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Date
23 September 2015

Our reference
MSB-2015-086-VOJ-kek

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Project number
060.14852/01.62.01

Your reference
Email. woensdag 2 september 2015

Copy to
H. Rahaoui, Secretary

Subject
Efficacy of disinfection by ozone generated in air distribution system

Dear Mr. Bakermans,

Please find attached the report on the analysis of the disinfection efficacy of the Diamond Solutions ozone based air disinfection system in a model air-distribution system.

Please feel free to contact me in case you need additional information.

Kind regards,

Jos van der Vossen, PhD
Senior Scientist Microbiology and Systems Biology

REPORT

Title
Efficacy of bacterial elimination by ozone generated in applying the Diamond Solutions system in air distribution.

Introduction
At the request of Diamond Solutions BV, located at Eindhoven, The Netherlands, the research group Microbiology and Systems Biology of TNO, Zeist, investigated the bacterial elimination efficacy of the Diamond air ozone generator system in an air distribution application for two types of bacteria.

Experimental
The experiments were basically conducted as described by Kowalski et al. (2003). In this investigation, 4 Diamond air ozone generators were placed in line in an air distribution channel theoretically keeping the concentration ozone at about 1ppm. Upstream a fan was inserted for transport of air and ozone into the air channel. Subsequently, the air channel contains a chamber for inserting 6 petri-dishes with bacteria of interest to study the efficacy of bacterial elimination. At the end of the air channel, the point of exhaust, an active carbon filter is present for ozone capturing and finally a HEPA filter preventing the release of possible viable bacteria. The condition of the air in the laboratory was 18°C and relative humidity of 65-68%.

In air the following reaction will proceed:

$$2\,O_3 \rightarrow 3\,O_2$$

In addition, at the carbon filter the following reaction will proceed:

$$C + 2\,O_3 \rightarrow CO_2 + 2\,O_2$$

In the investigation, the eliminating effect of ozone is determined on two types of bacteria:

- Gram positive bacterium, *Staphylococcus aureus* ATCC 6538;
- Gram negative bacterium *Escherichia coli* ATCC 8739;

These two bacteria are also included in the test as the species *S. aureus* represent potentially food borne pathogens as well as methicillin resistant nosocomial strains (MRSA) and the species *E. coli* comprises non-pathogenic gut commensals as well as zoonotic strains.

The two types of bacteria were present at low density and high density inoculation levels being about 10^2 colony forming units (cfu) per petri dish and a factor 100 higher level, about 10^4 cfu/petri dish containing tryptone soy agar.

The duration of exposure to the air with ozone generated by the 4 Diamond Solutions systems was 90 minutes and 150 minutes. The investigations were executed at two different days. For each day of investigation a fresh culture of the two bacteria were prepared and used for inoculation of the petri dishes prior exposure to ozone. After exposure to the plates were incubated at 37°C during about 16 hours. The number of colonies on the plates were counted and compared to the colony counts on petri dishes that were not exposed as control.

Results

The results demonstrate the eliminating effect of the generated ozone in the air on the two types of bacteria included in the test. The results are summarized in table 1. Moreover, the effects on cfu/plate are graphically presented in figure 1 to 4; in which figure 1 shows the results of bacterial elimination on petri dishes with low bacterial density after 90 minutes of exposure to ozone compared to the control without exposure, figure 2 shows the results of bacterial elimination with high bacterial density after 90 minutes of exposure to ozone compared to the control, figure 3 shows the results of bacterial elimination with low bacterial density after 150 minutes of exposure to ozone compared to the control, and, figure 4 shows the results of bacterial elimination with high bacterial density after 150 minutes of exposure to ozone compared to the control.

Table 1. summary of results showing the efficacy of bacterial elimination by ozone under the different conditions tested.

	Low density	High density	Average
Percentage reduction viability *Staphylococcus aureus* 90 minutes exposure time	94.5%	96.7%	95.6%
Percentage reduction viability *Escherichia coli* 90 minutes exposure time	98.5%	99.7%	99.1%
Percentage reduction viability *Staphylococcus aureus* 150 minutes exposure time	98.6%	99.3%	98.9%
Percentage reduction viability *Escherichia coli* 150 minutes exposure time	99.6%	99.9%	99.8%

Conclusions

- The ozone generated in the air distribution channel reduces the viability of bacteria present on the petri dishes.
- The dose of ozone applied reduces the number of cfu/plate with at least 94,5% in 90 minutes.
- The strongest eliminating effect of ozone is observed after 90 minutes of exposure. A longer period of exposure adds relatively less strong to the viability eliminating effect, as observed after 150 minutes of exposure.
- The Gram-negative *Escherichia coli* cells seem to be more sensitive to ozone as the Gram-positive *Staphylococcus aureus* cells.

Literature

Kowalski, WJ, Bahnfleth, WP, Striebig, BA, and Whittam, TS. 2003.
Demonstration of a hermetic airborne ozone disinfection system: study on E. coli.
AIHA J. (Fairfax, Va) 64:222-227.

Signature

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Figures

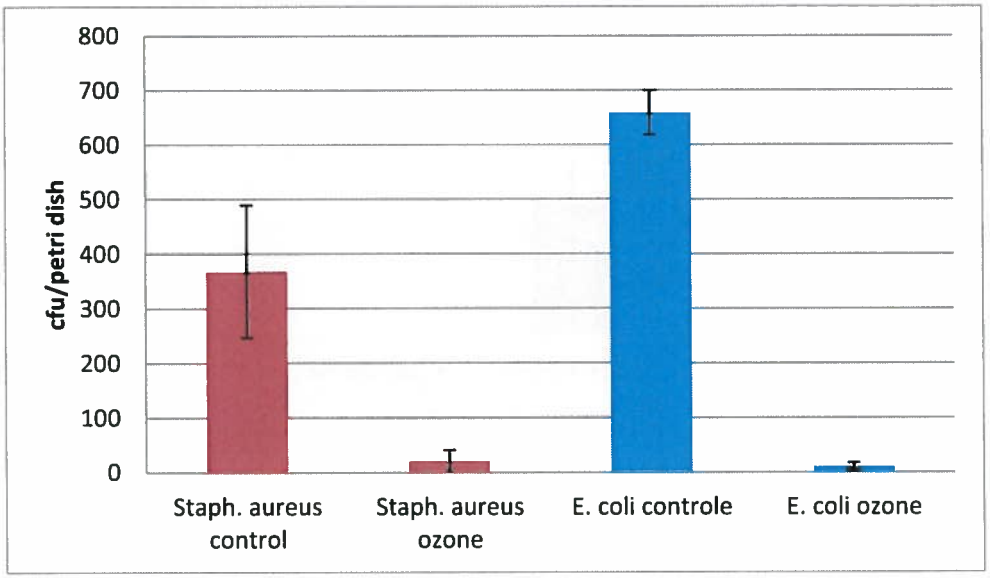


Figure 1. Effect of ozone on low bacterial density on petri dish in 90 minutes exposure

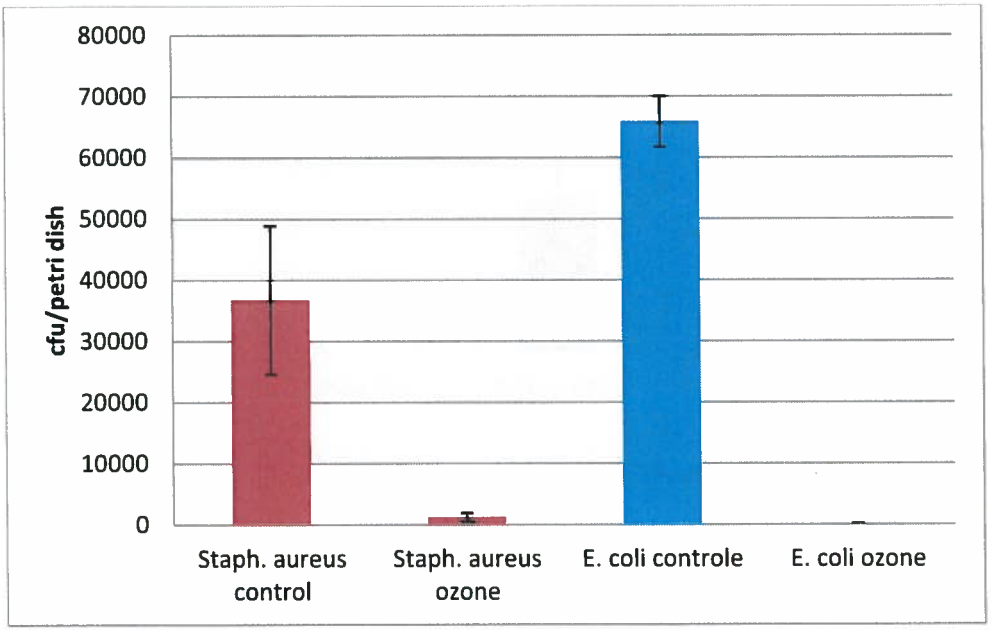
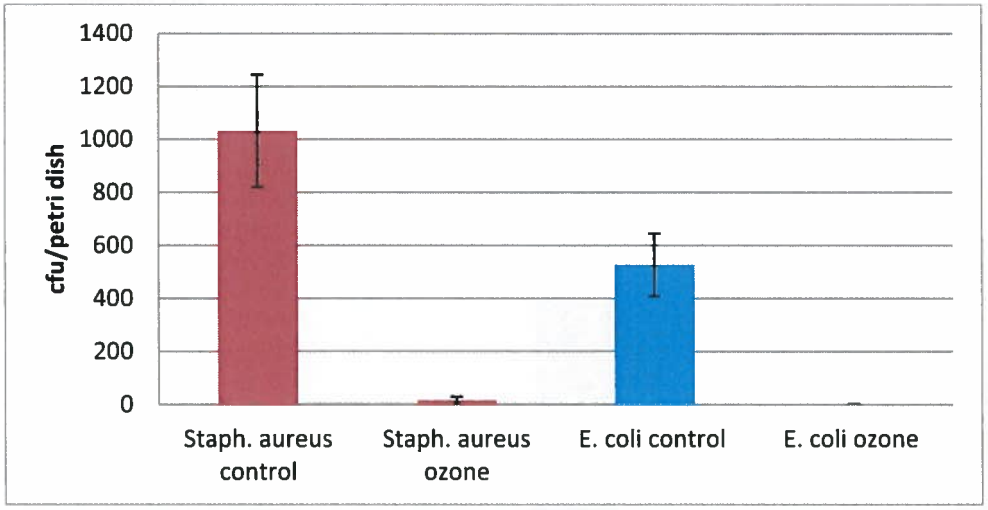
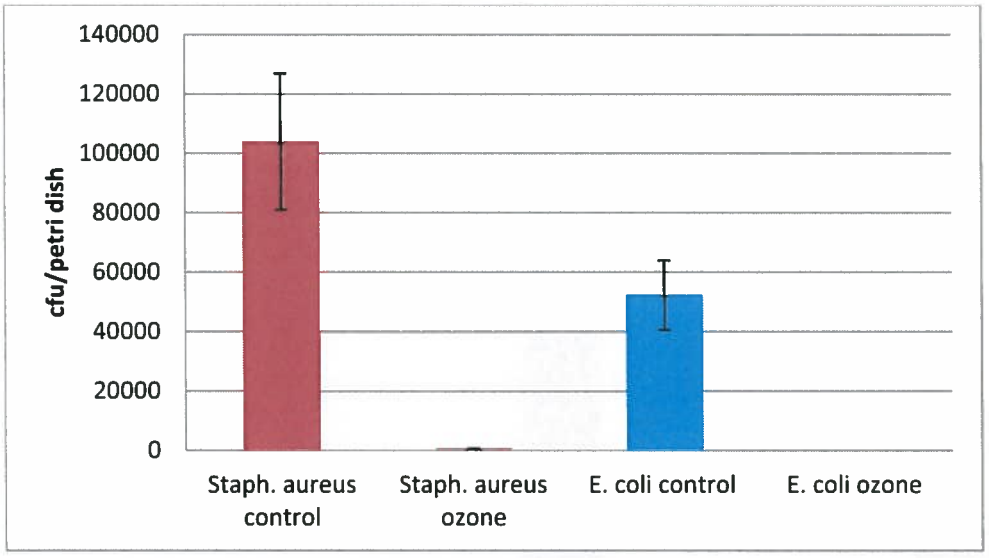


Figure 2. Effect of ozone on high bacterial density on petri dish in 90 minutes exposure



Figuur 3. Effect of ozone on low bacterial density on petri dish in 150 minutes exposure.



Figuur 4. Effect of ozone on high bacterial density on petri dish in 150 minutes exposure.