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:	March 13, 2003
Test Organism:	*Pseudomonas aeruginosa*
ATCC Strain:	15442
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. *Pseudomonas aeruginosa* ATCC 15442 was selected as a challenge organism. *P. aeruginosa* is a motile gram negative bacterium that is present in soils and water. It is an opportunistic pathogen which utilizes the respiratory tract as its route of infection. The testing of bottled water for the presence of *P. aeruginosa* is required in Canada and the European Union.

METHODOLOGY

A. Stock Culture Preparations:

The bacterial challenge strain was reconstituted in nutrient broth and passed 3 times. All culture tubes were incubated at 35°C for 24 hours. Twenty-four hours prior to testing, the bacterial challenge organism was inoculated into 500 ml of nutrient broth and incubated at 35°C. After incubation, the culture was centrifuged at 2500 rpm for 10 minutes. The supernatant was discarded and the remaining microbial pellet was washed three times in sterile buffered deionized water (SBDW). After the third centrifugation step, the pellet was resuspended in 50

mL of SBDW. An approximate cell density of the suspension was obtained either via direct staining with acridine orange or plating on growth media.

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 10^6 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 Pseudomonas Isolation Agar (PIA). The plates were incubated at 42°C for 24 hours prior to enumeration.

RESULTS

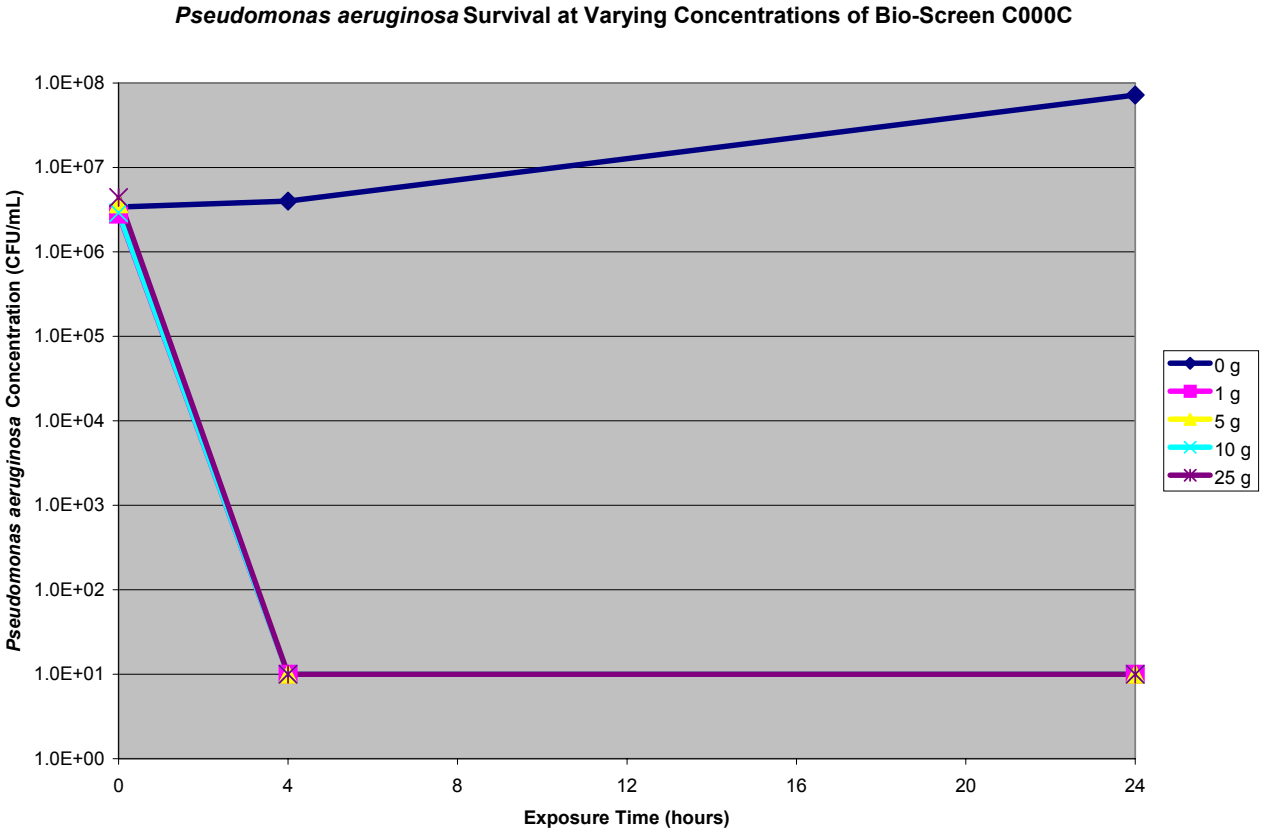
Table 1. Survivability of *P. aeruginosa*. Exposure time is provided in hours. The grams of Bio-Screen C000C added to 100 mL of the SBDW are provided. Microorganism concentrations are provided in Colony Forming Units per milliliter (CFU/mL). The results are means of three replicates.

Exposure Time	0 g	1 g	5 g	10 g	25 g
0	3.4×10^6	2.8×10^6	3.7×10^6	2.9×10^6	4.4×10^6
4	4.0×10^6	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
24	7.2×10^7	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$

Table 2. Log Reduction of *P. aeruginosa*. 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	5.4	5.6	5.5	5.6
24	5.4	5.6	5.5	5.6

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



SUMMARY

As is evident, all percentages of Bio-Screen C000C resulted in greater than 5 log inactivation of *P. aeruginosa* within 4 hours.

Laboratory Supervisor:_____Date:_____