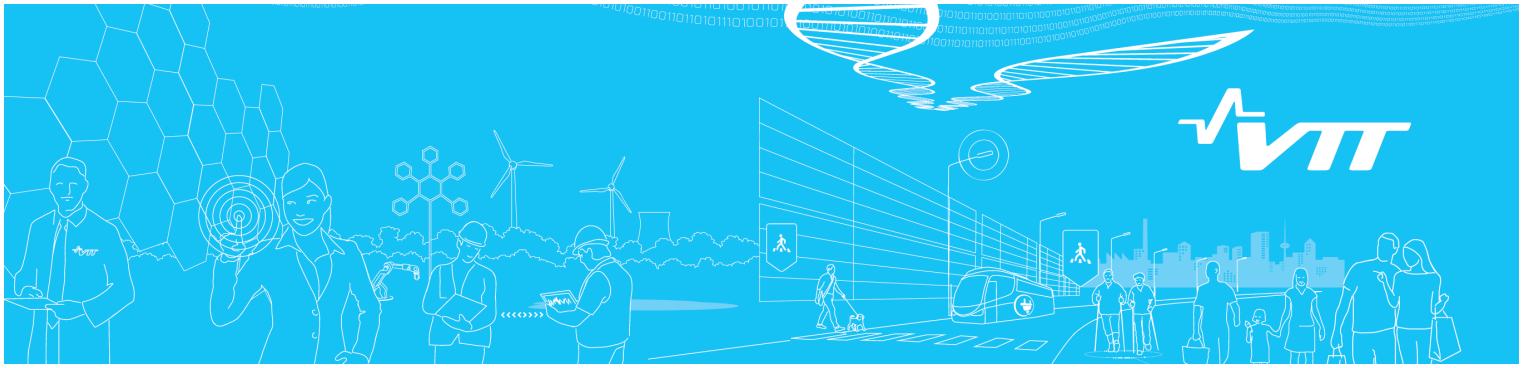


CUSTOMER REPORT

VTT-CR-03980-14 | 18.12.2014

Efficacy of treatments in ozone cabinet against microbes

Author: Satu Salo

Confidentiality: Confidential

Report's title

Efficacy of treatments in ozone cabinet against microbes

Customer, contact person, address	**Order reference**
Juha Punta Oy Riku Luomaniemi Teollisuustie 2 25460 Kisko Finland	E-mail 14.8.2013
Project name	**Project number/Short name**
Turvateho	84573

Summary

In this study the efficacy of ozone treatments in Hygio ozone cabinet against five different microbes in two materials using three ozonation programs was measured. Based on this study Hygio a40 is reducing microbial growth from test materials. The microbicidal efficacy was significantly improved when the ozone treatment lasted longer. The 10 min program is very mild but 60 min program significantly reduces microbial counts especially from laboratory coat material. Microbes were easier to kill from laboratory coat material than from insole material. Especially the counts of *Staphylococcus epidermidis* and *Escherichia coli* were significantly reduced from coat material during 60 min ozone treatment. More than 5 log units of *Staphylococcus epidermidis* was killed from coat material with 60 min ozone treatment which can clearly be considered as an efficient treatment.

Espoo 18.12.2014

Written by	**Reviewed by**	**Accepted by**
Satu Salo Senior Scientist	Maria Saarela Principal Senior Scientist	Johanna Maukonen Research Team Leader

VTT's contact address

VTT, P.O.Box 1000 (Tietotie 2 Espoo), 02044 VTT

Distribution (customer and VTT)

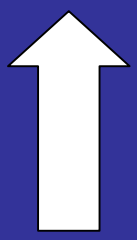
Juha Punta Oy, Riku Luomaniemi, 1 copy
VTT Archive, 1 copy

Contents

1. Description and objectives

Ozone is known to be toxic gas. In this study the efficacy of ozone treatments in Hygio ozone cabinet against various microbes was measured. For different applications the requirements for microbicidal efficacy vary. In this study tested parameters were all three ozonation programs in Hygio, five different microbes and two different materials. The ozonation programs of Hygio are fixed to 10 min, 30 min and 60 min programs and the ozone levels are not adjustable by user. Selected microbes for this study are *Bacillus atrophaeus, Escherichia coli, Staphylococcus epidermidis, Candida albicans* and *Cladosporium sphaerospermum; B. atrophaeus* is used as a spore suspension and it represents bacterial spore that is difficult to kill (table 1), *Escherichia coli* is gram-negative bacteria and commonly used as hygiene indicator, *Staphylococcus epidermidis,* common bacteria living on human skin is representing gram-positive bacteria, *Candida albicans* is a pathogenic yeast representing vegetative fungi and *Cladosporium sphaerospermum* is mould representing fungal spores. The two different test materials in this study were laboratory coat made of 35 % cotton and 65 % polyester for simulating ozonation of protective clothing and shoe insole simulating ozonation of shoes (Figure 1).

Table 1. Classification of microbes by their resistance to disinfection (McDonnell 2011).

	Microbe	Examples
Typically more resistant microbes  **Typically less resistant microbes**	Bacterial spores	*Bacillus spp., Clostridium spp.*
	Mycobacteria	*Mycobacterium tuberculosis*
	Non-enveloped, non-lipid viruses (hydrophilic)	*Norovirus, poliovirus, MS2*
	Fungal spores	*Aspergillus* spp. *Penicillium* spp.
	Gram-negative vegetative bacteria	*Pseudomanas* spp., *Escherichia coli*
	Vegatative fungi	*Aspergillus* spp., *Candida* spp.
	Large non-enveloped viruses	*Adenovirus, rotavirus*
	Gram-positive bacteria	*Staphylococcus* spp.
	Enveloped, lipid viruses (lipophilic)	*HIV, hepatitis B*

2. Methods

Hygio a40 ozone cabin was brought to VTT by customer. The time cycles and ozone production of ozonation programs were set by customer. The program number I lasted 10 min, number II lasted 30 min and number III lasted 60 min. The cabin was filled with four laboratory coats (35 % cotton and 65 % polyester). A test material pieces of laboratory coat and shoe insole (Finsole Mionetta fabric) were 15 cm x 20 cm and they were autoclaved before contamination with 5 ml inocula. Microbes used were *Bacillus atrophaeus* (former names *Bacillus subtilis* var. *niger*, *Bacillus globigii*, red strain) VTT E-052737 (spore suspension), *Staphylococcus epidermidis* VTT E-97768T (gram positive bacteria), *Escherichia coli* VTT E-97835 (gram negative bacteria), *Candida albicans* VTT C-96263

(yeast) and *Cladosporium sphaerospermum* VTT D-94423 (mould). Test pieces (three of each) were placed in ozone cabin between laboratory coats (figures 1 and 2). After ozone treatment each test piece was placed in stomacher bag with diluent, homogenized and cultured on Petrifilm AC (for bacteria) or YM (for yeasts and moulds). After incubation colonies were counted. Tests were performed with three replicate samples in two replicate test setup.

Figure 1. Test pieces ready to go to ozone treatment in Hygio.

Figure 2. Hygio was filled with four laboratory coats and test pieces.

3. Results

Results for both test materials and for tested microbes are shown in figures 3-12 and in table 2. The unit used is logarithmic value of colony forming units recovered from whole test piece (log CFU/test piece). Microbicidal efficacy is the difference between untreated test piece and treated test piece (reduction of growth, log CFU/test piece).

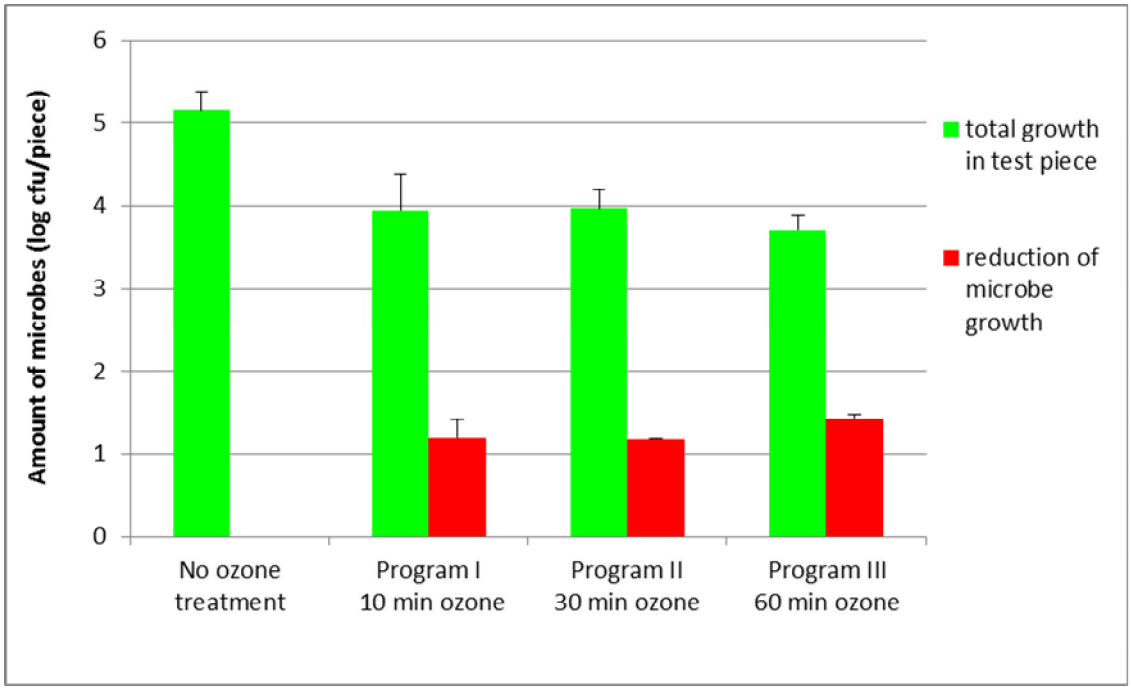


Figure 3. Efficacy of ozone treatments against *Bacillus* spores in coat material.

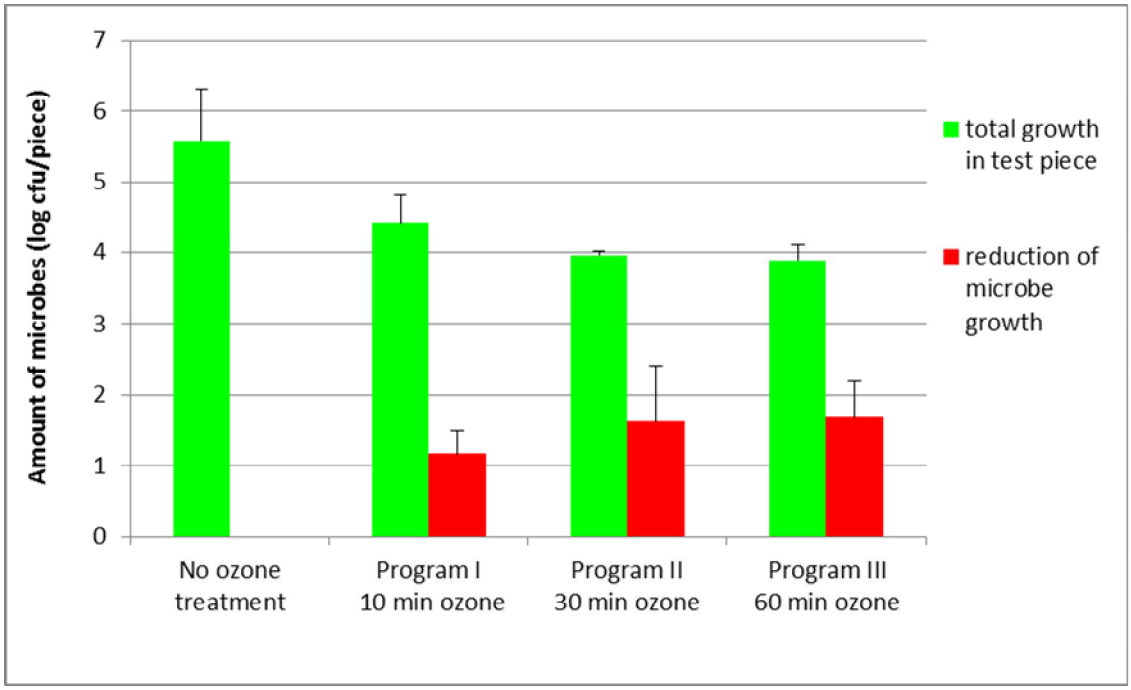


Figure 4. Efficacy of ozone treatments against *Bacillus* spores in insole material.

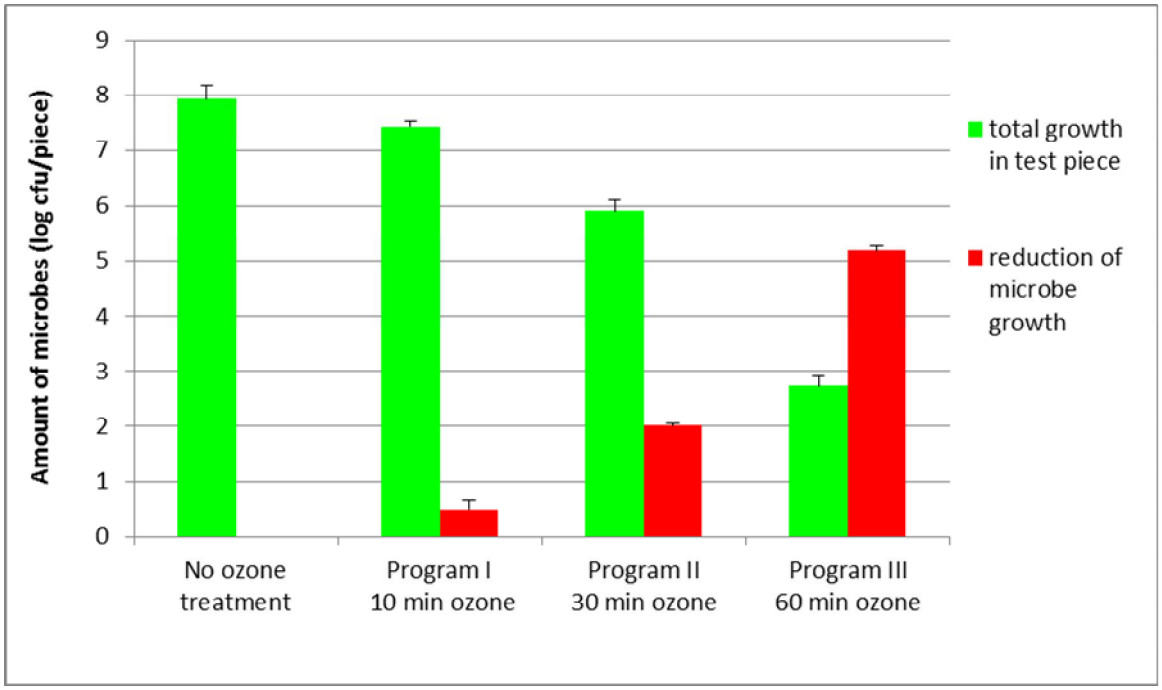


Figure 5. Efficacy of ozone treatments against *Staphylococcus epidermidis* in coat material.

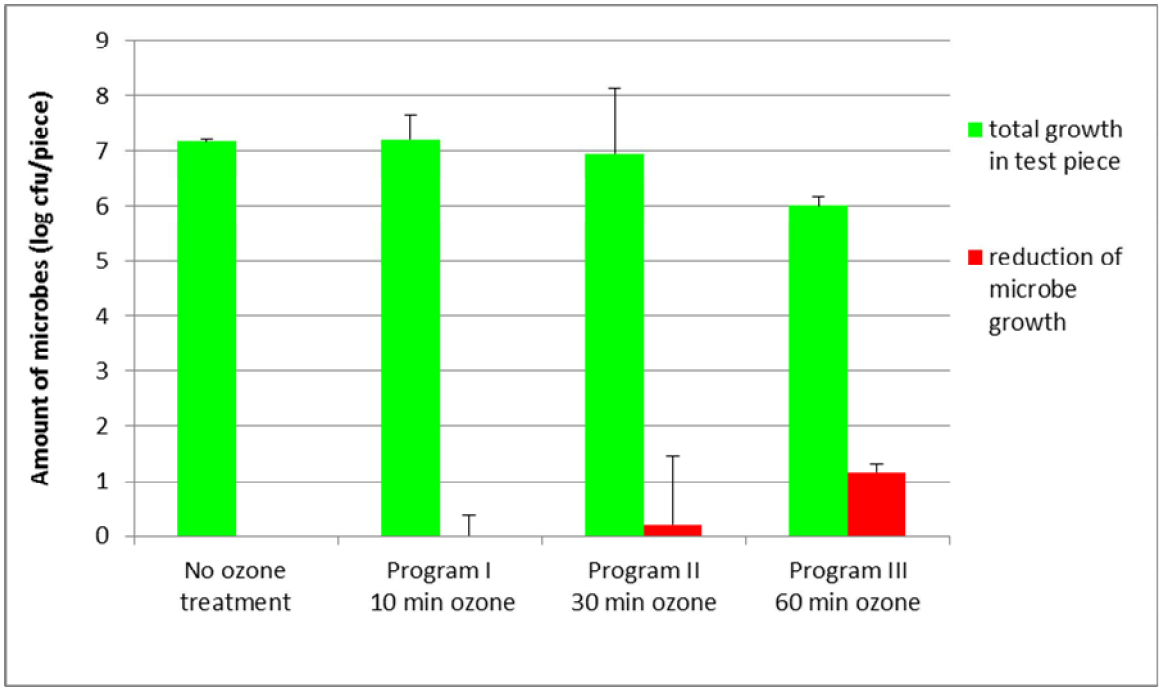


Figure 6. Efficacy of ozone treatments against *Staphylococcus epidermidis* in insole material.

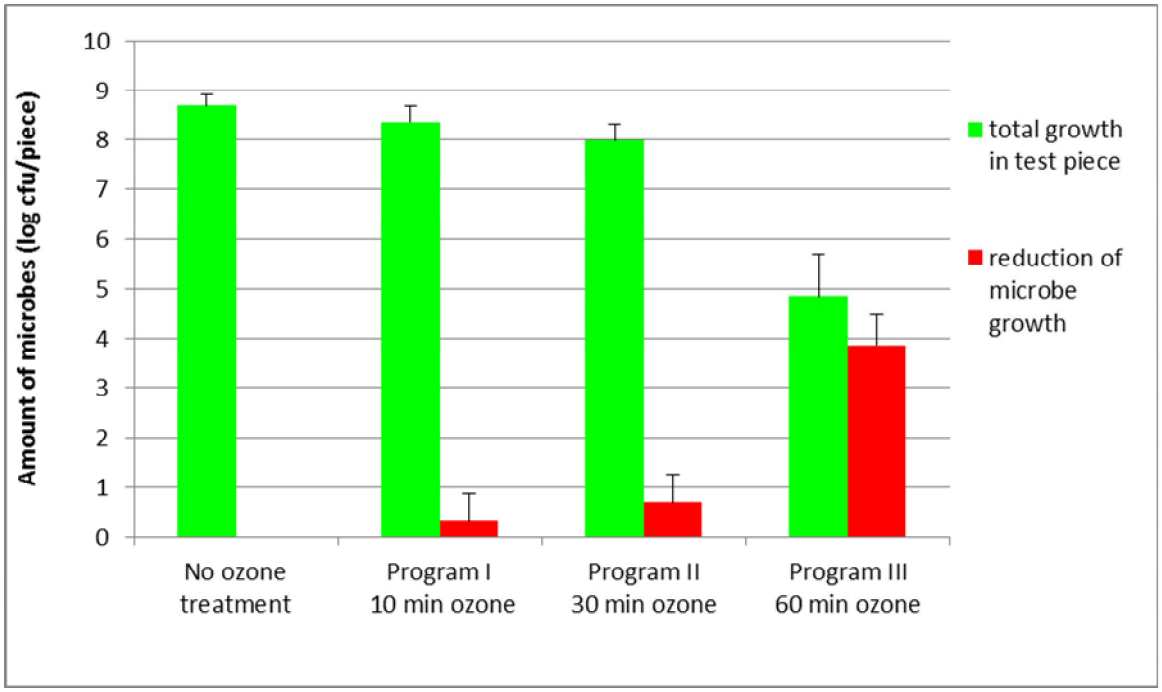


Figure 7. Efficacy of ozone treatments against *Escherichia coli* in coat material

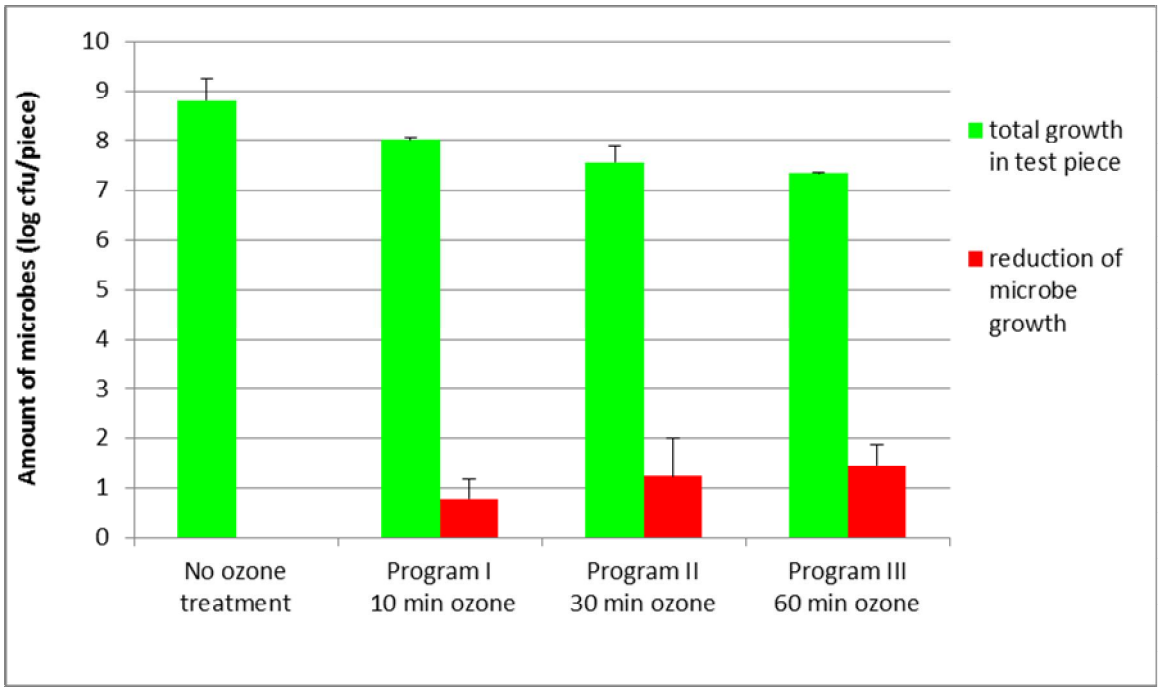


Figure 8. Efficacy of ozone treatments against *Escherichia coli* in insole material.

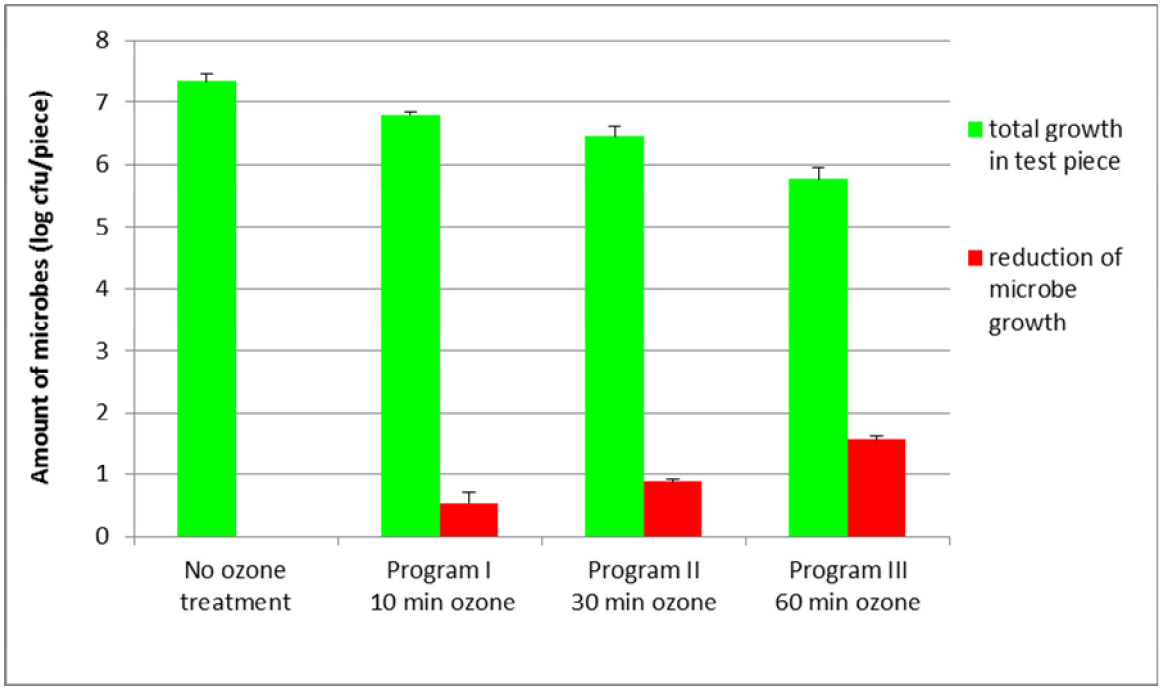


Figure 9. Efficacy of ozone treatments against *Candida albicans* in coat material.

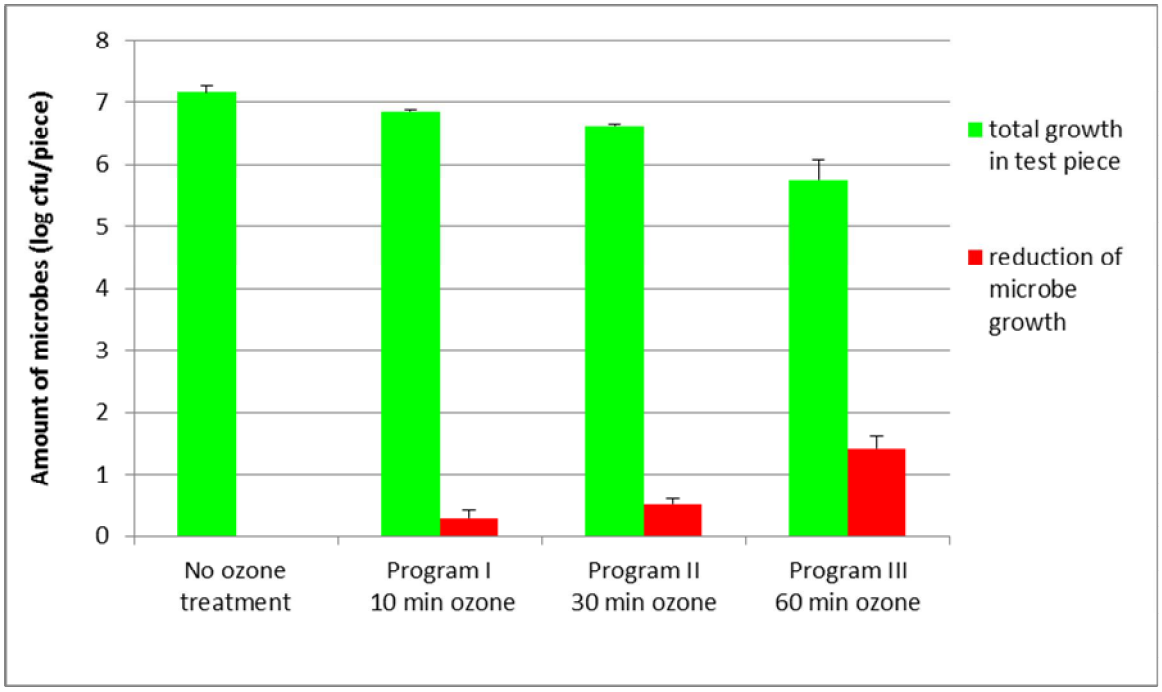


Figure 10. Efficacy of ozone treatments against *Candida albicans* in insole material.

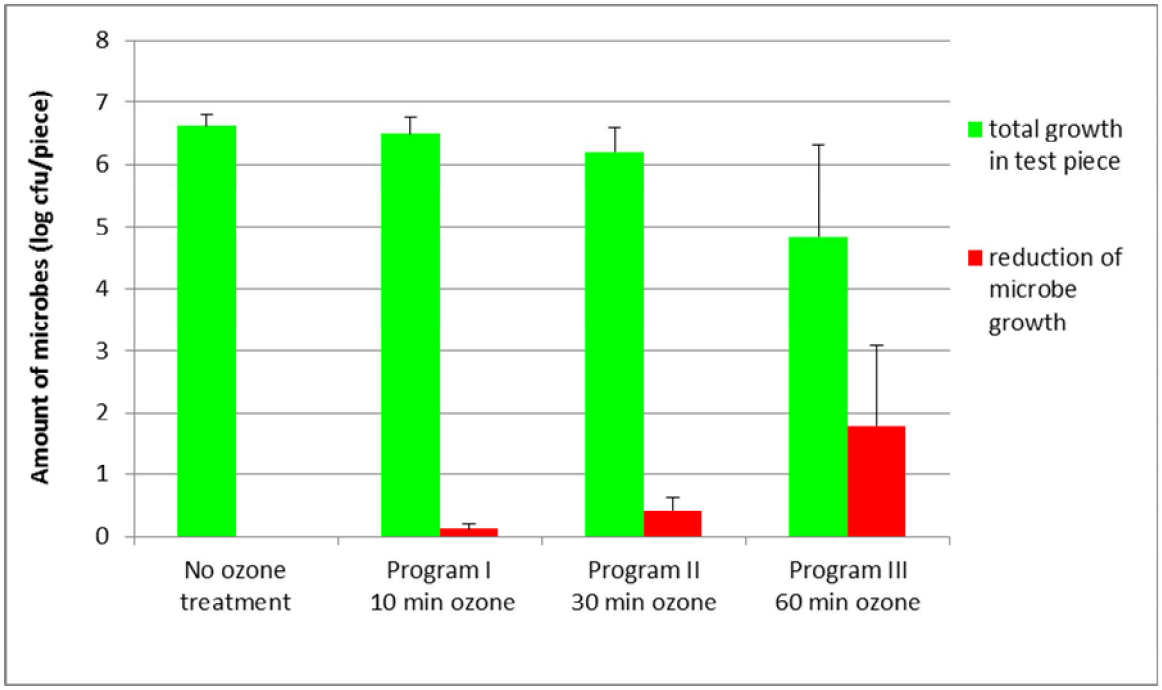


Figure 11. Efficacy of ozone treatments against *Cladosporium sphaerospermum* in coat material.

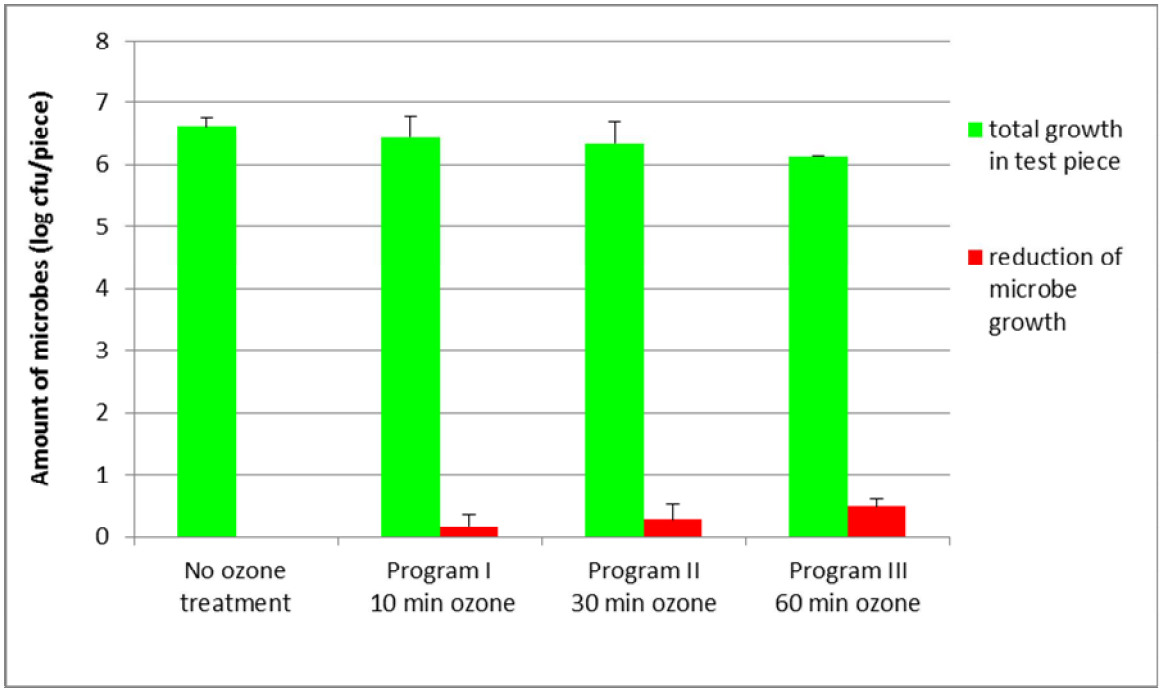


Figure 12. Efficacy of ozone treatments against *Cladosporium sphaerospermum* in insole material.

Table 2. Results of the test series of efficacy studies.

		Program		Program I				Program II				Program III			
		No ozone treatment		10 min ozone				30 min ozone				60 min ozone			
Microbe	Material	growth (log cfu/piece)	st. dev	growth (log cfu/piece)	st. dev	reduction of growth (log cfu/piece)	st. dev	growth (log cfu/piece)	st. dev	reduction of growth (log cfu/piece)	st. dev	growth (log cfu/piece)	st. dev	reduction of growth (log cfu/piece)	st. dev
Bacillus spores	Coat	5,2	0,2	3,9	0,4	1,2	0,2	4,0	0,2	1,2	0,0	3,7	0,2	1,4	0,1
	Insole	5,6	0,7	4,4	0,4	1,2	0,3	4,0	0,1	1,6	0,8	3,9	0,2	1,7	0,5
Staphylococcus	Coat	7,9	0,3	7,4	0,1	0,5	0,2	5,9	0,2	2,0	0,0	2,7	0,2	> 5,2	0,1
epidermidis	Insole	7,2	0,0	7,2	0,4	0,0	0,4	6,9	1,2	0,2	1,2	6,0	0,2	1,2	0,1
Escherichia coli	Coat	8,7	0,2	8,4	0,3	< 0,3	0,5	8,0	0,3	0,7	0,6	4,8	0,8	3,9	0,6
	Insole	8,8	0,4	8,0	0,0	0,8	0,4	7,6	0,3	1,2	0,8	7,3	0,0	1,5	0,4
Candida albicans	Coat	7,3	0,1	6,8	0,0	0,5	0,2	6,5	0,2	0,9	0,0	5,8	0,2	1,6	0,0
	Insole	7,2	0,1	6,9	0,0	0,3	0,1	6,6	0,0	0,5	0,1	5,7	0,3	1,4	0,2
Cladosporium	Coat	6,6	0,2	6,5	0,3	0,1	0,1	6,2	0,4	0,4	0,2	4,8	1,5	1,8	1,3
sphaerospermum	Insole	6,6	0,1	6,4	0,3	0,2	0,2	6,3	0,4	0,3	0,2	6,1	0,0	0,5	0,1

4. Conclusions

Based on this study Hygio a40 is reducing microbial growth from the test materials. The microbicidal efficacy of program I (10 min) was generally only slight. The reductions under 1 logarithmic unit were not considered significant in this study. More than 5 log units of *Staphylococcus epidermidis* was killed from coat material with 60 min ozone treatment. This can clearly be considered to be an efficient treatment. In the literature reduction of microbes over 5 log units in suspension test is typically considered to indicate sufficient microbicidal efficacy of the disinfectant. For microbes attached on surfaces three log unit reduction is a clear indication of microbicidal efficacy. Efficacy of disinfectants and antimicrobial agents is traditionally determined in free cell suspensions, which do not mimic the conditions on surfaces (Wirtanen, 1995). The efficacy testing based on microbial suspensions gives only an indication whether the disinfectant is effective against the microbe in question, but is not a proof of efficacy against biofilm growth. The agent must reduce the bacterial populations by 5 log units and fungal populations by 4 log units in suspensions in order to be considered effective. All disinfectants passing the efficacy test should reduce the number of bacterial spores by 1 log unit (Wirtanen, 1995). Often the goal for reduction of surface-attached bacteria with disinfectants is 3 log units (Mosteller and Bishop, 1993).

O_3 is a potent oxidant and an important disinfectant, acting on microorganisms by means of oxidation of their biological material (Belchor *et al*. 2012). According to literature the appropriate amount of ozone can exert effective microbicidal activity by destroying the bacterial cell membrane, subsequently producing intracellular leakage and eventually causing cell lysis (Thanomsub *et al*. 2002).

In the present study the microbicidal efficacy was significantly improved when the ozone treatment lasted longer. Microbes were easier to kill from laboratory coat material than from insole material. Especially the counts of *Staphylococcus epidermidis* and *Escherichia coli* were significantly reduced from coat material during 60 min ozone treatment.

References

Belchor, F., Cattani Heimbecker, A. M., Brito, G., Costa, S., van der Heijden, I., Levin, A., and Rasslan, S. (2012) Effect of low-dose gaseous ozone on pathogenic bacteria, BMC Infectious Diseases, 12:358.

McDonnell, G. and Burke P. (2011) Disinfection: is it time to reconsider Spaulding? Journal of Hospital Infection 78, 163-170

Mosteller, T.M. and Bishop, J.R. (1993). Sanitizer efficacy against attached bacteria in a milk biofilm. J. Food Prot., 56, pp. 34-41.

Thanomsub, B., Anupunpisit, V., Chanphetch, S., Watcharachaipong, T., Poonkhum, R. and Srisukonth, C. (2002) Effects of ozone treatment on cell growth and ultrastructural changes in bacteria, J. Gen. Appl. Microbiol., 48, 193–199

Wirtanen, G. (1995). Biofilm formation and its elimination from food processing equipment, VTT Publications 251. Espoo: VTT Offsetpaino. 106 p.