Preliminary Assessment of the Antimicrobial Properties of Bio-Screen C000C

Performed by NSF International Microbiology Laboratory for The Toxicology Group LLC

Client:	H.B.A. Sa.
Product:	Bio-Screen C000C
Date of Experimentation:	April 2, 2003
Test Organism:	*Stachybotrys chartarum*
ATCC Strain:	16275
Product Percentage:	1, 5, 10, 25%

BACKGROUND

The Client contracted the Toxicology Group, LLC to perform an initial assessment of the antimicrobial properties of their product Bio-Screen C000C. *Stachybotrys chartarum* ATCC 16275 was selected as a challenge organism. *S. chartarum* is a common airborne mold contaminant. It is strongly cellulolytic and has been found on paper, seeds, solid and textiles. It has been implicated in the damage of building materials and also has suspected health effects due to its production of toxic metabolites.

METHODOLOGY

A. Stock Culture Preparations:

The fungal challenge strain was reconstituted in potato dextrose broth and passed 3 times. All culture tubes were incubated at 22°C for 72 hours. Three Sabouraud Dextrose Agar (SDA) were inoculated with 0.5 mL of the third culture pass. The plates were incubated at 22°C for 72 hours. A spore suspension was prepared from the plates according to the methods outlined in AOAC 955.17. An approximate cell density of the suspension was obtained via direct counts using a hemocytometer and bright field microscopy.

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B. Protocol:
Five 250 mL sterile Erlenmeyer flasks containing 100 mL of SBDW were spiked with the challenge organism to achieve a minimum final concentration of 1 x 10^4 CFU/mL. The following product amounts were amended to individual test flasks: 1 g, 5 g, 10 g, and 25 g. An inoculum control containing no product was also setup. All flasks were placed on a rotary shaker set at 50 rpm at 25°C. 1 mL from each flask was aseptically removed at the following time points: 0 hours, 4 hours, and 24 hours and transferred to 1 mL of a neutralizer solution consisting of lecithin, Tween 80 and phosphate buffer. Serial dilutions were carried out in SBDW. 0.1 or 1 mL aliquots were pour-plated using Sabouraud Dextrose Agar (SDA). The plates were incubated at 22°C for 5-7 days prior to enumeration.

RESULTS

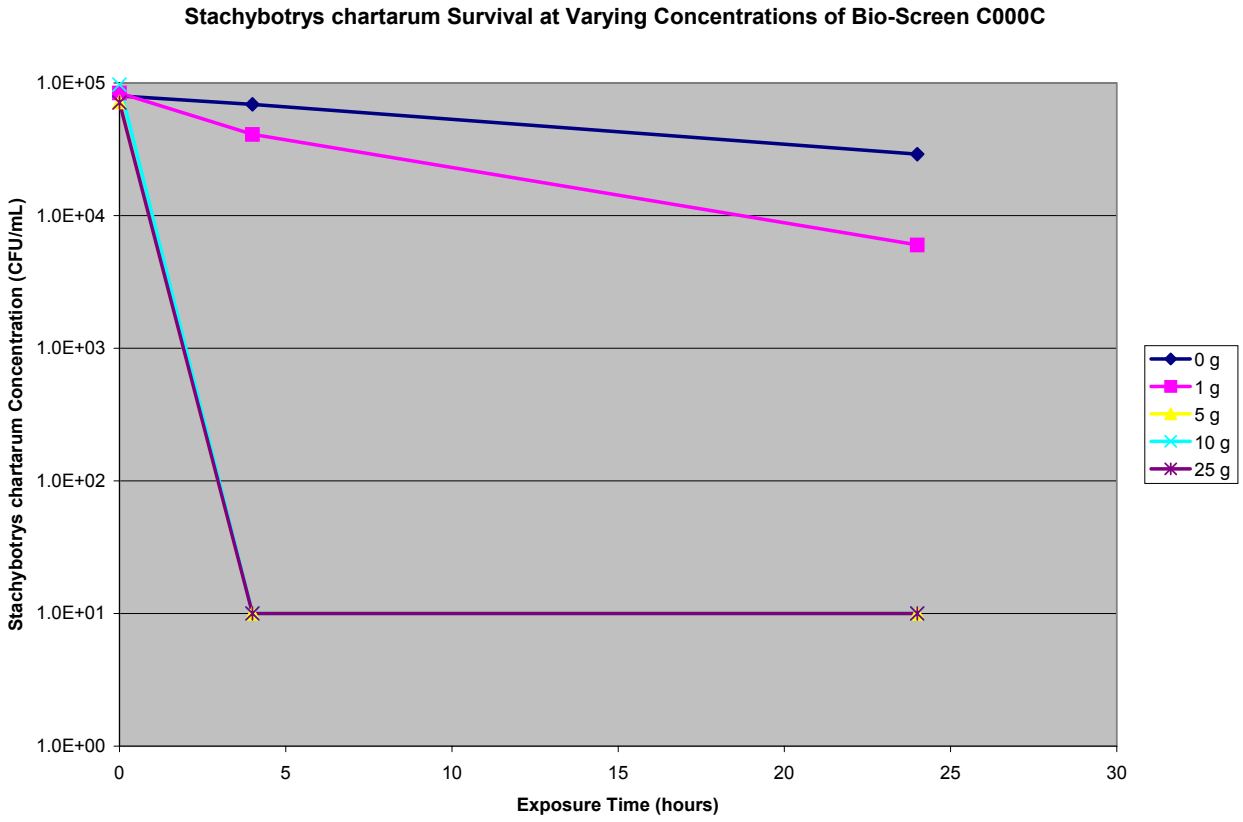
Table 1. Survivability of *S. chartarum*. Exposure time is provided in hours. The grams of Bio-Screen C000C added to 100 mL of the SBDW are provided. Microorganism concentrations are provided in Colony Forming Units per milliliter (CFU/mL). The results are means of three replicates.

Exposure Time	0 g	1 g	5 g	10 g	25 g
0	8.0×10^4	8.4×10^4	7.2×10^4	9.7×10^4	7.1×10^4
4	6.9×10^4	4.1×10^4	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
24	2.9×10^4	6.0×10^3	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$

Table 2. Log Reduction of *S. chartarum*. Exposure time is provided in hours. The grams of Bio-Screen C000C added to 100 mL of the SBDW are provided. The results are means of three replicates.

Exposure Time	1 g	5 g	10 g	25 g
4	0.3	3.9	4.0	3.9
24	1.1	3.9	4.0	3.9

Figure 1. Survival of *S. chartarum* at varying concentrations of Bio-Screen C000C. The limit of detection is 10 CFU/mL.



SUMMARY

As is evident, the 5, 10 and 25 percentages of Bio-Screen C000C resulted in greater than a 3.9 log inactivation of *S. chartarum* within 4 hours. 1 percent did not result in a significant inactivation of the challenge organism

Laboratory Supervisor:_____Date:_____