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# Investigating Process Conditions and Product Quality in the Recycling of Used Horse Bedding

**May 12, 2014**

## 1. Introduction

GreenScene Agritek Inc. (GSA) has developed a patented proprietary technology that recycles horse bedding to produce dried, shredded wood fibers. High temperature drying is able to render the pathogens non-viable. The GSA pre-commercial pilot plant in Delta, BC can process a maximum of 5 wet tonnes (2.5 dry tonnes) of used horse bedding per hour. Typically, used horse bedding in equestrian facilities in BC consists of about 80-90 percent wood shavings or wood pellets, and the remaining 10-20 percent as hay, grass and manure as organics. After the refinement, pasteurization and drying processes, the woody components are reclaimed and reused as a recycled bedding product, and the remaining fecal matter organics and small unusable wood fibres are separated and removed as fines.

Horses are allergic to organic particulate matter such as bedding dusts and forage-residing fungi and mold spores, resulting in chronic or acute respiratory and pulmonary diseases and other types of disorders. Moreover, bacteria and viruses are the causes of diseases, examples being herpes, strangles, botulism, diarrhea and neurological disorders that are of concern for horse owners.

In 2013, the Department of Chemical and Biological Engineering at the University of British Columbia (Vancouver, BC) conducted a research project with an aim to verify the ability and effectiveness of GSA's production process in exposing the bulk of the used bedding materials to the time-temperature conditions required for the eradication of human and horse pathogens.

## 2. Methodology and Procedure

### 2.1 Sampling and Measurement

The degree of thermal inactivation of pathogens is primarily a function of temperature and time. During the study, samples of used horse bedding (raw materials) and finished product in the form of dried wood fibers from the pilot plant have been regularly collected and sent to accredited laboratories for analysis and test results.

**2.2 Analysis**

Microbiological analysis was performed by the IG MicroMed Environmental Lab (Vancouver, BC, Canada), the Silliker JR Laboratories (Burnaby, BC) and the RES-Silliker Lab (Chicago, IL, USA) (Silliker is a Mérieux Nutrisciences Company) for the raw materials and the finished product, using standard procedures that are approved by Health Canada and FDA USA.

The microbial analysis includes the following: Fecal coliforms, *E. Coli*, *Salmonella*, *Staphylococcus, Clostridium perfringens*, *Clostridium botulinum*, mold and yeast. In general, fecal coliform is an indicator organism for testing the end product. *Clostridium botulinum,* which produces the lethal botulinum toxin and causes the botulism disease in humans and animals*,* is also a frequently used indicator organism in the sterilization of food material via thermal processing. The absence or low counts of these bacteria would indicate a decontamination of the product.

Multimycotoxins screen tests were also performed at the Silliker JR Laboratories. Mycotoxins are toxic secondary metabolites produced by fungi (commonly known as molds). They are produced as a result of fungal infection of crops that may be used as livestock feed. Mycotoxins might not be broken down during digestion. In general, one mold species may produce different mycotoxins, and it is possible that the same mycotoxin may originate from several species. The appendix to this report contains a list of mycotoxins tested.

**3. Results and Discussion**

Based on the measurements and record of the temperature of the hot air stream in the triple-pass dryer, we estimated that the bulk of the materials could achieve a temperature of 110-120$^{o}$C. Furthermore, the retention time of the particles in the dryer was estimated to be 2-3 minutes. These values are compatible with typical configuration and characteristics of a rotary dryer as well as the physical properties of the materials, which include the drum dimensions, drum speed, solids feed rate, air flowrate, feed moisture content, and particle size. The observed time-temperature relationship is deemed to be effective for pathogen inactivation.

Microbiological analysis results indicated that the following bacteria, mold and virus were consistently at an acceptable low level in the samples of the final dried product:

| | |
|---|---|
| Fecal coliforms | < 5-15 MPN*/g (gram sample) |
| *E. Coli* | < 3-15 MPN/g |
| *Salmonella* | negative/25 g |
| *Staphylococcus* | < 10-25 CFU/g |
| *Clostridium perfringens* | < 5-100 CFU/g |
| *Clostridium botulinum* | negative/8g |
| Mold | < 20 CFU/g |
| Yeast | < 10 CFU/g |
| Equine Herpes Types 1 and 4 | negative |
| Influenza | negative |
| * MPN: most probable number | CFU: colony forming units |

Since the outlet setpoint temperature was well maintained by the burner, the feed having moisture contents of 45-50% (wet basis) was evenly and consistently dried to a typical moisture content of 10-11% (wet basis) for the final product. At this moisture content of the final dried product, a level of fecal coliforms at 5 MPN/g sample is equivalent to 50 MPN/g sample. Although GSA's process does not involve composting, it may be useful to make reference to the CCME (Canadian Council of Ministers of the Environment) guidelines for compost quality. The value of 50 MPN/g sample is much lower than the 1000 MPN/g threshold criteria for Class A (unrestricted use) compost product derived from organic wastes which include manure.

Each type of fungi have their own ideal temperature and moisture level where they grow best, but none can grow well at low moisture levels of less than 15% (wet basis). Results show that the concentrations of a wide range of mycotoxins were below 200 ppb (parts per billion). The results have been validated and confirmed with "spike tests" on the samples.

Raw test results from the microbial and multimycotoxins analyses are also placed in the appendix to this report.

**4. Conclusions**

The results suggest that GSA's process is able to produce the required time and temperature to achieve effective elimination of human and horse pathogens. Hence, the dried and recycled horse bedding product may be deemed safe for both human and animals.

**Prepared by:**
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Chemical and Biological Engineering, University of British Columbia

# Appendix

## Mycotoxin screen test results

SILLIKER
a Mérieux NutriSciences Company

**Silliker, JR Laboratories**
12-3871 North Fraser Way, Burnaby, BC V5J 5G6
Tel. 604/ 432 9311 Fax. 604/ 432 7768

**CERTIFICATE OF ANALYSIS**

| | |
|---|---|
| **COA No:** | BRN-36544663-0 |
| **Supersedes:** | BRN-36477859-0 |
| **COA Date** | 10/24/13 |
| **Page 2 of 4** | |

**TO:**
Mr. Paul Cross
General Manager
GreenScene Agritek Inc.
40874 Yale Road West
Chilliwack, BC V2R 4J2

| | |
|---|---|
| **Received From:** | Chilliwack, BC |
| **Received Date:** | 9/9/13 |

**Location of Test: (except where noted)**
Burnaby, BC

### Analytical Results

**Desc. 1:** Sample 2
**Desc. 2:** Not heated

**Laboratory ID:** 341480573
**Condition Rec'd:** NORMAL
**Temp Rec'd (°C):** 23.8

| Analyte | Result | Units | Method Reference | Test Date | Loc. |
|---|---|---|---|---|---|
| Multimycotoxins Screen | | | BFCL-047, CFIA BLMM | 9/27/13 | |
| AcDON | <50 | ppb (w/w) | | | |
| Aflatoxin B1 | <3 | ppb (w/w) | | | |
| Aflatoxin B2 | <3 | ppb (w/w) | | | |
| Aflatoxin G1 | <3 | ppb (w/w) | | | |
| Aflatoxin G2 | <4 | ppb (w/w) | | | |
| Cyclopiazonic acid | <2 | ppb (w/w) | | | |
| Deoxynivalenol (DON) | <20 | ppb (w/w) | | | |
| Diacetoxyscirpenol | <10 | ppb (w/w) | | | |
| Ergocristine | <15 | ppb (w/w) | | | |
| Ergocryptine | <15 | ppb (w/w) | | | |
| Ergosine | <15 | ppb (w/w) | | | |
| Fumonisins B1 | <15 | ppb (w/w) | | | |
| Fumonisins B2 | <15 | ppb (w/w) | | | |
| Fumonisins B3 | <15 | ppb (w/w) | | | |
| Fusarenone-X | <20 | ppb (w/w) | | | |
| HT-2 toxin | <17 | ppb (w/w) | | | |
| Neosolaniol | <10 | ppb (w/w) | | | |
| Nivalenol | <60 | ppb (w/w) | | | |
| Ochratoxin A | <3 | ppb (w/w) | | | |
| Sterigmatocystin | <3 | ppb (w/w) | | | |
| T-2 toxin | <15 | ppb (w/w) | | | |
| alpha-Zearalenol | <20 | ppb (w/w) | | | |
| beta-Zearalenol | <20 | ppb (w/w) | | | |
| Zearalenone | <20 | ppb (w/w) | | | |

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## Microbiological analysis results

### Analytical Results

| | | | |
|---|---|---|---|
| **Desc. 1:** | Recycled Bedding | **Laboratory ID:** | 344928372 |
| **Desc. 2:** | Sample 6 | **Condition Rec'd:** | NORMAL |
| **Desc. 3:** | Date: 12/30/2013 | **Temp Rec'd (°C):** | 20 |
| **Desc. 4:** | Temp IN: 556; Temp OUT: 190, Time 14:02 | | |
| **Desc. 5:** | Dryer HZ 40, Blower HZ 60 | | |
| **Desc. 6:** | Moisture 10.35% | | |
| : | Non heated | | |

| Analyte | Result | Units | Method Reference | Test Date | Loc. |
|---|---|---|---|---|---|
| Clostridium perfringens | <100 | CFU/g | MFHPB 23 | 3/26/14 | MRK |
| E. coli - 3 tube MPN | <3 | MPN/g | Modified MFHPB-19 | 3/22/14 | |
| Faecal coliforms | <3 | MPN/g | Modified MFHPB-19 | 3/22/14 | |
| Salmonella | Negative | /25g | MFHPB 20 | 3/24/14 | |
| Staphylococcus aureus | <10 | CFU/g | MFHPB 21 | 3/22/14 | |
| Yeast and Mold | | | MFHPB 22 | 3/25/14 | |
| Yeast | <10 | CFU/g | | | |
| Mold | 20 est. | CFU/g | | | |

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**Microbiological analysis results**

# IG MicroMed Environmental Inc.

190 - 12860 Clarke Place, Richmond, B.C. V6V 2H1
Tel: (604) 279-0666 Fax: (604) 279-0663

## CERTIFICATE OF ANALYSIS

Attn: Anthony Lau, Ph.D., P. Eng.
Associate Professor
UBC – Chemical & Biological Engineering
2360 East Mall
Vancouver, B.C.
V6T 1Z3

31 January, 2013

Reference No: 253900

These are the results of the samples received January 20.

Product Sampled: Two dirt samples were received in the laboratory for analysis.

| Tests Performed: | Sample # 1 RAW (dirt) | Sample # 2 FINISHED (Product) |
|---|---|---|
| Total Coliforms: | ≥2.4 x $10^5$ | 14 |
| Fecal Coliforms: | ≥2.4 x $10^5$ | 14 |
| Escherichia coli: | ≥2.4 x $10^5$ | 14 |
| Salmonella species: | Negative | Negative |
| Staphylococcus aureus: | <25 | <25 |
| Clostridium perfringens: | <5 | <5 |
| Yeast: | 60 | <5 |
| Mold: | 40 | <5 |

All results are for CFU per gram, per gram, for 25 grams or for MPN per gram of sample (as is).