Prions Inactivation Through Composting Process

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Agriculture and Agri-Food Canada

Compost Matters in Alberta
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Edmonton Alberta
- Scrapie – Sheep and goats
- CWD – Deer and elk
- BSE – Cattle
- CJD – Humans
CWD and BSE in Canada

- 19 BSE cases in Canada
- 17 cases – typical BSE (Feed contamination)
- 2 cases – atypical BSE (Spontaneous mutation)
- 173 CWD cases in Alberta
- Economic losses
  1. Cattle industry – $6 billion
  2. Cervid industry – $43 million
Specified Risk Materials

- Tonsils
- Dorsal root ganglia
- Vertebral column
- Spinal cord
- Distal ileum
- Skull
- Brain
- Eyes
- Tonsils
- Trigeminal ganglia
Specified Risk Materials

- 250,000 tonnes SRM in Canada
- 74,000 tonnes SRM in Alberta
- 50,000 cervid carcasses from farms and road kills
- SRM are rendered, dehydrated and disposed of in landfills
- $300/tonne cost for disposal
Methods for Prion Inactivation

- **Alkaline or thermal hydrolysis**
  - pH 14 and 150-180°C at 4-12 atmospheric pressure for 1-3 h

- **Incineration**
  - Two stages at 850°C and 1000°C for 16 h

- **Sodium hydroxide**
  - 2 N for 1 h

- **Autoclaving**
  - 134°C for 1 h in saturated steam
<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Source microbes</th>
<th>Enzyme reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp. (°C)</td>
</tr>
<tr>
<td>Keratinase</td>
<td>Bacillus licheniformis</td>
<td>50</td>
</tr>
<tr>
<td>Alcalase</td>
<td>Bacillus licheniformis</td>
<td>35</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>Tritirachium album</td>
<td>50</td>
</tr>
<tr>
<td>Properase, protease M</td>
<td>Bacillus spp.</td>
<td>60</td>
</tr>
<tr>
<td>Alkaline proteinase</td>
<td>Streptomyces sp.</td>
<td>60</td>
</tr>
<tr>
<td>Protease E</td>
<td>Thermus sp.</td>
<td>80</td>
</tr>
<tr>
<td>Keratinolytic protease</td>
<td>Nocardiopsis sp.</td>
<td>60</td>
</tr>
</tbody>
</table>

Susuki et al. (2006)
- **Lab-Scale Composting**
  - Biocontainment laboratory level 3
  - Approx. 40 kg
  - Composted for 28 days; Two 14 day cycles
  - SRM; Scrapie 263K, CWD, BSE (WB & PMCA)

- **Field-Scale Composting**
  - Approx. 85,000 kg;
  - Composted for 230 days
  - 16 cattle mortalities; SRM; Scrapie 263K (Bioassay)
Laboratory Compost

Lethbridge Research Centre (AAFC)

Level 3 laboratory (CFIA)
Laboratory Compost - Sampling

- Polyester twine
- Mesh bag
- Nylon bag
- SRM or manure sphere
- Compost materials

1 ml 10% TSE BH

1 g dry manure sphere
Methods for Prion Detection

- Western blot (WB)
- Protein misfolding cyclic amplification (PMCA)

Colby and Prusiner (2011)
All dimensions in cm
Baker Retrieval Pyramid

- Scrapie Beads
- 263K Beads
- PVDF Low
- PVDF High
- Temperature Probe
- Backbone With Scrapie Beads
- Chain attachment
- Grated Base
Binding to Stainless Steel Beads

- 440C Stainless Steel; Hamster 263K
- Mass: ~0.5 mg; Diameter: 0.5 mm
- Surface area: 0.8 mm\(^2\); Density: 7.68 g cm\(^{-3}\)
- Syrian hamster model – IC implant

Clean beads

Beads coated with brain homogenate
Temperature

- Lab-scale compost
- Field-scale compost

![Temperature graphs for Lab-scale and Field-scale composting](image)
SRM Degradation

- Lab-scale
- Field-scale
Microbial Invasion Brain

Day 7 Brain

Day 14 Brain
Bacterial Characterization

Rubrobacter radiotolerans (U65647)
Actinomycetospora succinea (AB514514)
F-7-3 (JN596831)
Saccharomonospora viridis (AB588632)
C-7-1 (JN596829)
F-14-6 (JN596834)
F-7-4 (JN596832)
C-7-2 (JN596830)
Thermobifida fusca (AB562472)
F-21-10 (JN596838)
Actinomadura sp. (FN178409)
F-28-12 (JN596840)
Actinomadura hallensis (AM932278)
F-14-7 (JN596835)
Thermoactinomycetaceae bacterium (AB362280)
Thiohalospira alkaliphila (EU169227)
C-14-5 (JN596833)
C-21-9 (JN596837)
C-28-11 (JN596839)
Pseudomonas pertucinogena (EF673695)
C-21-8 (JN596836)
Enterobacter sp. (HM461153)
F-28-13 (JN596841)

Keratinase activity
Saccharomonospora viridis (Al-Zarban, 2002)
Actinomadura (Puhl et al., 2009)
Fungal Characterization

Lipase activity
*Trichaptum*
Goud et al. (2009)

Proteinase activity
*Chaetomium*
Breuil and Huang (1994)

Proteinase activity
*Cladosporium*
Li and Li (2009)
Lab-scale compost – WB

(a) % Dilution

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1.3</th>
<th>0.6</th>
<th>0.3</th>
<th>0.2</th>
<th>0.1</th>
<th>0.04</th>
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30 kDa

20 kDa

(b) Day 0 Day 14 Day 28 Day 2

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>IBH</th>
<th>C1</th>
<th>C2</th>
<th>F1</th>
<th>F2</th>
<th>C1</th>
<th>C2</th>
<th>F1</th>
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Scrapie 263K Degradation

2 $\log_{10}$
Scrapie 263K Degradation

- Lab-scale compost – PMCA

Day 0 VS Day 14

Day 0 Detected at 6 log
Day 14 Control Detected at 5 log
Day 14 Feather Detected at 4 log
Lab-scale compost – PMCA

Scrapie 263K Degradation

Day 0 VS Day 28

Day 0 Detected at 7 log

Day 28 Control Detected at 5 log

Day 28 Feather Detected at 5 log
## Steel Bead IC implant

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Clinical DPI (mean ± SD)</th>
<th>No. of sick hamsters/total no. of hamsters</th>
</tr>
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<tbody>
<tr>
<td>10^-1</td>
<td>107 ± 2</td>
<td>3/3</td>
</tr>
<tr>
<td>10^-2</td>
<td>119 ± 6</td>
<td>5/5</td>
</tr>
<tr>
<td>10^-3</td>
<td>109 ± 4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^-4</td>
<td>133 ± 10</td>
<td>4/4</td>
</tr>
<tr>
<td>10^-5</td>
<td>196 ± 38</td>
<td>3/4</td>
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<tr>
<td>10^-6</td>
<td>179 ± 32</td>
<td>3/5</td>
</tr>
<tr>
<td>10^-7</td>
<td>&gt;330</td>
<td>0/5</td>
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<tr>
<td>10^-8</td>
<td>&gt;330</td>
<td>0/4</td>
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Infectivity titre: $10^6$ ID50 / bead = 100 ng brain tissue (Reed and Muench 1938)
**Scrapie 263K Degradation**

- **Field-scale compost**

  - **Compost Day 0**: 11/11 clinical disease at 103 ± 3 dpi
  - **Compost Day 112**: 2/5 clinical disease at 123 ± 0 dpi
  - **Compost Day 230**: 1/5 clinical disease at 252 ± 0 dpi
## Summary - Scrapie 263K

<table>
<thead>
<tr>
<th>Lab-scale compost</th>
<th>Field-scale compost</th>
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<tbody>
<tr>
<td>Total 28 days</td>
<td>Total 230 days</td>
</tr>
<tr>
<td>≥ 55°C for 3 days</td>
<td>≥ 55°C for 75 days</td>
</tr>
<tr>
<td>Feather &gt; Control after 14 days</td>
<td>Effective to reduce infectivity</td>
</tr>
<tr>
<td>Approx. 2 log after 28 days</td>
<td>4.8 log* after 230 days</td>
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*Four-parameter logistic regression model (SAS)*
CWD Degradation

- Lab-scale compost – WB

(a)

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<td>% Dilution</td>
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30 kDa ➔

20 kDa ➔

(b)

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30 kDa ➔

20 kDa ➔

1-2 log$_{10}$
CWD Degradation

- Lab-scale compost – PMCA

Day 0 VS Day 14

Day 0 Detected at 9 log
Day 14 Control Detected at 8 log
Day 14 Feather Detected at 8 log
**CWD Degradation**

- **Lab-scale compost – PMCA**

Day 0 VS Day 28

Day 0 Detected at 9 log

Day 28 Control Detected at 6 log

Day 28 Feather Detected at <5 log
**Lab-scale compost – WB**

(a) Lab-scale compost – WB

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(b) Lab-scale compost – WB

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1 log₁₀
Composting degrades:

- SRM completely
- Prions at the logarithmic level
- CWD elk at field-scale after 230 days?
- BSE at lab-scale after 25 cycles (=230 days of field-scale composting)?
Brandon Gilroyed – University of Guelph
Tim Reuter – Alberta Agriculture
Norman Neumann, Mike Belosevic, Jerry Leonard – University of Alberta
Aru Balachadran, Stefanie Czub, Catherine Graham – Canadian Food Inspection Agency
APRI, AAFC, NSERC
Way to go. . .

Approved

COMPOSTING