STERILIZATION EFFICACY OF ULTRAVIOLET IRRADIATION ON MICROBIAL AEROSOLS UNDER DYNAMIC AIRFLOW BY EXPERIMENTAL AIR CONDITIONING SYSTEMS

BY

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ABSTRACT

In order to know the sterilization efficacy of ultraviolet irradiation on microbial aerosols, the size and the weight of the aerosol particles were evaluated, and these were irradiated under dynamic airflow created by an experimental air conditioning system. The experimental apparatus consisted of a high efficiency particulate air (HEPA) filter, an aerosol generator, spiral UV lamps placed around a quartz glass tube, an Andersen air sampler and a vacuum pump. They were connected serially by stainless steel ducts (85mm in diameter, 8m in length). Six types of microbial aerosols generated from an ultrasonic nebulizer were irradiated by UV rays (wavelength 254nm, mean density 9400 μW/cm²). Their irradiation time ranged from 1.0 to 0.0625 seconds. The microbial aerosols were collected onto the trypticase soy agar (TSA) medium in the Andersen air sampler. After incubation, the number of colony forming units (CFU) were counted, and converted to particle counts.

The diameter of microbial aerosol particles calculated by their log normal distribution were found to match the diameter of a single bacteria cell measured by a microscope. The sterilization efficacy of UV in standard airflow conditions (0.5 sec. irradiation) were found to be over 99.5% in Staphylococcus aureus, Staphylococcus epidermidis, Serratia marcescens, Bacillus subtilis (vegetative cell) and Bacillus subtilis (spore) and 67% in Aspergillus niger (conidium). In A. niger, which was the most resistant microbe to UV irradiation, the efficacy rose up to 79% when irradiated for 1.0 sec., and it was observed that the growth speed of the colonies was slower than that of the controls. It was thought that UV rays caused some damage to the proliferation of A. niger cells.

Key words: Microbial aerosol, Ultraviolet ray irradiation, Andersen air sampler, Diameter of aerosol, Weight of aerosol, Air sterilization, A. niger.

INTRODUCTION

For microbial organisms, air is not an inhabitation, but rather a medium of passage. They are said to exist as two forms in the air: "droplet nuclei" and "dust particles". To prevent air contamination by microbes, it is necessary to know the behavior and character of microbial aerosols. In aerosol studies there have been numerous reports made on the dust particles, but little has been done on the droplet nuclei of microbial organism (Wells and Sharp [1], [2]), since they are difficult to observe in the atmosphere or room air. In the clean rooms of operating centers, air contamination by microbial aerosols is a very serious problem. Nowadays HEPA filters are mainly utilized for purging the air of microbial particles inside the air conditioning duct. However the filter method has some weaknesses such as the

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possibility of air leak and the growth of mold on the filter surface. Recently infectious diseases mediated by air conditioning have appeared with the popularization of air conditioning systems.

Preliminary studies (Nakamura [3], [4]) and the following studies using experimental air conditioning systems were performed to investigate the microbial aerosols and their inactivation under UV irradiation.

MATERIALS AND METHODS

1. Experimental apparatus

The experimental apparatus basically consisted of the following components (Fig. 1, 2):

(1) Stainless steel air ducts (85 mm in diameter, 8 m in length)
(2) HEPA filter (Cambridge, Co., U. S. A.) 0.3 μm in diameter, η = 99.97%, Q = 1.4 m/min.
(3) Fan (The airflow velocity of the system was made so that it could be freely control-

led from 50 to 200% of the standard air conditioning system.)

(4) Aerosol generator: ultrasonic nebulizer (1.75 KHz, 20 W: EN-2, Acoma Co., Ltd., JAPAN)
(5) Andersen air sampler (MODEL 1100: 2000 INC., U. S. A.)
(6) UV ray irradiation light (wavelength 254 nm, intensity 9400 μW/cm², four spiral lights 25 cm in length around the quartz tube: Nippo Co., JAPAN)
(7) Filter holder (Quick holder: Chiyoda Safety Appliance Co., Ltd. JAPAN)

2. The challenge bacteria

The following organisms were used in the test:

(1) Staphylococcus aureus ATCC 10390
(2) Staphylococcus epidermidis ATCC 155
(3) Serratia marcescens K-1 (stock strain in Department of Microbiology, Tokyo Medical and Dental University)
(4) Bacillus subtilis (vegetative cell) ATCC 6633
(5) Bacillus subtilis (spore) ATCC 6633

Fig. 1. Schematic Representation of Flow Diagram of Experimental Apparatus Used.
(6) *Aspergillus niger* (stock strain in the National Health Laboratory, JAPAN)

The test organisms were cultured in tryptase soy broth (TSB; BBL Inc.,) at 27 to 30°C overnight and the cell concentrations were determined by standard bacteriological techniques. As for the *B. subtilis* (spore), the bacterial culture was kept in cold storage (4°C) for 48 to 140 hours, and the spore formation was identified by the Möller stain (Fig. 3A).

To prepare the *A. niger* conidium (Fig. 3B), the following filter technique was used:

1. *A. niger* with full proliferation of black conidium was cultured on a trypticase soy agar (TSA; BBL Inc.,) plate.
2. The plate was inverted above a filter paper with a funnel, which had been previously sterilized. The contents were gently knocked onto the filter. At this point, a coffee filter (Kalita Co., Ltd.,) was used for this.
3. A 0.005% sulfosuccinic acid diocetyl sodium was poured onto the *A. niger* on the filter, and the filtrate was collected in a flask under the funnel.
4. After shaking, the filtrate was stored for one night. The supernatant fluid was removed and distilled water added to the filtrate, and the flask was shaken again. This procedure was repeated several times.

Finally a water suspension of *A. niger* conidium was acquired. To prevent contamination of the filtrate by conidium scattered in the room, procedures 2) and 3) were done in a specially made sealed box.

3. Experimental procedures

(1) Microbial cultures were diluted to the final concentrations of $10^6$ to $10^7$ cells per millilitre with sterilized distilled water just before use.

(2) Ten ml of the microbial suspension were poured into the bottle of the ultraso-
nic nebulizer and converted to aerosol. The aerosol was then fed into the air duct, where it was mixed with filtered air and made to flow at a constant stream of one cubic foot per minute (0.028 m$^3$ per min) (1 cfm). To keep the generation of aerosol constant, the microbial suspension must be properly mixed to the right dilution and the power of the generator fixed at 10 watts.

(3) Sampling of test organisms

The aerosol in the air duct was drawn into the Andersen air sampler (Andersen [5]) (Fig. 4) through a sampling nozzle and collected on the Petri dishes. The aerosols were caught in the six stages of the sampler in the order of size by the impaction theory (Lanz and Wong [6]). Each petri dish contained 27 ml of TSA medium held horizontally. Sampling time was set at one minute to allow one cubic foot (0.028 m$^3$) of air pass through the Andersen air sampler.

(4) The dishes were then removed and inverted onto their covers, and kept at 32±1°C in a dark culture box.

(5) The colony-forming units (CFU) were counted with a colony counter after incubation for 24 to 48 hours.

(6) The number of theoretical microbial particles collected in the Andersen air sampler was calculated according to the “positive hole” method of Andersen [5]. The counts of CFU in stages 3–6 were converted to particle counts by the use of the basic formula of Feller (Andersen [5]).

$$Pr = N \left[ \frac{1}{N+1} + \frac{1}{(N-1)+1/(N-2)+\ldots+1/(N-r+1)} \right]$$

where

- $Pr$: expected number of viable particles
- $r$: positive holes (CFU)
- $N$: total number of holes per stage (400)

(7) Measurement of microbial weight

The sampling air was passed through two sheets of paper filter (dust sampling filter paper, TOYO HE-40T: Toyo Roshi Co., Ltd., Japan) fixed on a filter holder. The weight of the two sheets before and after sampling was measured by a weight scale (d=0.1mg; Kensei Kougyou Co., Ltd., JAPAN) and the difference noted. The weight of the TSB aerosol alone was also measured for calibration.

(8) Conditions created by UV irradiation

The stay time in the quartz tube in the UV light system, which equals the irradiation time, was controlled in the range of 1.0 to 0.0625 seconds by manipulating the airflow velocity and the number of lamps. In standard airflow, the irradiation time was 0.5 seconds and the conditions in the
duct were as follows: temperature 33°C, relative humidity 32–37% and concentration of ozone 0.01 ppm on the ozone monitor (EG2001; Ebara Co., Ltd., Japan).

(9) Efficacy

The UV-irradiated aerosols and the non-irradiated aerosols (control) were sampled alternately. The sterilization efficacy of UV irradiation was calculated as follows:

\[ E = \frac{(C - U)}{C} \times 100 \]

(2)

E: sterilization efficacy of UV irradiation (%)
C: total number of particles on the control dishes (s1+...+s6)
U: total number of particles on the dishes with irradiated aerosol (s1+...+s6)

(10) The SAS program of computer center (M680H: University of Tokyo) was used in the conversion and statistical analyses.

RESULTS

1. Particle size distribution of microbial aerosols

The Andersen air sampler gave the highest count in the fifth stage with S. aureus, S. epidermidis, Serratia, B. subtilis (veg. cell) and B. subtilis (spore). In A. niger, the highest count was recorded at the third stage. (Fig. 5)

Assuming that the particle size distribution in the experiment is approximately equal to the log normal distribution by its cumulative probability of particle counts, it was calculated by the aerodynamic mass diameter (AMD) and geometrical standard
Table 1. Parameter of Microbial Aerosol

<table>
<thead>
<tr>
<th>Organism</th>
<th>AMD(μm)</th>
<th>σg</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>B. subtilis (vegetative)</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>B. subtilis (spore)</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>A. niger (conidium)</td>
<td>3.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

AMD: aerodynamic mass diameter  
σg: geometrical standard deviation

Table 2. Size of microbial diameter

<table>
<thead>
<tr>
<th>Organism</th>
<th>AMD(μm)</th>
<th>Microscopic diameter(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>1.1</td>
<td>0.5×(0.5~1.0)</td>
</tr>
<tr>
<td>B. subtilis (vegetative)</td>
<td>1.1</td>
<td>5×0.7</td>
</tr>
<tr>
<td>B. subtilis (spore)</td>
<td>0.8</td>
<td>0.8 (Fig. 3A)</td>
</tr>
<tr>
<td>A. niger (conidium)</td>
<td>3.3</td>
<td>4.6 (Fig. 3B)</td>
</tr>
</tbody>
</table>

CMD: count median diameter in aerodynamic studies

deviation (σg) (Table 1). The count median diameter (CMD) of the aerosols was calculated by the following formula (Hatch and Choate [7]).

\[
\text{AMD} = \text{CMD} \times e^{3(\ln \sigma)^2}
\]  

Furthermore, the CMD was compared with the diameter determined by the microscope (Table 2). From this table, it can be seen that the size of the microbial aerosol particles is almost the same as that of the microscope measured microscopically, except for the B. subtilis (vegetative cell) which is not a coccid type.

2. Weight of microbial aerosols

The weight of microbial aerosols was calculated by the weight difference of the two paper filters. The weight of the pure TSB aerosol was subtracted from the weight of the aerosol containing the microbe. From these tests, the weight concentration of the microbial aerosols containing S. epidermidis, B. subtilis (spore) and A. niger was found to be 1 mg/m³ at the most (Fig.
3. UV irradiation light

The UV lamp was of 254nm wavelength, and the mean intensity of the UV lamp was 7500µW/cm² when the experiment was set up (Fig. 7). However, at the end of the experimental series, the mean intensity re-measured by the UV monitor (UVR254: Nippo Co., Ltd., JAPAN) was 9400µW/cm² per UV lamp.

1) Effect of UV irradiation on colony formation (B. subtilis spore) (Fig. 8).

The photograph shows the B. subtilis (spore) colonies growing on the Petri dishes, with labels indicating which stage of the Andersen sampler they were obtained from. Comparing the control group with the UV group, there is no proliferation of the colonies in all stages with the cells irradiated for 1/4 sec. Most cells were killed by 1/4 sec. irradiation, but some cells irradiated for 1/16 sec. survived.

2) Particle counts given by Andersen sampler of six organisms (Fig. 9)

These are the mean particle counts of the Andersen sampler of the six tested bacteria; those of the irradiated bacteria (254nm, 9400µW/cm², 0.5 seconds) and those of the control. Only the Serratia was irradiated for 0.33 seconds. With the S. aureus, S. epidermidis, Serratia, B. subtilis (vegetative cell) and B. subtilis (spore), the particle counts of the controls reached the peak in the fifth stage, but the particle counts of the irradiated bacteria were less than 1 in each stage. On the other hand, with the A. niger the particle counts showed the peak in the third stage, though the peak was lower in the irradiated sample than that in the control. This showed that A. niger had a stronger resistance to UV irradiation in comparison with the others.

3) Particle counts of test organisms exposed to various lengths of time (Table 3)

The table shows the number of tests carried out(n), the mean particle counts (mean) and the standard deviation (SD) for each irradiation time on a particular organism. From the stochastical two-way layout of the presence or absence of UV irradiation and the irradiation time, multivariate analyses were made for each
organism by the general linear model (GLM) procedure. These were significant differences in the UV irradiation efficiency on each test organism. 4) Observing survival rates by UV irradiation time and intensity on A. niger (Fig. 10)

With the A. niger, which is the most resistant to UV, UV efficiency was studied by varying UV intensity and irradiation time. The left graph, which is taken from a previous report (Nakamura [8]), indicates that UV (750µW/cm²) has no effect on A. niger. But with UV (9400µW/cm²), as was used in this experiment, the survival counts dropped with the lengthening of the irradiation time. There was observed a relationship between the irradiation time and sterilizing efficiency. 5) UV irradiation time and survival rate in six organisms (Fig. 11)

Under standard airflow (irradiation time 0.5 seconds), the sterilization efficiency was over 99% in S. aureus, S. epidermidis, Serratia, B. subtilis (veg. cell) and B. subtilis (spore). However in A. niger, it was 67%, and it rose to only 79% at 1.0 second irradiation. The survival rate curves indicate that it is necessary to use a more
Fig. 8. Effects of UV Irradiation on Colony Formation of *B. subtilis* (spore) as Defined by the Stages of Andersen Air sampler. The upper group of plates is control, the middle and the lower groups are UV irradiated (1/16, 1/4 seconds respectively).

Fig. 9. Particle Counts of Andersen Air Sampler in Six Organisms. The value of UV irradiation is marked as closed triangles and that of the control is marked as open circles.
Table 3. Particle Counts of Tested Organism by UV Irradiation time

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test</th>
<th>UV irradiation time (sec)</th>
<th>F VALUE (GLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td>S. aureus</td>
<td>C</td>
<td>2143.6±</td>
<td>3649.5±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1912.4(15)</td>
<td>2578.8(5)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.3±</td>
<td>0.4±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5(24)</td>
<td>0.7(8)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>C</td>
<td>3343.3±</td>
<td>86.1±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3223.8(14)</td>
<td>101.4(17)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>8.4±</td>
<td>0.5±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.3(24)</td>
<td>0.9(26)</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>C</td>
<td>1336.2±</td>
<td>4867.5±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1870.9(12)</td>
<td>704.6(5)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.7±</td>
<td>0.9±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1(20)</td>
<td>1.2(8)</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>C</td>
<td>240.9±</td>
<td>569.1±</td>
</tr>
<tr>
<td>(vegetative)</td>
<td></td>
<td>18.1(5)</td>
<td>511.7(7)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>1.0±</td>
<td>0.1±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9(8)</td>
<td>0.3(10)</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>C</td>
<td>2747.2±</td>
<td>80.1±</td>
</tr>
<tr>
<td>(sprre)</td>
<td></td>
<td>1029.3(6)</td>
<td>63.6(21)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.3±</td>
<td>0.6±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5(12)</td>
<td>0.9(40)</td>
</tr>
<tr>
<td>A. niger</td>
<td>C</td>
<td>768.5±</td>
<td>1658.4±</td>
</tr>
<tr>
<td>(conidium)</td>
<td></td>
<td>596.7(16)</td>
<td>1424.4(14)</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>164.8±</td>
<td>550.3±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>144.6(32)</td>
<td>620.3(24)</td>
</tr>
</tbody>
</table>

Sum of viable particles (mean±SD(n)) SD: standard deviation, n: number of tests
C: Control
U: UV irradiation (254nm, 9400μW/cm²)
GLM: General linear model procedure of multivariate analysis

<table>
<thead>
<tr>
<th>750(μW/cm²) 0.24s</th>
<th>9400(μW/cm²) 0.3s</th>
<th>9400(μW/cm²) 0.5s</th>
<th>9400(μW/cm²) 1.0s</th>
</tr>
</thead>
<tbody>
<tr>
<td>750(μW/cm²) 0.24s</td>
<td>9400(μW/cm²) 0.3s</td>
<td>9400(μW/cm²) 0.5s</td>
<td>9400(μW/cm²) 1.0s</td>
</tr>
</tbody>
</table>

Fig. 10. Number of Surviving Cells by UV Irradiation Time and Intensity (A. niger). The value of UV irradiated cells is marked as closed triangles, that of the control is marked as open circles.
ULTRAVIOLET STERILIZATION OF MICROBIAL AEROSOLS

Fig. 11. UV Irradiation Time and Survival Rate in Six Organisms

Fig. 12. Morphological Variations of A. niger Colonies After UV irradiation. In each pair of dishes, the left is the control and the right the UV irradiated. The upper left pair is after 24 hours, upper right 48 hours, lower left 72 hours and the lower right 96 hours after sampling.
powerful UV light or to irradiate for longer periods in order to destroy the A. niger.

6) Morphological variation of A. niger colonies with time (Fig. 12).

The colony growth of A. niger was investigated for over 24 to 144 hours. The colonies increased in number and size, but there was a large difference between the UV irradiated cell colonies and the non-irradiated cell colonies.

The number of CFU in the control was constant after 24 hours, but the irradiated cells continued to increase up to 48 hours.

The colony growth histogram (Fig. 13) shows that the non-irradiated cell colonies were increasing in size satisfactorily. In the irradiated cell colonies there were two types of colonies observed. Some of them stopped growing and remained as tiny colonies, while the others increased in size by the extra nutrient available. These results indicate that the UV rays affect the colony growth.

**DISCUSSION**

1. Particle size of microbial aerosols

It is impossible to observe directly the behavior of the microbial aerosols in air. In this experiment, in order to know the diameter of a microbial aerosol particle, the microbes were trapped on the dishes of the Andersen sampler according to the aerosol size and jet flow based on the sampler’s “impaction theory” (Lawz and Wong [6]). The resulting colonies were counted. Assuming the particle counts conterved by the “positive hole” theory were that of viable microbes, it was possible to deduce semi-quantitatively the number of viable microbial particles in the dynamic airflow. In this experimental system, the microbial aerosol generated by the ultrasonic nebulizer showed a normal distribution in size, and their count median diameter (CMD) was very close to the optically observed size of a bacterial cell, except for B. subtilis vegetative cells. From this, it can be deduced that most microbes in the aerosols in a dynamic airflow exist in single
particles rather than in clumps. In the example shown in the photograph (Fig. 8), a small number of colonies were observed in the first stage besides the numerous colonies in the fifth stage in some of the tests. This phenomenon was probably due to the disorderly flow in the sampler orifice creating a secondary concentration of cells and not to the biphasic peak in the size of surviving cells. In the well-known works by Andersen [5], the conversion table of the “positive hole” method is applicable only to CFU in the third stage to sixth stage. After conversion, the particle counts of the first stage and second stage turn out to be negligible. This is why the CMD of the microbial aerosol generated by the ultrasonic nebulizer is almost the same as the optically measured diameter found in many references. This indicates that the microbes in the aerosol act as single, separated cells.

2. Weight of microbial aerosols

It must be remembered that the weight of the microbial aerosol would include both the intracellular fluid and the fluid around the cell body. The filter methods used in this experiment does not make clear the true weight as it is probably influenced by the concentration and the viscosity of the medium presented in the aerosol. However, considering that the aerosol diameter was found to be approximately equal to the diameter of a single cell, it can be assumed that the fluid in the microbial aerosol exists mostly within the cell, or as a thin film around it. It is probable that in nature, when *A. niger* is scattered in the air, the conidium is much lighter and contains less water.

3. Ultraviolet irradiation as direct method of air sterilization

The bactericidal effects of UV have been well-known for a long time and there have been some excellent studies about UV sterilization of airborne bacteria from the 1930’s. Sharp [2] reported on his experiment on UV irradiation to the bacteria suspended in air as early as 1940, and his results of the efficiency of UV was similar to ours except for *A. niger* which was not examined, and *B. subtilis* which contained both the spore-forming and vegetative cells. In the practical use of UV irradiation to airborne particles, Hart, Goldner, Moggio and their coworkers [9]–[17] at Duke University, and the National Academy of Science [18] reported in their numerous studies from 1936 that the incidence of orthopedic wound infection of refined clean cases has been lower than 0.5% since the use of UV irradiation in the operating room. In the use of UV irradiation in the air conditioning systems, Robbins [19] had commercialized his aseptic air units with UV lights in 1962, but it was not popular because of the weak intensity of the UV light despite the system being a huge structure with a cooling system.

However, UV sterilization is utilized only in a limited place, due to the poor assessment of its efficiency. Recently UV has been drawing attention as a safety and efficient sterilization method (Carlson and Levenson [20], [21]) because of the development of powerful UV light and the conception of the secondary environmental pollution of sterilizing drugs and gas.

To control airborne contamination, clean ventilating methods are widely used in air conditioning systems, and the HEPA filter has proved highly efficient. These methods however have very serious problems such as the growth of molds on the filter surface and the risk of filter leak. On the other hand, there are direct sterilization methods such as heat, UV ray and X-ray, to name the physical methods, and chemical methods such as the vapor of glycol. If these are to be utilized, it is necessary that they are harmless and odorless to man, easily controlled in terms of
temperature and humidity.

Certain wavelengths of UV rays are harmful to the eyeball, conjunctiva or skin, and the ozone generated by UV rays can cause secondary harm to the human body. In applying this to the air conditioning system, it is easy to shut off the UV ray with glass or metal, and the 254 nm wavelength UV ray does not generate ozone.

Applying UV light to sterilize air, it has one advantage of UV irradiation that it does not cause dehydration and very little can escape its influence. In this experiment, it has been deduced that the microbial cells (droplet nuclei) in the aerosol exist as single cells almost in a “naked” state, making the microbes more vulnerable to UV rays in any of the other mediums (liquid or solid). This must be the reason that airborne bacteria are less resistant than those floating in the liquid suspension (Rentschler [22]). Furthermore, the spiral type of UV lamp used in the experiment enable the rays to reach even the bacteria that might hide in the shadow of the dust particles. Thus, UV sterilization is most suited for air sterilizing systems.

Now small but powerful UV lights are obtainable. The spiral type of UV light, used in this experiment, had a greater intensity than 9400μW/cm² when it was measured at one point within the quartz tube (Furumi and Sugawara [23], [24], [25]). Moreover it was found that a microbe can be irradiated in all directions, thus intensifying the irradiation. For this reason, only seconds are required to destroy the bacteria. The UV light will, therefore, prove effective when used as sterilizers in the air conditioning systems.

4. Resistance of microbes to UV light

Of the six bacteria, *S. aureus* and *S. epidermidis* are pathological epithelial bacteria and *A. niger* and *B. subtilis* are resis-tant to UV light (Murayama [26] [27]) and were therefore used as an index to UV efficiency.

In a previous experiment (Nakamura [6]), the sterilizing efficiency of UV (750μW/cm², 0.24 sec.) was found to be over 99% in *S. aureus*, *S. epidermidis*, *Serratia*, and *B. subtilis* (veg. cell), 69% in *B. subtilis* (spore) and 2% in *A. niger*. The recent experiment carried out in the dynamic airflow likened to the air conditioning system, the sterilization efficiency was over 99% in *S. aureus*, *S. epidermidis*, *Serratia*, *B. subtilis* (veg. cell) and *B. subtilis* (spore). The sterilization efficiency in *A. niger* rose up to 79% at 1.0 sec. irradiation. Nevertheless, this was the limit of efficacy in this experimental system, and in order to increase the efficiency further, it was necessary to use a more powerful lamp, or irradiate for longer periods.

Generally, the spore cells are said to be more resistant to the UV rays than the vegetative cells (Hachisuka and Horikoshi [28]). As for the reason why *A. niger* is resistant to UV rays, it could be due to the thick cortex or the special structure of the cell making it less vulnerable, or some action of the fluid contents in the cells or the spore photoprodut. The true reason, however, is still unknown.

Since there were no differences in the size distribution of the control and the irradiated cells, it is clear that resistance to UV rays is not due to the spore cell size.

5. Effect of UV on growth of *A. niger*

A change was observed in the colony formation of *A. niger* when irradiated. This shows that the dose of UV irradiation affected the growth of viable microbial cells, even though not enough to kill.

In molecular biology, UV is utilized for making mutant strains, by which the study of bacterial cells has progressed to analyzing the gene map and exchange of genes, especially on *E. coli*. In biochemistry, it has
been proven that the UV affects the DNA of the nuclei and that the photochemical products influence the viability of the cells or reactivation in vitro (Kondou [29]).

On the other hand in vivo, there are few reports on the effect of UV rays on A. niger, which is the most resistant to UV rays, and many things are yet unclarified. Furthermore, there have been very few studies made on the photoreactivation or DNA repair after UV irradiation, first taken up by Kelnar [30] in 1949. It is hoped that more studies will be made in this direction.

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