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中華民國實驗室認證體系
行政院環保署環境檢驗認證

Bactericidal Activity of BIO-ALPOSOL against *Legionella pneumophila*

Client	GreenTech Biotechnology Environmental Co., Ltd.	Report No.	SL93P9022 EN
Client Address	3F, No.97, Jingye 1st road, Taipei, Taiwan, R.O.C.		
Client Specimen I.D.	BIO-ALPOSOL	Date Received	May 17, 2004
Specimen I.D.	9022P01	Date Issued	May 25, 2004
Specimen description	Fluid packaged in blue bottle, 50mL/bottle	Specimen collector: ■ Research Contract	

Remarks：

1. This report includes: 4 pages totally. It will be in vain if separated and/or partially copied.
2. The results in this report are valid only to the specimen sent by client.
3. All report content is used as references, not for advertising, sales promotion and notarial purpose.

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Abstract

Evaluation of bactericidal activity for BIO-ALPOSOL (provided by GreenTech Biotechnology Environmental Co., Ltd.) *against Legionella pneumophila was performed. Tested bacterial suspension which containing Legionella pneumophila strain was added to 10% BIO-ALPOSOL fluid. After exposure for 15, 30, 60 and 90 minutes with 10% BIO-ALPOSOL fluid, the remaining total viable counts for samples with different exposure time were performed. Results indicated that 10% BIO-ALPOSOL fluid can kill 100% of Legionella pneumophila after exposure for 15 minutes. In the control group, after exposure for 15 minutes with sterile distilled water, the remaining viable counts showed in a reasonable range. In conclusion, this study has proved that 10% BIO-ALPOSOL fluid which provided by GreenTech Biotechnology Environmental Co., Ltd. is an effective bactericidal agent against Legionella pneumophila.*

Research Content

1. Objectives:

Evaluation of bactericidal activity for BIO-ALPOSOL against *Legionella pneumophila* was performed.

2. Specimen:

BIO-ALPOSOL (provided by GreenTech Biotechnology Environmental Co., Ltd.)

3. Tested species:

One tested bacterial strain of *Legionella pneumophila* was selected for this testing. The concentration of tested strain is approximately 1.5×10^4 ~ 1.5×10^5CFU/mL. The name of tested species, diluted broth and tested agar used for the total viable count (spreading method) is listed on Table 1.

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Table 1. Tested Species, Dilution Broth and Inoculated Agar Medium Used for the Total Viable Count.

No.	Name of tested species	Broth used for dilution	Agar used for the total viable count
1	*Legionella pneumophila*	BCYEα Broth	BCYEα Agar

4. Methods:

(1) The concentration of tested strain, *Legionella pneumophila*, is approximately 1.5×10^5 CFU/mL.

(2) The original fluid of BIO-ALPOSOL is diluted to 10% (1:10) with sterile distilled water.

(3) 1.0 mL of tested bacterial suspension was added to one sterile test tube which containing 9 mL of tested fluid (10% BIO-ALPOSOL). After mixed vigorously, time was calculated immediately, sterile distilled water was used in control group. After the reaction took 15, 30, 60 and 90 minutes, 1.0 mL of reactive solutions with different exposure time were taken from test tube and put into 9 mL of dilution BCYE α broth respectively, and followed by mixed homogenously. Ten-fold serial dilution was carried out if necessary.

(4) The total viable counts were performed by applying individually sterile 2 mL pipet to aspirate 0.2 mL of each dilute broth to plate containing Plate Count Agar (Difco, B-D Co., USA), we then spread the broth over the surface on each plate medium with sterile L-shaped glass rod.

(5) All culture plates are placed and incubated at a 35℃ incubator for 72 ± 2 hours, after that, the total viable count can be obtained by calculating the colony growth on each plate. The bacterial colony number between 30~300 CFU/plate was selected for calculation.

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5. Result and Conclusion:

Tested bacterial suspension which containing *Legionella pneumophila* strain was added to 10% BIO-ALPOSOL fluid. After exposure for 15, 30, 60 and 90 minutes with 10% BIO-ALPOSOL fluid, the total remaining viable counts for samples with different exposure time were performed. Results indicated that 10% BIO-ALPOSOL fluid can kill 100% of *Legionella pneumophila* after exposure for 15 minutes (Table 2). In the control group, after exposure for 15 minutes with sterile distilled water, the remaining viable counts showed in a reasonable range (Table 2). In conclusion, this study has proved that 10% BIO-ALPOSOL fluid (provided from GreenTech Biotechnology Environmental Co., Ltd.) is an effective bactericidal agent against *Legionella pneumophila*.

Table 2. Bactericidal activity of BIO-ALPOSOL against *Legionella pneumophila*

Experiment group/Exposure time		Remaining count after 15 minutes (CFU) /killing rate (%)	Remaining count after 30 minutes (CFU) /killing rate (%)	Remaining count after 60 minutes (CFU) /killing rate (%)	Remaining count after 90 minutes (CFU) /killing rate (%)
L. pneumophila	Tested concentration				
1.1×10^5 CFU	1 : 10	0 (100)	0 (100)	0 (100)	0 (100)

Table 2. Bactericidal activity of BIO-ALPOSOL against *Legionella pneumophila* (cont'd)

Control group/Exposure time		Remaining count after 15 minutes (CFU) /Remaining rate (%)	Remaining count after 30 minutes (CFU) /Remaining rate (%)	Remaining count after 60 minutes (CFU) /Remaining rate (%)	Remaining count after 90 minutes (CFU) /Remaining rate (%)
L. pneumophila	Tested Solution				
1.1×10^5 CFU	Normal saline	5.8×10^5 (>100)	4.8×10^5 (>100)	4.2×10^5 (>100)	4.5×10^5 (>100)

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May 25, 2004

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