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A NEW PROCESS OF DISINFECTION AND DEODORIZATION OF HELMET BY OZONATION

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Abstract

The cleanliness and microbiological safety of helmets is an important issue for many and frequent users whether they are used in a professional setting and / or for leisure activities. Today, chemical-based spray disinfectant solutions are or can be used. Although the real effectiveness of these solutions is still to be demonstrated, the persistence of these chemicals compounds after treatment can be a real security problem for exposed users. The CleanCasc[®] process, developed as part of this study, proposes an alternative method of decontamination and deodorization of helmets using ozone gas. The results obtained during this feasibility study (i.e. commercial apparatus used) and synthesized in the framework of this presentation demonstrate that, depending on the different process parameters (ozone concentration, gas flow rate, etc.), the device used is perfectly capable to induce a reduction of more than 5 log₁₀ (CFU/mL) of a standard strain of *Staphylococcus aureus* after 3 minutes of ozone gas application. Even though the CleanCasc[®] (Figure 1) process is being finalized before the start of its large-scale commercialization phase, it can now be seen that the implementation of ozone gas during treatment makes it possible to respond already to requirements in terms of disinfection.

Keywords: Helmet, Disinfection and deodorization process, Ozone Gas, *Staphylococcus aureus*.

Introduction

The skin is an organ that naturally has a microbiological system called the skin microbiome. This one, hosts a great variability of microbiological species with more than 1000 species [1]. These organisms include [2]: (i) anaerobic diphtheroids (*Propionibacterium*); (ii) aerobic diphtheroids (*Corynebacterium*); (iii) aerobic cocci (*Micrococcus* and *Staphylococcus*); (iiii) yeast and fungi. This complex ecosystem limits the risk of colonization by a pathogenic species and thus protects the skin from the risk of inflammation [3]. Nevertheless, *Staphylococcus aureus*, in case of excessive proliferation, remains one of the most common and troublesome strain of bacteria causing disease in humans [4]. Wearing a protective helmet (motorcycle helmet, bicycle helmet, pilot helmet) causes the accumulation of microbial load inside it. The helmet consists of a hard shell on the outside and a foam on the inside. This foam is sensitive to bacterial contamination and proliferation following repeated use of the helmet; this phenomenon being particularly true when the temperature and the humidity are high (i.e. combination of factors favoring the development of microorganisms). Ozone (O₃), a gas known for its ability to reduce the microbiological load, CleanCasc Company has designed, for commercial purposes, an innovative system of ozonation treatment of pilot helmets.

However, in order to optimize the performance of the initial process and to meet the most demanding disinfection markets (i.e. reduction of more than $5 \log_{10}(\text{CFU/mL})$ of a standard strain of *Staphylococcus aureus*), scientific collaboration and technology have been put in place with Unilasalle EA7519 (manager of LaSalleO3 R&D platform). Even if efficiency of ozone is well known for microbiological disinfection surface, the aim of the present study was to validate and to optimize the disinfection process using gaseous ozone inside this commercial device.



Figure 1 : CleanCasc® device

Material and methods

Pilot helmet foam sampling

The various tests were carried out on a foam sample resulting from the cap of the Roof brand helmets. The model of the cap corresponds to ROOF R05 INNER LINING (Ref 40200161). For the microbiological evaluation, the foam samples were taken using a Facom 245A.M1 punch with its 245A.T30 nozzle. The cap samples thus taken correspond to 30 mm diameter foam discs, i.e. a surface of 7.07 cm^2 . The prepared samples were sterilized by autoclaving (Commodore Ref STERICLAV LAB1073) at 121°C for 15 minutes in a dry sealed bag.

Preparation of a mother suspension of Staphylococcus aureus

A suspension of *Staphylococcus aureus* strain CIP 4.83 [Biological Resource Center of the Institut Pasteur (CRBIP)] was used for the artificial inoculation of the foam discs. The preparation of this suspension is carried out the day before (D-1) of the inoculation. *Staphylococcus aureus* is extracted from the strainer and inoculated into a 250 mL Erlenmeyer flask containing 100 mL of Brain Heart Broth - BCC (Biokar Diagnostics, Ref BK015). The proliferation in broth is performed at 37°C for 16h. At the end of this production, a centrifugation stage (Eppendorf centrifuge, model 5810R) at $5000 \times g$ for 20 minutes at 4°C makes it possible to concentrate the suspension.

Two washing phases (washing with sterile deionized water) followed by centrifugation were carried out in order to separate the nutrient medium from the bacterial biomass produced. Finally, a last resuspension in 10 mL of sterile deionized water is performed. An enumeration on Baird-Parker agar with potassium tellurite egg yolk is made from this concentrated suspension (Biokar Diagnostics, Ref BK055 / BM018). The concentration of the parent suspension is $9.04 \text{ Log}_{10} (\text{CFU} / \text{mL})$.

Contamination of foam discs

The standardized foam disks at 7.07 cm² are inoculated with 200 µL of concentrated suspension of *Staphylococcus aureus*. The volume is uniformly distributed over the disc surface by depositing small droplets. The foam rapidly absorbs the droplets thus deposited on the surface. A step of drying in the open air makes it possible to eliminate the humidity of the suspension. The drying is carried out under a laminar flow hood (CYTAIR, model 125) for 16 hours at room temperature (T ≈ 20 °C).

Detection and enumeration of Staphylococcus aureus

The reference method used in the laboratory for *Staphylococcus* is AFNOR Standard NF V08-057-1 (Routine Method for the Counting of Coagulase-positive *Staphylococcus* by Colony Counting at 37 °C). Treated or untreated (i.e. control) disks by ozone gas are placed in a sterile plastic pouch for stomacher. A volume of 20 mL of sterile tryptone salt (Biokar Diagnostics, Ref BK014) is added to the pouch. The samples are kneaded with stomacher for 1 minute. Cascade dilutions were then carried out until a 1/10 000 000 dilution (i.e. dilution determined by preliminary tests) was obtained according to the general rules for the preparation of dilutions for the microbiological examination of NF V 08-010 standard. From the dilution range, culture dishes (Baird Parker agar with potassium tellurite egg yolk) are inoculated on the surface with 200 µL of suspension of each of the dilution tubes. For each dilution tube, two boxes of cultures are thus inoculated. The culture dishes are then incubated for 48 hours at 37°C before reading and counting. The reading is done on the medium Baird Parker by the search of black colony convex, shining and having a lysis halo around the colony. Confirmation by serotyping, coagulase search, Gram stain, catalase search and API 20 STAPH gallery was performed and validated the presence of *Staphylococcus aureus* on the culture dishes. Colony count is valid when the total number of colonies for a box is between 15 and 150 for surface seeding.

Ozonation

The helmet foam discs standardized at 7.07 cm² were processed by ozone (Figure 2). The ozone first went through a bubbler immersed in deionized water. The wet gas obtained was measured at the inlet by an ozone analyser BMT 964 OG (BMT, Messtechnik GmbH, Germany) then injected into a cylindrical connection sleeve ¼" made of inox 316L. Ozone gas flow (CFS3-2G, Ozonia, France) was performed from 3 to 177g/m³, 0.6m³/h for 3 minutes, followed by 1 minute of purge with air flow. The ozone dabbling process is important. It allows the flow of ozone gas to enrich in moisture.

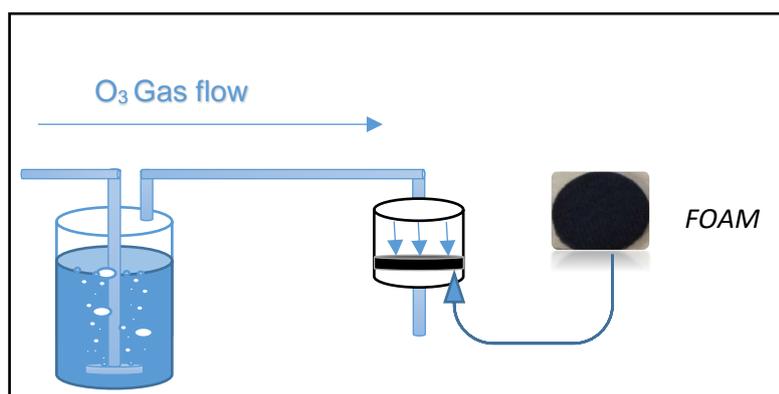


Figure 2 Ozonation process

Results and discussion

Ozone process was reported to be an effective method of bacteria and fungi disinfection [7]. Tests were conducted to observe the minimal ozone concentration useful to eliminate the strain of *Staphylococcus*. The ozone generator allowed to test a wide range ozone concentration from 3 to

177 g/m³ – 0.6m³/h – 3min. (Table 1). Destruction of *Staphylococcus aureus* was observed regardless of the concentration of applied ozone.

Table 1: Effectiveness of ozone treatment on *Staphylococcus aureus*

Ozone process (replicate n=2)	Ozone Concentration (g/m ³)					
	0	3	26	33	43	177
Staph Log ₁₀ (CFU/mL)	7.74	2.17	0	0	0	0

In this study, the highest antimicrobial efficacy of ozone was achieved with an ozone concentration of 26 g/m³. There is no interest here to increase the ozone concentration more than 26 g/m³. And although at 3g/m³ of ozone, the reduction of the number of *Staphylococcus* exceeds the criteria of disinfection of 5 Log₁₀(CFU/mL) of bacteria, the study was continued with the concentration of 26 g/m³. The various optimization tests that are in progress show that technical adaptations can be proposed to reduce the ozone concentrations used without limiting the power of decontamination. The late stage of this study was to confirm the effectiveness of the disinfection of bacteria in the aforementioned conditions. Table 2 presents by way of illustration the results obtained for the following experimental conditions: ozone concentration of 26 g/m³, ozone gas flow of 0.6 m³ for 3 minutes, followed by 1 minute of purge. Under these operating conditions, as can easily be seen from the results presented, the treatment with gaseous ozone of the contaminated helmet foams enables to reach an almost total decontamination (i.e. under the detection level) of the supports contaminated with *Staphylococcus aureus*. More than 7 Log₁₀(CFU/mL) *Staphylococcus aureus* were inactivated in 3 minutes of ozone exposition.

Table 2: Effectiveness of ozone treatment on *Staphylococcus aureus* at 26g/m³ Ozone

Ozone process (replicate n=5)	Ozone process 26 g/m ³ – 0.6m ³ /h – 3min				
	Number of <i>Staphylococcus aureus</i>				
	CFU/mL	Average (CFU/mL)	Standard Deviation (CFU/mL)	Log ₁₀ (CFU/mL)	Reduction Log ₁₀ (CFU/mL)
Contaminated Foam (Control)	4.40E+07 4.65E+07 5.36E+07 4.77E+07 7.16E+07	5.27E+07	1.11E+07	7.72	-
Contaminated Foam treated by ozone	0* 0* 0* 0* 0*	0	-	-	7.72

* Below the detection limit of 1500 CFU/mL or 3.17 Log₁₀ (CFU/mL) and no reviviscence in BCC medium

Conclusion

This study demonstrated that the CleanCasc unit achieves unrestricted the disinfection objectives for vegetative forms represented here by *Staphylococcus aureus* (greater than 5 log₁₀ CFU/mL). Nevertheless, the rest of the study should document the effect of the CleanCasc treatment on endospore forms and yeast and fungi strains. Other ozonation systems for workwear, footwear or agri-food equipment already exist on the market. However, these systems require a very long ozone

treatment time of several hours. For the first time, an autonomous system is able to achieve a reduction of the microbiological load in less than 4 minutes with a relatively small amount of ozone applied. This is where the originality of the CleanCasc process; the treatment can be applied routinely in the context of a community application: leisure helmet rental, transport helmet rental.

REFERENCES

1. Paller, A.S., Kong, H.H., Seed, P. Naik, S., Scharschmidt T.C. Gallo, R.L., Luger, T., Irvine, A.D... 2018. The microbiome in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, Volume 143, Issue 1, 26-35.
2. Kligman, A. M., Leyden, J. J., McGinley K. J. 1976. *Bacteriology. The journal of Inverstigative Dermatology*, Volume 67, 160-168
3. Hacard, F., Nosbaum, A., Huynh, V. A., Nicolas, J. F., Bérard, F. 2015. *Plus il y a de bactéries différentes, moins il y a d'inflammation : la révolution microbiotique*. In *Annales de Dermatologie et de Vénérologie* Volume 142, pp. S13-S17.
4. Otto, M. 2008. Staphylococcal biofilms. *Curr. Top. Microbiol. Immunol.* Volume 322, 207-228.
5. Zanotta, M., Winters,M. 2017. Helmet use among personal Bicycle riders and bike share users in Vancouver,B.C, *Am. J. Prev. Med.*, 53(4),465-472.
6. Öztekin, S., Zorlugenç B., Zorlugenç F. K. L. 2006. Effects of ozone treatment on microflora of dried figs. *Journal of Food Engineering*. Volume 75, 369-399.
7. Masotti, F., Vallone, L., Ranzini, S., Silveti, T., Morandi, S. 2019. Effectiveness of air disinfection by ozonation or hydrogen peroxide aerosolization in dairy environments. Volume 97, 32-38.