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Demonstration of a Hermetic Airborne Ozone Disinfection System: Studies on *E. coli*

An enclosed flow-through system using airborne ozone for disinfection and which removes the ozone with a catalytic converter was tested with a strain of *Escherichia coli*. Petri dishes containing the microorganisms were inserted in a chamber and exposed for 10–480 min to ozone concentrations between 4 and 20 ppm. Death rates in excess of 99.99% were achieved. Survival data is fitted to a two-stage curve with a shoulder based on the multihit target model. Ozone was removed from the exhaust air to nondetectable levels using a metal oxide based catalyst. The possibility of using ozone as an airborne disinfectant for internal building surfaces and catalytically removing the ozone on exhaust is demonstrated to be feasible. A model for the decay of *Bacillus cereus* under ozone exposure is proposed as an example for predicting the sterilization of buildings contaminated with anthrax. The potential for disinfecting airstreams and removing ozone to create breathable air is also implied by the results of this experiment.

Keywords: airborne pathogens, *Bacillus cereus*, decontamination, *E. coli*, multihit model, ozone

In a previous study the ability of high levels of airborne ozone (>300 ppm) to disinfect surfaces was demonstrated using *Escherichia coli* (*E. coli*) and *Staphylococcus aureus*.⁽¹⁾ Ozone levels of 300 ppm and higher were found to be effective for disinfecting surfaces within seconds. The present study used ozone at comparatively low levels (1–20 ppm) and simultaneously removed residual ozone through catalysis such that no trace of ozone remained in the exhaust airstream.

The ability of this system to disinfect plates of *E. coli* implies the potential for disinfection of airstreams. The development of an ozone-based air disinfection system offers a new alternative for the control of airborne disease that has potentially favorable economics in comparison with other technologies such as filtration and ultraviolet (UV) germicidal irradiation.

Because of its toxicity to humans as well as to bacteria, any ozone system used to produce breathable air must reduce ozone concentrations to ambient levels or lower. Several alternative methods for removing residual ozone after use as a disinfectant have been addressed in the literature, including ozone filters,^(2–5) ozone-destruction catalysts,⁽⁶⁾ UV irradiation, extended

residence time in mixing plenums,⁽⁷⁾ thermal decomposition,⁽⁸⁾ and the catalytic effect of glass and silica.⁽⁹⁾ Catalytic conversion appeared the most feasible due to the rapid removal rates and long life span of the catalytic material.^(10–12)

Various researchers have studied the biocidal properties of airborne ozone for the disinfection of air or surfaces.^(13–16) The results of studies on the biocidal effects of low-to-moderate levels of ozone on bacteria and fungi are summarized in Table I. The studies by Ishizaki⁽¹⁷⁾ demonstrated that relative humidity (RH) has a major impact on the biocidal effect of ozone, and results suggest that humidities above 80% result in efficient sporocidal activity. The RH in the Dyas⁽¹⁸⁾ study was not published.

The use of airborne ozone for disinfection of airstreams and surfaces is limited by the fact that residual ozone at any level is considered a hazardous pollutant. The Occupational Safety and Health Administration limit is set at 0.1 ppm for extended human exposure. A means of completely removing ozone is needed before any possible applications involving indoor air disinfection can be developed.

The demonstration of surface disinfection inside a chamber and the catalytic removal of

TABLE I. Summary of Previous Airborne Ozone Tests on Bacteria and Fungi

Test Microbe	Ozone (ppm)	RH%	Time (min)	Survival (%)	Researchers
<i>Staphylococcus aureus</i>	0.3–0.9	—	240	0.5	Dyas et al. (1983)
<i>Pseudomonas aeruginosa</i>	0.3–0.9	—	240	31	Dyas et al. (1983)
<i>Serratia</i> spp.	0.3–0.9	—	240	3.2	Dyas et al. (1983)
<i>Proteus</i>	0.3–0.9	—	240	0.9	Dyas et al. (1983)
<i>Aspergillus fumigatus</i>	0.3–0.9	—	240	8	Dyas et al. (1983)
<i>Streptococcus salivarius</i>	0.6	60–75	100	2	Elford & van de Eude (1942)
<i>Bacillus cereus</i>	3	95	60	0.013	Ishizaki et al. (1986)
<i>Fusarium oxysporum</i>	0.1	35–75	240	2	Hibben & Stotzky (1969)
<i>Aspergillus niger</i>	0.1	35–76	240	84	Hibben & Stotzky (1969)
<i>Rhizopus stolonifer</i>	0.1	35–77	240	43	Hibben & Stotzky (1969)
<i>Penicillium chrysogenum</i>	3–9	90	1380	0.1	Foarde et al. (1997)

ozone from the exhaust simulate the decontamination of a building and the removal of ozone in the air exhausted outdoors. Methods of decontaminating buildings have been under study recently as a means of remediating anthrax spore contamination. The use of chlorine dioxide to decontaminate buildings has met with some limitations, and ozone offers a new and workable alternative for such applications.

MATERIAL AND METHODS

Bacterial cultures grown on solid media in petri dishes were ozonated in an experimental ozone chamber of approximately 72 L in volume (see Figure 1). Sensors monitored air temperature and RH, which remained between 25–27°C and 18–21%, respectively, throughout the trials. The test apparatus was located inside an exhaust hood, which contained any ozone leakage. A sliding drawer in the test chamber allowed rapid insertion and removal of the bacterial samples without significantly disturbing internal ozone concentrations.

The catalytic material used was Carulite 200, which is composed of magnesium dioxide, copper oxide, and aluminum oxide.⁽¹⁹⁾ The catalytic material, which resembles activated carbon, has a density of 0.93 g/cc and a surface area of at least 175 m²/g. The catalyst has no upper limit of ozone concentration, but to achieve a high rate of ozone removal it has an optimal residence time of 0.36 sec and an optimal face velocity of 0.671 m/sec (2.2 ft/sec) in dry air. Under these conditions performance is stated

to be predictable and independent of RH. The catalytic converter unit, which is depicted atop the ozone chamber in Figure 1, is approximately 36 cm long and has a maximum width of approximately 18 cm at the outlet. It contains Carulite held in place with screening, much like charcoal adsorbers.

E. coli ancestral strain DEC 5D (055:H7) was used for this experiment. This is a weak strain in comparison with other variants of *E. coli* and was selected to ensure that a measurable response would be obtained with the low levels of ozone used here.

Cultures for inoculation were grown overnight (for 18–20 hours) in Bacto Nutrient Broth (Difco Laboratories, Detroit, Mich.) by shaking at 37°C to concentrations of approximately 10⁸ cells/mL. Approximately 0.1 mL of pure (or diluted) culture was uniformly spread by an automatic spiral plater on the surface of petri dishes of Mueller-Hinton brown agar, which were prepared per the manufacturer's instructions. Control cultures were diluted to a titer of 10³ cells/mL, whereas ozonated cultures were diluted to either 10⁷ cells/mL or 10⁶ cells/mL.

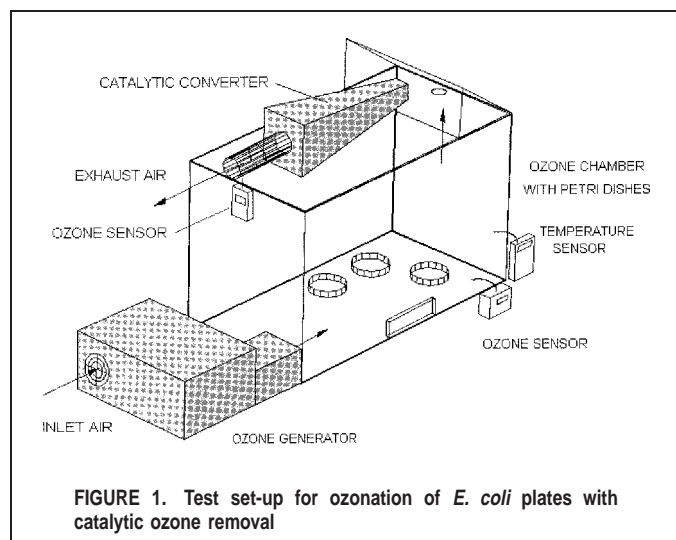
Petri dishes were plated using a spiral plater from Spiral Biotech (Norwood, Mass.). Plates were spread in a uniform logarithmic spiral with 250 µL of solution per plate. After plating, the cultures were allowed to dry on the agar surface for 2–3 hours. Controls received no ozonation. Test samples were inserted in groups into the ozone chamber with continuous ozone injection. The ozonated samples were exposed for intervals of 10 to 120 min, as timed by a digital stopwatch.

Exposed cultures were then incubated for a period of 12 to 16 hours at 37°C, after which the colonies had become fully grown. The plates were then digitally imaged. The bacterial colonies were counted using the QCount digital imaging system from Spiral Biotech. Results were expressed in terms of colony forming units (CFUs).

Three ozone generators were used in the course of this experiment. Ozone levels from 5–20 ppm were generated using an assembly of UV lamps (model G36T8), which was part of an ozonation system belonging to the Penn State Applied Research Laboratories (ARL). The ARL system generated ozone at concentrations between 50–100 ppm and at a rate of 2–10 L/min. Ozone levels were reduced to 5–20 ppm and approximately 9 L/min by mixing the supply flow with building air in stages.

Flow rates for the ARL ozone generator were measured using rotameters. These included one series GR 150 mm flowmeter and two model MR3000 flowmeters from Key Instruments (Trevose, Pa.). The series GR flowmeter had a rated accuracy of ±3% of full scale, whereas the model MR3000 meters had a rated accuracy of ±4% of full scale.

Ozone levels between 0.25–4 ppm were generated using a specially built prototype system (Biozone Scientific, Vero Beach, Calif.).



This prototype unit, which used a UV lamp for ozone generation, had adjustable airflow and power levels.

An Oxyfresh AC300 corona discharge ozone generator (Oxyfresh Worldwide, Spokane, Wash.) with adjustable output was used to generate ozone in the range of 1–3 ppm. Although this unit had a rated output of 1.3 ppm, at the highest settings the output was slightly higher during the test due to increased air resistance through the test apparatus.

Three ozone sensors were used during the course of this experiment. Levels between 20–100 ppm were measured using an Orbisphere 3600 sensor (Orbisphere Labs, Neuchatel, Switzerland), which had an accuracy of $\pm 1\%$. Levels inside the test chamber between 0.5–20 ppm were measured with an Ecosensor model OS-2 (Eco Sensors, Inc., Santa Fe, N.M.), which had an accuracy of 10–20% in the range 0.01–0.3 ppm and ± 2 ppm outside this range below 20 ppm. Concentrations between 0–10 ppm were measured using an Ecosensor A-21Z, which had an accuracy of $\pm 20\%$ in the range 0.5–20 ppm.

Airflow rates for the Biozone and AC300 ozone generators were measured using a 129GN hot wire anemometer attached to a model 510e Multi-function Monitor from Solomat. Total accuracy for this unit was $\pm 3\%$.

RESULTS

The experiment was run in three phases. First, the ozone sensors were calibrated. Second, the catalytic converter was tested to establish performance under various flow conditions and ozone concentrations. Finally, a series of bioassays were run at various ozone concentrations, with the catalytic converter operating throughout the tests.

Ozone Sensor Calibration Results

A series of calibration tests was run to establish the operating characteristics of the Ecosensor A-21Z. These were necessary because the sensor was operating above its ideal operating point of < 1 ppm most of the time, and operating characteristics in the 1–10 ppm range were not well established. Because the A-21Z is not suited for continuous exposure to ozone levels above 1 ppm, it was given a timed exposure.

In the calibration tests the Orbisphere sensor was used to measure supply ozone concentrations that were then diluted down to 1–10 ppm. The diluted concentrations were then measured with the A-21Z. Although the design operating range of the A-21Z is below 1 ppm, it was determined that the A-21Z was accurate within approximately ± 2 ppm when measuring concentrations up to 10 ppm if an exposure time of 22 sec was used. The A-21Z was used in this high range only for corroborating the Orbisphere.

The Ecosensor OS-2 was internally set to the expected ozone levels. It required some 12–24 hours of warm-up operation in ozone before all transient effects abated. The internal set point was 1.3 ppm for the bioassays that were performed between 1–3 ppm.

Ozone Catalysis Results

Twenty trials, including 11 bioassays, were run to evaluate catalytic converter unit performance. The A-21Z ozone sensor was placed at the catalytic converter outlet, but was periodically inserted into the chamber to check readings against the OS-2 sensor. Because its accuracy is greatest at low ozone levels (i.e., < 1 ppm), it was ideal for detecting the presence of low levels of ozone or

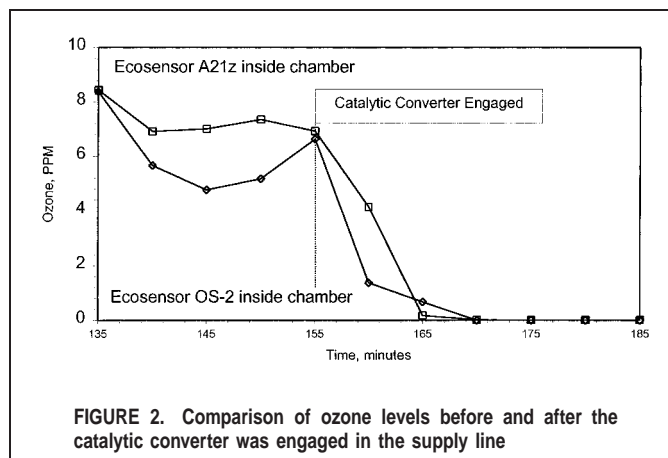


FIGURE 2. Comparison of ozone levels before and after the catalytic converter was engaged in the supply line

the absence thereof. The OS-2 ozone sensor was used to continuously monitor internal ozone concentrations inside the chamber as shown in Figure 1.

No ozone or other odors could be smelled in the exhaust airstream. Humans are capable of sensing ozone at levels below 0.01 ppm, and this corroborated the sensor readings in a subjective but appropriate manner. In 20 tests the catalytic converter completely removed any measurable ozone from the airstream at flow rates from 5–102 L/min and ozone concentrations of 0.3–15.6 ppm.

Figure 2 shows one test in which the catalytic converter was bypassed during a calibration test and then engaged. Supply air containing ozone was routed through the catalytic converter at the 155-min mark of the test, and chamber levels dropped as the chamber was completely purged in about 15 min.

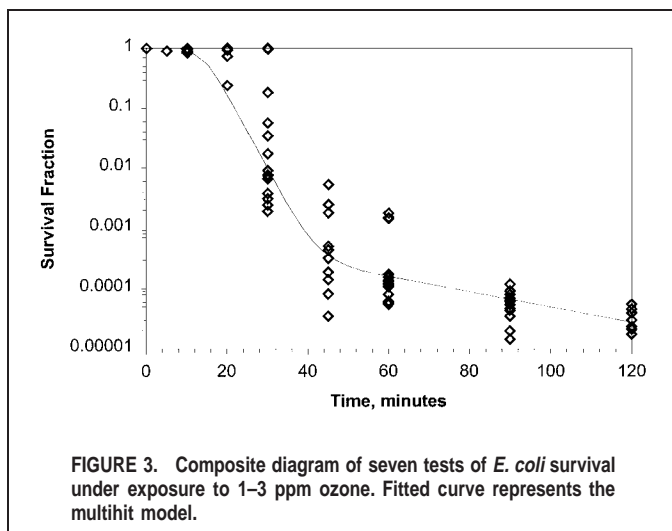
In the course of these tests the catalytic converter was run for well over 50 hours, during which it did not exhibit diminished capacity to remove ozone. The capacity and operational limits of metal oxide catalysts have been studied and reported at length elsewhere.^(12,20) Per the manufacturer's product information the catalyst has no concentration limit, but performance is limited by the residence time implicit in the air velocity. The recommended minimum residence time for the catalyst is 0.36 sec under normal humidity, but is 0.72 sec under 100% RH.⁽¹⁹⁾ In this experiment the residence time varied from 0.9–10 sec.

Bioassay Test Results

Nine successful tests were run with the *E. coli* strain. Table II summarizes the number of bacterial samples tested, the mean CFU of the control plates, the ozone concentrations used, flow rates, and the survival fraction after 60 min of exposure. Control

TABLE II. Summary of Bioassay Results

Test No.	Ozone (ppm)	Flow Rate (L/min)	No. of Controls	Control CFU	No. of Trials	% Survival at 1 Hour
1	5.7	10	2	4.08E+11	12	0.10
2	0.3	102	3	1.02E+11	6	58.0
3	1.2	22	3	3.60E+03	8	0.01
4	1.4	43	4	8.83E+09	5	0.014
5	1.4	43	4	3.89E+09	16	0.010
6	1.6	43	3	3.14E+09	11	0.011
7	1.2	43	3	8.6E+09	20	0.156
8	1.7	43	1	1.23E+09	9	0.011
9	1.2	43	5	9.74E+08	23	0.010



CFU were varied for tests at different ozone concentrations, but the variation in the control CFU was approximately $\pm 8\%$. This relatively tight spread is credited to the precision of the spiral plater. A limited number of plates, typically 1–4, were placed in the ozone chamber at any one time, because ozone was apparently being consumed when too many plates were added at one time.

Dose dependence is implied by the data in Table II, but the results are insufficient to establish quantitative conclusions. An ozone dose could be conceptualized as the ozone concentration multiplied by the time of exposure. However, the flow rate of the ozone is also a factor, although it is unlikely to be a simple linear relationship. That is, higher flow rates at some constant concentration of ozone over the surface of the petri dishes will likely increase the disinfection rate up to a point.

Some of the scatter in the data is due to various factors such as variations in dilution concentration, variations in ozone levels during the course of the experiment, variations in air currents within the test apparatus, consumption of ozone within the test chamber when too many plates were ozonated simultaneously, and measurement error.

Data from tests 4 through 9 are plotted in a composite chart in Figure 3. In spite of the scatter, it is clear that both a two-stage curve and a significant shoulder are present. The shoulder causes a delay in the response of approximately 20 min at this ozone level. The second stage of the curve represents a resistant population of approximately 0.1%.

Overlaid on the data in Figure 3 is a two-stage curve with a shoulder based on the multihit target model. The first stage of the curve represents the data between 20 and 45 min, which yields the following fitted single-stage equation:

$$S = 207.25e^{-0.295t} \quad (20 \leq t \leq 45) \quad (1)$$

The second stage of the curve is based on a fit of the data between 60 and 120 min. The resulting single-stage equation is:

$$S = 0.0009e^{-0.0294t} \quad (60 \leq t) \quad (2)$$

It can be observed that the intercept of the second stage occurs at $S=0.0009$. This represents the fraction of the population that is resistant and implies that the fraction of the population for the first stage is 0.9991. The combined two-stage equation is simply the addition of Equations 1 and 2, with the first stage multiplied by the population fraction, or:

$$S = 207.0635e^{-0.295t} + 0.0009e^{-0.0294t} \quad (3)$$

In Equation 3 the survival S has a maximum value of 1.0. The shoulder threshold occurs at approximately 20 min. The second stage curve intercepts the y-axis at approximately $S=0.001$, which implies a resistant fraction of $f=0.001$. A more convenient way of expressing the shoulder mathematically is the multihit target model, which is often used in UV water disinfection. The multihit target model⁽²¹⁾ can be written as follows:

$$S(t) = 1 - (1 - e^{-kD})^n \quad (4)$$

The parameter n represents the number of discrete critical sites that must be hit to inactivate the microorganism, which is unique for each species. The parameter D represents the dose, and k represents the rate constant for UV exposure. In the case of ozone dosing the dose is simply the ozone concentration multiplied by the time and is the same for both population fractions.

Although the rate constants for ozone exposure could be defined at this point, it is unnecessary because only a mathematical solution is being sought. Because there are already least squares curve fits for the two stages in Equation 3, one can simply say $k_1D=0.295t$ for the first stage and $k_2D=0.0294t$ for the second stage.

Given the population fractions noted above, the complete two-stage decay curve can be written based on analogy with the multihit target model as follows.

$$S(t) = (1 - f)[1 - (1 - e^{-kD})^{n_1}] + f[1 - (1 - e^{-kD})^{n_2}] \quad (5)$$

The values of n_1 and n_2 can be determined only by computing the least squares error between Equation 5 and the data shown in Figure 3. Trial and error results in a value of $n_1=65$ and $n_2=1$. No attempt is made here to explain the physical significance of these target sites in relation to ozone. It suffices that these values result in the best fit of the curve to the data as shown in Figure 3. Inserting the appropriate parameter values, including the dose coefficients based on Equation 3, the equation for the ozonation curve can be written in simplified form as:

$$S(t) = 0.9991[1 - (1 - e^{-0.295t})^{65}] + 0.0009e^{-0.029t} \quad (6)$$

Sterilization often is defined as a six-log (base 10) reduction in microbial population. Based on Equation 6, a six-log reduction would be achieved by about 240 min.

Figure 4 shows a time-lapse series of digital images of the spiral plated petri dishes exposed to ozone. Although the 30-min exposure is hardly distinguishable from the control plate in these images, it was countable by the digital imaging system. Note the drastic change in CFU between the 30- and 60-min marks. To improve counting accuracy, it ultimately proved necessary to subdivide the dilutions into three sets and bracket the zones from 0–30, 30–60, and 60–120 min.

It would appear that the net flow rate has as much to do with the survival rate as the ozone level in this range. That is, 5.72 ppm at 10 L/min is an order of magnitude less effective than 1.3 ppm at 43 L/min. This suggests the possibility that surface disinfection could be enhanced through increased air velocity, or perhaps increased turbulence, without increasing ozone levels.

Figure 5 shows a comparison of the present results on *E. coli* with those of the previous researchers from Table I. This chart has been formulated to reflect the theoretical ozone dose, in which dose is the product of concentration and exposure time, so as to provide a better comparative basis. This comparison ignores variations in the percentage of RH. This comparison assumes that dose dependence exists at low levels of ozone, a hypothesis for which some evidence exists.^(15,17,22) In this chart the test microbe

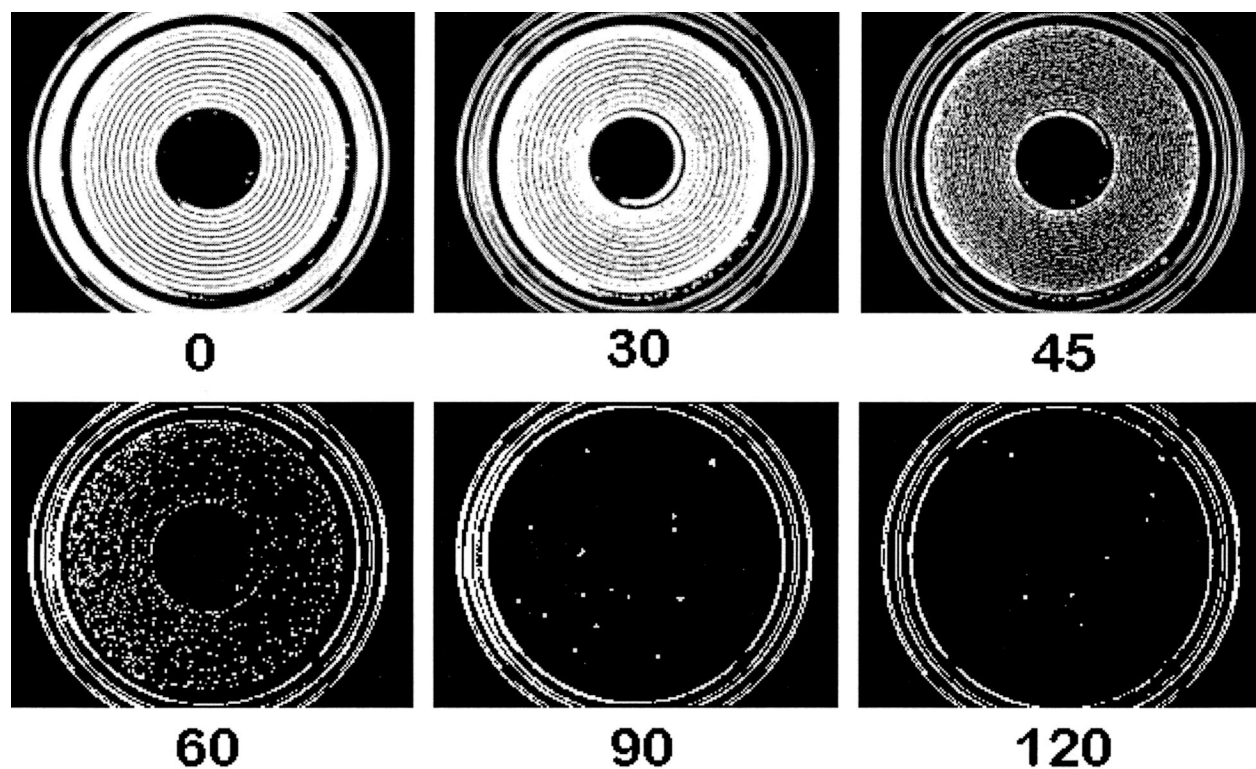


FIGURE 4. Spiral plated samples of *E. coli* with ozone exposure time indicated in minutes. Images have been contrast enhanced to highlight the colonies.

E. coli is clearly more vulnerable to ozone than most microbes, which is to be expected because a weak strain was used.

DISCUSSION AND CONCLUSIONS

By demonstrating that surface disinfection can be obtained with levels of airborne ozone that can be completely removed by catalytic conversion, the possibility of developing a microbial growth control system has been demonstrated. It has further been shown that the decay curve from ozonation displays the characteristics of a two-stage curve with a shoulder. The multihit target

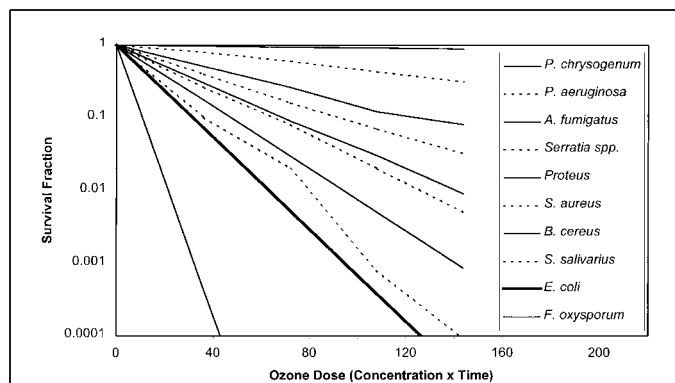


FIGURE 5. Comparison of present results on *E. coli* (dark line) with results of other researchers. Microbe names are associated with lines from top to bottom.

model, adapted from UV disinfection modeling, has been successfully applied to the ozone disinfection data.

Enhancement of the biocidal effect is possible through humidity control. According to the Ishizaki et al.⁽¹⁷⁾ study the biocidal effects of ozone are markedly increased above about 50–70% RH. High humidity results in increased production of short-lived hydroxyl radicals that are thought to account for a large part of the biocidal effects of ozone. Humidity was uncontrolled in this experiment but remained in the 18–22% range, and although the *E. coli* used in this experiment is a weak strain, it could be expected that higher humidity would have resulted in biocidal effects on a normal strain of *E. coli*, or on other bacteria like *Legionella pneumophila*. Such levels of humidity would be normal in indoor environments and inside air handling units. Increasing RH% may enable even lower ozone levels to be used.

A six-log reduction is often used as the technical definition of sterilization. Extrapolation of Equation 6 suggests that a six-log reduction of this strain of *E. coli* could be achieved in 3 hours at 1–3 ppm and 18% RH.

Hypothetically, a 24-hour exposure at some level of ozone that could be completely removed catalytically would be sufficient to sterilize surfaces of all microbial contamination. Applications involving air handling units, for example, in which ozone was injected at the inlet and catalytically removed downstream, may be a feasible means of controlling microbial growth. There are material considerations, of course, because not all materials used inside air handling units will tolerate ozone exposure. Other applications may include the sterilization of unoccupied rooms or buildings for remediation, or the use of ozonation for sterilizing medical equipment and safety cabinets.

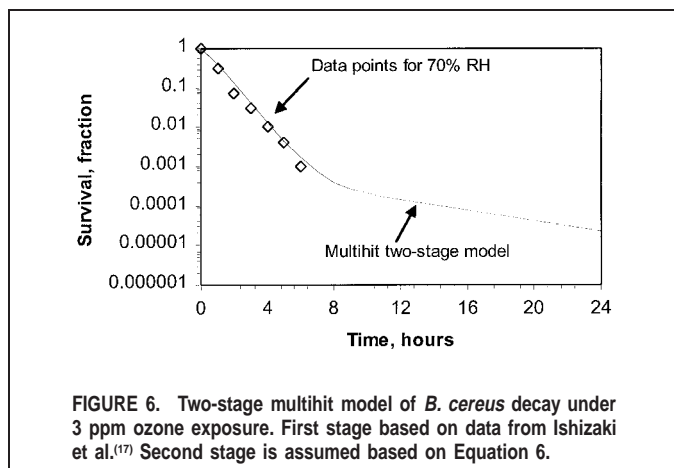


FIGURE 6. Two-stage multihit model of *B. cereus* decay under 3 ppm ozone exposure. First stage based on data from Ishizaki et al.⁽¹⁷⁾ Second stage is assumed based on Equation 6.

According to the product literature the catalytic process produces only oxygen and carbon dioxide, with no hazardous by-products except trace amounts of carbon monoxide.⁽¹⁹⁾ The ozonation of organic materials such as microbial samples may possibly produce byproducts of an unknown nature.⁽²³⁾ These matters are beyond the scope of the current experiment.

Further study remains to be done to determine whether microbes that are more resistant to ozone than the variant of *E. coli* studied here can be controlled by the same levels of ozone but with higher humidities, higher ozone levels, or longer exposures. Studies are also needed to determine the effectiveness of ozone against airborne microorganisms and thereby establish the design basis for an ozone airstream disinfection system.

The possible use of ozone for the disinfection of buildings contaminated with anthrax is illustrated by the graph of the *B. cereus* data from Ishizaki et al.⁽¹⁷⁾ in Figure 6. *B. cereus* is virtually identical to *B. anthracis* except that it is a nonvirulent strain. The multihit model developed for *E. coli* in Equation 6 was used with the predicted first stage rate constant for *B. cereus* based on the Ishizaki et al.⁽¹⁷⁾ data at 70% RH and 3 ppm. The second stage was assumed to have 10% of the first stage decay rate, similar to the *E. coli* data presented here. No significant shoulder was evident in the data, so an exponent of only 1.3 was used. The decay equation at 3 ppm used in Figure 6 is as follows:

$$S(t) = 0.9991[1 - (1 - e^{-1.153t})^{1.3}] + 0.0009e^{-0.153t} \quad (7)$$

Based on this model, sterilization could be achieved within 48 hours. More rapid decontamination is possible at higher ozone levels. No data exists to demonstrate the second stage decay rate for *B. cereus*, but data from Hibben and Stotzky⁽²²⁾ suggests that a second stage exists for some spores. If no second stage exists, this model is conservative. Further research is needed to determine the characteristic second stage of *B. cereus* under ozone exposure to verify this model.

Further research also is needed to determine the response of airborne microorganisms to different levels of ozone and the impact of RH before a full-scale system can be designed for the disinfection of airstreams.

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