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Use of Ozone to Reduce Bacteria and Moulds in the Air and on Surfaces

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Key Words

Indoor air · Micro-organisms · Ozone · Surfaces · UV radiation

Abstract

The concentrations of bacteria and moulds in the air decreased to undetectable levels in experiments with ozone and UV radiation. When exposed, in all of the species tested, viability on surfaces varied depending on the concentration and species of micro-organisms. At a concentration of less than 50 cm^{-2}, all species of micro-organisms were susceptible to the ozone and UV treatments at the laboratory table. At the concentration of 10^5 cm^{-2}, the most effective was ozone for *Escherichia coli* and *Pseudomonas aeruginosa*, while *Bacillus subtilis* was relatively more resistant. Compared with ozone treatment, the application of UV radiation was less effective on micro-organisms including methicillin-resistant *Staphylococcus aureus* when the micro-organisms were placed under the laboratory table.

Introduction

Ozone (O_3) is a toxic colourless and markedly acrid smelling gas. It is not flammable and is a strong oxidising agent. Because of its strong oxidising action ozone is used for disinfection. Its most common application is in the disinfection of water. However, it is being tested more and more for the disinfecting of air, and experiments are being conducted on the disinfection of surfaces. In view of our favourable experience with the decontamination of denture surfaces from methicillin-resistant *Staphylococcus aureus* (MRSA) in stomatology [1], we have tested the effects of ozone on micro-organisms in the ambient air and on bacteria including MRSA and yeasts on solid surfaces. We tested the application of ozone together with an air humidifier, as ozone is more effective in a humid environment [2].

We also compared the effect of ozone on micro-organisms in the air and on solid surfaces with that of UV radiation.

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Material and Methods

Micro-organisms

Bacillus subtilis CCM 4062, *Pseudomonas aeruginosa* CCM 1961, *Escherichia coli* CCM 4517, *Staphylococcus aureus* CCM 4516, MRSA ATTC 43300 and *Candida albicans* CCM 8215.

Apparatus

Ozone generator (Lifetech) at a 25% output (ozone concentration 0.2 ppm) with a throughput volume of 120 m^3 per hour.

Air humidifier (Bionaire).

UV source (Opting service) with a built-in ventilator with air flowing through a pipe with 253.7 nm/2 × 15 W incandescent tubes with 30 m^3 exposed air per hour.

The various apparatus were in operation for 12 hour periods.

The air was contaminated by spray using drinking water that had been left standing for 14 days (25 $ml \cdot m^{-3}$).

The air was investigated microbiologically with an A/AIR/010 (Agea) aeroscope impinging on agar in Petri dishes for the determination of the overall number of micro-organisms and of the overall number of moulds and yeasts.

Contamination and investigation of solid surfaces

Three concentrations of each micro-organism were applied. Microbial suspensions in peptone water were pipetted onto microscope slides. On each slide there was 0.1 ml of suspension covering an area of 1 cm^2. Each slide was prepared in a series of nine pieces. Each time three identical slides were placed in the room under investigation: (1) 2 m to the left of the generator on the laboratory table, and (2) 2 m from the generator to the right below the laboratory table. Three control slides were placed in another room under the same initial temperature and humidity (control).

Following a 12-hour exposure the slides containing micro-organisms were washed in sterile physiological saline solution and seeding was carried out by appropriate methods for the determination of each microbial species. Microbial growth was assessed upon incubation. The initial concentrations of micro-organisms inoculated on the slides were calculated upon seeding the original suspensions and their dilutions.

For calculating the percentage of surviving micro-organisms the following equation was applied: $S_{1,2}(\%) = (N_{1,2} : N_c)$. 100, in which N_1 is the mean number of micro-organisms detected from slides placed at site 1, and N_2 is the mean number of micro-organisms detected from slides placed at site 2, and N_c is the mean number of micro-organisms detected from slides placed in the control room.

The experiments were carried out in a closed room 22 m^3 in volume.

Results

Results on the concentration of micro-organisms in the ambient air along with temperatures and relative humidity are presented in Tables 1, 2, and 3.

Table 1. Microbial concentrations in the ambient air in the application of an ozone generator and humidifier

	Before contamination	Upon contamination	After 12 h of the experiment
Temperature (°C)	23	23	25
Relative humidity of the air (%)	26	51	44
Bacteria (CFU·m^{-3})	105	1960	Nd
Moulds (CFU·m^{-3})	20	60	Nd

CFU – colony forming unit; Nd – not detected.

Table 2. Microbial concentrations in the ambient air in the application of a UV source and humidifier

	Before contamination	Upon contamination	After 12 h of the experiment
Temperature (°C)	22	23	24
Relative humidity of the air (%)	25	49	43
Bacteria (CFU·m^{-3})	95	1280	Nd
Moulds (CFU·m^{-3})	15	42	Nd

CFU – colony forming unit; Nd – not detected.

Table 3. Microbial concentrations in the ambient air on application of a humidifier alone

	Before contamination	Upon contamination	After 12 h of the experiment
Temperature (°C)	23	23	25
Relative humidity of the air (%)	26	51	44
Bacteria (CFU·m^{-3})	105	2010	1450
Moulds (CFU·m^{-3})	25	85	75

CFU – colony forming unit.

Table 4. Mean numbers of surviving micro-organisms inoculated on microscope slides upon action of ozone and humidifier

Micro-organism/initial concentration (CFU·cm^{-2})	Surviving micro-organisms	
	S_1 (%)	S_2 (%)
Bacillus subtilis		
5.10^4	95	88
5.10^2	28	55
5	0	0
Pseudomonas aeruginosa		
8.10^4	35	32
8.10^2	0	0
8	0	0
Escherichia coli		
6.10^4	33	36
6.10^2	0	0
6	0	0
Staphylococcus aureus		
3.10^5	72	74
3.10^3	27	65
30	0	0
MRSA		
4.10^5	70	75
4.10^3	32	50
37	0	0
Candida albicans		
5.10^4	65	74
5.10^2	5	0
5	0	0

Table 5. Mean numbers of surviving micro-organisms inoculated on microscope slides upon action of UV source and humidifier

Micro-organism/initial concentration (CFU·cm^{-2})	Surviving micro-organisms	
	S_1 (%)	S_2 (%)
Bacillus subtilis		
3.10^4	89	97
6.10^2	32	75
7	0	0
Pseudomonas aeruginosa		
8.10^4	31	42
8.10^2	0	17
8	0	5
Escherichia coli		
5.10^4	30	46
6.10^2	0	14
6	0	0
Staphylococcus aureus		
4.10^5	88	94
2.10^3	25	85
19	0	5
MRSA		
1.10^5	83	92
4.10^3	34	71
42	0	7
Candida albicans		
6.10^4	58	84
7.10^2	5	25
9	0	4

Results on the mean numbers of surviving micro-organisms inoculated on slides after the action of ozone and UV radiation are presented in Tables 4 and 5.

Discussion

In the present experiments disinfection of the ambient air in a closed room by the action of ozone proved to be just as effective as disinfection with UV radiation. Results on the efficacy of ozone on bacteria and moulds in ambient air have already been published [3,4]. Higher concentrations of ozone acting for a shorter time (4 and 20 ppm, 10–480 min.) were almost 100% efficient against *E. coli* in ambient air, and the authors of the present study are of the opinion [5] that such a mode of disinfecting ambient air could be applied in buildings in the event of contamination with *Bacillus anthracis*.

Micro-organisms are very often tested on surfaces following inoculation on agar plates. In such cases, after the action of ozone, about 30% of *E. coli* bacteria survived [6]. The same numbers of *E. coli* likewise survived in our experiment when they had been inoculated at the highest concentrations on microscope slides. Glass surfaces were inoculated with *Streptococcus mutans* and *Streptococcus sobrinus* [7], and also in that case the effect

of ozone was marked. Ozone was very effective against methicillin-resistant *Staphylococcus aureus* on the surfaces of dentures [1]. In some cases the efficacy of ozone was not sufficient, e.g. against moulds on surfaces [3] or for the complete decontamination of the surface of eggs inoculated with *Salmonella enteritidis* bacteria [2].

Of the strains tested, gram-negative rods of *E. coli* and *P. aeruginosa* were the most sensitive, a finding also presented by others [e.g. 8].

In agreement with other authors [4] ozone disinfection can be recommended for closed spaces. In such a way micro-organisms can be removed not only from the ambient air but also from less accessible solid surfaces. Bacterial contamination of surfaces in the indoor environment is at levels similar to the lowest tested concentrations. In hospitals, we have found a maximum of $4\,CFU\cdot cm^{-2}$ on surfaces in the rooms of in-patients [9].

Before entering premises that have been treated with ozone, its remnants have to be removed by ventilation, or degraded down to permitted exposure limits. Those in force for the working environment in the Czech Republic are: $PEL = 0.1\,mg\cdot m^{-3}$, $MPEL\text{-}Wk = 0.2\,mg\cdot m^{-3}$ [10] and $0.1\,mg\,m^{-3}$ in the indoor environment of residential premises [11].

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