



FINAL REPORT

Disinfectant Efficacy Testing

PROTOCOL
Specification of Bactericide in a Microcosm Study

ORDER Number
371011467

PREPARED FOR:

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Certificate of Analysis

Client: Microgen Inc.

Contact: Robert G. Prince

Project: Product Efficacy 2010-1

Product : D-125, US EPA Reg. No. 61178-1, Production Lot No. 2991

EMSL NO: 371011467

Sample received: 9/28/2010

Aerobic Bacteria Study

Start date: 9/29/2010

Report date: 10/7/2010

Challenge Bacteria: Aerobic bacteria – *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Microbacterium* species

Sulfate Reducing Bacteria Study

Start date: 9/29/2010

Report date: 10/26/2010

Challenge Bacteria: Consortium of Sulfur-reducing Bacteria (anaerobic and aerobic SRB combined)

Experimental Summary: The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, Microgen, Inc. The testing procedure is based on Annex II of the Specification of Bactericide in a Microcosm Study, with the testing conducted on a cleaning solution for its ability to sterilize and disinfect Aerobic bacteria and Sulfur-reducing Bacteria. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Aerobic Bacteria Study

The testing was performed to determine the effectiveness of the D-125 cleaning solution against the challenge aerobic bacteria, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Microbacterium* species. The organisms were grown on Tryptic Soy agar (TSA) at 35°C for 24 h prior to testing. A 0.5 McFarland standard was used to create a 10^6 solution of each bacterial species in phosphate buffer. From this solution 3 dilutions were created for each species. Bacterial solutions were then mixed by adding 150 μ L of each to 13 mL of phosphate buffer, and 1.5 mL of the testing solution or 14.5 mL of



phosphate buffer plus solutions for controls, giving a total of 15 mL. This was repeated for each dilution created. The mixed solution was then added to a heterotrophic aerobic bacterial microcosm complete with media and growth indicator and incubated at room temperature for 7 days. Each microcosm was checked daily and observations were recorded.

Sulfur-reducing Bacteria Study

The testing was performed to determine the effectiveness of the D-125 cleaning solution against the challenge consortium of Sulfur Reducing Bacteria (SRB). The organisms were obtained from a 10^6 enrichment of SRB that included both anaerobic and aerobic microorganisms. From this solution, 3 dilutions were created. The SRB solution was then mixed by adding 500 μ L to 13 mL of phosphate buffer and 1.5 mL of the testing solution or 14.5 mL of phosphate buffer for controls; giving a total of 15 mL. This was repeated for each dilution created. The mixed solution was then added to an SRB microcosm complete with media and growth indicator and incubated at room temperature for 28 days. Each microcosm was checked daily and observations were recorded.

Experimental Results:

Table 1.1 Aerobic Bacteria Study

Dilutions	Testing Solution CFU/mL	Control CFU/mL
10^{-1}	ND	7.0×10^6
10^{-2}	ND	5.0×10^5
10^{-3}	ND	5.0×10^5

ND = None Detect <10 CFU/mL

Table 1.2 Sulfate-reducing Bacteria Study

Dilutions	Testing Solution CFU/mL	Control CFU/mL
10^{-1}	ND	1.8×10^4
10^{-2}	ND	1.2×10^3
10^{-3}	ND	2.0×10^2

ND = None Detect <10 CFU/mL



Conclusions/Observations:

The D-125 cleaning solution provided by Microgen, Inc. was tested using a bactericide microcosm approach against aerobic bacteria and SRB. At the completion of this study the D-125 solution demonstrated the ability to disinfect (kill) all aerobic bacteria and SRB inoculated into the microcosm.

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