Technical Report

20CE00202

Client:

Normann srl

Via G. Oberdan 79, Fontanafredda (Pn)

Progect:

Measure microbial abatement of ozone treatment with “EONOS F 60” equipment.

Date:

July 20, 2020

Authors:

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Introduction

On June 23, 2020, trials were performed to measure the effectiveness of microbial abatement of a sanitization treatment with an ozone base executed with "EONOS F 60" equipment ("purifier cabinet"). Such test was led by measuring the value of the microbial loads of strains innoculated on tissue samples; the test was conducted measuring 4 types of treatment:

- **Treatment 1:** Cycle 20 min (5 min ozone emission + 15 min forced-air circulation)
- **Treatment 2:** Cycle 30 min (10 min ozone emission + 20 min forced-air circulation)
- **Treatment 3:** Cycle 2hr (5 min ozone emission + 10 min forced-air circulation + 5 min ozone emission + 10 min forced-air circulation + 5 min ozone emission + 75 min forced-air circulation)

All the treatments were compared with counterparts of contaminated fabric but not subjected to treatment.

Preparation of the trials

First, liquid-soil suspensions were prepared of bacterial strains that were chosen on the basis of their normal presence on human skin (*Staphylococcus, Candida*), common pathogens in an acquatic environment (*Pseudomonas*) and bacteria with the ability to form spores (*Bacillus subtilis*). The selected strains are shown in the following table:

Microorganisms	Strain utilized
Staphylococcus aureus	ATCC 6538
Pseudomonas aeruginosa	ATCC 9027
Candida albicans	ATCC 76615

Table 1: Tested microbial strains

Preparation of the samples

The test was performed by measuring the efficacy of abatement on 10x10 cm test specimens of sample fabric (cotton). The tested specimens had not undergone preventative treatments and the contaminations were performed on the samples "as such."

Figure 1: Equipment tested “EONOS F60”

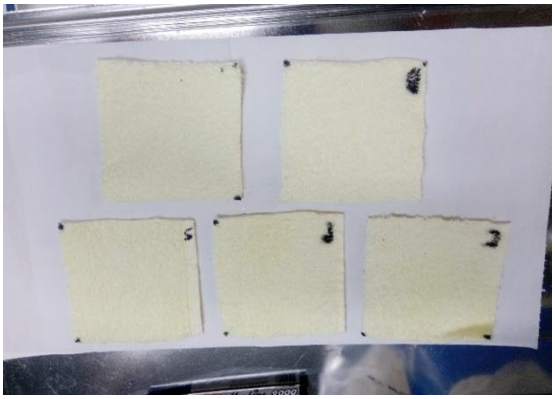

Figure 2: Exhibition of the specimen samples

Figura 3: Placement method of samples inside the cabinet (hung at half height)

Figure 4: Equipment "EONOS F60" - Particular source of ozone emission

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Performance of the trials

Exhibition of the test

Once the above-mentioned bacterial suspensions were placed, they were transported to the client's headquarters and utilized for the contamination of the specimens. The inocula were distributed both maintaining isolation among them (one microbial strain per sample specimen) and mixing together in a single media. This latter mode was performed for the purpose of measuring the microorganisms as "total microbial count."

In total four series of samples corresponding to the three types of treatment were prepared, plus a series, not subjected to treatments, that has represented the non-treated of reference.

Analyses procedure

Retrieval of the microrganisms

At the end of each treatment each specimen was gathered individually and inserted in a sterile baggie conveniently labled and then transported to the laboratory for the analyses phase.

The retrieval of the residual microorganisms from the samples was performed by inserting 100 ml of saline solution (Peptone-salt) inside the baggies and then subjected to mechanical shaking with a Stomacher. At the end of this phase the solvent was filtered with serial dilutions on a filter membrane (0.45 µm porosity), which was successively placed on a TSA agar culture medium for each microorganism; the culture plates were placed lastly to incubate for 24 hours at +37.0 ºC (at the exclusion of *Candida* seeded on Sabouraud Dextrose Agar incubated at +30.0 ºC for 72 hours).

Results

	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Candida albicans***	***Microrganismi totali a 30°C***
Non-treated sample	$2.4 \cdot 10^8$	$1.1 \cdot 10^8$	$3.1 \cdot 10^6$	$1.5 \cdot 10^9$
Treatment 1 "20-minute Cycle"	$3.3 \cdot 10^2$	$7.5 \cdot 10^6$	$1.7 \cdot 10^3$	$4.3 \cdot 10^7$

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Abatement	99.90%	93.20%	99.90%	97.10%
Abatement (Log)	5.9	1.2	3.3	1.5
Treament 2 "30-minute Cycle "	$4.7 \cdot 10^2$	$5.6 \cdot 10^6$	$6.2 \cdot 10^3$	$4.3 \cdot 10^7$
Abatement	99.90%	94.90%	99.80%	97.10%
Abatement (Log)	5.7	1.3	2.7	1.5
Treatment 3 "2-hour Cycle"	$< 2 \cdot 10^1$	$5.6 \cdot 10^5$	$4.4 \cdot 10^3$	$6.3 \cdot 10^6$
Abatement	99.90%	99.50%	99.90%	99.60%
Abatement (Log)	7.1	2.3	2.8	2.4

Table 2: Outcome of the tests – Comparison of treatments.

Conclusions

All the types of treatments considered result in having a good ability to abate the innoculated microbial strains.

The comparison of the treatments indicates a greater efficacy for Number 3 ("2-hour Cycle") while Number 1 ("20-minute Cycle"), though only slightly, shows that it has a relatively minor abatement efficacy. The performances of the various treatments appear, however, comparable even in a view to comparison of the efficacy/time relationship.

Faithfully,
Dr. Luigi Fratini

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