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 1, 2003
Test Organism:	*Penicillium oxalicum*
ATCC Strain:	1126
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. *Penicillium oxalicum* ATCC 1126 was selected as a challenge organism. Members of the Genus *Penicillium* are common airborne mold contaminant. Various species of this genera have been implicated in the damage of building materials.

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 Potato Dextrose Agar plates (PDA) 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.

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

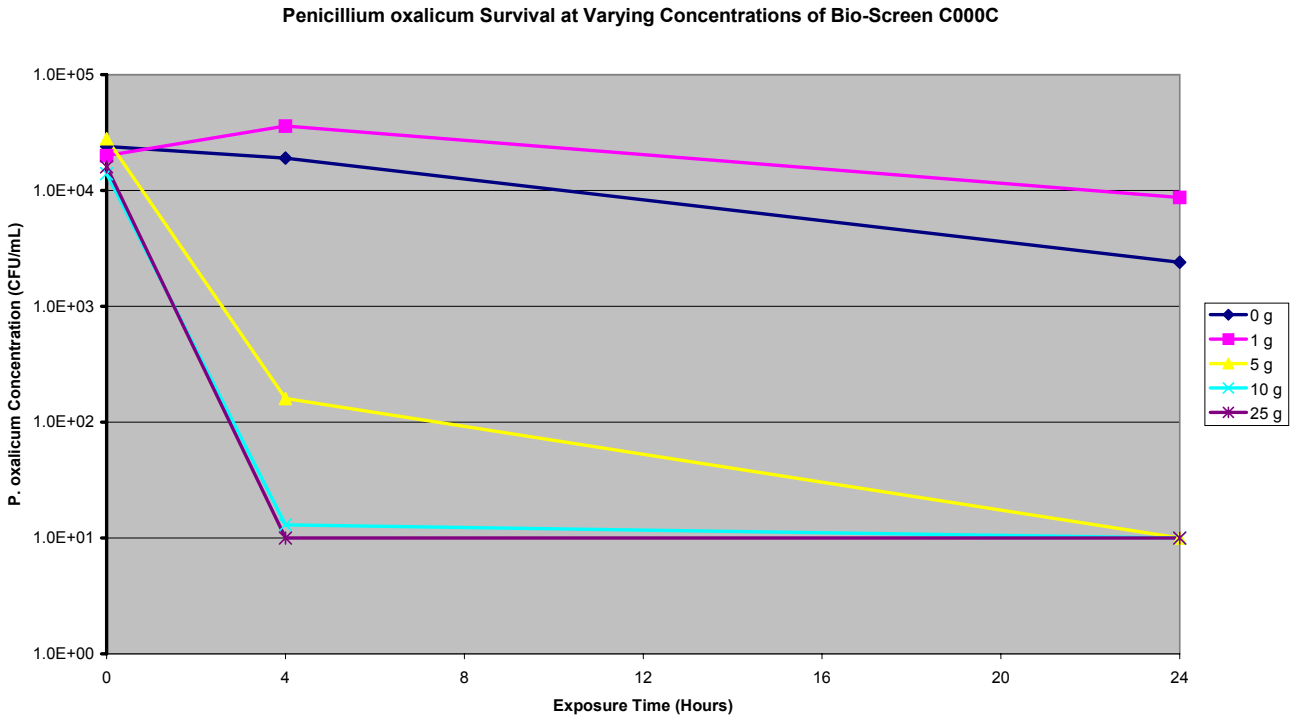
Table 1. Survivability of *P. oxalicum*. Exposure time is provided in hours. The grams of Bio-Screen C000C added to 100 mL of the SBDW are provided. Microorganism concentrations are provided in Colony Forming Units per milliliter (CFU/mL). The results are means of three replicates.

Exposure Time	0 g	1 g	5 g	10 g	25 g
0	2.4×10^4	2.0×10^4	2.8×10^4	1.4×10^4	1.6×10^4
4	1.9×10^4	3.6×10^4	1.6×10^2	1.3×10^1	$< 1.0 \times 10^1$
24	2.4×10^3	8.7×10^3	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$

Table 2. Log Reduction of *P. oxalicum*. 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	2.2	3.0	3.2
24	0.4	3.4	3.1	3.2

Figure 1. Survival of *P. oxalicum* 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 3.1 log inactivation of *P. oxalicum* within 24 hours. 1 percent did not result in a significant inactivation of the challenge organism

Laboratory Supervisor:_____Date:_____