Evaluation of Microbial Inactivation by Caustic Digesters Using Embedded Test Samples

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ABSTRACT

INTRODUCTION – Caustic digestion is based on alkaline hydrolysis, which involves the use of sodium or potassium hydroxide to catalyze the hydrolysis of biological tissue materials at elevated temperatures and pressure. The studies presented are the first to most closely simulate actual digester operations by using biological indicators in stainless steel capsules embedded in adult large animal carcasses (horses and cattle). Previous tests of the use of digesters in treating carcasses potentially contaminated with pathogens involved suspending the indicators along the inner wall of the digester. The present investigations were conducted using Progressive Recovery Inc. (PRI) thermal caustic digesters with capacities of 80 lbs (CDU-80) and 500 lbs (CDU-500) per cycle.

METHODS – High concentrations of *Geobacillus stearothermophilus* spores (10e6) and *Mycobacterium terrae* vegetative cells (10e8) were seeded onto paper strips which were inserted into specially fabricated, sealed, stainless steel capsules. The latter were implanted into holes drilled into adult large animal heads or surgically inserted deep into the musculature, near the bones of larger portions of animal carcasses. Three separate test runs, each with a 3 hour residence time at 275°F, were conducted with each of the units. At the conclusion of each run, the biological indicator strips were removed from the capsules and packaged for shipment to an independent laboratory for quantitative evaluation.

RESULTS – Field controls, i.e., strips containing both types of indicators that were shipped untreated to and from the test site, yielded from log 4.57 to 5.48 viable spores and from log 7.23 to 7.67 *mycobacterial* vegetative cells. Greater than a 4.0 log reduction of viable *G. stearothermophilus* spores was observed with all 10e6 spore strips treated within the carcasses. Similarly, greater than a 6.0 log reduction was noted with all 10e8 treated *M. terrae* samples embedded in animal tissue and bone.

CONCLUSIONS – Since the stainless steel capsules prevented the exposure of the biological indicators to the caustic solution, the inactivation of the biological indicators is directly attributable to the heat and duration of exposure in the CDU-80 and CDU-500 PRI caustic digesters. These are the first tests to separate the treatment parameters responsible for microbial decontamination. In addition, these are the first in which indicators were implanted in animal carcasses to simulate digester operations.

INTRODUCTION

The principals of alkaline hydrolysis employed in caustic digesters dates back to the 19th century. The first reference, to the authors' knowledge, is contained in a patent (#394,982) issued to Amos Herbert in 1888, in which Herbert states:

My invention related to the treatment of bones and animal waste and the horns, hoofs, skins, cartilages, and meat of animals…If the bones be treated with from 5 to 10 per cent of their weight of caustic potash or its equivalent, the …matter of the bone is dissolve…especially if heated to or above boiling-point.

In the modern versions of Herbert's invention, sodium or potassium hydroxide catalyze the hydrolysis of animal carcasses, at an elevated temperature and pressure, into an aqueous solution consisting of small peptides, amino acids, sugars, and soaps which may be disposed of, if permitted by local ordinance, through the sanitary sewerage system. The only solid by-products are the mineral constituents (calcium phosphate) of the bones and/or teeth of the animals.

The objective of these investigations was to evaluate the efficacy of caustic digesters in reducing the bioload of potential pathogens associated with pathologic waste, i.e., portions of adult large animal carcasses (horses and cattle). Paper strips seeded with 10e6 *Geobacillus stearothermophilus* (ATCC 7953) spores and strips with 10e8 *Mycobacterium terrae* (ATCC 15755) dried vegetative cells were placed into specially fabricated stainless steel capsules. The latter were then implanted into holes drilled into adult large animal heads which were treated in a Progressive Recovery, Inc. (PRI) Thermal Caustic Digester (CDU) with an 80 lb capacity. To broaden the studies, similar capsules were surgically inserted deep into the musculature, near the bones of larger portions of animal carcasses which were processed in a PRI CDU with a 500 lb capacity.

Since the stainless steel capsules prevent the exposure of the biological indicators to the caustic solution, these are the first tests to assess heat as the sole operating condition responsible for microbial decontamination. In addition, by implanting the biological indicators within the animal carcasses, these are the first studies to be conducted under conditions that most closely simulate the digester operations.

METHODS

All *G. stearothermophilus* seeded strips were purchased from Sterilator Company, Inc. (Cuba, NY) and shipped directly to the test site. Analytical Services, Inc. (ASI, Williston, VT) prepared the *M. terrae* test strips, as well as provided the microbiological laboratory services for this study. In brief, the test isolate was grown on Middlebrook 7H11 agar plates at 35±1°C for 14-21 days, the plates washed twice with sterile phosphate buffered water, and the resulting cell suspension concentrated by centrifugation to obtain an inoculum solution of 1.0 x10e8 CFU/0.1ml. Sheets of colored construction paper were cut into pieces that measured approximately 7.5 by 1.0 cm and each piece inoculated at a rate of 0.1 ml concentrated stock. The inoculated strips were allowed to dry in a biological safety cabinet, individually packaged in separate glassine envelopes, which were stapled shut and then forwarded to the test site via FedEx Priority delivery.

At the test site, samples of both types of test strips were randomly selected to serve as Field Controls and were returned, untreated in plastic "baggies" to ASI. Two *Geobacillus* spore strips (10e6 spores per strip) were inserted into each of 2 stainless steel capsules, along with approximately 0.5 ml of water and the capsules sealed. Similarly, 2-10e8 strips with dried *mycobacterial* vegetative cells were placed into each of 2 steel capsules, along with the same volume of water and the capsules sealed. Two capsules with each type of biological indicator were then inserted into holes drilled into horse heads for the CDU-80 or surgically inserted deep into the musculature, near bones of larger portions of adult animal carcasses in the CDU-500. All *Geobacillus* strips were treated under two conditions, i.e., exposed either within or outside their glassine envelopes, whereas all strips seeded with *mycobacteria* vegetative cells were treated within glassine envelopes. At the conclusion of each of three test runs, treated samples were placed into plastic "baggies" for return shipment to ASI. All samples, including field controls, from each day of testing were shipped back to ASI by FedEx Priority delivery in the same insulated cooler with cold packs.

Upon receipt at ASI, *M. terrae* and *G. stearothermophilus* samples were initially eluted by vortexing in Middlebrook 7H9 broth (modified by the addition of PANTA; BD 8049759) and sterile DI water, respectively. *Geobacillus* field control samples were heat treated at 95-97°C for 15 minutes in sterile DI prior to plating. Serial dilutions of all *Geobacillus* samples were prepared and plated on Tryptic Soy Agar (TSA) in duplicate and incubated at 55 – 60° C for 7 days. *M. terrae* samples were prepared and plated onto M7H11 with oleic acid albumin dextrose complex in duplicate and incubated at 35 ± 0.5°C for 21 days.

The untreated samples served as positive controls. M7H9 broth with PANTA was diluted and plated at the highest and lowest dilutions to serve, along with sterile deionized water, as negative controls for *M. terrae* and *G. stearothermophilus*, respectively. Results for each biological indicator were calculated, and the log reduction (LR) achieved by treatment for each sample in each treatment run were calculated using the following equation:

$$LR = \text{Log}_{10}(\text{Untreated Field Control, in CFU/ml}) / (\text{Treated Sample, in CFU/ml})$$

All dilutions were plated in duplicate. Negative control samples were processed concurrently.

RESULTS: The results from these investigations are summarized in the following tables:

Geobacillus stearothermophilus **–Three Separate Tests[a]**

[a] Data represents the average of results obtained in three separate tests of each of the PRI units
[b] N/A = Not applicable

The following are the specific results with both CDU-80 and CDU-500 units from each of the test cycles involving *Geobacillus stearothermophilus*:

- Greater than 4.09 log reduction was noted on Day 1 as compared to Field Controls;
- Greater than 4.44 log reduction was noted on Day 2 as compared to Field Controls;
- Greater than 5.00 log reduction was noted on Day 3 as compared to Field Controls.

Therefore, greater than 4 log reductions of *G. stearothermophilus* was observed for all spore strip samples treated in both units and no *G. stearothermophilus* colonies were detected in any of the cultures prepared from the 24 treated samples.

Client ID / Description	Average CFU/strip	Average Log	Average Log Reduction
Field Control 10e6	8.91X10e4	4.95	N/A[b]
PRI Model CDU-80			
10e6 Spore Strip w/in Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/in Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/out Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/out Envelope	<3	<0.48	>4.51
PRI Model CDU-500			
10e6 Spore Strip w/in Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/in Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/out Envelope	<3	<0.48	>4.51
10e6 Spore Strip w/out Envelope	<3	<0.48	>4.51

Mycobacterium terrae **– Three Separate Tests [a]**

a Data represents the average of results obtained in three separate tests of each of the PRI units
b N/A = Not applicable

The following are the specific results with both CDU-80 and CDU-500 units from each of the test cycles involving *Mycobacterium terrae*:

- Greater than 6.82 log reduction was noted on Day 1 as compared to Field Controls;
- Greater than 7.13 log reduction was noted on Day 2 as compared to Field Controls with both CDU-80 and CDU-500 units. However, a 7.13 log reduction was found with one treated sample, while a 6.35 log reduction was noted with a second sample. A single plate in the dilution series prepared from each of these samples contained one or more *M. terrae* colonies. Given the results (>4 log reduction) obtained with the *Geobacillus* samples as part of the test run in the same unit, these colonies probably indicate contamination during sample handling and processing;
- Greater than 7.09 log reduction was noted on Day 3 as compared to Field Controls.

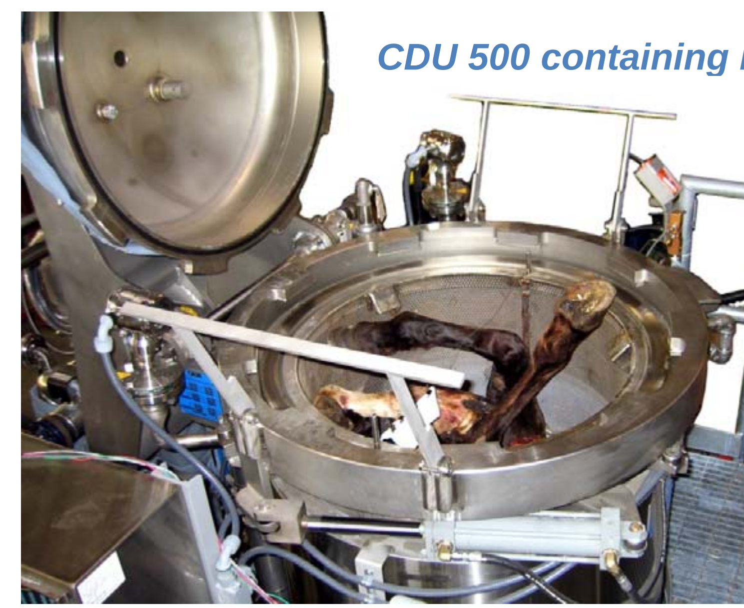
In summary, greater than 6.0 log reduction was observed in all 24 treated *M. terrae* samples.

Client ID / Description	Average CFU/strip	Average Log	Log Reduction
Field Control 10e8	3.25X10e7	7.50	N/A[b]
PRI Model CDU-80			
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
PRI Model CDU-500			
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00
10e8 Mycobacteria w/in Envelope	<3	<0.48	>7.00

CONCLUSIONS

While caustic digester vendors have conducted efficacy studies of their technologies to meet federal and/or state regulatory requirements, to the authors' knowledge there have been no previous published investigations of the decontamination capabilities of digesters. The data from our tests indicate that under the test conditions described, the Progressive Recovery, Inc. (PRI) Thermal Caustic Digester units (CDU-80 and CDU-500 model digesters) exceed the State and Territorial Association on Alternate Treatment Technology's performance criteria of 4 log reduction of viable *Geobacillus stearothermophilus* spores and 6 log reduction in the concentration of *Mycobacterium terrae* vegetative cells. However, results from other studies (unpublished – personal communication) have indicated that the digesters can cause a 6 log reduction of *G. stearothermophilus* spores.

Since the stainless steel capsules prevented the exposure of the biological indicators to the caustic solution, the inactivation of the biological indicators is directly attributable to the heat and duration of exposure in the CDU-80 and CDU-500 PRI units. The earlier unpublished tests conducted by manufacturers for regulatory purposes reviewed by the authors involved suspending the indicators either within or above the caustic solution. Therefore, these are the first tests to separate the effect of the two treatment parameters (heat and caustic) responsible for microbial decontamination and to document the effect of heat treatment alone. In addition, this is the first study in which indicators were implanted in animal carcasses to simulate digester operations.



CDU 500 containing horse and cow limbs.

Horse skull with steel capsule after digestion cycle.

PRI's Caustic Digester uses known technology and incorporates design refinements to: enhance the mechanics of dissolving the matter, reduce pH and odor, and facilitate the methods of disposal.

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