

*AIR MICROBIAL LOAD REDUCTION RESEARCH STUDY
FOR XXX-001*



FIGURE 1- TEST
EQUIPMENT

SUBMITTED TO
DETAILED ADDRESS OF ORDERING PARTY

Testing Facility
Biocare Research (I) Pvt. Ltd.
1st floor, Kanth Complex, Paldi cross roads
Ahmedabad - 07

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Air microbial load reduction research study for XXX-001

Objective

This study aimed to evaluate the bacterial, fungal and viral filtration and inactivation efficiency of submitted instrument

Product Process Details

Not provided by Customer/Client

Test Microorganism

Laboratory Identification

LAB_ID

Test Report completed on

dd/mm/yyyy

Customer/Contact personDetails

CUSTOMER DETAILS

TEST SUMMARY

This study aimed to evaluate the bacterial, fungal and viral filtration and inactivation efficiency of submitted instrument called, "TEST EQUIPMENT" (Test article reference no LAB_ID). The instrument submitted is fitted with UV light visibly identifiable at the front lower bottom of the instrument. Additionally, it has two PP side attachment to cover the air flow. The instrument was kept in a pre-cleaned closed test chamber(17 m³) along with aerosol capture device and temperature, humidity meters. The challenge solution was prepared using freshly transferred pure culture of bacteria, or fungi or virus. The strength of the suspension was 6X10¹¹ cfu or pfu /ml and then further diluted for aerosolization. The organism controls were found satisfactory. All the test organisms were aerosolized (aerosol size 3μ ± 0.5μ) to charge the test area with desired test organisms using externally operated nebulizer. The aerosols were allowed to stabilize before any operations or procedure. The test instrument was operated as per manufacturer's instruction for different time interval for intermittent (TEST EQUIPMENT) efficacy analysis (at fan speed 3). The bioaerosols were captured using AGI impinger / multi stage active air sampling method in buffer/saline/nutrient rich media according to organisms tested. **The reduction in microbial load was enumerated in triplicates.** The test chamber was disinfected after each run using fogger using commercial disinfectant solution (H₂O₂+AgNO₃). Throughout the analysis the internal chamber condition was maintained at 25°C and RH <60%. The list and details of microorganisms used are described in table 1 below.

Table 1: Test organism details

Sr. no	Name of microorganism	ATCC reference Number	Type of Microorganisms
1	<i>Staphylococcus aureus</i>	6538	Gram positive cocci
2	<i>Escherichia coli</i>	8739	Gram negative rods
3	<i>Pseudomonas aeruginosa</i>	9027	Gram negative rods
4	<i>Candida albicans</i>	10231	Yeast
5	<i>Aspergillus niger</i>	16404	Filamentous fungus
6	<i>Phi X 174</i>	13706B1	ss DNA Virus
7	<i>MS 2</i>	15597 B1	ss RNA virus

Growing organisms *in vitro* and suspension preparation

1. **Bacteria:** *S. aureus*, *E.coli* and *Pseudomonas aeruginosa*. were grown on nutrient agar plates. Axenic cultures were inoculated in TSB broth and were incubated for 12-16 h at 37° C. After achieving required optical density (approx. 0.8 at 560 nm) bacterial suspension containing 6.0×10^{11} cells was prepared in PBS from TSB broth. The prepared bacterial suspensions were used for aerosolization in chamber.
2. **Fungi** –*Candida* and *Aspergillus* were cultivated on SDA. Culture was transferred to get 6×10^{11} fungal cell or spore count/ml in suspension and was diluted for use in aerosolization in chamber.
3. **Virus** –Phi X 174 and MS2 viruses were propagated using *E. coli* (ATCC 13706) and *E.coli* (ATCC 15597) host culture respectively. After scrapping and centrifugation, suspension containing 6×10^{11} pfu/ml was further used for aerosolization in chamber.

Experimental condition

1. The chambers, pipes and fittings were disinfected before and after conducting individual set of experiment with each bacteria/fungi/viruses.
2. The culture suspensions prepared as mentioned in above section were inoculated in nebulizer to generate aerosols of 3μ (Approximately $\pm 0.5\mu$) inside 17 m^3 test chamber.
3. The aerosols were allowed to stabilize.
4. The “TEST EQUIPMENT” was run for different time intervals like, 15 mins, 30 mins, 45 mins, 60 mins, 90 mins, 120 mins and 150 mins and 180 mins after stabilization of aerosols in chamber.
5. The treated air was captured after active running of instrument using AGI impinger using active air sampler for enumeration of bacterial, viral and fungal survival on appropriate growth medium (with host in case of virus enumeration) for cultivation.
6. Plates were inoculated in triplicate. Plates were incubated at 37°C for 72 hours in case of bacteria, at 25°C for 5 days in case of fungi, and at 37°C for 4 days in case of PhiX 174 and MS2, and colonies or plaques were counted for calculation.

CALCULATIONS

Percent Reduction

The percent reduction in titer was calculated as follows:

$$\text{Percent Reduction} = (1 - 10^{-LR}) \times 100$$

where:

$$LR = \text{Log Reduction} = (\text{Initial Log value} - \text{Final Log value})$$

Result

Table -2: Results for *S.aureus* ATCC 6538

Product	Test parameters	Initial Log /cfu	Exposure Duration in min	After exposure Log /cfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>S.aureus</i>	6.778 (6.0×10^6 cfu)	15	5.389 (245000 cfu)	1.39	95.9167
			30	5.089 (122667 cfu)	1.69	97.9556
			45	4.643 (44000 cfu)	2.13	99.2667
			60	3.784 (6083 cfu)	2.99	99.8986
			90	3.611 (4083 cfu)	3.17	99.9319
			120	2.969 (932 cfu)	3.81	99.9845
			150	1.763 (58 cfu)	5.01	99.9990
			180	0.845 (07 cfu)	5.93	99.9999

Figure 1: Graphical representation for log reduction in *S.aureus*

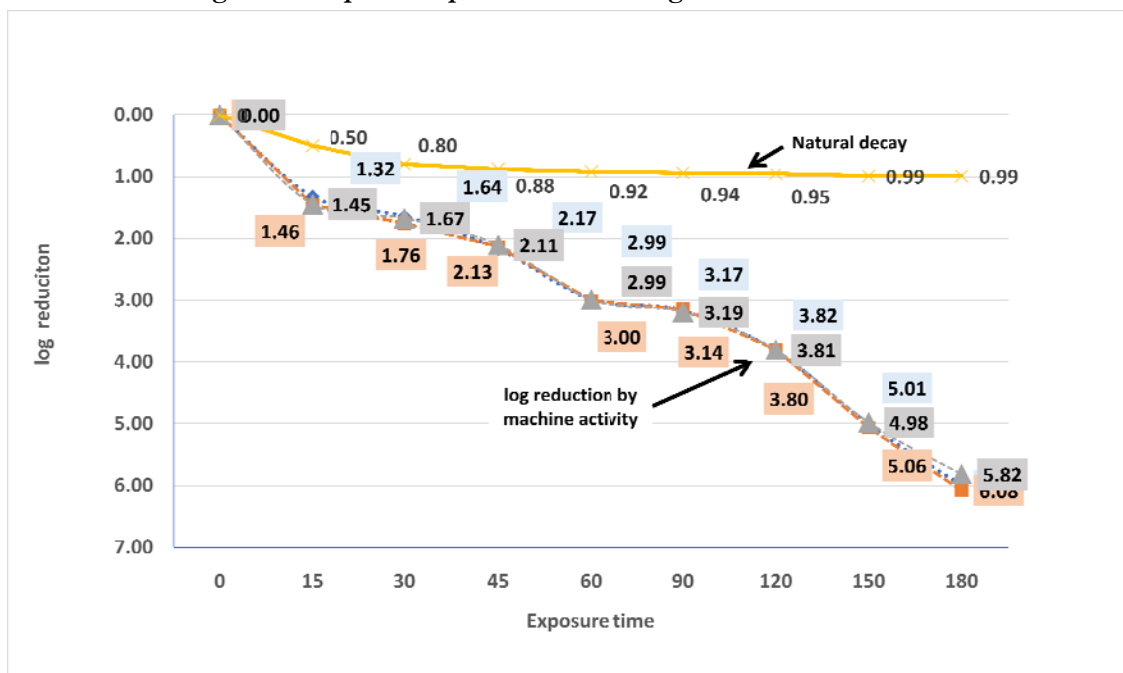


Table -3: Results for *E.coli* ATCC 8739

Product	Test parameters	Initial Log /cfu	Exposure Duration in min	After exposure Log /cfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>E.coli</i>	6.770 (5.9 x10 ⁶ cfu)	15	5.401 (251667 cfu)	1.37	95.7345
			30	5.154 (142667 cfu)	1.62	97.5819
			45	4.686 (48500 cfu)	2.09	99.1780
			60	3.772 (5917 cfu)	3.00	99.8997
			90	3.615 (4117 cfu)	3.16	99.9302
			120	2.940 (872 cfu)	3.83	99.9852
			150	1.841 (69 cfu)	4.93	99.9988
			180	0.845 (07 cfu)	5.93	99.9999

Figure 2: Graphical representation for log reduction in *E.coli*

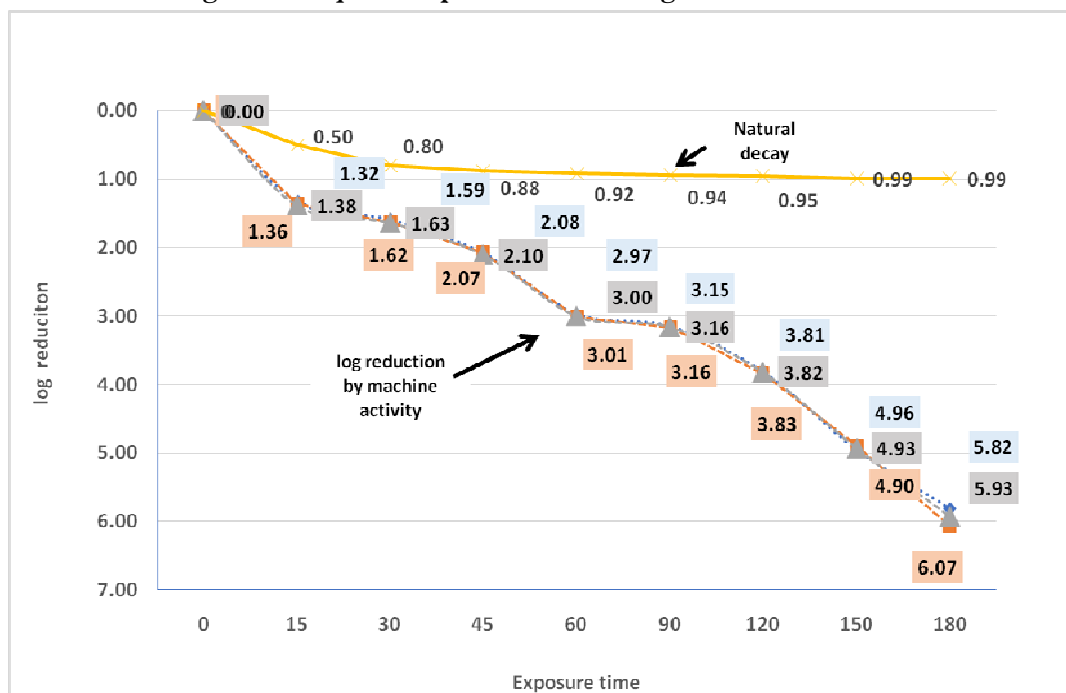


Table -4: Results for *P.aeruginosa* ATCC 9027

Product	Test parameters	Initial Log /cfu	Exposure Duration in min	After exposure Log /cfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>P.aeruginosa</i>	6.778 (6.0×10^6 cfu)	15	5.505 (320000 cfu)	1.27	94.6667
			30	5.261 (182333 cfu)	1.52	96.9611
			45	4.817 (65667 cfu)	1.96	98.9056
			60	3.894 (7833 cfu)	2.88	99.8694
			90	3.616 (4133 cfu)	3.16	99.9311
			120	3.035 (1083 cfu)	3.74	99.9819
			150	1.951 (89 cfu)	4.83	99.9985
			180	0.921 (08 cfu)	5.86	99.9999

Figure 3: Graphical representation for log reduction in *P.aeruginosa*

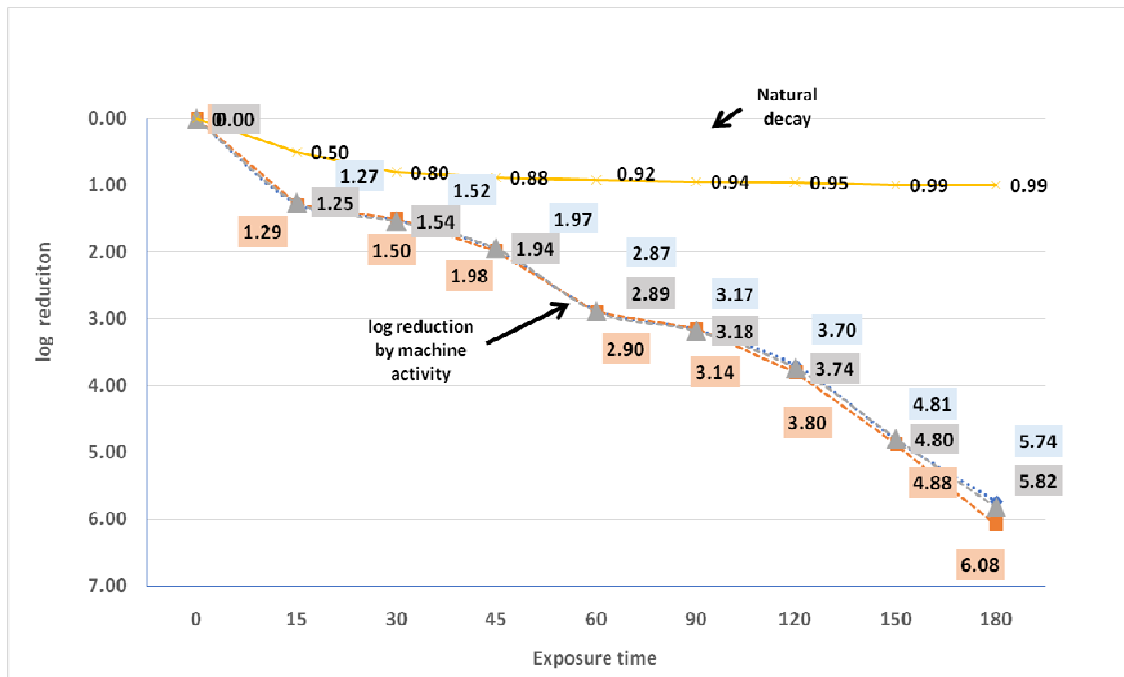


Table -5: Results for *Phi X 174 ATCC 13706B1*

Product	Test parameters	Initial Log /pfu	Exposure Duration in min	After exposure Log /pfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>Phi X 174</i>	6.778 (6.0×10^6 pfu)	15	5.795 (623333pfu)	0.98	89.6111
			30	4.870 (74167 pfu)	1.91	98.7639
			45	3.768 (5867 pfu)	3.01	99.9022
			60	2.979 (953 pfu)	3.80	99.9841
			90	2.738 (547 pfu)	4.04	99.9909
			120	2.171 (148 pfu)	4.61	99.9975
			150	1.781 (60 pfu)	5.00	99.9990
			180	0.921 (08 pfu)	5.86	99.9999

Figure 4: Graphical representation for log reduction in *Phi X 174*

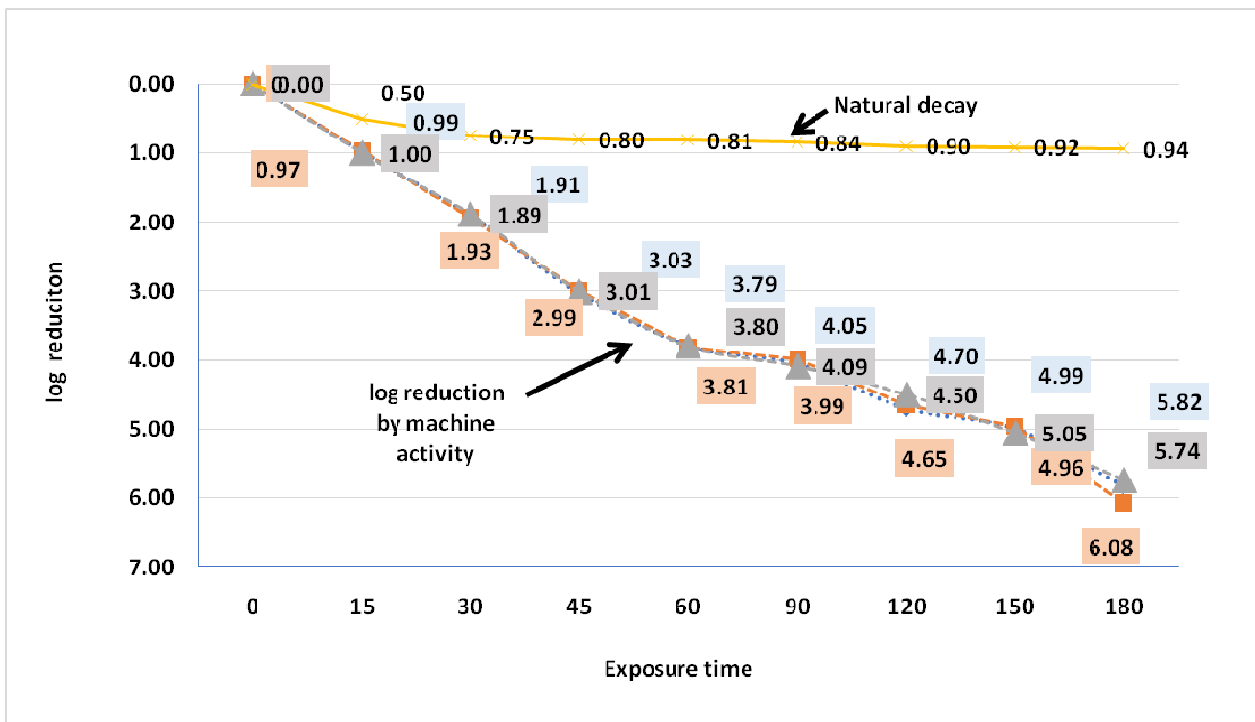


Table -6: Results for *Candida albicans* ATCC 10231

Product	Test parameters	Initial Log / cfu	Exposure Duration in min	After exposure Log / cfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>C. albicans</i>	6.778 (6.0×10^6 pfu)	15	5.401 (251667cfu)	1.38	95.8056
			30	5.154 (142667 cfu)	1.62	97.6222
			45	4.686 (48500 cfu)	2.09	99.1917
			60	3.772 (5917 cfu)	3.01	99.9014
			90	3.615 (4117 cfu)	3.16	99.9314
			120	2.940 (872 cfu)	3.84	99.9855
			150	1.841 (69 cfu)	4.94	99.9988
			180	0.845 (07 cfu)	5.93	99.9999

Figure 5: Graphical representation for log reduction in *C. albicans*

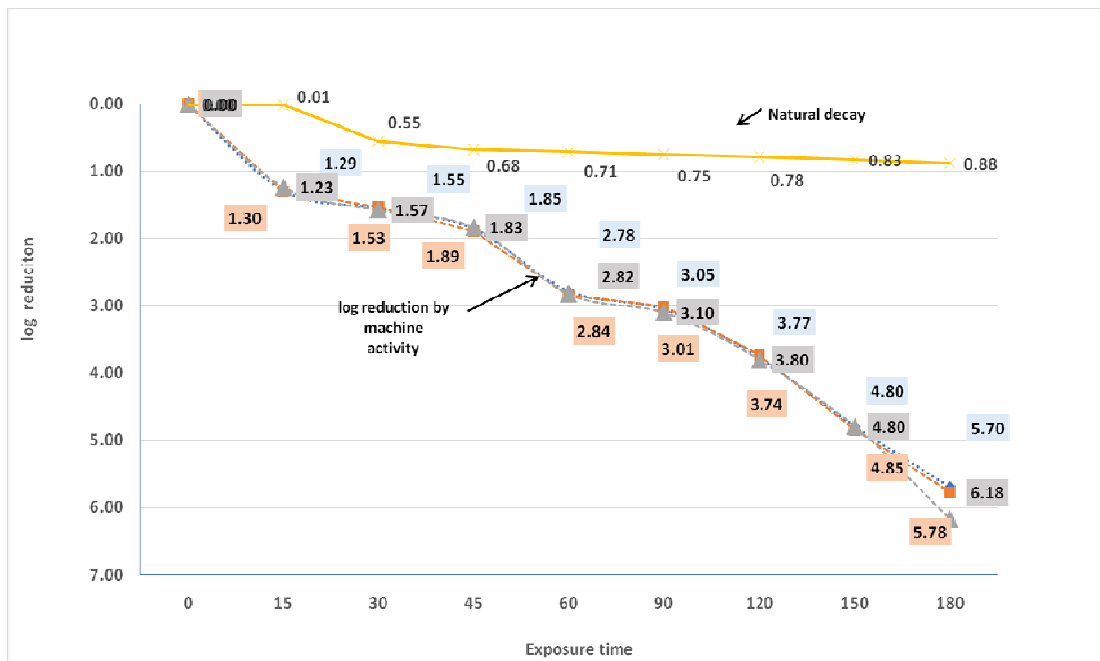


Table -7: Results for *Aspergillus niger* ATCC 16404

Product	Test parameters	Initial Log / cfu	Exposure Duration in min	After exposure Log / cfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	<i>A.niger</i>	6.778 (6.0×10^6 cfu)	15	5.591 (390000cfu)	1.11	92.2000
			30	5.236 (172333 cfu)	1.46	96.5533
			45	4.967 (92667 cfu)	1.73	98.1467
			60	3.929 (8500 cfu)	2.77	99.8300
			90	3.687 (4867 cfu)	3.01	99.9027
			120	3.097 (1250 cfu)	3.60	99.9750
			150	2.368 (233 cfu)	4.33	99.9953
			180	1.279 (19 cfu)	5.42	99.9996

Figure 6: Graphical representation for log reduction in *A.niger*

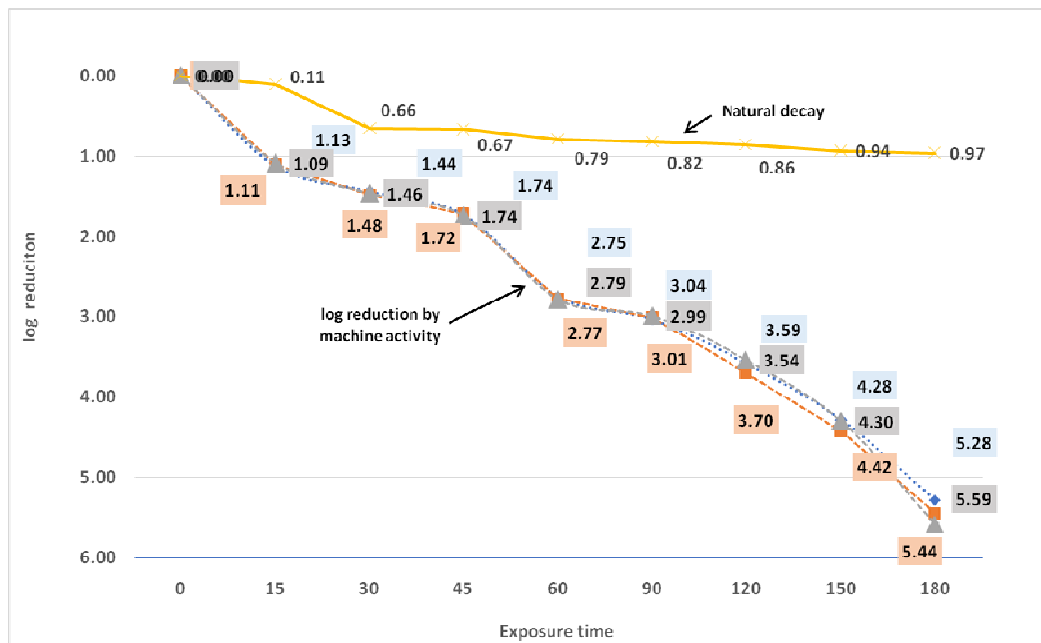
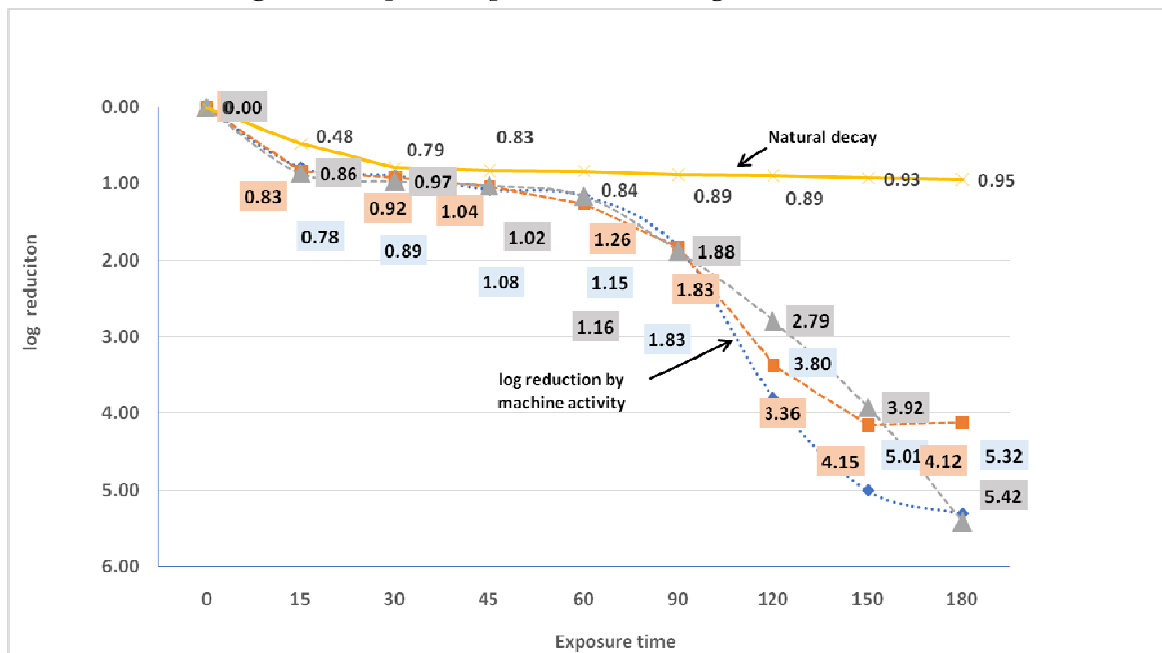


Table -8: Results for *MS 2 ATCC 15597 B1*

Product	Test parameters	Initial Log /pfu	Exposure Duration in min	After exposure Log /pfu	Log Reduction after exposure to Test Material	Percent Reduction (%)
TEST EQUIPMENT	MS 2	6.778 (6.0x10 ⁶ pfu)	15	5.954 (899333pfu)	0.82	85.0111
			30	5.852 (711000 pfu)	0.93	88.1500
			45	5.733 (540333 pfu)	1.05	90.9944
			60	5.588 (387667 pfu)	1.19	93.5389
			90	4.933 (85667 pfu)	1.85	98.5722
			120	3.649 (4453 pfu)	3.13	99.9258
			150	2.604 (402 pfu)	4.17	99.9933
			180	2.230 (170 pfu)	4.55	99.9972

Figure 7: Graphical representation for log reduction in MS 2



Remarks: Air sampling method: Active air sampling

Note: The above instrument was kept in the center of the testing chamber (When in operation)

Above tested parameter is not part of NABL/ Accreditation Scope.

----- End of Test Report -----

Dr. D. N. Zaveri
Authorized signatory