

Identification and Screening of Infectious Carcass **Pretreatment Alternatives**

technical BRIEF

Introduction

Managing the treatment and disposal of large numbers of animal carcasses following a foreign animal disease (FAD) outbreak is a challenging endeavor. Pretreatment of the infectious carcasses might facilitate the disposal by simplifying the transportation,

On-Site Size

Reduction

reducing the pathogen load, or by isolating the pathogen from the environment to minimize its spread. This brief summarizes information contained in U.S. **Environmental Protection Agency** (EPA) report (EPA/600/R-15/053) entitled Identification and Screening of Infectious Carcass Pretreatment Alternatives. This brief describes how each of eleven pretreatment methods can be used prior to, and in conjunction with, six commonly used large-scale carcass disposal options (Figure 1).

The six disposal options considered are:

 rendering • burial

The eleven pretreatment methods considered are:

- on-site size reduction digestion
- alkaline hydrolysis sterilization •
- bioreduction
- encapsulation
- addtitive/sorbent • • packaging.
- incineration
 - burning
- physical inactivation
- chemical inactivation
- freezing

The advantages and disadvantages of the pretreatment methods are listed in Table 1.

Disposal and Pretreatment Methods

Animal carcasses considered here include whole bodies or body parts of dead animals, which could be inseparably mixed with manure and bedding or other organic materials. Regulation of carcass management vary from state to state. Treatment and disposal can require special permit(s) approved by federal (e.g., United States Department of Agriculture), tribal, state and

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Bioreduction

Burning

Burial

Alkaline

Hydrolysis

Sterilization

Digestion

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and Potential Pre-treatment Technologies landfill • composting •

local agencies. For all disposal and pretreatment methods, applicable federal, state, local, and tribal laws and regulations must be followed.

Throughout the U.S., the disposal of animal carcasses is regulated by state laws that vary according to animal species. While there are several methods for disposal of animal carcasses, the most common are the following six disposal options (Figure 1):

- **Rendering** for the purpose of disposal of contaminated carcasses involves a series of processes using high temperature and pressure to treat large animal and poultry carcasses or their by-products. The processes include a combination of blending, cooking, pressurizing, fat melting, water evaporation, and microbial and enzyme inactivation. A prerendering process involves size reduction and conveying, and post-rendering process involves screening the protein and fat materials, sequential centrifugations for separation of fat and water, and drying and milling of protein materials.
- **Burial** refers to the placing of the infectious carcasses within the ground at the site of the incident. Many states advise that this option should only be used based on an environmental assessment of site characteristics as well as the implementation of proper environmental controls to protect groundwater, surface water, and soil from leachate.
- Landfilling involves carefully designed structures built into or on top of the ground in which waste is isolated from the surrounding environment. There are different types of landfills, each designed to handle particular waste streams. Generally, each landfill is permitted or licensed for particular kinds of waste. A landfill generally cannot accept waste that falls outside the scope of its permit.
- **Composting** for the purpose of disposal of contaminated carcasses is the controlled biological decomposition of biomass in the presence of air to form a humus-like material. Controlled methods of composting include mechanical mixing and aerating, ventilating the materials by dropping them through a vertical series of aerated chambers, or placing the compost in piles out in the open air and mixing it or turning it periodically. This treatment option is distinct from backyard composting, which is conducted by individuals on their own property. Instead, composting, as a treatment option, is used to decompose large quantities of waste either on a farm in association with animal disease control activities or at off-site composting facilities. Off-site composting will trigger transportation considerations.
- **Incineration** for the purpose of disposal of contaminated carcasses burn the biomass at high temperature under controlled conditions. Different incinerators are permitted for different kinds of waste.
- **Burning**, i.e., the deliberate outdoor burning of waste, can be done in open drums, in fields, and in large open pits or trenches. The use of this option is highly restricted; many states and local communities have laws regulating or banning open burning. Under certain conditions, emergency waivers may be issued.

Review of Pretreatment Options

ON-SITE SIZE REDUCTION

On-site size reduction is the manual or mechanical cutting, grinding, or crushing of the carcass to decrease the dimensions of the resultant parts for ease of handling, to decrease volume, or to enhance further processing. Key size reduction processors include crushers, shredders, and grinders (Figure 2). Onsite grinding for size reduction is advantageous for rendering, composting, burial, landfill, and incineration and

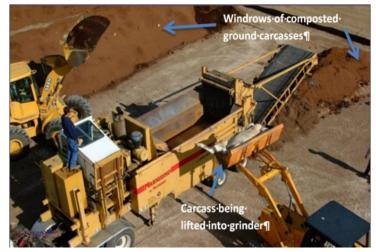


Figure 2. Large-Scale Mobile Carcass Grinder

reduces the risk associated with transporting whole carcasses. For example, decomposition of the carcasses can be sped up by up to 50 percent by grinding them before composting.

DIGESTION

Digestion is a process that liquefies carcasses under acidic conditions, either using lactic acid or phosphoric acid (Figure 3). Lactic acid fermentation uses bacteria to ferment the material primarily into methane, carbon dioxide, and water. Phosphoric acid preservation essentially pickles the carcasses or biomass.



Figure 3. Carcass in Digester (Photo courtesy - Jeff Miller, University of Wisconsin-Madison)

Lactic acid fermentation is a process by

which lactic acid bacteria are added to ground carcasses with fermentable carbohydrates to produce lactic acid under anaerobic conditions. These bacteria can produce volatile acids, hydrogen peroxide, and antibiotic-like compounds that inhibit many bacteria. A variety of animal carcasses can be treated with lactic acid fermentation, including cattle, swine, poultry, sheep, goats, fish, and wild birds. In the phosphoric acid preservation process, phosphoric acid is added directly to ground or small pieces of carcasses. The phosphoric acid disrupts the membrane functions of the microorganisms, reducing their disease-causing activity.

BIOREDUCTION

Bioreduction is the biodegradation of animal by-products or whole carcasses in a partially sealed vessel, where the contents are mildly heated and aerated. Bioreduction is a method that simultaneously permits storage and reduction in the volume of carcasses and that relies on internal enteric microorganisms and enzymes to drive decomposition. Carcass material is placed in a watertight vessel, where the contents are heated (to 40 ± 2 °C) and actively aerated with a pump. Bioreduction is also described as complete bio-digestion and liquefaction of

carcasses. The bioreduction vessels can be buried in the ground, so the overall footprint of the operation is reduced. Transmissible spongiform encephalopathy (TSE) agents are not destroyed at the operational temperatures of bioreduction.

ALKALINE HYDROLYSIS

Alkaline hydrolysis occurs when sodium hydroxide or potassium hydroxide is mixed with biological materials such as protein, nucleic acids, carbohydrates, and lipids (Figure 4). Heat can be applied (150 °C or ~300°F) to significantly accelerate the process. The result is a sterile aqueous solution consisting of small peptides, amino acids, sugars, and soaps. As the process is generally conducted at 150 °C in a 1N potassium hydroxide (KOH) for greater than 6 hours, the resulting effluent (pH 9-10) needs to be cooled and neutralized prior to disposal. When the alkaline solution is properly treated, it may be safe for disposal in wastewater or sewer systems.



Figure 4. Representative Alkaline Hydrolysis Units

STEAM STERILIZATION

Steam sterilization is the process of destroying microorganisms and infective agents with heated water under pressure. The steam sterilization process is time-, temperature-, and pressure-dependent. In this wet thermal treatment, the waste is first shredded and then exposed to high-pressure, high-temperature steam. Steam sterilization has similarities to the process of autoclave sterilization. The sequence of operations can vary from manufacturer to manufacturer. For example, STI (Biosafe Engineering, Brownsburg, Indiana) first performs shredding, but there is no pressure under their current STI models. The Rotoclave® rotating autoclave (Tempico Manufacturing, Hammond, Louisiana) includes a pressurized autoclave system, but there is no "pre-shredding" of the waste. The Rotoclave system rotates so that cutting blades can chop up the waste while it is being steamed under pressure. Application of steam can be with or without pressure, and with or without shredding, depending on the system. Given a suitable temperature and contact time, most varieties of microorganism are inactivated by wet thermal disinfection (for example, sporulated bacteria require 121 °C [249.8 °F]).

FREEZING

Freezing animal carcasses can be done in fixed facilities or mobile units. Freezer types include chest freezers, crust freezers, mobile freezer units, and refrigerated industrial trucks. For large-scale applications, industrial trucks can be used on-site to store and transport carcasses. Although freezing of carcasses might have little implication for decreasing pathogens, this method can be effective in extending the storage time and helping transportation while eliminating or minimizing the decomposition process.

PHYSICAL INACTIVATION

Physical inactivation is the process of eliminating pathogenic microorganisms (excluding bacterial spores) from inanimate objects. Different inactivation methods have different target ranges, not all methods can kill all microorganisms. Inactivation is different from sterilization, which is an absolute condition where all the microorganisms, including bacterial spores, are killed. Of the potential physical methods only steam was considered a potential for carcass treatment.



Figure 5. Handling and Processing of Carcasses

CHEMICAL INACTIVATION

Chemical inactivation is the use of chemical agents to kill/destroy pathogens, including bacteria, bacterial spores, and to inactivate viruses and prions. A wide variety of chemicals are available and these chemicals include but are not limited to oxidizers (chlorine, hypochlorite, ozone, and peroxide), organic acids (lactic acid, acetic acid, and gluconic acid), organics (benzoates, propionates), bacteriocins (nisin, magainin [antimicrobial peptides]), and acidic and basic electrolyzed water. Chemical inactivation can be used in conjunction with other carcass treatment processes, such as size reduction. Depending on the overall treatment scheme, chemical inactivation can be performed during size reduction by addition of chemical additives and mixing or can be applied on the surface of the whole carcass (Figure 6). Surface chemical inactivation would allow for increased biosafety during loading and transport of carcasses, however, it would not serve to reduce pathogens released during decomposition while in transit. There are numerous vendors who supply these chemicals.

	Type of Microorganism							
Biocide	Vegetative bacteria (Gram +)	Vegetative bacteria (Gram –)	Mycobacteria (Gram +)	Fungi	Viruses	Bacterial Spores	Mode of Action	
Oxidizing								
Halogen con	itaining compo	ounds			~			
Chloramines	\bigcirc	8		\mathfrak{P}	\mathcal{O}	ð	Similar to hypochlorite but less active.	
Iodine Compounds	\bigcirc	8	IJ	Ş	\bigcirc	Q	Attacks N-H and S-S/S- H protein bonds.	
Sodium hypochlorite	0	8		Ŷ	\bigcirc	Q	Oxidizer of biological molecules (e.g., proteins, nucleic acids).	
Non-haloger	n containing c	ompounds					, i i i i i i i i i i i i i i i i i i i	
Hydrogen peroxide	\bigcirc	8	IJ	$\mathbf{\hat{m}}$		\bigcirc	Generates hydroxyl fre radicals, which attack biological molecules.	
Non-oxidizing	ţ							
Cationic surfactants	\bigcirc	0	2	\mathcal{O}			Affects proteins metabolic reactions, cell permeability, etc.	
Formalin (37% formaldehyde)	\bigcirc	8	U	\mathcal{O}	\bigcirc		Affects the cell wall and denatures amino proteins.	
Glutaraldehyde	\bigcirc	8	ß	Ŷ	\bigcirc	Q	Affects proteins (e.g., enzymes, transport of nutrients, cell wall, etc.)	
Peraclean® (peracetic acid)	0	8		സ്റ്റ	\bigcirc	Q	Potent oxidizer	
Phenol	0	8		Ŷ			Combines with and denatures proteins.	
\bigcirc	Susceptible	Resis	\cup	sus	newhat ceptible	0	Susceptible at high concentrations	

Figure 6. Effects and Mode of Action of Selected Chemical Inactivation Agents¹ (Chattopadhyay, S. et al., 2004. Evaluation of Biocides for Potential Treatment of Ballast Water. U.S. Department of Homeland Security, U.S. Coast Guard, Report No. CG-D-01-05.)

¹ Prions may not be considered to be what are currently defined as microorganisms, but at the same time they are transmissible and usually resistant to physical and chemical inactivation. Environ LpH (Steris Corp., St. Louis, Missouri), a commercial disinfectant, has been effectively inactivated prions. Prion inactivation occurs with a 1 percent solution of LpH for 10 hours or with a 10 percent LpH solution for one hour. Environ LpH is not as corrosive to surfaces as bleach or NaOH. It should be thoroughly mixed to prepare a treatment solution until uniform consistency can be achieved. User must observe the precautions and safety requirements on the registered product label.

ADDITIVE/SORBENT

Additive/sorbent is a supplemental material mixed with or otherwise added to create a favorable condition by keeping away insects and rodents, increase movement of oxygen throughout the processed material, and absorb excess liquid produced by the decomposing carcass. Additive/sorbent materials (like wood chips, corn silage, straw/manure, rice hulks, and ground cornstalks) help keep the processed material porous, and permeable to gas. Smaller materials (like, sawdust) help absorb the liquid due to water holding capacities and contribute more compaction properties. These additives are also a carbon source needed to sustain the microbes. A combination of suitable additives with appropriate water holding capacities, porosity, gas permeability, and compaction can allow optimal oxygen passage while absorbing any excess liquid. In addition, additives/sorbents reduce potential spread of organisms during transport of processed or unprocessed material. Additives and sorbents are widely available from numerous local stores and vendors.

ENCAPSULATION

On-site carcass foam encapsulation is the treatment of the carcass with cementaceous materials (such as Portland cement, gypsum cement, pozzolanic fly ash, aluminum, and dolomitic lime matrix), Plaster of Paris, polyurethane foam, or commercial encapsulant. These materials, when fully reacted, will encase the carcass in a solid protective matrix.

PACKAGING

Packaging, i.e., on-site carcass packaging or wrapping, is containment of the carcass within a flexible or rigid container. Packaging can be done by rigid, leak-proof, break-proof packaging, or permanently closed, with sufficient absorbent material included to sorb and retain the liquid present.

Advantages	Disadvantages					
On-site Size Reduction						
 Mobile on-site Low environmental impact Very high throughput capacity Few safety issues for operators Ease in handling and transport of processed material Accelerated decomposition 	 Cost of capital equipment Operating cost of machinery Potential aerosol production Groundwater contamination if untreated effluent is released Soil pollution if carcasses accumulate on the ground faster than the processing rate 					
Digestion						
 Long-term storage Kills pathogenic bacteria Cost of storage is relatively low compared to cold storage. Increased biosecurity while minimizing the need for frequent transportation Produces several co-products: biomethane, combined heat and power, compressed natural gas, soil amendments 	 If a digester is not available on site, carcasses must be transported, increasing risk of spreading pathogen. Transmissible spongiform encephalopathy agent is not inactivated; Lactic acid fermentation fails over 10 percent of the time. The capacity is relatively low Carcass pre-processing, such as grinding, is recommended. Higher capital cost than composting. Operation requires skilled technicians. 					

 Table 1. Advantages and Disadvantages of Carcass Pretreatment Technologies

Bioreduction	
 Field and laboratory results showed that the bacterial load is significantly reduced and some pathogens are eliminated. The entire pretreatment and disposal process could be performed on-site with no need for transporting carcasses. Provides a method for storing carcasses thereby reducing the number of collections and transports Overall biomass is reduced. 	 Only research data are available for small-scale operations. Not known to destroy prions (e.g., TSE agents) The geographical location and/or terrain limits where vessels can be installed Can require additive (wood chip) to reduce malodor and to reduce leaching to soil/groundwater No commercially available units were identified.
Alkaline Hydrolysis	
 Inactivation of viruses, bacteria, spores, and TSE agents Sterilization and digestion in one unit Reduction of waste volume and weight by as much as 97% No air emission 	 Relatively low capacity Potential issues with disposal of effluent High pH of effluent must be neutralized prior to disposal in a sewer system
Steam Sterilization	
 Inactivates most pathogens Low environmental impact Few safety issues for operators Facilitates safe transport Creates value-added product 	 High capital cost Requires pre-configured and constructed systems An inadequate shredder might retard efficiency. Requires fuel and water logistics more than other technologies discussed in this report Operational conditions have a pronounced influence on the efficiency of disinfection. Might not inactive TSE agents
Freezing	
 Mobile and on-site freezing facilities are available Increases biosecurity for transportation Prolongs storage for delayed disposal Low cost rental units are available Allows for flexibility of choosing one or more disposal options 	 Mobile units may not be feasible for large-scale die- offs of large animals Thawing step required before size reduction, rendering, burning, or incineration Limited bacterial reduction; surface reduction only for some methods of freezing
	 Energy cost and overall operating cost can be high
Physical Inactivation	
	 Energy cost and overall operating cost can be high Some steam applications alone do not reduce surface bacteria Significant wastewater/environmental impact Potential for aerosolizing infectious agents Slow/labor intensive, and it only removes surface pathogens. As decomposition progresses, internal pathogens will also be exposed.
 Physical Inactivation Equipment readily available Moderate equipment cost Moderate safety issues Potential for reduction of surface infectious agents 	 Some steam applications alone do not reduce surface bacteria Significant wastewater/environmental impact Potential for aerosolizing infectious agents Slow/labor intensive, and it only removes surface pathogens. As decomposition progresses, internal
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Physical Inactivation • Equipment readily available • Moderate equipment cost • Moderate safety issues • Potential for reduction of surface infectious agents • Low environmental impact Chemical Inactivation • Commercially available • Ease of application with little training of personnel • Flexible to apply on site or centralized	 Some steam applications alone do not reduce surface bacteria Significant wastewater/environmental impact Potential for aerosolizing infectious agents Slow/labor intensive, and it only removes surface pathogens. As decomposition progresses, internal pathogens will also be exposed. Environmental concerns on spillage and final disposal Surface treatment might not be effective Some of the chemicals can be harmful Storage prior to use and treatment of large volume of
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 Enhances efficacies of burial, landfill, composting, and incineration Few safety issues for operators Facilitates safe transport and disposition of carcass material Low to moderate cost per carcass 	 Hard materials (wood chips) might not be rendered. Dependent on the amount of sorbent addition, increase in volume of material can increase disposal cost
Inorganic Sorbents	
 Low environmental impact Enhances efficacies of burial and landfill Moderately safe for operators Facilitates safe transport and disposition of carcass material Moderate cost per carcass 	 Eliminates rendering as a disposal option Does not inactivate infectious agents Unknown impact on composting. Volatile toxics, if present, may not be suitable for incineration and burning
Commercial (Chemical) Sorbents	
 Certain active ingredients can kill pathogens Low environmental impact Enhances efficacies of burial, landfill, and incineration Moderately safe for operators 	 Chemical neutralizers, if present, can negatively impact rendering and composting High cost Several of these additives do not inactivate infectious agents
Encapsulation	
 Both mobile and on-site treatment facilities are available Properly encased (stabilized and unbreached) material can prevent disease spread during transport Pathogen inactivation possible through lime/alkaline treatment 	 High cost per carcass Low throughput No significant pathogen inactivation
Packaging	
 Mobile and on site packaging are available Low environmental impact Moderate throughput capability Few safety issues for operators Moderate cost per carcass 	 Unwrapping of carcasses may be needed prior to certain disposal procedures If not sealed properly, there might be potential for leakage It aids the transport and handling, however, it does not reduce the infectivity

Conclusions

Each of the potential pretreatment methods was defined and evaluated based on present status and potential applications, advantages and disadvantages, scale of operations, environmental effects, availability from vendors and typical cost range. The evaluation revealed that many pretreatment options are available, and research studies are ongoing to evaluate the effectiveness of these methods and technologies to pretreat carcasses and the impact of these treatments on the air, soil, and water systems.

Based on identification and evaluation, Table 2 provides a qualitative ranking of eleven pretreatment alternatives to foster proactive protection, response, and recovery to dispose animal carcasses in the event of animal disease outbreak. Each of the eleven pretreatment options offers unique advantages and disadvantages. None of these treatments, individually or in combination, should be considered absolute. The pretreatment scheme should be approached on a case by case basis. Two or more

pretreatment/disposal methods can be selected so as not to overburden a processing site. Parallel treatment schemes can be considered by using treatment of part of the feed material by selected methods while treating remaining parts of the feed material by other method(s). Table 3 highlights key attributes of each of these technologies.

Disposal Option	On-site Size Reduction	Digestion	Bioreduction	Alkaline Hydrolysis	Sterilization	Freezing	Physical Inactivation	Chemical Inactivation	Additives/ Sorbents	Encapsulation	Packaging
Rendering	+++	++	++	-	++	++	++	++	++	-	-
Incineration	+++	+	+	-	+++	++	++	++	+++	+	++
Composting	+++	+++	+++	-	-	++	++	-	+++	-	-
Burial	++	+	-	+	+++	++	++	++	+++	++	++
Burning	+++	-	-	-	+++	-	++	++	+++	+	++
Landfill	++	+	-	+	+++	++	++	++	+++	++	++

 Table 2. Carcass Pretreatment Options Matrix

Note: Several of the pretreatments may have overlapping processes. Some of the activities can be conducted at centralized or mobile locations. +++, ++ and + denote qualitative importance of the criteria (+++ > ++ > +), and - indicate not applicable.

Color Key



Subject to acceptability of characteristics of feedstock by the processing facility/plant

Not Suitable

Pretreatment Options	Favorable Applications			
On-site Size Reduction	Grind carcasses to reduce size for transport and to use in subsequent processes such as composting, rendering, and digestion; high throughput applications.			
Alkaline Hydrolysis	Destroys prions; reduces waste volume and weight by as much as 97%. However, it generates significant amount of liquid waste that require additional treatment.			
Steam Sterilization	Sterilizes for shredded mass.			
Encapsulation	Safe handling; protection of the immediate environment (not during process of wrapping).			
Digestion	Reduce total volume under certain conditions and may take long time.			
Additives/Sorbents	Enhance or accelerate disposal processes.			
Bioreduction	Reduce total volume and some bacterial pathogens; dispose of animals over time without the need to transport off site; effective for small quantities of biomass.			
Freezing	Delay decomposition; safe transportation; large capacity transport to disposal site(s); decontamination of freezer may be necessary.			
Inactivation	Eliminates most pathogenic microorganisms for safe transport and handling.			
Packaging	Safe transportation to disposal site; safe handling.			

Table 3. Favorable Applications of the Pretreatment Options

Reference

U.S. EPA. 2016. Identification and screening of infectious carcass pretreatment alternatives. Cincinnati, OH: U.S. Environmental Protection Agency. EPA/600/R-15/053

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