Alkaline hydrolysis of prion-positive materials for production of non-ruminant feed

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The sterilizing effects of autoclaving and alkaline hydrolysis are being evaluated as a single commercial process for destroying thermophilic bacterial spores and vegetative cells, and TSE prions in a safe and timely manner. An alkaline hydrolysis unit (Hydrol-Pro Technologies Inc., Zephyrhills, FL) located at the Colorado State University Veterinary Diagnostic Laboratory in Fort Collins, CO, is being utilized to hydrolyze samples with potassium hydroxide (KOH) and subjecting the mixture to 150°C heat at 4.14 Bars for 6 hours. Initially, five replicates of *Geobacillus stearothermophilus* (ATCC 10149) spore suspensions were evaluated under the chemical, environmental, and complete process conditions present in the alkaline hydrolysis process. Chemical conditions were investigated by incubating *G. stearothermophilus* spore suspensions with 50% (conc.) potassium hydroxide (KOH; pH 14) added aseptically at 8% of sample weight at room temperature (25°C) for an equivalent amount of time to the samples undergoing hydrolysis. Environmental conditions were evaluated by subjecting spore suspensions of *G. stearothermophilus*, sealed within self-contained stainless steel modules, to the hydrolysis vessel's heat and pressure. Complete process conditions were investigated by hydrolyzing *G. stearothermophilus* spore suspensions with KOH sealed within self-contained stainless steel modules. From start to finish each hydrolysis cycle lasted approximately 3 – 5 days. This included the heating, cooking, and cooling stages, which occurred over an approximate 24 hour period. The final process, the vacuuming stage, was responsible for consuming the remainder of the cycle time. This final process is responsible for reducing load weight to 30 – 35 % of its original weight by removing excess available water.

Samples exposed to the chemical conditions associated with the alkaline hydrolysis process (Table 1) demonstrated total detectable spore inactivation (<1.0 log CFU/ml detection limit). Samples exposed to the environmental conditions of the alkaline hydrolysis unit (Table 1) demonstrated total spore inactivation (<0.0 log CFU/ml detection limit). Combining both steps into a single complete process, all *G. stearothermophilus* spores were destroyed below the 1.0 log CFU/ml detection limit (Table 1).

Currently, additional validation research is being performed by investigating the efficaciousness of alkaline hydrolysis to destroy *Mycobacterium terrae* (ATCC 15755). Much like *G. stearothermophilus*, *M. terrae* is recognized in the scientific and medical industries as a valuable organism for validating stem sterilization. We are proposing to investigate the *M. terrae* validation in a manner similar to what was performed for the *G. stearothermophilus*.

Secondary testing will concentrate on hydrolyzing infectious CWD-positive deer lymph node (Bio-Rad TSE ELISA optical density of 200 mg lymph node = 1.600 or higher) and testing the hydrolysate for the presence of abnormal prion proteins with an identical Bio-Rad TSE ELISA diagnostic assay.

Lastly, a mouse bioassay has been initiated with the USDA-APHIS National Wildlife Research Center in Fort Collins, CO, to investigate the infectivity of rendered and hydrolyzed mouse-adapted scrapie brain. One hundred and twenty mice (C57Bl/6) were randomly divided into 4 groups. Each mouse was injected intraperitoneally with 500 µl of their designated inoculum. Group 1 received normal mouse brain to serve as the negative control. Group 2 was inoculated with untreated scrapie-positive mouse brain to serve as the positive control. Group 3 received hydrolyzed scrapie-positive mouse brain. Group 4 was inoculated with rendered scrapie-positive mouse brain to evaluate the efficacy of current rendering procedures to destroy prions. Three mice from each group will be euthanized at 2, 4, and 6 months after inoculation for examination. The remaining mice will be monitored for overt signs of disease and those showing symptoms will be euthanized to prevent undue suffering. Brain and spleen tissue will be collected from mice that die (from any cause) or are euthanized and analyzed using Bio-Rad TSE ELISA and Western Blot diagnostic assays for the presence of abnormal prion proteins ($PrP^{Sc)}$. Positive control mice are expected to show clinical signs of disease around 200-220 days after injection. Mice showing no signs of disease will be euthanized at 18 months after injection and analyzed for the presence of PrP^{Sc}. In addition, the relative feed value and composition of hydrolyzed carcass material will be investigated for its potential inclusion in non-ruminant animal feeds or other alternative uses.

Table 1. Initial and final log CFU/ml concentrations of *Geobacillus stearothermophilus* (ATCC 10149) culture suspensions before and after testing and enrichment results of treatment 3

Treatment	Replicate	Initial Concentration Log CFU/ml	Final Concentration Log CFU/ml	pH of Lowest Plated Dilution	Enrichment
	1	6.45	< 1.0*	7.8	-
Chemical	2	6.96	< 1.0	7.4	-
	3	7.00	< 1.0	7.7	-
	4	6.43	< 1.0	7.0	-
	5	6.30	< 1.0	7.2	-
	1	6.45	0.0	7.5	-
Environmental	2	6.96	0.0	7.3	-
	3	7.00	0.0	7.2	-
	4	6.43	0.0	8.0	-
	5	6.30	0.0	6.8	-
	1	6.45	< 1.0	7.8	Negative
Complete	2	6.96	< 1.0	7.0	Negative
Process	3	7.00	< 1.0	7.5	Negative
	4	6.43	< 1.0	6.8	Negative
	5	6.30	< 1.0	7.2	Negative

* The detection limit of the analysis was <1.0 log CFU/ml for treatments 1 and 3 because they had to be neutralized in double strength D/E Neutralizing Broth. The detection limit for treatment 2 was <0.0 log CFU/ml because it did not require a neutralization step.