



Laboratory technical report

**Study of the capacity of ozone as a disinfectant for living spaces.
Equipment: PURO₃ 150 W unit**

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0. EXECUTIVE SUMMARY

This report presents the results of the *Escherichia coli* (*E. coli*) disinfection tests on melamine surfaces inoculated with a known initial concentration of *E. coli* of $1.65 \cdot 10^9$ colony formation unit (CFU)/ml using an ozone generator.

Surfaces have been placed on the floor inside a closed room and exposed during 30 min to ozone generation, using the PURO₃ ozone generator (by DGR-Green) of 150 W, placed at around 1.25m from the floor.

The disinfection capacity of this system is around 94.4% for *E. coli*, achieving an ozone concentration inside the testing room up to 4.3 ppm.

1. GOAL

The objective of this offer was to evaluate the disinfection capacity of the 150 W PURO₃ ozonation system on a certain surface (melamine) located in a 78.7 m³ close room at 30 min of exposure time, using the *E. coli* bacteria as a reference microorganism.

2. EXPERIMENTAL PART

2.1. DESCRIPTION OF THE DISINFECTION SYSTEM

The PURO₃ ozone disinfection system developed by DRG Green has a power of 150 W (Figure 1).



Figure 1. PURO₃ ozone disinfection system of 150 W.

2.2. TEST DESCRIPTION

This system was located in the central part of an empty space of 6.7 x 4.7 x 2.5 m (78.7 m³) (Figure 2) at 1.25 m of the floor. The melamine surfaces (specimens of 2 x 2cm) inoculated with a known concentration of *E. coli* were placed on the floor of the test room, at a horizontal distance of approximately 3m from the generator (Figure 3).

The disinfection capacity of the generator was evaluated during 30 min in a testing room closed without any fan inside to move the air. After 30 min of treatment, melamine surfaces were removed to be analyzed. Subsequently, an air suction pump that passed through an activated carbon column provided by Eurecat was used to accelerate the ozone degradation.



Figure 2. Ubication of the ozone generator (PURO₃ 150 W) and ozone sensor in the testing space of 78.7 m³.



Figure 3. Melamine surfaces inoculated with *E. coli* placed on the floor of the testing space.

The evolution of ozone concentration inside the testing room during the experimental time was quantified by an ozone gas detector from Dino Purification Co, Ltd., in a range from 0 to 100 ppm of O₃ (Figure 4). The sensor was placed at approximately 0.8 m from the floor.



Figure 4. Ozone sensor used during experiments.

For the performed assay, a total of 6 plates (3 positive controls for melamine surface and 3 for the experiment) were inoculated with an *E. coli* culture, dropping 50 µl of stock at a known concentration of approximately $1.65 \cdot 10^9$ CFU / mL.

The experimental procedure carried out on the different surfaces is detailed below:

- 3 positive controls: surfaces inoculated and without application of the treatment, with nomenclature C (+) SurfaceA 1, C (+) SurfaceA 2, C (+) SurfaceA 3.
- Melamine: samples inoculated and subsequently treated by ozone, with nomenclature M1, M2, M3.

Once the samples were inoculated, they were dried for 60 minutes in a laminar flow cabinet.

Once dry, the different surfaces are treated in triplicate under the operational conditions specified above.

2.3. METHODOLOGY FOR ANALYSIS

Extraction of *E. coli* from the surfaces

The inoculated surfaces were inserted into a sterile tube containing 15ml of LB culture medium. The tubes were vortexed for 2min and 18min at 1000 rpm (Thermomixer, Eppendorf), to obtain the extract for analysis.

E. coli analysis

The concentration of *E. coli* in each extract was determined by two techniques, depending on the limit of quantification required:

- Plate culture in solid LB medium, with incubation at 37°C for 24 ± 4 hours (UNE-EN-ISO 6222: 1999).
- Colilert-18 assay with Cuanti-Tray / 2000 trays (IDEXX), which uses the technology of substrate defined with fluorescence for the quantification of *E. coli*, with incubation at 37°C between 18-24 hours (ISO 9308-2: 2012).

3. RESULTS

The results of the *E. coli* analysis on each extract are detailed in Table 1 in terms of most probable number (MPN) / ml in the extract. Each analysis has been carried out in duplicate and in different dilutions, and the average values obtained are shown in the table.

Table 1. *E. coli* count in the positive controls of the extracts of the melamine surfaces.

Ozone system	Surface type	Sample abbreviation	[<i>E. coli</i>] (NMP/ml)
PURO ₃ 150 W	Melamine	C (+) M1	2.22 · 10 ⁵
		C (+) M2	2.05 · 10 ⁵
		C (+) M3	2.48 · 10 ⁵

Positive controls show the initial reference concentration and they are used to calculate the reduction of logarithmic units according to the following formula:

$$\text{Log reduction} = \log [E. coli]_{\text{initial}} - \log [E. coli]_{\text{final}}$$

Where: $\log [E. coli]_{\text{initial}}$ corresponds to the extract concentration of the positive controls.

$\log [E. coli]_{\text{final}}$ corresponds to the extract concentration after the disinfection tests.

For achieving the results, the logarithmic reduction value is calculated from the arithmetic mean of the three replicates analyzed. Results are shown in Table 2.

Table 2. Disinfection results for the operational conditions evaluated.

Conditions	Type of surfaces	[<i>E. coli</i>] initial (MPN/ml) mean	[<i>E. coli</i>] final (MPN/ml) mean	Reduction (logarithmic units)	Reduction (%)
30 min O ₃ generation	Melamine	$2.25 \cdot 10^5$	$1.27 \cdot 10^4$	1.4	94.4

The evolution of ozone concentration through the time is depicted in Figure 5.

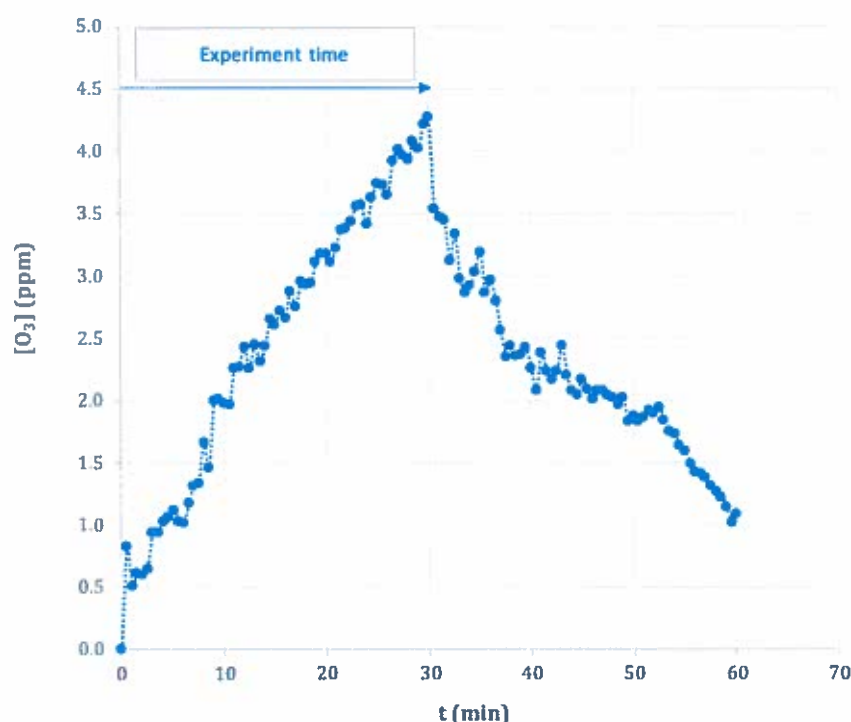


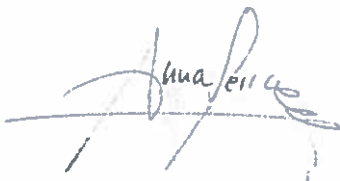

Figure 5. Time evolution of ozone concentration inside the testing space.

Obtained results show a removal of *E. coli* from melamine surfaces of 94.4% (reduction of 1.4 logarithmic units) in 30 min of ozone generation. The ozone concentration inside the testing space increased up to 4.3 ppm O₃, being the time where ozone has a concentration higher to 4 ppm of approximately 3 min. It is worth to say that the concentration of ozone on the floor, where melamine surfaces are placed could be higher than at 0.8 m (the distance of ozone sensor), thus higher than the values of Figure 5.

4. CONCLUSIONS

The disinfection capacity of PURO₃ ozone system with a power of 150 W is around 94.4% for *E. coli* removal from melamine surfaces inoculated with an initial concentration of $1.65 \cdot 10^9$ CFU / mL after 30 min of ozone generation in a closed room of , aching an ozone concentration inside the testing room up to 4.3 ppm O₃ and a concentration higher than 4 ppm O₃ during 3 min.

5. SIGNATURES

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6. CHANGE CONTROL

Version	Data	Modified section	Description
1	06-08-2020		

