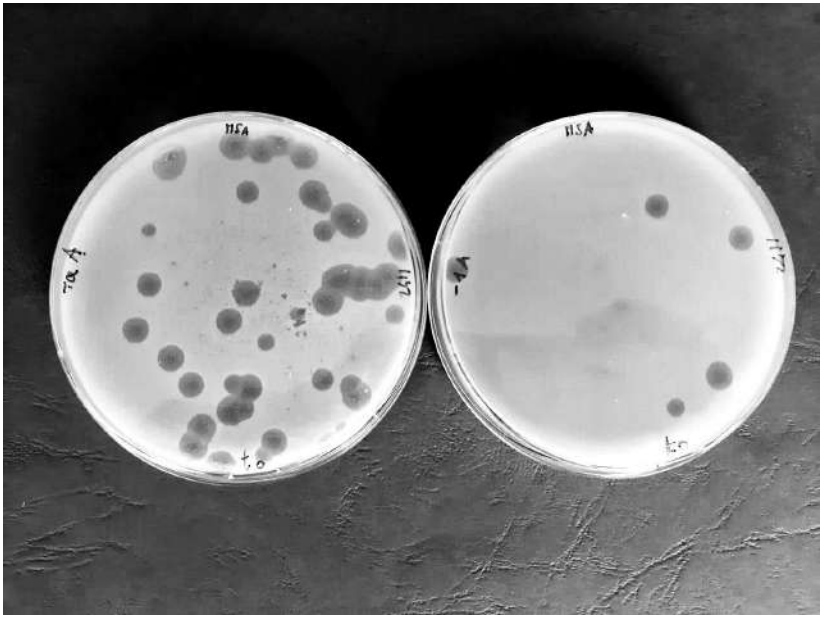
HEXAFAN



ENG

THE MICROBIAL LOAD REDUCTION IN INDOOR ENVIRONMENTS THROUGH A PHOTOCATALYTIC TECHNOLOGY DISINFECTION DEVICE.

Date	Revision	Protocol
28/04/2023	01	82 rev.01-2022

HEXAFAN

The microbial load reduction in indoor environments through a photocatalytic technology disinfection device.

PASALABS
Green testing and analysis

INTRODUCTION

This study presents the findings of two tests to assess the efficiency of microbiological reduction in the indoor air of a technology device called Hexafan. The device was created by Fan Motors Italia Srl.

In order to measure the reduction of bioaerosols, the experiment involved using two different microbiological solutions to contaminate the premises:

- Test 1: a solution containing a strain of *Escherichia coli* and a strain of *Saccharomyces cerevisiae.*
- Test 2: a solution with a viral strain, the φX174 bacteriophage.

Each test was conducted independently.

To evaluate the effectiveness of the photocatalytic device, we counted the number of live bacteria and viruses before and after using Hexafan. The results are presented in logarithmic reduction format.

INSTRUMENTATION

ID	Brand	Model
01	Temptop	M2000C
02	FLAEM	Vaporizer
03	Trotech	BH30
04	XPower	F-8 350W
05		AGI-30
06	Aquaria	CF 20 Alfa

TEST 1- BACTERIAL AND FUNGAL CONTAMINATION

1.1 EXPERIMENTAL CONDITIONS

The experiment took place in a 72 m3 room typically used for meetings. The doors and windows remained shut throughout the test, and operators wore appropriate personal protective equipment to prevent *cross-contamination.*

The relative humidity was kept between 39-59% using a humidifier connected to a hygrostat. A pre-prepared microbiological solution was sprayed into the entire room using an atomizer. After the vaporization, the first air sample was taken (t0, Hexafan off). Then, the Hexafan device was switched on, and air samples were taken at predetermined intervals throughout the 8-hour test.

Before the test, a blank test was conducted under the same conditions, but the device was not activated between the withdrawals at time 0 and the following withdrawals. The blank test and test sampling times were the same, except for t4, t8, and t11. The blank test showed the need to increase the withdrawals. Table 1.2 shows all air sampling times.

Sample ID	Sampling Time	Air	
		Blank	Test
C1	t0	✔	✔
C2	t1	✔	✔
C3	t2	✔	✔
C4	t4	-	✔
C5	t6	✔	✔
C6	t8	-	✔
C7	t11	-	✔
C8	t24	✔	✔

Table 1.2 - Blank and test sampling frequencies.
In yellow: Hexafan off; in light blue: Hexafan on

1.2 ANALYTICAL PARAMETERS AND DATA PROCESSING

After collecting samples, they were taken to the laboratory for analysis. The following parameters were examined: Total Bacterial Load (CBT), Total Fungal Load (CMT), Viable Escherichia coli count, and Vital count of Saccharomyces cerevisiae. The efficacy of microbiological inactivation in the air matrix was expressed as a logarithmic reduction calculated by comparing the colonies found before treatment (t0) to those found at all other sampling times on a logarithmic scale.

Logarithmic reduction R = log10 (A/B)

A= n° viable cells before treatment (t0) B= n° viable cells after treatment

1.3 RESULTS

1.3.1 Total Bacterial load

The graphs presented below illustrate the CFU/m3 levels over time in the white, blue, and orange line treatment tests (Figure 1.3), while Figure 1.4 compares the logarithmic reduction achieved in the two tests.
The CBT reduction of the treatment test appears to be greater than that of the blank after just one hour of treatment.

The most impressive result is observed two hours after turning on the Hexafan device, with a reduction of 6.1 logs, corresponding to a 99.9999% reduction percentage.

This reduction is maintained for all subsequent sampling times, even after turning off the photocatalytic action device. The maximum log reduction achieved in the blank is 4.5 logs after six minutes. At time t24, the battery charge seems to increase again.

Table 1.3 compares the log reduction values of the CBT for the blank and the treatment test.

TOTAL BACTERIAL LOAD

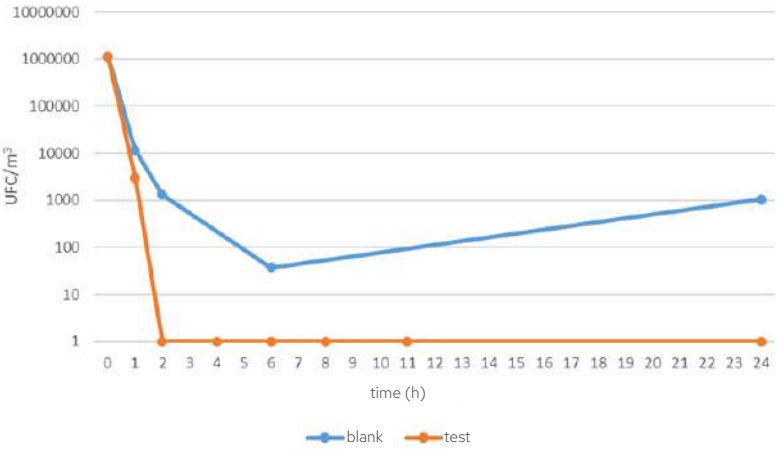


Fig.1.3 Total bacterial load for each sampling time, expressed in CFU/m3.

REDUCTION LOG TBL AIR

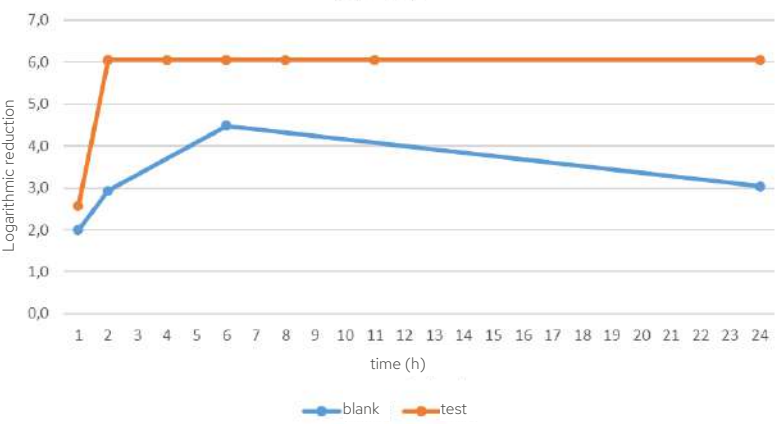


Figure 1.4 shows logarithmic reduction over time in the samples of the blank (blue) and the test (orange).

Time (h)	Logarithmic reduction Total Bacterial Load	
	Blank	Test
t1	2,0	2,6
t2	2,9	6,1
t4	-	6,1
t6	4,5	6,1
t8	-	6,1
t11	-	6,1
t24	3,0	6,1

Table 1.3, CBT log reduction values between blank and test with treatment at various withdrawal times.

1.3.2. TOTAL MYCOTIC LOAD/ viable S.cerevisiae count

The graph in Figure 1.5 displays the CFU/m3 detected in both the blank and treated tests. Figure 1.6 depicts the log reduction observed in CMT over time, with the white and treatment tests shown in blue and orange lines, respectively.

Table 1.4 presents the numerical values of the logarithmic reduction of the CMT. After conducting various analyses, no other mycotic species were found apart from S.cerevisiae, which was artificially injected into the room air. Therefore, the curves of S.cerevisiae and Total Fungal Load (CMT) inevitably overlap.

The maximum logarithmic abatement of 4.9 log is observed after one hour of treatment (t1) and remains constant throughout the test, even after the photocatalytic device is turned off.

In the white test, the same blast chilling value is achieved after two hours but reduces at t24, at which point the CFU/m3 increases again.

TOTAL MYCOTIC LOAD

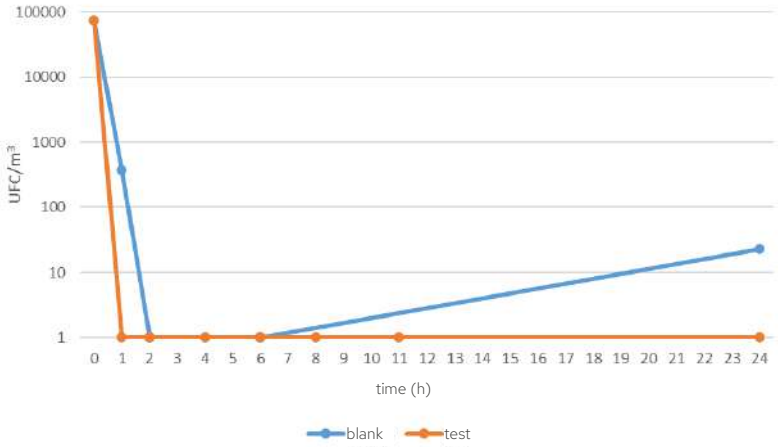


Figure 1.5- Total Fungal Load for each sampling frequency, expressed in CFU/m3

LOGARITHMIC REDUCTION OF TOTAL MYCOTIC LOAD OVER TIME

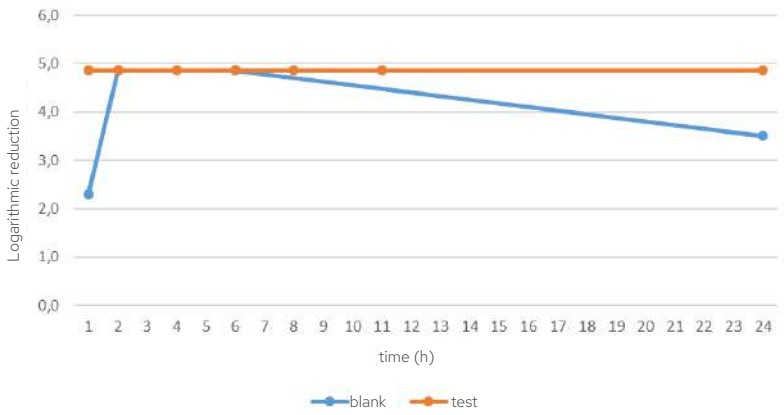


Figure 1.6, the graph shows the reduction of total mycotic load over time. The blue line represents the blank, while the orange line represents the test.

Time (h)	Logarithmic reduction Total Mycotic Load	
	Blank	Test
t1	2,3	4,9
t2	4,9	4,9
t4	-	4,9
t6	4,9	4,9
t8	-	4,9
t11	-	4,9
t24	3,5	4,9

Tabella 1.4- CBT log reduction values between blank and test with treatment at various withdrawal times.

2. TEST 2 - VIRAL CONTAMINATION

2.1. VIRAL STRAIN CHOICE

The selected strain was φX174, a single-stranded DNA virus. This strain has been compared to viruses that are more resistant and harmful to humans, such as poliovirus and parvovirus. Additionally, it's commonly used in aerobiology and proposed as a model for studying viral disinfection. The host microorganism used was *Escherichia coli* strain C, ATCC 13706.

2.2 VIRAL SOLUTION PREPARATION

The bacteriophage was kept in a 1 ml liquid suspension (figure 2.2) in the dark at a temperature of 4°C until use. When it was time to use it, the phage suspension was diluted in sterile demineralized water, poured into an atomizer tank, and then vaporized throughout the entire volume of the experimental room (figure 2.2).

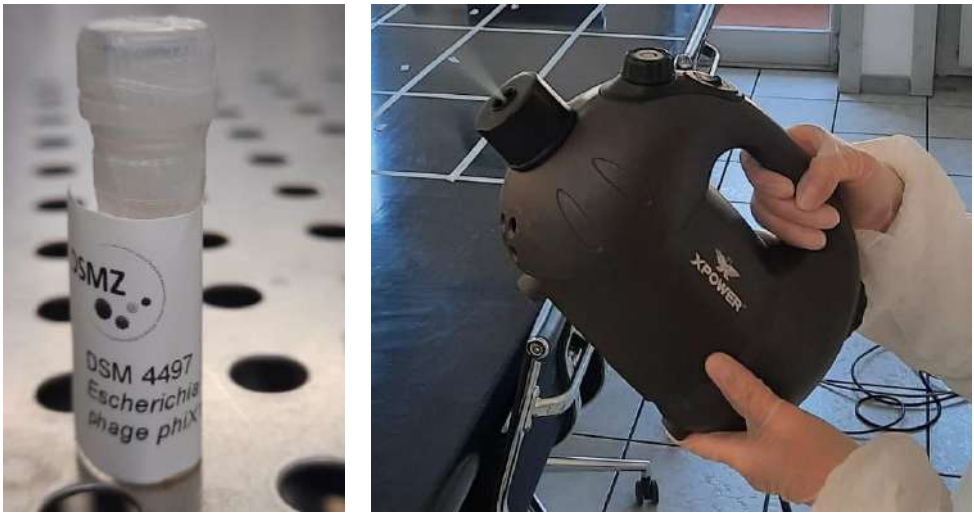


Figure 2.2, the left side shows a liquid suspension containing the bacteriophage φ X174, while the right side displays the vaporization phase of the viral solution.

2.3 HOST STRAIN CULTURE PREPARATION

To prepare the host strain culture, we used Modified Scholten's Broth (M.S.B.) as required by ISO 10705.

2.4 CONDITIONS

For the experiment, we used a closed room with a volume of 52 m3, which is typically used as an office. The technicians wore appropriate personal protective equipment to avoid cross-contamination, and the Hexafan sanitizing device was positioned at the same distance as the first test conducted on bacterial and fungal strains.

We maintained a relative humidity of 39-45% using a humidifier with hot steam connected to a hygrostat.
The air samples were taken at time 0, i.e., after vaporization of the phage suspension, with Hexafan off, and at different sampling frequencies after turning on the Hexafan (table 2.1).

To ensure accuracy, we conducted a blank test under the same conditions without activating the device to understand if the possible reduction of coliphages in the air matrix was due to a gravitational fallout.

Sample ID	Sampling time (min)	Sampled matrices	
		Air	Surfaces
		Inpinger	Buffer
C1	t0	✓	✓
C2	t10	✓	✓
C3	t20	✓	✓
C4	t30	✓	✓
C5	t40	✓	✓
C6	t50	✓	✓
C7	t60	✓	✓
C8	t90	✓	✓
C9	t120	✓	✓
C10	t150	✓	✓
C11	t180	✓	✓

Table 2.1 shows the sampled matrices and their corresponding sampling frequencies. The samples taken with the Hexafan turned off are highlighted in yellow, while the ones taken with the Hexafan turned on are highlighted in light blue.

2.2 TEST 2 – RESULTS

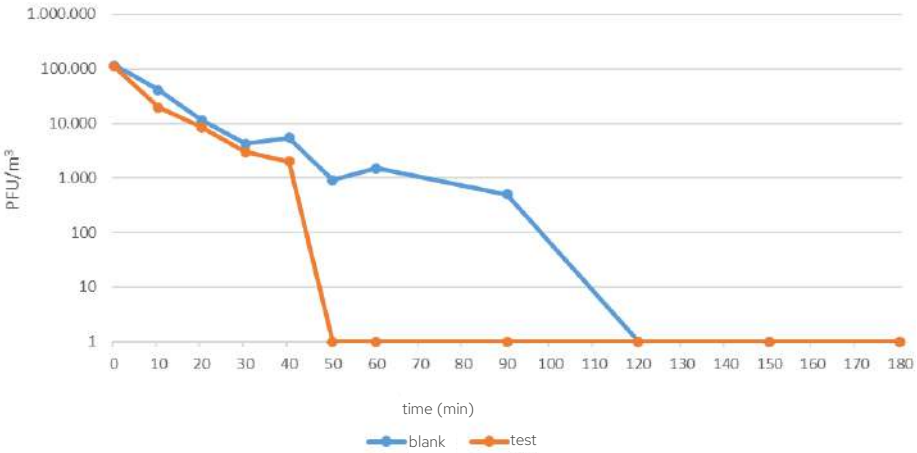
2.2.1 AIR MATRIX

The data for air quality is presented in Figure 2.5 and Table 2.3. The treatment method shows a significant decrease in the amount of PFU/m3 compared to the control group at all sampling times. Specifically, the photocatalytic treatment method resulted in a complete reduction of ELTs/m3 at t50, while the control group took until t120 to reach the same level.

Time (mi)	PFU /m³ ϕ X174	
	Blank	Test
t0	116.500	109.500
t10	41.500	20.000
t20	11.468	8.458
t30	4.286	3.000
t40	5.473	2.000
t50	913	0
t60	1.500	0
t90	500	0
t120	0	0
t150	0	0
t180	0	0

Table 2.3 shows the PFU/m3 detected during various sampling times, both in the blank and in the test with treatment.

VIRAL CHARGE ϕ X174 ARIA



The chart in Figure 2.5 shows the number of plaque-forming units per cubic meter of air of the φX174 bacteriophage at various sampling times.

Time (min)	log reduction ϕ X174	
	Blank	Test
t10	0,4	0,7
t20	1,0	1,1
t30	1,4	1,6
t40	1,3	1,7
t50	2,1	5,0
t60	1,9	5,0
t90	2,4	5,0
t120	5,1	5,0
t150	5,1	5,0
t180	5,1	5,0

Table 2.4, logarithmic reduction of the ϕ X174 bacteriophage at various sampling times in both the blank and the test with treatment.

LOGARITHMIC REDUCTION ϕ X174 ARIA

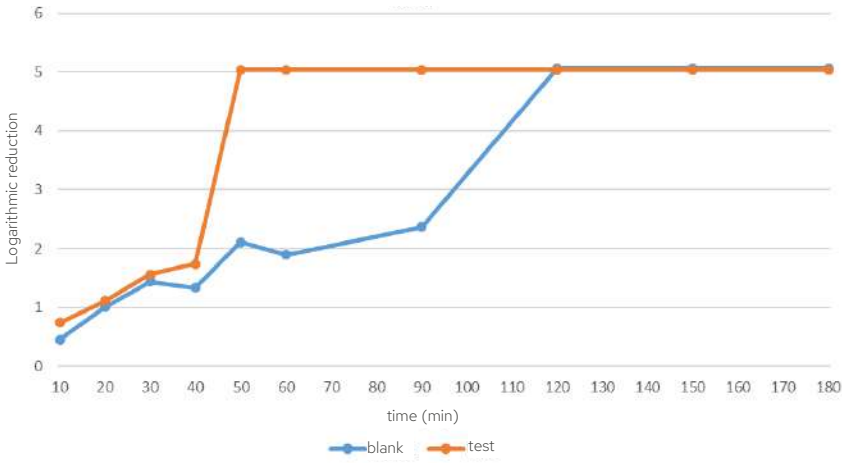


Figure 2.6- Logarithmic reduction of the φX174 bacteriophage over time at different sampling times, in the blank and the test with treatment.

During treatment, the viral load decreases significantly just 10 minutes after turning on the device.

The most significant reduction (5.0 log) occurs at t50 when the viral load reaches zero. However, in the blank test, it takes longer to get zero viral loads, specifically t120, as shown in tab- 2.4 and fig 2.6.

2.2.2 SURFACES

The effectiveness of the device has not been assessed through this matrix. This device is only designed to treat the air and for no other purposes. To rule out any significant leakage from the air matrix through sedimentation, surface sample analysis was conducted to evaluate the deposition of virus particles due to gravity. Figure 2.7 displays a graph comparing the data between the blank and the test with treatment. The superimposability of the two curves confirms an almost equivalent sedimentation loss in both tests.

ϕ X174 SURFACES

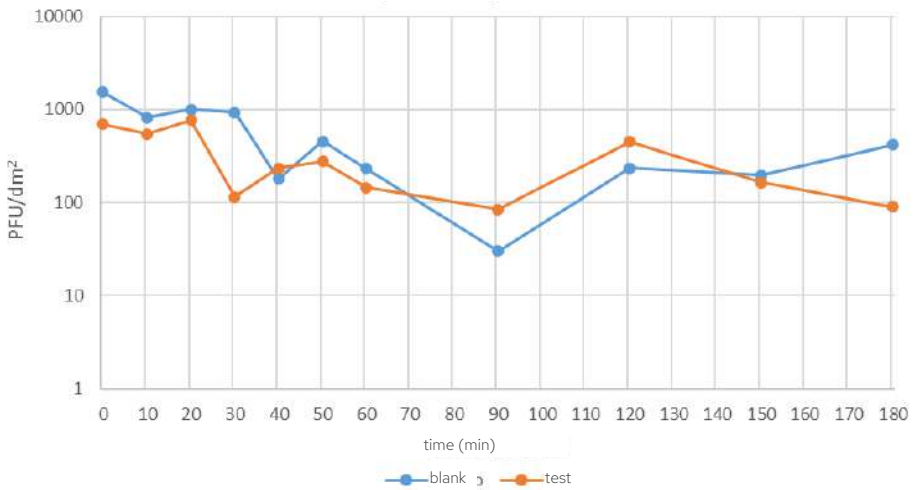


Figure 2.7- PFU/dm2 surfaces, comparison between blank and test with treatment

3. CONCLUSIONS

Based on the results of two experimental tests, the device was found to be effective against bacterial, fungal, and viral species.
The device was able to eliminate microbiological organisms within the first hour of treatment, except for the Total Bacterial Load, which required two hours to reach maximum reduction. The sanitizing action of the device persisted even after it was turned off.

These results are consistent with the unique features of photocatalytic treatments, which include fast action and long-lasting effects.
The samples taken from surfaces during the experiment, particularly in the test on the ϕ X174 viral stem, were essential in demonstrating that the loss of viral particles due to sedimentation did not affect the interpretation of the results regarding the efficacy of airborne treatment.

We Care, We Take Care!

Safe air, always.

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