

## Original Paper

# Microwave Disinfection of Biohazardous Carcasses

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### **Abstract**

*A Medister microwave device specifically developed for thermal disinfection of infectious waste was assessed concerning its efficacy for the disinfection of biohazardous animal carcasses. Employment of animals for the experiments was avoided by simulation of carcasses with deep frozen nutrient agar contaminated with bacillus subtilis spores. At a disinfection time of 45 minutes, the reduction rate of the spores was 7.90-8.58 decimal power levels. Controlled microwave disinfection is thus a valuable alternative tool for the treatment of contaminated carcasses.*

### **Keywords**

*microwave, animal carcasses, inactivation, disinfection, biohazardous waste*

### **1. Introduction**

The risks and pitfalls associated with the disposal of microbial contaminated carcasses are enormous. This not only applies for animals infected in laboratory experiments, but rather potential pathogenic outbreak events such as avian influenza (Pollard et al., 2008). Assessment of critical control points for risk management during carcass disposal demonstrated multiple opportunities for exposures to human health, animal health, and the environment (Delgado et al., 2010; Pollard et al., 2008). Therefore, safe and effective disposal is required in order to protect the surrounding environment as well as population. In general, early stages of the disposal chain (e.g., farm or research facility) pose greater opportunities for exposure to hazardous agents than later stages, where carcasses are contained and treated by regulated processes (Pollard et al., 2008). On the other hand, controlled treatment of carcasses off-site bears the risk of uncontrolled transport (Wu et al., 2014). Risks from (relatively short) uncontrolled transport inside a respective facility from the site of generation to the treatment site should also not be disregarded.

In general, a variety of waste treatment technologies are available and there is not one, which is optimal

for every need (UNEP, 2012). For carcass disposal, there are also a number of treatment opportunities, from which incineration plays a major role (Hill, 1999; Sander et al., 2002). Thermal incineration treatment is recognized as a safe elimination process and because animal wastes have a high calorific value even the transformation of the ashes into a slow release fertilizer is considered (Sharrock et al., 2009).

Alternatively, for the digestion and decontamination of infectious animal waste, alkaline hydrolysis based tissue dissolvers are commercially available tools (Homer et al., 2012).

Autoclaves are widely utilized for disinfection of biohazardous materials, however, certain waste materials are difficult to decontaminate in the autoclave because they insulate and protect the contaminating organisms from heat and steam penetration (National Research Council, 1989). Examples include not only animal carcasses, but also human body parts, and large volumes of contaminated clothing. Increased sterilization times of 4 hours might address this issue (Santacroce et al., 2015).

In order to improve the range of decontamination processes for infectious animal waste, alternative methods and especially technologies, which can be carried out in low-scale dimensions and on-site are needed. Furthermore, employment of animals for the experiments should be avoided solely testing disinfection capabilities. Thus, new experimental set-ups for testing the inactivation of microbial contamination of animal carcasses are required as well.

As long as properly controlled, special microwave technology is an emerging technology for the treatment of biohazardous waste including even frozen animal carcasses (Zimmermann, 2017). Unlike in autoclaves, the heat in microwave devices is generated inside the wet waste material, which is thus an attractive treatment possibility for carcasses as well. To prove this assumption, the inactivation capability of microwave devices for infected animal carcasses was assessed in the present study with an experimental set-up avoiding animal experiments.

## 2. Method

### 2.1 Contamination of Simulated Carcasses with *Bacillus Subtilis* Spores

The carcass of a rabbit was simulated by Caseinpepton-Sojapepton-Agar (CSA). Four L of melted CSA with a temperature of 55°C were contaminated with 55 mL suspension of *bacillus subtilis* spores ATCC 6633 with a density of  $8.2 \times 10^8$  colony forming units (CFU)/mL. The contaminated CSA was transferred to autocleavable bags, which were positioned in plastic containers. For determination of the initial amount of *bacillus subtilis* spores, 50 µL were removed and transferred to 5 mL caseipepton-sojapepton-bouillion. Portions of 1 mL were incubated for 48 hours at a temperature of  $36 \pm 1^\circ\text{C}$  in culture stocks. In addition to the contaminated CSA, 4 L portions of non-contaminated ballast tap water in autocleavable bags were utilized. Together these two bags met the dimension and the weight of a full-grown 8 kg rabbit. CSA and water bags were both frozen at  $-18^\circ\text{C}$  before the process.

## *2.2 Microwave Device*

For the disinfection of simulated animal carcasses, a microwave device specifically developed for the thermal disinfection of biohazardous waste (Medister 160, Meteka, Judenburg, Austria) was chosen (Katschnig, 1993; Dragas et al., 1994). With this device, the moisture already contained in the waste is supplemented by addition of water to an optimal steam content. Consequently, also low moisture waste types can be disinfected. Furthermore, the devices assure even heat distribution. The maximum volume for disinfection is 60 L, and maximum power consumption is 6.5 kW. In addition, a puncture-proof waste container is used for collection, transportation, and disinfection of the waste (Meditainer, Meteka). In addition to infectious solid waste, the microwave device has also specific programs for treatment of animal carcasses and fluids.

## *2.3 Disinfection Program*

The disinfection program is processor controlled and consists of a variable heating time until the disinfection temperature point, followed by an automatic redemption time of 30 min. After the automatic addition of 1 L water, the disinfection takes place with a holding-time of 45 min. Finally, with a cooling down period of 5 min the process is finalized. The disinfection cycle is documented by an integrated recorder including the complete temperature curve of the steam.

The temperature inside the simulated carcasses is measured by electronic data loggers. Two programmable loggers are fixed to the bottom in containers containing contaminated CSA and tap water.

## *2.4 Evaluation of the Disinfection Process*

After the disinfection process, ten 50 mL portions of the CSA are removed from each container and transferred to Petri dishes. After solidification of the agar, the Petri dishes are incubated at a temperature of  $36 \pm 1^\circ\text{C}$  for 24 hours and then at ambient temperature for 3 days. Finally, the germ colonies grown are counted.

## **3. Result and Discussion**

Contaminated CSA and water bags were taken from  $-18^\circ\text{C}$  freezers and placed into the Meteka microwave device. Because animal carcasses utilized in animal experiments are frequently frozen until further treatment the bags were also frozen to simulate a “worst case” scenario. Two disinfection experiments were carried out. In the first experiment, one frozen container containing the contaminated CSA was placed at the bottom of a Meditainer. The frozen tap water was placed on top of it. The overall duration of the disinfection process was 151 min (71 min heating time, 30 min redemption time and water addition, 45 min disinfection time, 5 min cooling time). In the second experiment, the contaminated CSA was placed on top of the frozen tap water block. For the second treatment process the overall process duration was 149 min (change of heating time to 69 min).

The temperature curves recorded by the loggers showed that during the disinfection process a temperature of about  $99^\circ\text{C}$  was hold for a duration of 45 min in both experiments. The evaluation of the

Petri dishes revealed a growth rate of 5-27 CFU/50 mL equivalent to 0.10- 0.54 CFU/mL (Tables 1 and 2). As the initial amount of germs was  $3.8 \times 10^7$  CFU/mL (first experiment) and  $4.3 \times 10^7$  CFU/mL (second experiment) the reduction rate of the spores thus reached 7.90-8.58 decimal power levels.

**Table 1. Results Experiment 1 (CSA Container Bottom)**

Sample number	Initial amount of germs		Amount of germs after disinfection		Log <sub>10</sub> reduction
	CFU/mL	Log <sub>10</sub> /mL	CFU/mL	Log <sub>10</sub> /mL	
1			0.26	-0.59	8.17
2			0.26	-0.59	8.17
3			0.22	-0.66	8.24
4			0.10	-1.00	8.58
5	$3.8 \times 10^7$	7.58	0.22	-0.66	8.24
6			0.50	-0.30	7.88
7			0.14	-0.85	8.43
8			0.26	-0.59	8.17
9			0.42	-0.38	7.96
10			0.22	-0.66	8.24

**Table 2. Results Experiment 2 (CSA Container Top)**

Sample number	Initial amount of germs		Amount of germs after disinfection		Log <sub>10</sub> reduction
	CFU/mL	Log <sub>10</sub> /mL	CFU/mL	Log <sub>10</sub> /mL	
1			0.32	-0.49	8.12
2			0.54	-0.27	7.90
3			0.34	-0.47	8.10
4			0.20	-0.70	8.33
5	$4.3 \times 10^7$	7.63	0.28	-0.55	8.18
6			0.32	-0.49	8.12
7			0.34	-0.47	8.10
8			0.46	-0.34	7.97
9			0.22	-0.66	8.29
10			0.32	-0.49	8.12

The special microwave device Medister with the recommended disinfection program applying a hold time of 45 min is thus appropriate for the disinfection of animal carcasses with a weight of up to 8 kg. The reduction rate reached levels far beyond 7.00 log<sub>10</sub> levels, which is well within established guidelines recommended for example by WHO.

In spite its obvious advantages, microwave technologies are rarely utilized for treatment of infected carcasses and not at all addressed in peer reviewed literature. In one example presented by Devine et al. (2007) microwave radiation was also coupled with steam heat to treat culled turkey carcasses. The experiment was designed to simulate a poultry mortality event. Inoculated chicken liver samples were mixed with the turkey for testing the decontamination of poultry waste. The system generated an approximate seven-log reduction in the microbial load of *salmonella* and a five-log reduction in *bacillus* spores. Though there is a major difference to the system utilized in the present study, i.e., that it is designed to treat much larger amounts of waste/carcasses and additionally employing shredding, the results are comparable.

From this example and our results, we conclude that controlled microwave disinfection is a valuable alternative tool for the treatment of contaminated carcasses.

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