rajion, Z.; Ab Kadir, M. O., Optimizing supercritical carbon dioxide in the inactivation of bacteria in clinical solid waste by using response surface methodology. *Waste Manag* **2015,** *38*, 462-73.
47. Wang, B. S.; Li, B. S.; Du, J. Z.; Zeng, Q. X., Combined pressure-thermal inactivation effect on spores in lu-wei beef--a traditional Chinese meat product. *J. Appl. Microbiol.* **2015,** *119*, (2), 446-54.

48. Wei, J.; Setlow, P.; Hoover, D. G., Effects of moderately high pressure plus heat on the germination and inactivation of *Bacillus cereus* spores lacking proteins involved in germination. *Lett. Appl. Microbiol.* **2009,** *49*, (5), 646-51.
49. Oliveira, E. A.; Nogueira, N. G. P.; Innocentini, M. D. M.; Pisani, R., Microwave inactivation of *Bacillus atrophaeus* spores in healthcare waste. *Waste Manage.* **2010,** *30*, (11), 2327-2335.
50. Wu, Y.; Yao, M., Inactivation of bacteria and fungus aerosols using microwave irradiation. *J. Aerosol Sci* **2010,** *41*, (7), 682-693.
51. Singh, R.; Singh, D., Sterilization of bone allografts by microwave and gamma radiation. *Int. J. Radiat. Biol.* **2012,** *88*, (9), 661-6.
52. Celandroni, F.; Longo, I.; Tosoratti, N.; Giannessi, F.; Ghelardi, E.; Salvetti, S.; Baggiani, A.; Senesi, S., Effect of microwave radiation on *Bacillus subtilis* spores. *J. Appl. Microbiol.* **2004,** *97*, (6), 1220-1227.
53. Siemer, C.; Toepfl, S.; Heinz, V., Inactivation of Bacillus subtilis spores by pulsed electric fields (PEF) in combination with thermal energy II. Modeling thermal inactivation of *B. subtilis* spores during PEF processing in combination with thermal energy. *Food Control* **2014,** *39*, 244-250.
54. Reineke, K.; Schottroff, F.; Meneses, N.; Knorr, D., Sterilization of liquid foods by pulsed electric fields-an innovative ultra-high temperature process. *Front. Microbiol.* **2015,** *6*, 400.
55. Barbosa-Canovas, G. V.; Pierson, M. D.; Zhang, Q. H.; Schaffner, D. W., Pulsed Electric Fields. *J. Food Sci.* **2000,** *65*, 65-79.
56. Walter, M. H., Efficacy and durability of *Bacillus anthracis* bacteriophages used against spores. *J. Environ. Health* **2003,** *66*, (1), 9-15.
57. Rashid, M. H.; Revazishvili, T.; Dean, T.; Butani, A.; Verratti, K.; Bishop-Lilly, K. A.; Sozhamannan, S.; Sulakvelidze, A.; Rajanna, C., A *Yersinia pestis*-specific, lytic phage preparation significantly reduces viable *Y. pestis* on various hard surfaces experimentally contaminated with the bacterium. *Bacteriophage* **2012,** *2*, (3), 168-177.
58. Omotade, T. O.; Bernhards, R. C.; Klimko, C. P.; Matthews, M. E.; Hill, A. J.; Hunter, M. S.; Webster, W. M.; Bozue, J. A.; Welkos, S. L.; Cote, C. K., The impact of inducing germination of *Bacillus anthracis* and *Bacillus thuringiensis* spores on potential secondary decontamination strategies. *J. Appl. Microbiol.* **2014,** *117*, (6), 1614-1633.
59. Shigeta, Y.; Aoyama, Y.; Okazaki, T.; Hagura, Y.; Suzuki, K., Hydrostatic Pressure-Induced Germination and Inactivation of Bacillus Spores in the Presence or Absence of Nutrients. *Food Sci. Technol. Res.* **2007,** *13*, (3), 193-199.