Investigation of Gas-Phase Ozone as a Potential Biocide

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Ozone has been used as a germicidal agent for drinking water since 1903, and its activity in the aqueous phase is well documented. However, despite the wide use of ozone generators for indoor air treatment, there is little research data on ozone's biocidal activity in the gas phase. This article presents experimental data on the effect of ozone on both vegetative and spore-forming fungi as well as a spore-forming bacterium. Dried suspensions of the test organisms were exposed to a range of ozone concentrations from 3 to 10 ppm in 50-L Teflon-coated stainless steel chambers. A two-phase study was performed. The first phase was an extensive series of tests on the efficacy of ozone itself. Tests using organisms deposited on glass slides to minimize losses of ozone were carried out under conditions of high (90%) and low (30%) relative humidity (RH). For the organisms used in this study, ozone concentrations in the range of 6 to almost 10 ppm were required for significant kill. Organisms exposed under high RH conditions were generally more susceptible to ozone. The second phase of tests employed actual building materials as the test surfaces. No microbial kill was demonstrated on any of the building materials even at 9 ppm ozone. FOARDE, K.K.; VANOSDELL, D.W.; STEIBER, R.S.: INVESTIGATION OF GAS-PHASE OZONE AS A POTENTIAL BIOCIDE. APPL. OCCUP. ENVIRON. HYG. 12(8):535–542; 1997. PUBLISHED 1997 BY AIH.

Ozone (O_3) is an oxidizing agent and potent germicide that has been used extensively for the disinfection of drinking water and municipal wastewater. It was first used in The Netherlands for the treatment of drinking water in 1893, and has been the subject of recent studies in the United States as an alternative to chlorination of drinking water. In this application, ozone has been shown to effectively inactivate bacteria, fungi, viruses, and protozoa.[1] Gas-phase ozone has long been used as a deodorant in the remediation of smoke-damaged buildings. As an important reactive species in the atmosphere, the chemistry of gas-phase ozone and volatile organic compounds (VOCs) has also been widely studied.[2] While both the germicidal activity of ozone in aqueous phase and ozone's role in atmospheric chemistry have been investigated at length, there are little research data on the biocidal activity of gas-phase ozone.

Recently there has been an upsurge of interest in using ozone to "clean" or "treat" indoor air, and room and household ozone generators are being marketed and used for indoor air purification to destroy odors, VOCs, and microorganisms. In addition, gas-phase ozone is currently used for some kinds of remediation of biocontamination in buildings.[3] Use of the room devices has been controversial from the viewpoint of the potential health effects of the ozone concentrations that can be generated. Shaughnessey and Oatman[4] reviewed the performance of room-size ozone generators and the potential health impacts of adding ozone to indoor air, concluding that safe use requires monitoring and prudence. The threshold limit value (TLV) for ozone is a STEL (short-term exposure limit)/ceiling limit of 0.1 ppm.[5]

The actual biocidal capability of gaseous ozone with regard to the microorganisms primarily responsible for indoor biocontaminant problems is largely unknown. Companies producing ozone generators claim significant germicidal capabilities.[6] Early literature describes differing results. Some studies indicate that ozone is effective against airborne vegetative bacteria but not bacterial spores or bacteria associated with organic (particulate) matter.[8,9] The *Indoor Air Quality Update*[7] recommended using ozone at concentrations of 100 to 250 ppb in cleaning interior wall surfaces for remediation purposes.

The objective of this study was to evaluate the effect of gaseous ozone on selected microorganisms in laboratory chamber studies under controlled conditions representative of reported ozone use in the field. The organism challenge consisted of spores or cells dried on surfaces. The study was conducted in two phases. First, a series of tests on the efficacy of ozone itself were conducted in which ozone losses in the chambers were minimized by using low reactivity materials wherever possible. Second, a brief series of tests were performed employing building materials as the test surfaces to evaluate their effect on both chamber concentration and ozone efficacy.

Materials and Methods

Test Organisms

Candidate organisms were selected based on two criteria. First, they had to be commonly isolated from the indoor environment; and second, reports of adverse health effects associated with the organism had to be documented in the literature. The four organisms selected for testing in this study were *Rhodotorula glutinis*, *Penicillium chrysogenum*, *Penicillium glabrum*, and *Streptomyces sp.*

Penicillium glabrum, purchased from the American Type Culture Collection (ATCC) as *P. aragonense* (#+228), was reidentified by R.A. Samson of the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. *Rhodotorula glutinis* (#6275) was also purchased from ATCC. *P. chrysogenum*, isolated from contaminated building material, is being maintained in the

University of Texas Medical Branch Fungus Culture Collection as UTMB3491. The actinomycete was isolated from a problem environment and identified as a member of the genus *Streptomyces*.

Preparation of Organisms for Testing and Inoculation of Substrates

The test organisms were first inoculated onto solid media: trypticase soy agar (TSA) for the actinomycete and Sabouraud dextrose agar (SDA) for the fungi. The cultures were allowed to grow for 5 to 10 days, until mature confluent growth covered the surface of the plate. A sterile swab wetted with sterile distilled deionized water was manually rubbed across the surface of the petri dish to collect the growth. The material collected on the swab was eluted into sterile water. The procedure was repeated until a reading of 80 percent transmittance at 520 nm (Milton-Roy Spec 20D) was achieved on a 1:100 dilution of the suspension, and then 50 μL of the organism suspension was pipetted onto a glass microscope slide, for a final concentration between 1×10^5 and 1×10^6 colony-forming units (CFUs)/ml. The concentration of the inoculum was determined by dilution and plating on TSA or SDA. The inoculated slides were dried under sterile conditions. These slides supported the test organisms during the ozone exposure.

Test Substrates

Each test for each organism at each exposure level included eight glass microscope slides. Seven slides were inoculated as described above. Four of the inoculated slides and one uninoculated slide were placed in the chamber and exposed to ozone. Three of the inoculated slides served as unexposed control slides. The control slides (inoculated, positive controls) not exposed to ozone were retained to serve as a baseline and for measurement of inoculum viability. During the experiments they were maintained in the appropriate constant relative humidity (RH) chambers. Uninoculated slides (negative controls), exposed simultaneously with the inoculated slides, were employed as a measurement of possible contamination. None was detected. Following exposure, all of the slides were processed simultaneously.

Slides were processed for recovery and quantitation of surviving test organisms by suspending the entire slide in 10 ml sterile phosphate-buffered saline containing 0.1 percent Tween 80 and agitating on a wrist-action shaker for at least 15 minutes. All necessary dilutions were made in the same buffer, and aliquots of the suspension were plated in duplicate. SDA was employed for tests with *P. glabrum*, *P. chrysogenum*, and *R. glutinis*, and TSA for the actinomycete. The plates were incubated at 25°C for at least 7 days for the fungi and 32°C for the bacterium. The minimum number of detectable CFUs per slide was approximately 100 (1 colony/plate ÷ 0.1 ml plated × 10 ml suspension = 100 CFUs).

The effects of the ozone on the organisms were evaluated by comparing the number of spores recovered from the exposed slides and those recovered from the unexposed slides. CFUs were counted, numbers per glass slide computed, and the data transformed to their logarithmic ($base_{10}$) value. The mean log CFUs of the triplicate inoculated, unexposed controls and of the four replicate inoculated exposed slides were computed and standard deviations calculated. The log reduction was calculated as follows:

$$\text{log reduction} = \log \text{CFUs}_C - \log \text{CFUs}_E \quad (1)$$

where:

$\log \text{CFUs}_E$ = mean log CFUs of exposed samples ($n = 4$)

$\log \text{CFUs}_C$ = mean log CFUs of control samples ($n = 3$)

Since the initial target concentration on the test substrates was approximately 5 logs (10^5) and the minimum detection limit 2 logs (10^2), the maximum reduction in CFUs that could be detected was 3 logs (or one thousandfold).

In addition to the tests on glass slides, the two *Penicillium* species were inoculated onto replicate samples of actual building materials. Small blocks of the materials measuring 3.8 × 3.8 cm were used. The same sample inoculation, processing protocol, and controls were performed as described for the glass slides.

The materials tested included three different types of heating, ventilation, and air conditioning duct-associated materials and two different types of ceiling tile. All duct materials were purchased new from a local commercial vendor. Specifically, they included fiberglass duct liner from two different manufacturers, fiberboard duct, and galvanized steel. Although fiberglass duct liners manufactured by two different companies were used as test surfaces, the compositions of both were similar and one description encompasses both materials. Two types of suspended ceiling tiles were used: class C and class A. Both were between 5 and 10 years old and had been collected from building offices. The class C tile was fire retardant, washable, standard white, and textured faced. The class A tile was a fire-resistant acoustical tile. A summary of the compositions of the materials compiled from the material safety data sheets is presented in Table 1.

TABLE 1. Composition of Test Materials

Material	Composition
Galvanized steel	70 to 99% iron, <1 to 30% zinc
Fiberglass duct liners	>44 to 98% fibrous glass, 1 to 18% urea–polymer of phenol and formaldehyde or urea extended phenol–melamine–formaldehyde resin, <0.1% formaldehyde
Fiberboard duct	85 to 96% fiberglass wool, 4 to 15% cured binder, <1% formaldehyde
Class C ceiling tile	0 to 90% mineral fiber, 10 to 15% gypsum, 10 to 15% starch, 10 to 15% paper fiber, 0 to 25% clay, 0 to 30% perlite, 0 to 12% silica, 0 to 12% styrene acrylic polymer, 0 to 8% phenolic resin
Class A ceiling tile	20 to 60% mineral wool fiber, 4 to 10% hydrous aluminum silicate

Experimental Apparatus

Figure 1 provides a schematic overview of the experimental apparatus used to maintain stable ozone exposure environ-

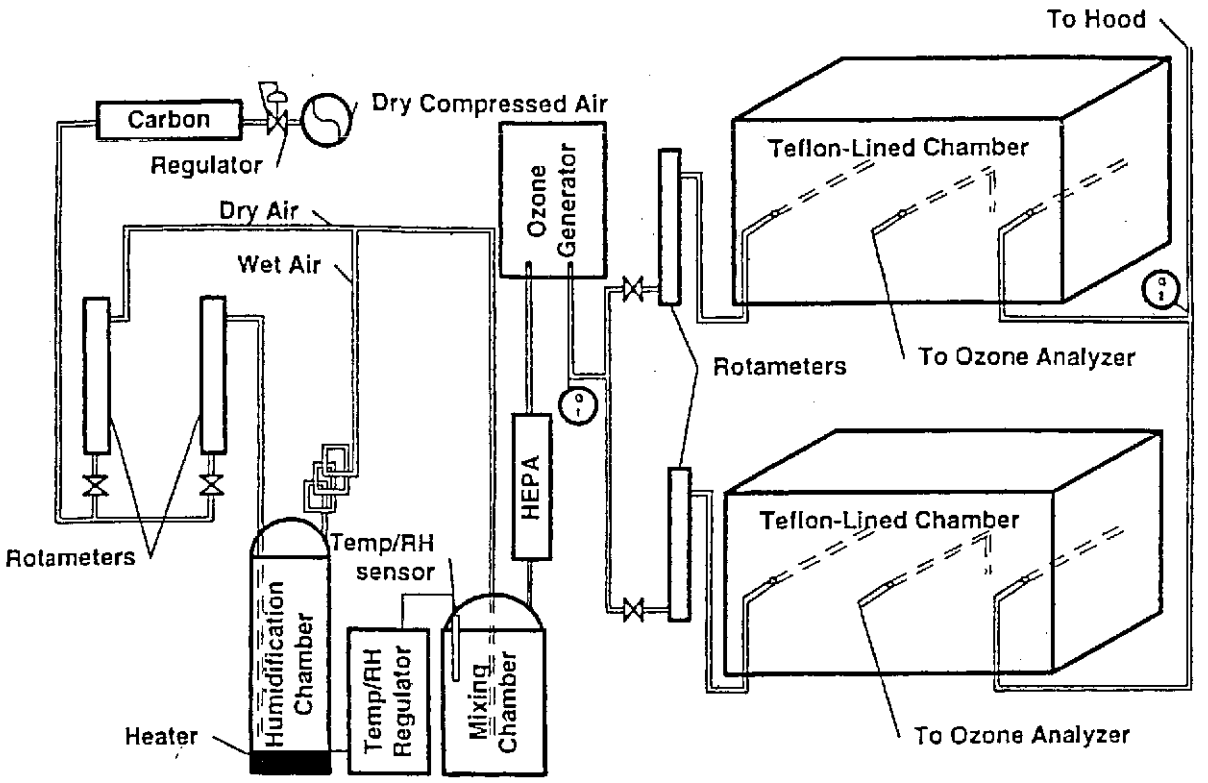

FIGURE 1. Schematic of ozone apparatus.

ments. Two identical chambers were used either in series or in parallel for these experiments. Their design was a modification of those chambers employed in small-scale chamber determinations of organic emissions from indoor materials and products.[10] Each was constructed of stainless steel and was Teflon-coated on the inside of the chamber and the inside of the lid. The chamber resembled a deep, narrow, seamless, stainless steel sink with rounded corners. A flat stainless steel cover was bolted to the rim to seal the chamber. The rim-top seal was made by an engineered Teflon over Viton gasket held in a groove in the chamber cover. The inside of each chamber was approximately 51 × 25 × 41 cm, with the cover being on one 51 × 25-cm end. The total volume was ~50 L. For use as an ozone exposure chamber, the chamber was placed on its side as shown in Figure 1. The ozone-laden air flowed generally horizontally within the chamber, and the test substrates inoculated with microorganisms were placed near the center of the 51 × 41-cm rectangular floor of the chamber.

Test gas was introduced into and removed from the chambers through 1.25-cm diameter Teflon distributor tubes that penetrated the cover and reached almost to the back of the chamber. The distributor tubes were straight and plugged at the back ends. Holes were drilled along the horizontal centerline of the tubes in the side away from the chamber center so the entering gas was required to make a 180° turn as it left the distributor. The hole size varied along the length of the distributor tube to encourage even distribution. For all tests, the test gas was injected into the chamber at 3.0 L/min.

Test Air Conditioning

The test air for the experiments was conditioned using the apparatus shown in Figure 1. Dry compressed air was treated in an activated carbon capsule filter for removal of hydrocarbons prior to mixing with the ozone. The cleaned air stream was split into two streams using rotameters and needle valves. The wet air stream was bubbled into heated water and mixed with the dry air, and the now humidified air entered the mixing chamber. The sensor for the temperature/humidity controller was positioned in the mixing chamber and the controller operated the heater on the water reservoir. Temperature was monitored but not controlled directly. The test gas was controlled to within ±1 percent RH once the system was stabilized at a particular operating condition. The humidified air was passed through a high efficiency particulate air filter as it left the mixing chamber.

Ozone Generation and Concentration Measurement

Following humidification, the test gas was passed into the ozone generator, which was a steel chamber to which a commercially available in-duct corona-type ozone generator (model D-100A; Kleen-Air Co. Inc., Carmel, Indiana) had been fitted. This unit is designed to mount on the side of a duct with the power supply and controls on the outside and the high voltage section inside the duct exposed to the moving air. The unit was controlled by manually increasing the voltage to obtain the desired concentration within the test chambers.

The air leaving the ozone generator was split into two 3-L/min streams using the rotameters shown in Figure 1 and

TABLE 2. Range of Ozone Exposure Levels in Parts per Million

Test Organism	30 Percent RH	90 Percent RH
Penicillium chrysogenum	3–9.9	4.2–9
Penicillium glabrum	3–9.9	0.5–6.2
Rhodotorula glutinis	2.5–9.9	1.5–9.9
Streptomyces	2.5–9.9	2.8–7.3

routed to the test chambers. All tubing downstream of the ozone generator was 0.64-cm diameter Teflon to minimize transport losses. The air leaving the chambers was discharged into an exhaust hood for disposal.

The ozone concentration in the chamber was measured by extracting a sample stream and analyzing that stream with a model 560 portable ozone analyzer (Thermo Environmental Instruments, Inc.). The analyzer was operated in accordance with the manufacturer's operating manual. It had a minimum detection capability of 1 ppb and a maximum concentration of 10 ppm. The instrument was calibrated prior to beginning the experiments and the calibration was checked periodically throughout the study.

As shown in Figure 1, the air sample for ozone concentration measurement was extracted near the horizontal center of the chamber about 2.5 cm above the bottom of the chamber using a 0.64-cm diameter stainless steel tube, and drawn from this sample tube to the analyzer through 140 cm of 0.64-cm diameter Teflon tubing. Except for this 30-cm length of stainless steel tubing, all surfaces in contact with the test gas were Teflon. For all tests, the sample extraction rate was 1 L/min for the ozone concentration measurement. The sampling frequency varied. Samples were normally extracted from the chambers for approximately 5 minutes every 2 to 4 hours during the day. This diversion of about one third of the chamber flow to the sample line for short periods was not found to significantly alter the chamber concentration.

Preliminary Chamber Mixing Characterization

Prior to beginning the ozone exposure experiments in the chambers, their mixing characteristics were evaluated to ensure that all test samples were exposed to the same ozone concentrations. The samples were contained in 100-cm diameter glass petri dishes, and for all experiments the petri dishes were clustered in the center of the bottom of the chamber. The maximum number of samples in a chamber at one time was 12, which occupied about half the total floor area of a chamber. The ozone samples were drawn from the center of the chamber about 2 cm above the floor.

Chamber mixing was evaluated by (1) comparing measurements made at different locations within the chamber, and (2) conducting ozone buildup and decay experiments. An ozone sample probe having approximately 40 cm of possible free movement from the chamber lid to a position near the back of the chamber was used for taking measurements at different locations. Movement of this probe from near the lid to the back of the chamber, once the chamber had stabilized at the target concentration, did not reveal significant concentration gradients near the central plane of the chamber.

A static decay experiment was used to evaluate losses to the walls of the chamber. Wall losses were found to be below 1 percent of the ozone charge rate. The chamber decay and

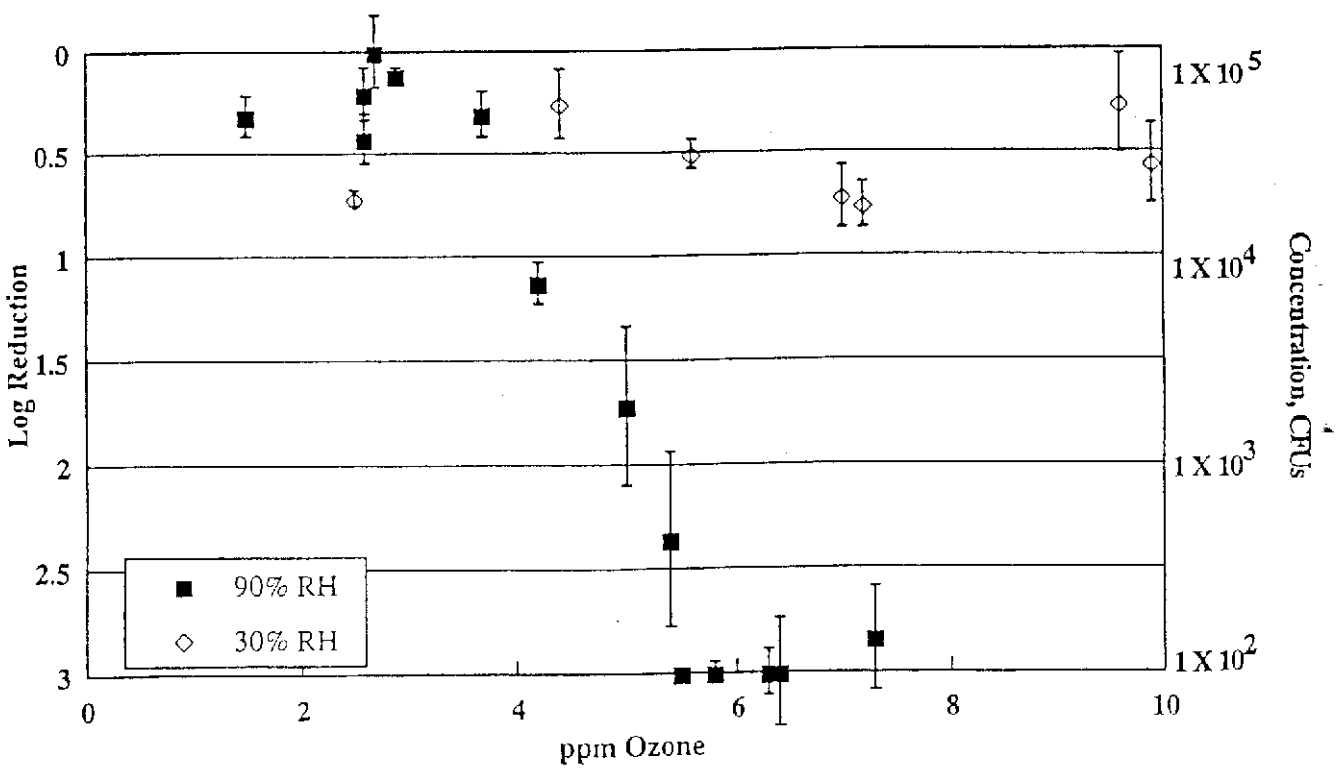

FIGURE 2. *Rhodotorula glutinis* exposed to gas-phase ozone.

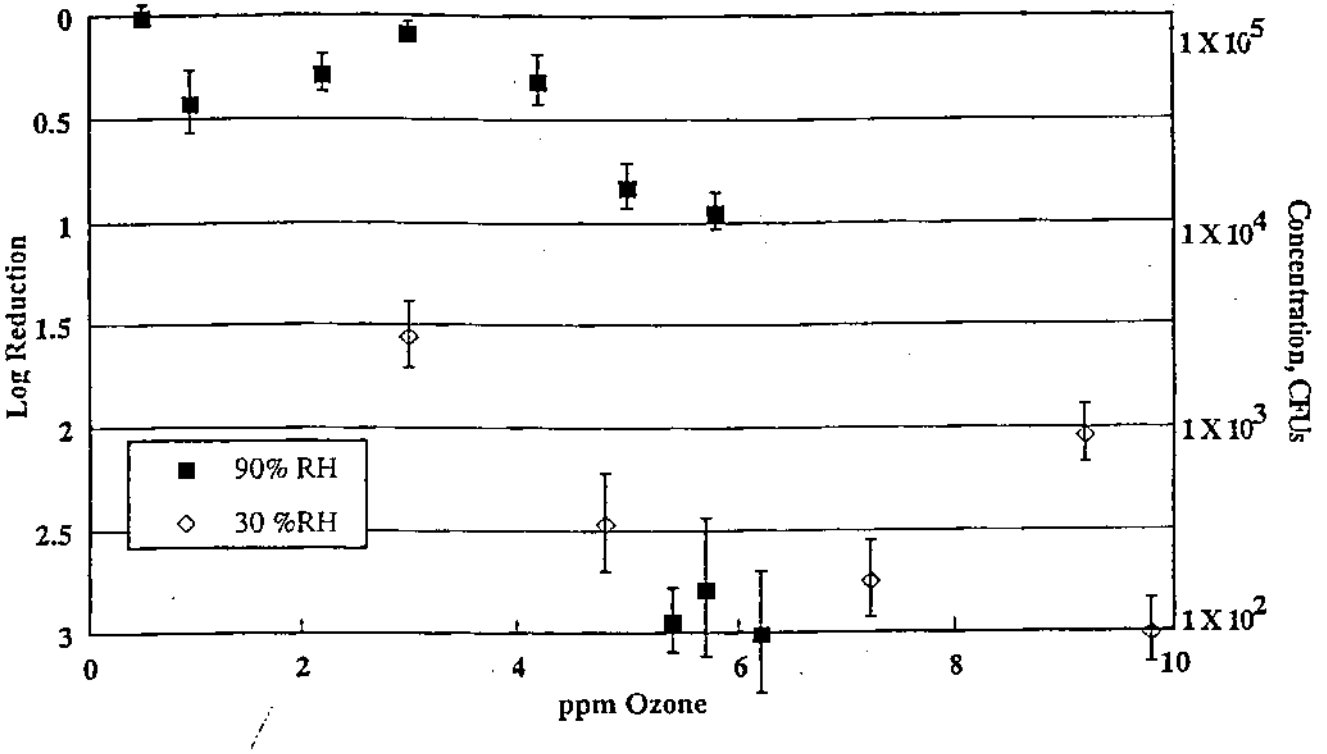

FIGURE 3. *Penicillium glabrum* exposed to gas-phase ozone.

buildup concentration curves were close to those expected of a completely mixed tank, and the ozone concentration reached its final value within 1 hour or less from the beginning of the experiment. Insertion of empty glass petri dishes as surrogate test materials did not appreciably affect the ozone concentration.

Results

Table 2 shows the overall range of ozone levels between which each test organism was exposed at each of the two RHs studied. The considerably lower levels at which testing was initiated for the *P. glabrum* reflect the early study design. Individual runs at specific levels of ozone are shown as the points plotted in Figures 2 through 5.

Ozone Activity Against Spores on Glass Slides

Figures 2 through 5 show the concentration of the organisms and the log reduction of CFUs at the different ozone exposure levels in parts per million for each of the test organisms. For each figure, the x axis is the range of ozone levels in parts per million. The log reduction, calculated using Equation 1, is on the left, and normalized concentration ranges from 10^5 to 10^2 CFUs are on the right. As discussed earlier, a 3 log reduction, the maximum reduction detectable given the minimum detection limit, translates to 100 CFUs.

Figure 2 presents the data for the yeast, *R. glutinis*. At 90 percent RH, a decline in the number of CFUs began with an exposure of 4 ppm, and by 5.5 ppm a 3 log reduction in CFUs was attained. In other words, at 5.5 ppm there were ≤100 survivors, or a decrease of at least one thousandfold between the control and the exposed samples. The samples exposed to ozone at 30 percent RH never attained a 1 log decrease. If a full 1 log decrease had been achieved, it would have been equivalent to a 90 percent kill, or 1×10^4 CFUs remaining.

Figures 3 and 4 show the data from the two *Penicillium* species, *P. glabrum* and *P. chrysogenum*, respectively. For *P. glabrum*, there was a gradual decrease in the numbers of CFUs at both RHs. At 90 percent RH, no survivors (3 log reduction) were detectable starting at 6.2 ppm, while at 30 percent RH, 9.8 ppm were required for the CFU levels to fall below the minimum detection limit. As seen in Figure 5, similar results were obtained for *P. chrysogenum* at both 30 and 90 percent RH.

Figure 5 presents the data for the *Streptomyces* isolate. Although some reduction in CFUs was demonstrated, at all ozone levels tested for both the high and low RH test conditions, organisms were always recoverable. At none of the ozone levels tested was a full 3 log reduction achieved. This indicates that actinomycete spores may be even more resistant to the effects of gaseous ozone than the fungal spores.

Efficacy of Ozone Against Spores on Building Materials

All exposures on building materials were at 90 percent RH, and the ozone generator was set to maintain a level of 9 ppm in the chambers. Only the two species of *Penicillium* were tested.

Table 3 presents the results for three duct materials inoculated with 1×10^6 *P. glabrum* spores and exposed to 9 ppm ozone for 23 hours compared with controls prepared at the same time. No decrease in CFUs was detected.

Table 4 shows the data for three other materials inoculated with 1×10^7 *P. chrysogenum* spores. Once again the desired

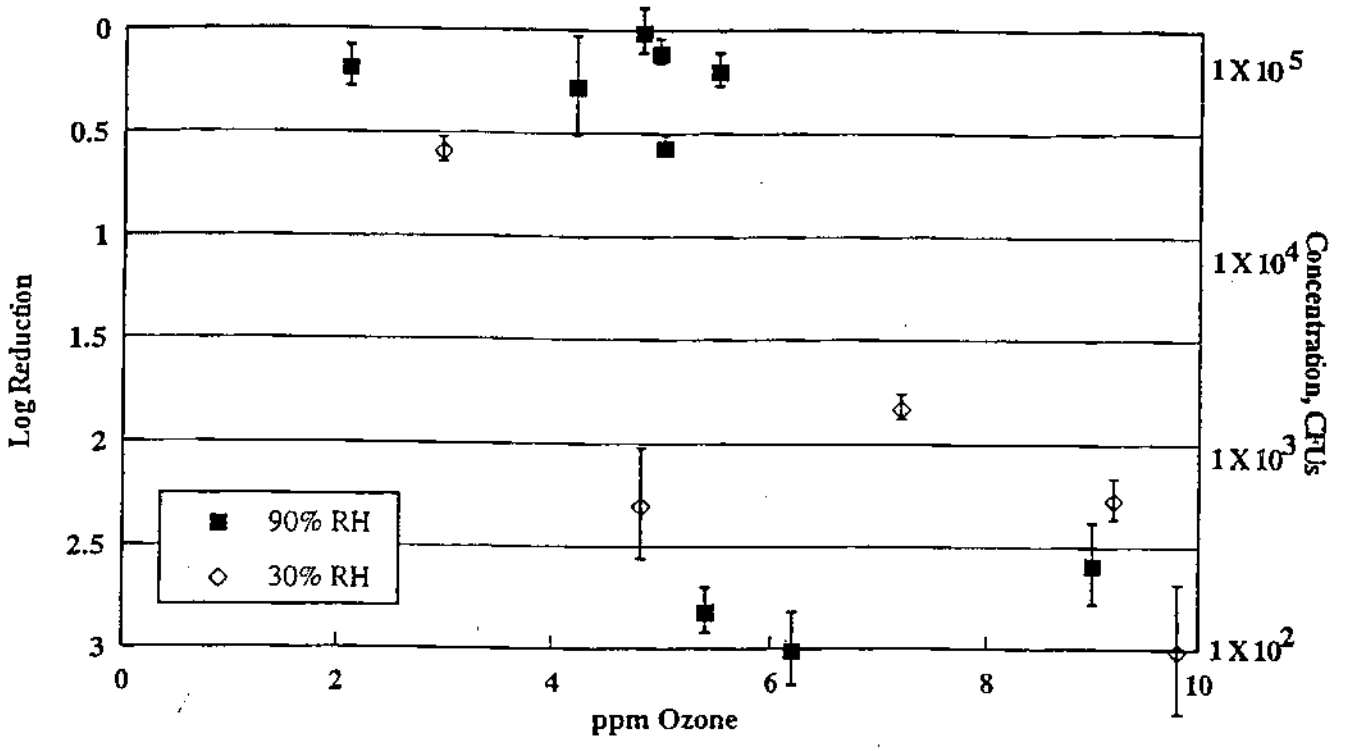

FIGURE 4. *Penicillium chrysogenum* exposed to gas-phase ozone.

experimental conditions were 90 percent RH and 23 hours exposure to 9 ppm ozone. As can be seen from Table 4, a level of 9 ppm ozone was never attained in the chambers during the ceiling tile sample exposure. The generator was set to provide 9 ppm and the chambers were preconditioned to that level, but when the material was added to the chambers, levels of 9 ppm could not be reached. The highest level attained was 5 ppm. Evidently the ozone reacted with the denser ceiling tile at a faster rate than it did with the duct materials, for which the 9-ppm exposure was reached at the center of the chamber.

As with the *P. glabrum*, no reduction in CFUs was attained. These results indicate that not only was the material probably protecting the spores on the surface, but in the case of the ceiling tiles, reacting with the ozone to the extent that the desired test levels could not be reached.

Discussion

The experimental design was to select a number of commonly isolated organisms representing the different groups of common microbial biocontaminant sources, conduct tests at a range of ozone concentrations, and evaluate the effect of different RHs. Initially, experiments were conducted at a series of relatively low ozone concentrations with two exposure times: 90 minutes and 23 hours. This design was abandoned when it became clear that there was no effect at the low ozone concentrations even at 23 hours exposure, and experiments were redesigned to go to higher exposures for 23 hours.

P. chrysogenum and *P. glabrum* were selected because they are frequently isolated indoor molds.[11] *P. chrysogenum* has frequently been isolated from problem environments and has been shown to be a cause of allergic alveolitis.[12] *P. glabrum* has been associated with asthma in occupational environments.[13] *R. glutinis*, a yeast, was chosen because it has been isolated from problem environments and associated with adverse health effects.[14] Mesophilic actinomycetes, such as *Streptomyces*, have also been isolated from problem indoor environments.[15] The thermophilic actinomycetes have been suggested as the causative agent in hypersensitivity pneumonitis and have produced serious lung disease in farmers (farmer's lung disease) and sugar cane workers (bagossis).[16]

The chamber RHs during the exposures were important. Many factors, including temperature, pH, RH, and organic load affect the susceptibility of microorganisms to ozone.[17] Increasing RH increases the biocidal capability of ozone,[18] and materials in buildings are exposed to a range of RHs. Two RHs were selected for use in this study: 30 ± 3 and 90 ± 5 percent. These two levels bracketed the use conditions for ozone in most buildings.

The form of the challenge was considered to be a critical element in the design of the study. Typically, biocides are evaluated employing the most difficult test conditions under which they may be expected to function. The method of ozone application during remediation was also a factor. Organisms dried on surfaces were selected because this challenge met both of the criteria: it was a sufficiently difficult challenge, and building surfaces are frequent targets of ozone during remediation. The number of challenge organisms was based on levels of contamination that have been reported on surfaces in buildings. Levels ranging from 10^3 to 10^7 CFU/cm^2 have been isolated from surfaces of contaminated buildings ranging from ceiling tile to wallboard.[19]

An extended series of experiments were performed using

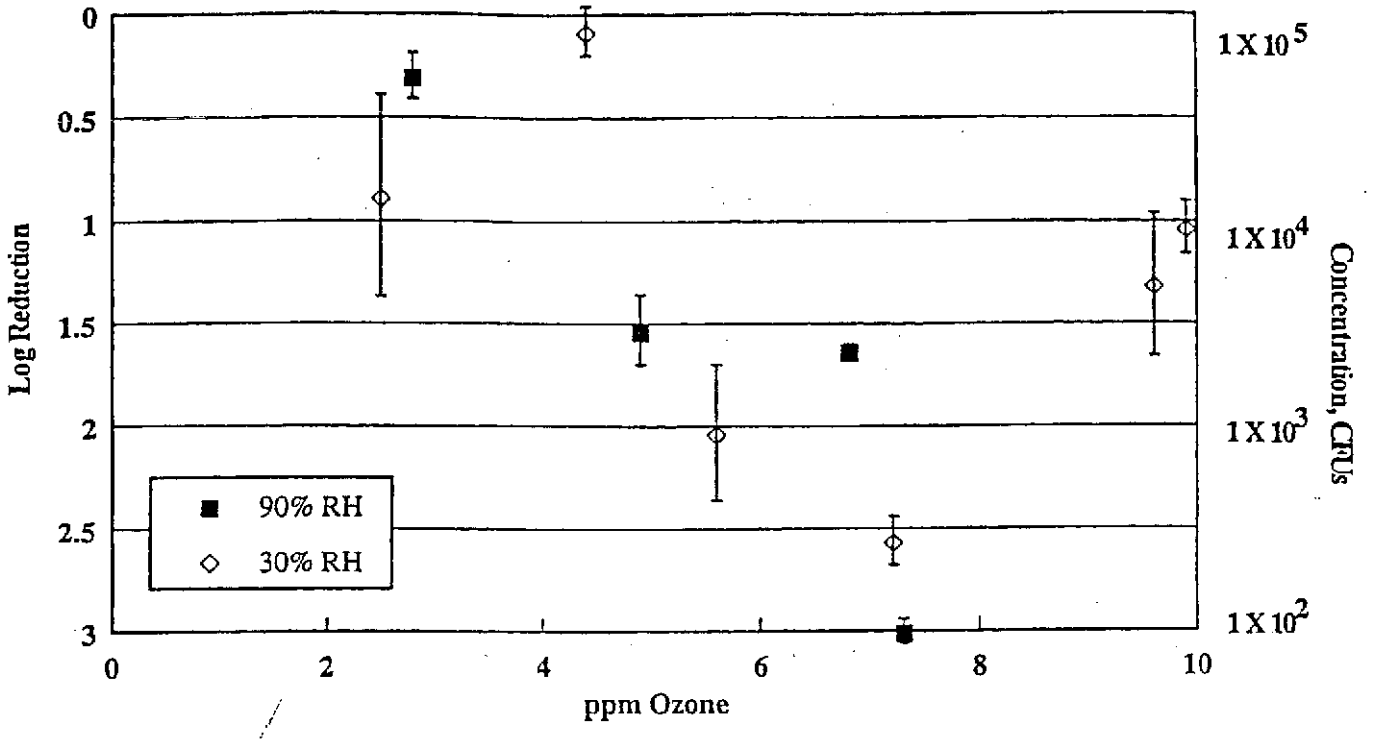

FIGURE 5. *Streptomyces sp.* exposed to gas-phase ozone.

glass slides as the test surface to minimize the loss of the ozone at the surface where the spores were deposited, thereby maximizing the probability of detecting an effect. To evaluate ozone under more realistic use conditions, spores were inoculated onto surfaces of actual building materials. The materials tested included two different fiberglass duct liners, fiberboard duct, galvanized steel, and two different types of ceiling tiles. The duct materials were all newly purchased from commercial vendors and the ceiling tiles were removed from offices.

A key issue in evaluating the efficacy of any biocide, including ozone, is to determine what would be an acceptable number of CFUs remaining after treatment. For example, if the initial concentration was 1×10^5 CFUs/sample, a 1 log reduction (90% kill) would mean that 1×10^4 CFUs remained after exposure; or if a 3 log reduction (99.9% kill) was attained, there would be 100 CFUs. However, if the initial concentration of organisms was 1×10^7 CFUs/sample, a 3 log reduction (99.9% kill) would be equivalent to 1×10^4 CFUs/sample. For these studies a 3 log reduction in CFUs was considered substantial.

The ozone exposure experiments, during which ozone loss was minimized, demonstrated some distinct differences between the various test organisms. *R. glutinis*, *P. chrysogenum*, and *P. glabrum* are fungi, while the *Streptomyces sp.* is a bacterium. Although the *Rhodotorula* and the two penicillia are all fungi, the *Rhodotorula* is a yeast and the other two are molds. Molds (filamentous fungi) reproduce by forming spores, which are resistant to unfavorable environmental conditions and can remain dormant for long periods of time, while yeasts are single-celled organisms that reproduce by budding and do not form spores. The fourth test organism, *Streptomyces sp.*, belongs to a group of gram-positive, spore-forming bacteria called actinomycetes. They are referred to as "fungus-like bacteria" because they are slow growing and form branching filaments leading to the development of mycelial colonies. In this study, the vegetative phase of the molds and actinomycete was not examined; therefore, the yeast represented the vegetative organisms. Since vegetative organisms are generally more susceptible to the effect of biocides than spore-forming organisms and bacterial spores are usually more resistant than fungal spores, the anticipated order of increasing resistance to a biocide for these organisms would be expected to be the yeast (most susceptible), the fungal spores (less susceptible), and the actinomycete spores (least susceptible).[20]

TABLE 3. Log CFU (Mean ± Standard Deviation) for *Penicillium glabrum* Spores

O_3, ppm	Material	Control	Exposed
9	Fiberglass duct liner 1	6.0 ± 0.2	6.2 ± 0.1
9	Fiberboard duct	6.8 ± 0.2	6.0 ± 0.1
9	Galvanized steel	6.9 ± 0.1	6.9 ± 0.2

TABLE 4. Log CFU (Mean ± Standard Deviation) for *Penicillium chrysogenum* Spores

O_3	Material	Control	Exposed
5.0	Used class A ceiling tile	7.0 ± 0.3	7.0 ± 0.2
5.0	Used class C ceiling tile	6.8 ± 0.4	7.0 ± 0.1
9.0	Fiberglass duct liner 2	6.8 ± 0.2	6.7 ± 0.1

Gaseous ozone concentrations of 6 to 10 ppm were required to obtain the full 3 log reduction for the three fungi tested. The full reduction was never achieved for the actinomycete. At 90 percent RH, significant kill of the only vegetative organism in the study, the yeast *R. glutinis*, was attained with only slightly lower levels of ozone than those required for the *Penicillium* spores. Across the range of ozone levels used, the impact of RH on the spore-forming organisms was not notable except when determining attainment of 3 log reductions. For the *Penicillium* spores, over 9 ppm ozone was required at 30 percent RH, while approximately 6 ppm was required at 90 percent RH. The impact of RH on *R. glutinis* was dramatic. While significant kill was achieved with 5.8 ppm at 90 percent RH, at 30 percent RH levels as high as 9.9 ppm did not result in even a 1 log reduction.

The results from the second phase of the study, where spores of the two *Penicillium* species were deposited on actual building material surfaces, showed no reduction in CFUs after a 23-hour exposure to 9 ppm of ozone. This result differs from those when the inoculated surface was the glass slide, and at 90 percent RH a 3 log reduction was attained with exposures greater than 6 ppm. Direct measures of ozone concentration at the exact site of a spore are not possible. However, it is reasonable to assume that the difference in ozone efficacy between the materials was due at least in part to the ability of the building material surfaces to react with the ozone and thus to protect the spores deposited on its surface. For the denser materials (ceiling tiles), test levels of ozone were not attainable.

Conclusions

These results show that at concentrations below 6 to 10 ppm gas-phase ozone would not be likely to achieve a significant reduction of surface biocontamination. Such concentrations are high relative to the TLV for ozone. These experiments show that 5 to 10 ppm ozone would be difficult to maintain near or at the surface of several commonly contaminated building materials.

Acknowledgments

The authors thank Dr. D.S. Ensor of Research Triangle Institute (RTI) and Dr. L.E. Sparks of the U.S. Environmental Protection Agency, who acted as technical reviewers. Within RTI, Mr. E. Myers and Ms. P. Dulaney actually conducted the tests, Ms. T.D. Webber provided additional technical assistance, and Dr. E.C. Cole provided additional technical review.

Mention of commercially available products does not imply their endorsement by the U.S. Environmental Protection Agency.

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