

Ozone - The Future Agent for Surface Disinfection?

Matts Ramstorp^{1,2} and Ronny Kammer²

1) BioTekPro AB, Malmö, Sweden

2) Institute of Design Sciences, Aerosol Technology, University of Lund, Sweden

Summary

The purpose of this project was to investigate the potential of airborne ozone to inhibit the growth of microorganisms on surfaces. The investigation was performed by studying the microbiological reduction in relation to ozone concentration, relative humidity as well as time of exposure. Lower concentration of ozone together with increased relative humidity was shown to be much more effective in reducing the number of microorganisms as compared to using higher concentration of ozone at lower levels of humidity.

In order to study the effect on highly microbiologically contaminated surfaces various microorganisms were exposed to high concentrations of ozone for shorter time periods. In less than 5 minutes a tremendous effect was observed with all microorganisms tested, including the very tolerant *Aspergillus niger*.

The overall result from this study clearly indicate that airborne ozone works well as disinfecting agent and may also be used for sterilization in closed systems, i.e. process equipments.

Introduction

Ozone is traced back to ancient Greece where its characteristic smell, created during thunderstorms, was known but not understood. In the literature the odour of ozone is often referred to as "sulphurous". In 1840 F.C.Schönbein observed the same characteristic smell from an anodic gas formed during electrolysis of water. He named this gas ozone, from the Greek word ὄζειν, to smell. Five years later de la Rive and de Marignac stated that ozone was another form of oxygen gas. They also produced ozone by applying electric discharge to pure oxygen. The first ozone generator, a discharge tube for laboratory use, was developed by W. von Siemens in 1857. The same principal is still used in modern generators. In 1865 J.L. Soret showed, by indirect determination, that ozone consists of 3 oxygen atoms. The bactericidal effect of ozone was first discovered by Ohlmüller in 1890 and during the following years large capacity ozone generators were produced by Siemens and Halske.

Disinfection of drinking water with ozone has been in use since the beginning of the 20th century. The use of ozone increased in the 1950's mainly due to the development of more effective generators. Since then, ozone has been used in several applications such as for cleaning water in swimming pools, bleaching of paper in the pulp and paper industry as well as for the removal of undesired odours, for example after fires. Promising applications for the future are expected in the food and beverage as well as in the pharmaceutical industry, mainly due to the microbiological reducing effect of ozone (1, 2, 3).

Microbiological control

The control of microbial growth includes removal, inhibition as well as killing of organisms from surfaces and (or) products. Three important definitions that are often used are:

- *Decontamination* – A treatment that renders an object or a surface **clean**. This treatment is aimed to remove potential microbial nutrients and contaminating microorganisms and thereby preventing their growth.
- *Disinfection* - A process of **reducing** the number of microorganisms, from objects and (or) surfaces. This is achieved with the aid of chemical and (or) physical methods aiming at destroying microorganisms.
- *Sterilization* - The elimination or destruction of **all** living microorganisms. Microorganisms are not killed instantly when exposed to a lethal agent. A microbiological population decreases in a

logarithmic way and microorganisms are considered dead when they are unable to grow at conditions that would normally support their growth.

Microbial destruction with ozone

Ozone is the 4th most oxidizing agent known. Its oxidizing potential is 2.07 mV compared to ethylene oxide (0.699 mV) and chlorine gas (1.36 mV). Ozone has been shown to be strong, rapid and has a broad antimicrobial spectrum, towards bacteria, bacterial spores, viruses, fungi, fungal spores as well as protozoa. Unlike many other sterilizing agents ozone is quite easy and fast to destroy and, furthermore, ozone does not leave any residues, odours and (or) taste. Another advantage of ozone is that it can be produced on site, either from the surrounding air or from pure oxygen.

The inactivation of microorganisms by ozone is due to the oxidizing of fatty acids in the cell-membrane and macromolecules in the cell, i.e. proteins and DNA. These reactions are irreversible and results in cell lysis. Ozone also has an effect on spores and viruses due to the oxidization of DNA and proteins.

The microbiological effect of ozone in water is well documented and is reported to be more effective as compared to dry applications, i.e. in air and other gases. Several studies have also shown that ozone is more effective at elevated humidity as compared to lower humidity (4, 5, 6, 7, 8).

Effects of ozone on humans

Ozone does not only affect microorganisms, it is also quite hazardous to humans. The National Board of Occupational Safety and Health in Sweden has established a permissible level of 0.1 ppm ozone in the air for eight hours exposure, and a short-term limit of 0.3 ppm for exposure less than 15 minutes. Studies indicate that exposure to ozone is quite harmful to humans, but the results, i.e. concentration and time of exposure, are not fully conclusive (9).

Effects of ozone on treated material

Ozone may also cause oxidation of exposed materials. No specific data are, however, available on how different materials acts upon exposure to ozone. It is therefore of great importance to study how materials reacts upon exposure (10).

Test methods

I. Ozone treatment in a small pressure chamber

These tests were all performed in a specially designed pressure chamber, as is schematically illustrated in Figure 1. Various microorganisms, obtained from the laboratory environment, were subjected to two tests, on 90 mm agar disks and on dry stainless steel plates, respectively.

Each agar plate was supplied with 500 µl of cell suspension under LAF protection. The agar plate was allowed to equilibrate for one hour before being placed in the test chamber and exposed to ozone at varying concentrations and exposure times. After this treatment the agar plate was removed from the test chamber, incubated and the number of CFU established.

Since ozone is such a strong oxidizing agent there is a strong possibility that the growth medium will be affected, resulting in false results. Experiments were therefore also performed under dry conditions with microorganisms placed on stainless steel plates. A pre-cleaned stainless steel plate was supplied with 20 µl of a cell suspension and allowed to dry for 30 minutes under LAF protection. The steel plate was then placed in the test chamber and exposed to ozone. After exposure, the plate was removed and sampled with a contact plate. The contact plate was subsequently incubated and the number of CFU counted.

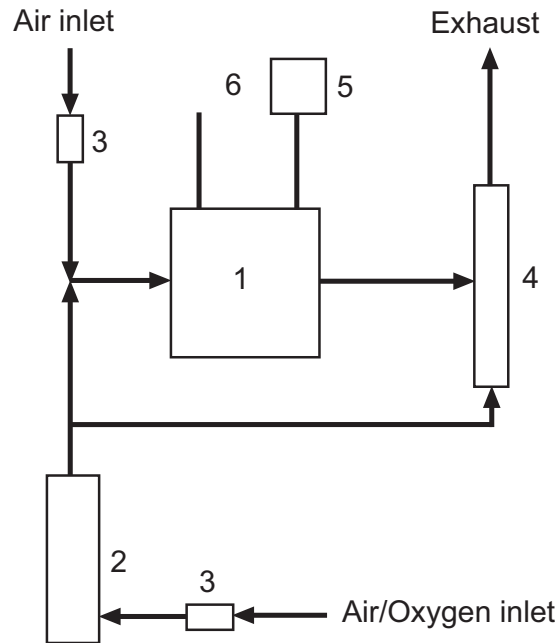


Figure 1. Schematic representation of the experimental set-up for ozone treatment in a small pressure chamber. 1) The pressure chamber, 2) Ozone generator, 3) Airflow- regulator, 4) Catalytic Material, 5) Temperature Probe, 6) Ozone Sensor

II. Ozone treatment at different relative humidity's in a controlled environment

This investigation was performed in order to study the killing effect of ozone on microorganisms at varying relative humidity's. All tests were performed in a laboratory cleanroom, in which temperature as well as humidity could be controlled.

500 µl of a cell suspension was poured onto an agar plate and 20 µl on a stainless steel plate, respectively. The samples were exposed to ozone during 60, 120 and 180 minutes, respectively. The agar plate was after ozone treatment incubated at the appropriate temperature and time for the specific microorganism. The steel plate was subsequently sampled with contact plates.

Low ozone concentration was initially used, 0.3 ppm, and the humidity was increased from 15-25 %, 50-60 % and up to 85-95%.

III. The effect of high concentration of ozone in air against microorganisms during short time exposure

This investigation was performed in order to study the growth inhibition of microorganisms when exposed to high ozone concentrations (approx 5.000 ppm) during short exposure times. Solutions of microorganisms were poured on agar plates and allowed to dry for 60 minutes under LAF protection. The agar plates were then placed in the test chamber, Figure 1, and exposed to high concentrations of ozone during a predetermined time. The agar plates were then incubated for the appropriate temperature and time. In these experiments higher concentration of microorganisms were exposed to ozone.

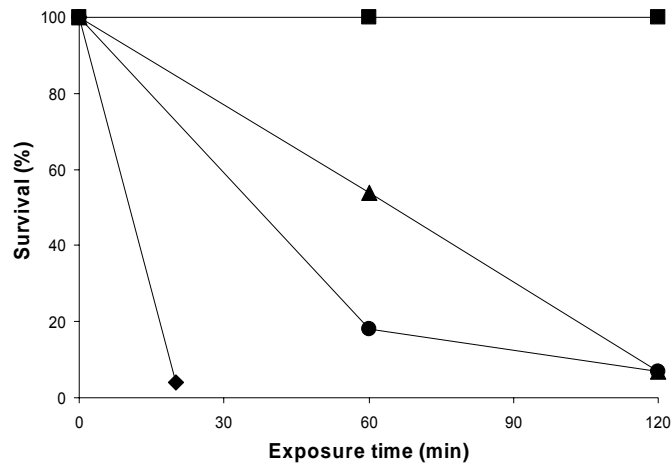
Results and discussion

I. Ozone treatment in a small pressure chamber

Several experiments were performed with ozone concentration of 0.1 ppm. The time of exposure ranged from 20 minutes up to several days and different types of microorganisms collected from the laboratory were tested. No significant effect was observed at this low concentration.

With increased ozone concentration the killing effect increased, Figure 2. More than 90 % of the bacteria were destroyed after 90 minutes exposure at 1.0 ppm of ozone and 120 minutes at 0.5 ppm,

respectively. When higher ozone concentrations (50 ppm) were used the killing effect increased dramatically.



Figur 2. Reduction of an unknown bacteria, collected from the laboratory, at different ozone concentrations. Tests performed on agar plates. Ozone concentration: ■ 0.1 ppm, ▲ 0.5 ppm, ● 1.0 ppm, ◆ 50 ppm.

No significant difference could be observed when microorganisms on agar plates and stainless steel plates, respectively, were subjected to ozone in these experiments, Figure 3.

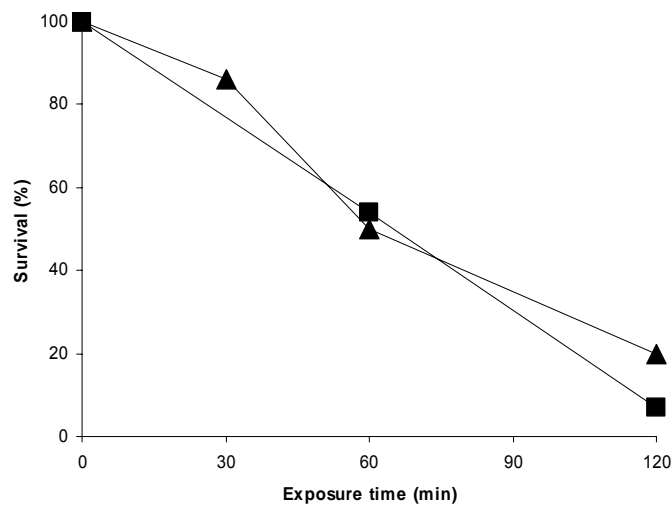


Figure 3. The effect of 0.5 ppm ozone on an unknown bacteria (#1) on agar plate (■) and on stainless steel plate (▲) respectively.

II. Ozone treatment at different humidity in a controlled environment

0.3 ppm ozone showed no effect on *Aspergillus niger* spores. No significant effect was even obtained at 0.6 ppm. Only a small effect was obtained at elevated humidity, 85-95 % RH, and after an exposure of at least 2 hours. The effect of 1.2 ppm ozone is shown in Figure 4.

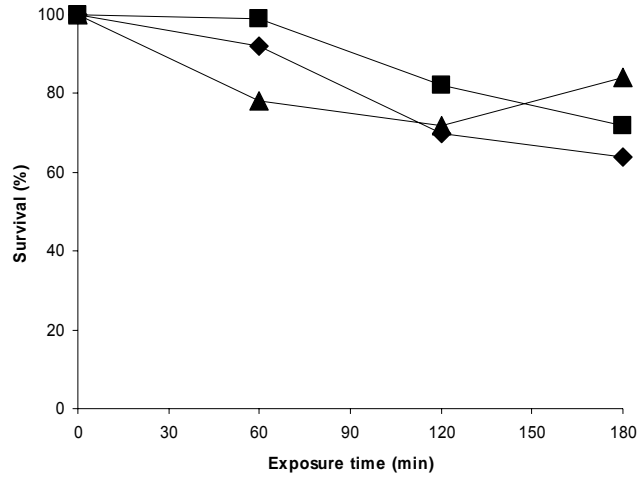


Figure 4. The survival rate of *Aspergillus niger* when exposed to an ozone concentration of 1.2 ppm and varying humidity. ■ 15 – 25 % RH, ▲ 50 – 60 % RH and ◆ 85 – 95 % RH.

Aspergillus niger placed on stainless steel plates was only slightly affected at elevated humidity. Tests performed on agar plates showed that ozone had an effect on *Aspergillus niger*, although the difference in humidity did not seem to have but a marginal effect.

With *Pseudomonas aeruginosa*, 0.3 ppm of ozone showed an increasing effect with increasing humidity. The effect was greater for bacteria on stainless steel plates than on agar plates Figure 5 and Figure 6.

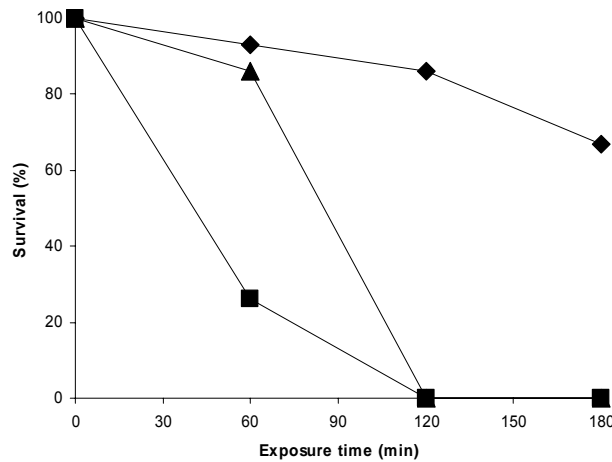


Figure 5. The survival of *Pseudomonas aeruginosa* on stainless steel plates exposed to 0.3 ppm ozone at varying relative humidity. ◆ 15 – 25 % RH, ■ 50 – 60 % RH and ▲ 85 – 95 % RH.

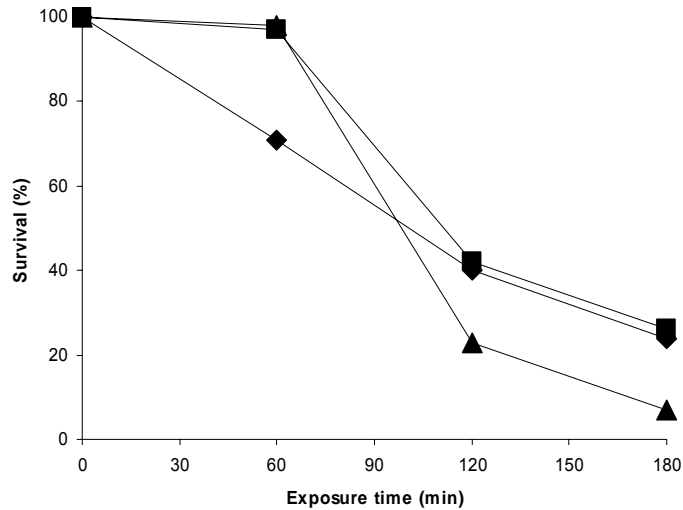


Figure 6. The survival of *Pseudomonas aeruginosa* on agar plates exposed to 0.3 ppm ozone at varying relative humidity. ◆ 15 – 25 % RH, ■ 50 – 60 % RH and ▲ 85 – 95 % RH.

When exposed to 0.6 and 1.2 ppm ozone, respectively, the killing effect of *Pseudomonas aeruginosa* on stainless steel plates increased dramatically with increasing humidity.

The same results were obtained when exposing *Staphylococcus aureus* for ozone at increasing relative humidity.

III. The effect of high concentration of ozone in air against microorganisms during short time exposure

When high concentrations of microorganisms, on agar plates placed in the test chamber are exposed to high levels of ozone, 5.000 ppm, the destruction was enormous. The results are shown in Table 1.

Exposure time (minutes)	Microorganism	Control (CFU)	Survival (%)
0.33	<i>Aspergillus niger</i>	$3 \cdot 10^4$	To numerous to count
5			0.27
20			0.19
0.33	<i>Pseudomonas aeruginosa</i>	$1 \cdot 10^6$	0.004
5			0.0004
20			0
0.33	Mix of Microorganisms	$5 \cdot 10^5$	To numerous to count
5			0.0018
20			0.0016

Table 1. The survival of high concentrations of microorganisms exposed to high ozone concentration during shorter exposure times.

Conclusion

The purpose of this project was to study the potential of using ozone to control microorganisms. Different aspects were considered, ozone concentration, humidity as well as exposure time. The results obtained indicate that ozone may work as a sanitizing agent for disinfection and maybe even for sterilization purposes. However, high concentrations of ozone was needed to obtain major reduction of the number of *Aspergillus niger*.

At very low ozone concentration (0.1 ppm) the reduction of microorganisms was almost zero but this result was obtained at low relative humidity. The role of humidity has been showed to be of great importance, especially for certain types of bacteria. Even at low ozone concentrations (0.3 ppm) elevated humidity (85-95 % RH) showed noticeable effect within 2-3 hours exposure. In some cases total reduction of microorganisms were obtained.

Furthermore, this study shows that lower ozone concentrations at higher relative humidity are much more effective in reducing bacteria as compared to higher ozone concentrations at lower relative humidity. The assumption that ozone has a better killing effect on bacteria on agar plates as compared to on stainless steel plates were confirmed in some cases. The increasing reduction of bacteria on steel plates observed at increasing humidity, was not observed with bacteria on agar medium. The reason for this might be the water in the agar medium has an impact on the killing effect even at lower humidity. Furthermore, the increased effect of high humidity on agar plates are much smaller as compared to the results obtained on stainless steel plates. Another reason for this difference might be that ozone also affects the agar medium in some way. This could be the reason why, in some cases, a higher survival rate is obtained when performing ozone treatment of bacteria on agar medium are exposed to ozone at increased relative humidity.

Finally, experiments to study the effect of high concentrations of ozone on high concentration of microorganisms during short time exposure, was performed. The results from these studies indicate an extremely high killing effect. An exposure time of 5 minutes reduced almost all of the microorganisms tested, including the very tolerant *Aspergillus niger*.

The overall conclusion of this study shows that ozone works well as a decontaminating agent at low concentration against the bacteria's used in this study, but higher ozone concentration were needed for reducing the spores of *Aspergillus niger*. Further studies are now in progress at our laboratory.

References

1. "Use of Ozone in the Food Industry" (2004) Z. B. Guzel-Seydima, A. K. Greeneb and A. C. Seydim, Lebensmittel-Wissenschaft und-Technologie, **37**, 453 – 460
2. "High-Density Ozone Disinfection of Medical-Care Materials for Dental Surgery", H. Ohkawa, M. Tsuji, K. Ohtsuki, M. Ohnishi and T. Akitsu (2005) J. Plasma Processes and Polymers, **2**, 112-119
3. "Making High-purity Water", A. Baird and R. Williams (2005) J. Chemical Engineering, **112**
4. "Bactericidal Effects of High Airborne Ozone Concentrations on *Escherichia coli* and *Staphylococcus aureus*" (1998) W. Kowalski, W. Bahnflethand T Whitam, J. Ozone Science and Engineering, **20**, 205 – 221
5. "Demonstration of Hermetic Airborne Ozone Disinfection System: Studies on E.coli"(2003) W. Kowalski, W. Bahnfleth, T. Whitam and B. Striebig, AIHA J., **64**, 222 - 227
6. "Inactivation Kinetics of Foodborne Spoilage and Pathogenic Bacteria by Ozone" (2000) J. Kim and A. Yousef, J. Food Science, **65**, 521-528, 2000
7. "Ozone and its Current and Future Application in the Food Industry" (2002) J. Kim, A. Yousef and A. Khadre, Adv. Food and Nutrition Research
8. "Inactivation of *Bacillus spores* by Gaseous Ozone" (1986) K. Ishizaki, N. Shinriki and H. Matsuyama, J. Appl. Bact., **60**, 67-72
9. "Health Aspects of Air Pollution with Particulate Matter, Ozone and Nitrogen Dioxide" (2003) Report on a WHO Working Group, Bonn, Germany
10. "Ozone sterilization" (1989) E. L. Karlson, J. Health-Mater-Manage, **7**,43 - 45,