Carcass Disposal: A Comprehensive Review

National Agricultural Biosecurity Center Consortium
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Chapter

6

Alkaline Hydrolysis

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Table of Contents

Abbreviations

APHIS	USDA Animal and Plant Health Inspection Service
ARS	USDA Agricultural Research Service
BOD	biochemical oxygen demand
BSE	bovine spongiform encephalopathy
CWD	chronic wasting disease
DNA	deoxyribonucleic acid
psig	pounds per square inch, gauge
STAATT	State and Territorial Association on Alternative Treatment Technologies,
TSE	transmissible spongiform encephalopathy
US	United States
USDA	United States Department of Agriculture
WR^2	Waste Reduction by Waste Reduction, Inc.

Section 1 – Key Content

Alkaline hydrolysis represents a relatively new carcass disposal technology. It has been adapted for biological tissue disposal (e.g., in medical research institutions) as well as carcass disposal (e.g., in small and large managed culls of diseased animals). One company—Waste Reduction by Waste Reduction, Inc. (WR^2)—reports that it currently has 30 to 40 alkaline hydrolysis digestion units in operation in the United States (US), several of which are used to dispose of deer carcasses infected with chronic wasting disease (CWD) (Grady, 2004).

1.1 – Process Overview

Alkaline hydrolysis uses sodium hydroxide or potassium hydroxide to catalyze the hydrolysis of biological material (protein, nucleic acids, carbohydrates, lipids, etc.) into a sterile aqueous solution consisting of small peptides, amino acids, sugars, and soaps. Heat is also applied (150°C, or ~300°F) to significantly accelerate the process. The only solid byproducts of alkaline hydrolysis are the mineral constituents of the bones and teeth of vertebrates (WR^2, 2003). This undigested residue, which typically constitutes approximately two percent of the original weight and volume of carcass material, is sterile and easily crushed into a powder that may be used as a soil additive (WR^2, 2003).

Proteins—the major solid constituent of all animal cells and tissues—are degraded into salts of free amino acids. Some amino acids (e.g., arginine, asparagine, glutamine, and serine) are completely destroyed while others are racemized (i.e., structurally modified from a left-handed configuration to a mixture of left-handed and right-handed molecules). The temperature conditions and alkali concentrations of this process destroy the protein coats of viruses and the peptide bonds of prions (Taylor, 2001a). During alkaline hydrolysis, both lipids and nucleic acids are degraded.

Carbohydrates represent the cell and tissue constituents most slowly affected by alkaline hydrolysis. Both glycogen (in animals) and starch (in plants) are immediately solubilized; however, the actual breakdown of these polymers requires much longer treatment than is required for other polymers. Once broken down, the constituent monosaccharides (e.g., glucose, galactose, and mannose) are rapidly destroyed by the hot aqueous alkaline solution (WR^2, 2003). Significantly, large carbohydrate molecules such as cellulose are resistant to alkaline hydrolysis digestion. Items such as paper, string, undigested plant fibers, and wood shavings, although sterilized by the process, are not digestible by alkaline hydrolysis.

Alkaline hydrolysis is carried out in a tissue digester that consists of an insulated, steam-jacketed, stainless-steel pressure vessel with a lid that is manually or automatically clamped. The vessel contains a retainer basket for bone remnants and other materials (e.g., indigestible cellulose-based materials, latex, metal, etc.). The vessel is operated at up to 70 psig to achieve a processing temperature of 150°C (~300°F). According to WR^2, one individual can load and operate an alkaline hydrolysis unit. In addition to loading and operation, personnel resources must also be devoted to testing and monitoring of effluent (e.g., for temperature and pH) prior to release into the sanitary sewer system (Powers, 2003). Once loaded with carcasses, the system is activated by the push of a button and is thereafter computer-controlled. The weight of tissue in the vessel is determined by built-in load cells, a proportional amount of alkali and water is automatically added, and the vessel is sealed pressure-tight by way of an automatic valve. The contents are heated and continuously circulated by a fluid circulating system (WR^2, 2003).

The process releases no emissions into the atmosphere and results in only minor odor production. The end product is a sterile, coffee-colored, alkaline solution with a soap-like odor that can be released into a sanitary sewer in accordance with local and federal guidelines regarding pH and temperature (Kaye, 2003). This can require careful monitoring of temperature (to ensure release of the effluent at or above 190°C [374°F], a temperature below which the effluent solidifies), pH, and biochemical oxygen demand (BOD) (Powers, 2003).

The pH of undiluted hydrolyzate is normally between 10.3 and 11.5. For those sewer districts that have upper limits of pH 9 or 10, bubbling carbon dioxide into the hydrolyzate at the end of the digestion lowers the pH to the range of pH 8 or less (Kaye, 2003). As an example of the quantity of effluent generated by the process, WR^2 (2003) estimates that a unit of 4,000 lb capacity would generate approximately 1,250 gal (2,500 L) of undiluted hydrolyzate, and approximately 2,500 gal (9,466 L) of total effluent (including hydrolyzate, cooling water, rinse water, and coflush water).

The average BOD of undiluted hydrolyzate is approximately 70,000 mg/L. However, WR^2 indicates that in many instances the digester is located in a facility that releases in excess of 1,900,000 L (500,000 gal) per day, and, therefore, the added BOD is a fraction of the material being presented to the sewer district daily (Kaye, 2003). WR^2 also suggests that although the BOD is high, the carbon-containing molecules in the hydrolyzate have been broken down to single amino acids, small peptides, and fatty acids, all of which are nutrients for the microorganisms of sanitary treatment plants (Kaye, 2003). These aspects notwithstanding, disposal of effluent from alkaline hydrolysis units is a significant issue and must be so treated when considering this technology. In fact, some operators are contemplating alternative means of handling effluent, including solidification of effluent prior to disposal.

The total process time required for alkaline hydrolysis digestion of carcass material is three to eight hours, largely depending on the disease agent(s) of concern. For conventional (e.g., bacterial and viral) contaminated waste, four hours is sufficient. However, for material infected (or potentially infected) with a transmissible spongiform encephalopathy (TSE) agent, six hours is recommended (European Commission Scientific Steering Committee, 2002; European Commission Scientific Steering Committee, 2003). WR^2 notes that mobile-trailer units consisting of a digester vessel, boiler, and containment tank have a capacity of digesting 4,000 pounds of carcasses every 8 hours, or approximately 12,000 pounds (5,443 kg) in a 24-hour day. Others, however, note that loading and unloading of the digester can take time—as much as one hour in between processing cycles. Furthermore, temperature and pH monitoring of effluent takes time (Powers, 2003).

WR^2 estimates the cost of disposal of animal carcasses via alkaline hydrolysis at $0.02 to $0.03 per pound ($40 to $60/ton) of material (excluding capital and labor costs) (Wilson, 2003). Others have estimated the cost to be $0.16 per pound ($320/ton) including labor and sanitary sewer costs (Powers, 2003). WR^2's mobile trailer unit capable of digesting 4,000 pounds of carcasses every 8 hours has a capital cost of approximately $1.2 million (Wilson, 2003).

1.2 – Disease Agent Considerations

The alkaline hydrolysis process destroys all pathogens listed as index organisms by the State and Territorial Association on Alternative Treatment Technologies (STAATT I and STAATT II), which require a 6-log (99.9999%) reduction in vegetative agents and a 4-log (99.99%) reduction in spore-forming agents. Significantly, the alkaline hydrolysis process has been approved for the treatment of infectious waste in all states in which specific application for such approval has been made (Taylor, 2000; Taylor, 2001b).

The efficacy of alkaline hydrolysis was evaluated against pure cultures of selected infectious microorganisms during processing of animal carcasses in a digester at the Albany Medical College. The organisms tested included *Staphylococcus aureus, Mycobacterium fortuitum, Candida albicans, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus fumigatus, Mycobacterium bovis* BCG, MS-2 bacteriophage, and *Giardia muris.* Animal carcasses included pigs, sheep, rabbits, dogs, rats, mice, and guinea pigs. The tissue digester was operated at 110-120°C (230-248°F) and approximately 15 psig for 18 hours before the system was allowed to cool to 50°C (122°F), at which point samples were retrieved and submitted for microbial culture. The process completely destroyed all representative classes of potentially infectious agents as well as disposing of animal carcasses by solubilization and digestion (Kaye et al., 1998).

A study conducted at the Institute of Animal Health at the University of Edinburgh examined the capacity of alkaline hydrolysis to destroy bovine spongiform encephalopathy (BSE) prions grown in the brains of mice. Two mice heads were digested for three hours and one head for six hours. Samples of the hydrolyzate from each digestion were neutralized, diluted, and injected intracerebrally into naïve mice known to be susceptible to the effects of BSE. After two years, mice were sacrificed and their brains examined for signs of TSE. Evidence of TSE was found in the brains of some mice injected with hydrolyzate taken from three-hour-long digestions. Significantly, no evidence of TSE was found in the brains of mice injected with hydrolyzate from the six-hour-long digestion. The persistence of infectivity in the three-hour samples may have been due to the fact that material was introduced into the digestion vessel in a frozen state and was contained inside a polyethylene bag (i.e., the actual exposure of the prion-containing samples to the alkaline hydrolysis process may have been much less than 3 hours) (Taylor, 2001a). Based on these experiments, the European Commission Scientific Steering Committee has approved alkaline hydrolysis for TSE-infected material with the recommendation that TSE-infected material be digested for six hours (European Commission Scientific Steering Committee, 2002; European Commission Scientific Steering Committee, 2003). As a safety measure, one US-based facility disposing of CWD-infected carcasses uses an eight-hour-long digestion process to ensure destruction of any prion-contaminated material (Powers, 2003).

1.3 – Advantages & Disadvantages

Advantages of alkaline hydrolysis digestion of animal carcasses include the following:

- Combination of sterilization and digestion into one operation,
- Reduction of waste volume and weight by as much as 97 percent,
- Complete destruction of pathogens, including prions,
- Production of limited odor or public nuisances, and
- Elimination of radioactively contaminated tissues.

Disadvantages of alkaline hydrolysis process of animal carcass disposal include the following:

- At present, limited capacity for destruction of large volumes of carcasses in the US and
- Potential issues regarding disposal of effluent.

Section 2 – Historical Use

Alkaline hydrolysis technology has been and is currently being used in many institutions, laboratories, and animal disease diagnostic facilities to dispose of carcasses and other forms of biological waste. Table 1 below lists several sites where alkaline hydrolysis has been employed since 1993. Alkaline hydrolysis technology has not been adopted for large-scale, *catastrophic* carcass disposal events. Nevertheless, alkaline hydrolysis has been relied upon for carcass disposal related to small and large *managed* culls of animals infected with chronic wasting disease (CWD) and other transmissible spongiform encephalopathies (TSEs). One company—Waste Reduction by Waste Reduction, Inc. (WR^2)—reports that it currently has 30 to 40 alkaline hydrolysis digestion units in operation in the United States (US). Many of these units are used to dispose of CWD-infected deer carcasses (Grady, 2004).

TABLE 1. Biomedical research institutes, pharmaceutical companies, health care facilities, veterinary facilities, mortuaries, government agencies, and agricultural facilities that use alkaline hydrolysis processing for animal tissue disposal (Kaye, 2003).

Company	Installation Date	Use	Cycle Capacity	Operating Frequency
Albany Medical Center	Oct 1993	rodents, lagomorphs, sheep, pigs, goats	500 lbs.	1x/day
Allergan, Inc.	Jan 2001	rodents, lagomorphs	280 lbs.	1x/day
Biocon, Inc.	Oct 2002	rodents	~11 lbs.	2x/week
Colorado State University	Feb 2002	teaching hospital anatomic material and TSE-infected deer, elk, and sheep	2,000 lbs.	2x/day
Genentech, Inc.	Oct 2003	rodents, lagomorphs	280 lbs.	2x/week
Smithkline Beecham, Glaxo	Feb 1997	rodents, lagomorphs	600 lbs.	2x/week
Health Canada, Winnipeg	July 2000	rodents from TSE studies	30 lbs.	
Illinois Department of Agriculture	Feb 2003	livestock, roadkill, deer	2,000-3,000 lbs.	1x/day
Florida Division of Animal Industry	Mar 2003	necropsy tissue wastes	~11 lbs.	1x/day
Lexicon Genetics, Inc.	Jun 2002	rodents	80 lbs.	1x/day
Methodist Hospital	Mar 2001	pigs, sheep, human anatomic waste	280 lbs.	
Research Foundation for Mental Hygiene	Dec 2003	rodents from TSE studies	30 lbs.	3x/week
Sierra Biomedical, Inc.	May 2002	monkeys, bedding and food waste, animal waste	500 lbs.	1x/day
Immunex	Jun 2003	rodents, lagomorphs	80 lbs.	
South Dakota State University	Aug 2003	necropsy tissue wastes	~11 lbs.	1x/day
Humane Society of St. Joseph County, Inc.	Sep 2002	cats, dogs, euthanized animals	2,000-3,000 lbs.	1x/week
State University of New York, Binghamton	Jan 2002	rodents, lagomorphs, anatomic teaching wastes	80 lbs.	4x/week
Smithkline Beecham Pharmaceuticals, Rennes	Jul 1998	rodents (unit sold with plant when Glaxo divested SB labs)	80 lbs.	
Texas A&M Research Foundation	Aug 2002	livestock, horses	2,000-3,000 lbs.	1x/day
Tranxenogen, Inc.	Jul 2002	chicks	~11 lbs.	1x/day
Tulane University Medical Center	May 2003	monkeys	200 lbs.	1x/day
University of Florida	Apr 1998	horses, cattle, sheep, pigs, teaching hospital anatomic material	3,000 lbs.	1x/day
USDA-APHIS, Ames	Apr 2003	Belgian TSE-infected sheep, awaiting new building for reinstallation	7,000 lbs.	
USDA-ARS, Laramie	Jan 2000	being upgraded for new building	1,500 lbs.	
State of Wisconsin and USDA-APHIS	Nov 2003	undergoing acceptance tests, livestock, CWD-infected deer	4,000 lbs.	
WR2	(in stock)	demonstration unit for Europe, livestock, sheep, etc.	280 lbs.	
Seiko International-Obahiro University, University of Tokyo	Feb 2003	rodents from TSE studies	30 lbs.	
Institute for Animal Health, Edinburgh	Mar 2000	sheep heads doped with 301V BSE	30 lbs.	
Florida State Anatomical Board	Apr 1996	Human cadavers from medical education	1,000 lbs.	1x/day

Section 3 – Principles of Operation

3.1 – General Process Overview

A hydrolytic process

Hydrolysis is a process whereby chemical bonds are broken by the insertion of a water molecule. Hydrolysis can be catalyzed by enzymes, metal salts, acids, or bases. Alkaline hydrolysis relies upon bases—typically, water solutions of alkaline metal hydroxides such as sodium hydroxide or potassium hydroxide. Heat significantly accelerates hydrolytic processes; in this way, alkaline hydrolysis uses elevated temperatures (150°C, or ~300°F) to hasten the conversion of biological material (protein, nucleic acids, carbohydrates, lipids, etc.) into a sterile aqueous solution consisting of small peptides, amino acids, sugars, and soaps. The only solid byproducts of alkaline hydrolysis are the mineral constituents of the bones and teeth of vertebrates (WR^2, 2003).

Protein degradation

Alkaline hydrolysis ultimately leads to the degradation of proteins—the major solid constituent of all animal cells and tissues. Sodium or potassium salts of free amino acids are generated by the hydrolytic reaction, while oligopeptides (small chains of amino acids) are generated as reaction intermediates. Some amino acids (e.g., arginine, asparagine, glutamine, and serine) are completely destroyed while others are racemized (i.e., structurally modified from a left-handed configuration to a mixture of left-handed and right-handed molecules). Meanwhile, carbohydrate side chains are released from glycoproteins. The protein coats of viruses are destroyed and the peptide bonds of prions are broken courtesy of the temperature conditions and alkali concentrations used in the alkaline hydrolysis process (Taylor, 2001a).

Lipid degradation and the formation of "soaps"

Simple fats consist of three fatty acid chains bound through ester bonds to a molecule of glycerol. During alkaline hydrolysis, these ester bonds are hydrolyzed, yielding "soaps" (i.e., the sodium and potassium salts of fatty acids). Meanwhile, polyunsaturated fatty acids and carotenoids (pigments) undergo molecular rearrangements and are also destroyed by alkaline hydrolysis (WR^2, 2003).

Carbohydrate degradation

Carbohydrates are the cell and tissue constituents most slowly affected by alkaline hydrolysis. Both glycogen (the most common large polymer of glucose in animals) and starch (the most common large polymer of glucose in plants) are immediately solubilized. However, the actual breakdown of these polymers requires much longer treatment than is required for other polymers. Once broken down, the constituent monosaccharides (e.g., glucose, galactose, and mannose) are rapidly destroyed by the hot aqueous alkaline solution (WR^2, 2003).

Significantly, large carbohydrate molecules such as cellulose are resistant to alkaline hydrolysis digestion. Paper, string, undigested plant fibers and wood shavings are among the cellulose-based materials which may be associated with animal carcasses but which are not digestible by alkaline hydrolysis. However, these indigestible materials are completely sterilized by the alkaline hydrolysis process. They may be removed from the basket of the digester and disposed of as ordinary waste at a sanitary landfill.

Nucleic acid degradation

Nucleic acids (e.g., deoxyribonucleic acid, or DNA) are large, unbranched linear polymers held together by phosphodiester bonds. Like the ester bonds of lipids, nucleic acids' phosphodiester bonds are hydrolyzed by alkaline hydrolysis.

Undigested inorganic residue

Alkaline hydrolysis of animal tissues and carcasses yields an undigested residue—namely, the dry inorganic component of bones and teeth. This material typically constitutes approximately two percent of the original weight and volume of carcass material. It is sterile and easily crushed into a powder that may be used as a soil additive (WR[2], 2003).

3.2 – Operation, Resource, and Personnel Requirements

Alkaline hydrolysis is carried out in a tissue digester that consists of an insulated, steam-jacketed, stainless-steel pressure vessel with a lid that is manually or automatically clamped. An example digester is shown in Figure 1. The vessel contains a retainer basket for bone remnants and other materials (e.g., indigestible cellulose-based materials, latex, metal, etc.). The vessels are pressure-rated by the American Society of Mechanical Engineers to operate at 100 pounds per square inch, gauge (psig), but are operated at less than 70 psig to achieve a processing temperature of 150°C (~300°F).

FIGURE 1. Example alkaline hydrolysis tissue digester with 2,000 lb capacity (Powers, 2003).

According to WR[2], one individual can load and operate an alkaline hydrolysis unit. Once the digester vessel has been loaded with carcasses, the operating system is activated by the push of a button. The process is then computer-controlled. During the operation, a measured amount of alkali and water, proportional to the amount of tissue in the vessel, is automatically added. The concentration is calculated with tissue weight determined by built-in load cells. Water is added in an amount proportional to the tissue weight, and the vessel is sealed pressure-tight by way of an automatic valve. The contents are heated and continuously circulated. There are no moving parts inside the vessel; high-level agitation is provided in the fluid circulating system (WR[2], 2003). In addition to the requisite alkaline solutions and water, energy (for steam generation) and accommodation capacity (for emptying effluent) are necessary (Wilson, 2003).

In one facility, a necropsy technician who took an interest in alkaline hydrolysis technology has been sufficiently trained to operate the digestion unit. However, training other substitute personnel to operate the digestion unit would take considerable time. In addition to loading and operation, personnel resources must be devoted to testing and monitoring of effluent (e.g., for temperature and pH) prior to release into the sanitary sewer system (see related discussion in sections 3.5, 3.6, and 5) (Powers, 2003).

3.3 – Location Considerations

The largest alkaline hydrolysis unit currently available has a capacity of 10,000 pounds of biological material. The unit is eight feet in diameter and just over eight feet high. This unit requires a minimum room height of 24 feet; the actual footprint of the unit is 102 x 168 inches. Other digesters, with a capacity of 4,000 pounds, are mountable on mobile semi-trailers (Wilson, 2003).

As section 3.5 elaborates, alkaline hydrolysis units can give off a soapy odor. However, concerns about this odor are primarily limited to the period of time devoted to loading and unloading (Powers, 2003). Consequently, odor does not overly influence where digester units should or should not be placed.

3.4 – Time Considerations

The total process time required for alkaline hydrolysis digestion of carcass material is three to

six hours (see related discussion in section 4). The precise processing time largely depends on the disease agent(s) of concern. For conventional (e.g., bacterial and viral) contaminated waste, three hours is sufficient. However, for TSE-infected (or potentially TSE-infected) material, six hours may be preferred.

WR[2] notes that mobile-trailer units consisting of a digester vessel, boiler, and containment tank have a capacity of digesting 4,000 pounds of carcasses every 8 hours, or approximately 12,000 pounds (5,443 kg) in a 24-hour day. Others, however, note that loading and unloading of the digester can take time—as much as one hour in between processing cycles. Furthermore, temperature and pH monitoring of effluent takes time (Powers, 2003).

3.5 – Disposal of Effluent

Alkaline hydrolysis results in a sterile, coffee-colored, alkaline solution with a soap-like odor. This solution can be released into a sanitary sewer in accordance with local and federal guidelines regarding pH and temperature (Kaye, 2003). In at least one facility, this has demanded careful monitoring of temperature (to ensure release of the effluent at or above 190°C (374°F), a temperature below which the effluent solidifies), pH, and biochemical oxygen demand (BOD) (Powers, 2003).

The pH of the undiluted hydrolyzate is essentially that of a solution of the sodium or potassium salts of the amino acids and small peptides remaining after digestion. This is normally between pH 10.3 and 11.5. For those sewer districts that have upper limits of pH 10 or even pH 9, bubbling carbon dioxide into the hydrolyzate at the end of the digestion lowers the pH to the range of pH 8 or less. The advantage of using carbon dioxide to adjust the pH is that it will not overcompensate and drive the hydrolyzate into the acid range (Kaye, 2003). The estimated quantity of effluent generated from the process is shown in Table 2.

The average BOD in the undiluted hydrolyzate is approximately 70,000 mg/L. While this is a high BOD, WR[2] notes that the largest digester has a total undiluted hydrolyzate volume of 9,100 liters (2,400 gal); and, again according to WR[2], in many instances, the digester is located in a facility that releases in excess of 1,900,000 L (500,000 gal) per day so that the added BOD is a fraction of the material being presented to the sewer district daily (Kaye, 2003).

TABLE 2. Approximate volume of hydrolysate and total effluent produced per cycle from the alkaline hydrolysis process (WR[2], 2003).

Unit Capacity (lb/kg)	Hydrolysate (undiluted effluent) produced per cycle (gal / L)[a]	Total effluent (hydrolysate, cooling water, rinse water, and coflush water) (gal / L)[a]
500 / 227	160 / 606	320 / 1,212
1,500 / 680	440 / 1,666	960 / 3,635
2,000 / 907	580 / 2,196	1,160 / 4,392
4,000 / 1,814	1,250 / 4,733	2,500 / 9,466
8,000 / 3,629	2,500 / 9,466	5,000 / 18,931
10,000 / 4,536	3,150 / 11,927	6,300 / 23,853

[a]Assumes unit loaded at full capacity. Hydrolysate produced is a function of the amount of tissue being processed. For example, processing at half capacity would generate half the amount of coflush water and cooling water. Cooling water (which is approximately 25% of total water used) can be saved in an optional tank to be reused as processing water for the next cycle.

WR[2] indicates that although the BOD is high in the hydrolyzate, the carbon-containing molecules have already been broken down from the large protein and fat molecules to single amino acids, small peptides, and fatty acids; all of these are nutrients for the microorganisms of sanitary treatment plants. In fact, reportedly some sewer districts prefer to receive the hydrolyzate at night to keep the bacteria active so they are ready to go to work when the bolus of waste arrives first thing the following morning (Kaye, 2003).

Despite this technical information and the fact that effluent exudes very little odor (Powers, 2003), disposal of effluent from alkaline hydrolysis units is a significant issue and must be so treated when considering this technology. In fact, some operators are contemplating alternative means of handling effluent, including solidification of effluent prior to disposal (Powers, 2003).

3.6 – Cost Considerations

WR^2 estimates the cost of disposal of animal carcasses via alkaline hydrolysis at $0.02 to $0.03 per pound of carcass material ($40 to $60/ton of carcass material) (excluding capital and labor costs) (Wilson, 2003). Others experienced with alkaline hydrolysis have estimated $0.16 per pound ($320/ton), a cost estimate that has been broken down in Table 3.

TABLE 3. Cost estimates for operation of an alkaline hydrolysis tissue digester with 2,000 lb capacity (Powers, 2003).

Item	Cost ($ per lb of carcass material processed)
Steam, water, electricity	$0.01/lb.
Chemicals (NaOH, KOH)	$0.02/lb.
Personnel (4 hours/day for 2 cycles)	$0.04/lb.
Sanitary sewer costs	$0.07/lb.
Maintenance & repair	$0.02/lb.
Total	**$0.16/lb.**

WR^2's mobile trailer unit consisting of a digestion vessel, boiler, and containment tank costs approximately $1.2 million. This unit would be capable of digesting 4,000 pounds of carcasses every 8 hours, or approximately 12,000 pounds (5,443 kg) in a 24 hour day (6 tons/day) (Wilson, 2003).

3.7 – Other Considerations

At present, research is being conducted on systems that would combine the alkaline hydrolysis process with a shredder–steam sterilizer technology. Such a system would theoretically allow processing of up to 25,000 to 30,000 pounds of animal carcasses per hour (12 to 15 tons/hr) for disposing of large volumes of biological waste (Kaye, 2003).

Section 4 – Disease Agent Considerations

4.1 – Conventional Disease Agents

The alkaline hydrolysis process destroys all pathogens listed as index organisms by the State and Territorial Association on Alternative Treatment Technologies (STAATT I and STAATT II). These reports call for a system to be able to prove efficacy in the destruction of infectious agents by producing a 6-log (99.9999%) reduction in vegetative infectious agents and a 4-log (99.99%) reduction in spore-forming agents. Significantly, the alkaline hydrolysis process has been approved for the treatment of infectious waste in all states in which specific application for such approval has been made (Taylor, 2000; Taylor, 2001b).

The efficacy of alkaline hydrolysis has been evaluated by testing for the destruction of samples of pure cultures of selected infectious microorganisms during processing of animal carcasses in a digester at the Albany Medical College. The organisms tested included *Staphylococcus aureus, Mycobacterium fortuitum, Candida albicans, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus fumigatus, Mycobacterium bovis* BCG, MS-2 bacteriophage, and *Giardia muris.* Animal carcasses included pigs, sheep, rabbits, dogs, rats, mice, and guinea pigs. The tissue digester was operated at 110-120°C (230-248°F) and approximately 15 psig for 18 hours before the system was allowed to cool to 50°C (122°F), at which point samples were retrieved and submitted for microbial culture. None of the samples obtained yielded indicator bacteria or fungi. Even *Giardia* cysts were completely destroyed; only small

fragments of what appeared to be cyst wall material could be recognized with light microscopic examination. No plaque-forming units were detected with MS-2 bacteriophage after digestion. Furthermore, samples of the hydrolyzate did not yield growth on culture media. Animal carcasses were completely solubilized and digested, with only the inorganic components of the bones and teeth remaining after draining and rinsing of the digestion vessel. Alkaline hydrolysis completely destroyed all representative classes of potentially infectious agents as well as disposing of animal carcasses by solubilization and digestion (Kaye et al., 1998). The protein coats of viruses are destroyed and the peptide bonds of prions are broken under the extreme conditions of temperature and alkali concentration used in the alkaline hydrolysis process (Taylor, 2001a).

4.2 – TSE Disease Agents

A study, funded in 2000 by the United Kingdom Ministry of Agriculture, Fisheries and Food and carried out by Dr. Robert Somerville at the Institute of Animal Health at the University of Edinburgh, specifically examined the capacity of alkaline hydrolysis to destroy bovine spongiform encephalopathy (BSE) prions grown in the brains of mice. Two mice heads were digested for three hours and one head for six hours. Samples of the hydrolyzate from each digestion were neutralized, diluted, and injected intracerebrally into naïve mice known to be susceptible to the effects of BSE. The mice were kept for nearly two years, at which time they were sacrificed and their brains examined for signs of TSE. Evidence of TSE was found in the brains of 5 out of more than 200 mice; these five mice had been injected with hydrolyzate taken from three-hour-long digestions. Significantly, no evidence of TSE was found in the brains of mice injected with hydrolyzate from the six-hour-long digestion. The persistence of infectivity in the three-hour samples may have been due to the fact that material was introduced into the digestion vessel in a frozen state and was contained inside a polyethylene bag (i.e., the actual exposure of the prion-containing samples to the alkaline hydrolysis process may have been much less than 3 hours) (Taylor, 2001a). Based on these experiments, the European Commission Scientific Steering Committee has approved alkaline hydrolysis for TSE-infected material with the recommendation that TSE-infected material be digested for six hours (European Commission Scientific Steering Committee, 2002; European Commission Scientific Steering Committee, 2003). As a safety measure, one US-based facility disposing of CWD-infected carcasses uses an eight-hour-long digestion process to ensure destruction of any prion-contaminated material (Powers, 2003).

4.3 – Radioactivity

WR^2 reports that alkaline hydrolysis technology is effective in eliminating radioactively contaminated tissues.

Section 5 – Implications to the Environment

Alkaline hydrolysis releases no emissions into the atmosphere and results in only minor odor production. However, as alluded to in section 3.5, there are legitimate concerns about the temperature, pH, and BOD of the effluent produced by alkaline hydrolysis.

Section 6 – Advantages, Disadvantages, & Lessons Learned

6.1 – Advantages

Advantages of alkaline hydrolysis digestion of animal carcasses include the following:

- Combination of sterilization and digestion into one operation,
- Reduction of waste volume and weight by as much as 97 percent,
- Complete destruction of pathogens, including prions,
- Production of limited odor or public nuisances, and
- Elimination of radioactively contaminated tissues.

6.2 – Disadvantages

Disadvantages of alkaline hydrolysis process of animal carcass disposal include the following:

- At present, limited capacity for destruction of large volumes of carcasses in the US and
- Potential issues regarding disposal of effluent

6.3 – Lessons Learned

A common question facing animal disease regulators is whether to use alkaline-hydrolysis digestion or incineration to dispose of TSE-infected animals. While alkaline-hydrolysis digestion has been widely reported to be the most robust method for dealing with TSEs, fixed-facility incineration is also an effective means by which to dispose of TSE-infected material (see chapter regarding incineration). While high-temperature, fixed-facility incineration may be as effective as alkaline hydrolysis in destroying the prion agent, it is nonetheless laden with unique public-perception problems. This has been evident recently in Colorado, where state wildlife officials have been pushing for the construction of a fixed-facility incinerator to dispose of CWD-infected deer and elk heads. Despite the need, officials in Larimer County, Colorado, have heeded local, anti-incinerator sentiments and, for the moment, have successfully blocked approval of the incinerator. Meanwhile, the alkaline-hydrolysis digester at Colorado State University has generated fewer concerns. Throughout the debate, citizens assembled as the Northern Larimer County Alliance have voiced public health and wildlife concerns about the proposed incinerator—including concerns that the prion agent might actually be spread through the air by the fixed-facility incineration process (de Yoanna, 2003a, 2003b; Olander & Brusca, 2002), a contention that is highly questionable in light of an existing UK risk assessment (Spouge & Comer, 1997) and preliminary studies in the US demonstrating the low risk of TSE spread via fixed-facility incinerator emissions (Rau, 2003).

In Larimer County, Colorado, officials are most interested in recent deliberations by Region 8 of the Environmental Protection Agency whereby fixed-facility incineration might be more clearly endorsed as a technology for managing CWD-infected carcasses (O'Toole, 2003; Anonymous, 2003, p.4). According to Dr. Barb Powers of Colorado State University, more clear studies and regulatory rulings like this are needed to ensure adequate consideration of all available technologies by which to dispose of TSE-infected carcasses (Powers, 2003).

Section 7 – Critical Research Needs

1. Investigate environmentally suitable and publicly acceptable options for effluent disposal.
2. Investigate other uses for the alkaline hydrolysis effluent (e.g., as a form of fertilizer, as a nutrient

cocktail for improving sewage treatment plant performance, etc.)

3. Carry out engineering studies to ascertain how to use alkaline hydrolysis technology to accommodate large numbers of animal carcasses.

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