Method for Generating Fungal Spores Using Dry Dispersion and Ultrasonic Device

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Abstract

To date, the methods for producing aerosols have included atomization of liquids and suspensions, the dispersion of powders, and condensation methods. Because each of these methods were developed for the purpose of generating aerosols, they are not very effective for generating bioaerosols, such as low-weight fungal spores.

In this study, the method for generating fungal spores using dry dispersion and an ultrasonic device was developed. This method was capable of generating polydisperse fungal spores with a size distribution that approximates that of actual fungal spores in an indoor environment.

The results indicated that the performance of air cleaners could be evaluated using the fungal spores generated by the developed ultrasonic device.

Key Words: fungal spores generator, ultrasonic device, dry dispersion method

1. Introduction

Aerosols are used for various types of inhalation studies, including animal exposure for toxicological testing and respiratory deposition studies in humans or animals, as well as for administration of therapeutic agents. It is widely known that the distribution of aerosols is greatly influenced by their physiochemical characteristics, static electricity, and other environmental factors. To date, techniques for generating aerosols have included atomization of liquids and suspensions, the dispersion of powders, and condensation methods.

The simplest way to generate a droplet aerosol is the compressed air nebulization. During nebulization, an atomizer or nebulizer, produces an aerosol of small particle size from the impact of larger droplets within the device. Atomization of liquids and suspensions is a simple method for generating monodisperse solid-particle aerosols. A common way to check the size calibration of instruments is the nebulizing a suspension containing monodisperse solid particles of known size. For the dispersion of powders, the most widely used method for generating solid-particle test aerosols is the pneumatic redispersion of a dry powder. This method, often referred to as dry dispersion, can accommodate a wide range of powdered materials and dust feed rates. This method for generating aerosols has been applied in filter and air-cleaning research, as well as in animal
inhalation toxicology studies\textsuperscript{3}).

Recently, the number of reports on the heightened performance of air cleaners regarding the removal performance of airborne microbes has increased. However, these reports were released from the air cleaner manufacturers, and the evaluation methods used failed to include microbes. To effectively evaluate the microbe removal performance of the air cleaner, test methods employing viable particles are required. Therefore, it is essential that a method for generating these particles be established. The purpose of the present study is to develop a method for generating fungal spores that are indispensable for evaluating the fungal spores removal performance of an air cleaner.

In this paper, the method for generating fungal spores using dry dispersion and an ultrasonic device, capable of generating polydisperse fungal spores with a size distribution comparable to that found in an indoor environment, is discussed.

\section{2. Methods and Materials}

\subsection{2.1 Ultrasonic device}

\textbf{2.1.1 Diffusion of fine particles in an acoustic tube}

A prototype experiment was conducted in an acoustic tube. The acoustic tube, 50 cm long and 5 cm in diameter, placed on a level surface and a driver unit was connected to one end of the tube. The high intensity standing wave field of 660 Hz was produced in the tube and a sound pressure level, up to 155 dB, was obtained at the closed end.

Talc 1.0 g, median diameter in 8.2 μm, was put at the loop and node of the sound pressure distribution, the dynamic motion of the talc was captured by a camera. The diffusion of talc captured at 33 ms after application of the 660 Hz wave field is shown in Fig. 1. The results showed that particles on the wall of acoustic tube were diffused in the medium at the node of sound pressure distribution i.e. at the loop of vibrational velocity of sound.

The next step was focused on the particles those were directory released inside the medium of acoustic tube. A nichrome wire, 0.3 mm in diameter, was set in the acoustic tube, it was tensioned to the radial direction. The liquid paraffin was coated on the wire and the white fine particles were released in the medium when the instantaneous DC voltage was applied to the wire. The is called a Smoke Wire Method. High speed camera\textsuperscript{4} was introduced to capture those particles movement. In this experiment, the setup was shifted as follows; the acoustic tube was perpendicularly holded because the particles were not contacted on the wall of acoustic tube, and the frequency was also shifted to 1000 Hz. This setup was not influenced to the movement of fine particles in the medium, because the sound pressure distribution was not affected by the way of coupling method of acoustic tube and also the shift of frequency.

Subsequently, movement of medium in the time domain was measured by high speed camera at 500 frames per second. Examples of captured frames are shown in Fig. 2.

The results indicated that paraffin particles at 9 and 27 cm of the node of the sound pressure distribution diffuse radially along the tube; however, little movement occurred at the wire located at the loop. The medium in the wave front at the node was instantaneously diffused toward to the wall of the tube. The velocity of the particles at the node, measured from the captured frames, was approximately 10 cm/s. This velocity was estimated to be 1/10 of the particle velocity of sound. As shown in Figs. 1 and 2, not only the talc powder but also the finer liquid paraffin particles were diffused in high intensity airborne sound field.
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2.1.2 High intensity ultrasound field in chamber

Two nodal circle mode stepped circular vibrating plate, 60 mm in diameter, was driven by a bolted Langevin transducer (BLT) with a 113 mm long exponential horn to generate a frequency of 27 kHz, as shown in Fig.3 left. A half wavelength resonance rod, 93 mm long and 10 mm diameter, was connected to the horn and extended the length of the sound source. The vibrating plate was connected to the tip of the rod and both the plate and rod were set in a glass chamber (83.4 mm width and 170 mm depth as shown in Fig.3 center). A high intensity ultrasound was generated inside the chamber. Measuring the sound pressure distribution inside the chamber proved to be difficult, a numerical calculation of the sound pressure distribution inside the chamber was completed using COMSOL Multiphysics as shown in Fig.3 right. The results indicated that the distribution was extremely complicated due to the interaction of sound waves with the chamber wall, however, this was desirable for obtaining a homogeneous dispersion of particles.

Inside the chamber, the fungal spores were affected by the strong alternative oscillation and the particle acceleration of ultrasound as the square of angle frequency times the displacement also made rapidly expanding the dispersion. The particles were then suspended inside the chamber without any heat damage. This small chamber was important to produce the airborne microorganism in live with homogenous dispersion.

2.2 Fungal spores generating test

The following two experiments were conducted to generate fungal spores. The experiment set up are shown in Fig.3. A stepped circular vibrating plate was placed inside the diffusion chamber. High intensity ultrasound field was obtained inside the chamber at the frequency of 27 kHz. MIDDLE: It shows the dimension inside the chamber. There is a beaker, 56 mm diameter and 70 mm high, for the stage of dummy particles. RIGHT: It shows a sample of sound pressure distribution inside the chamber calculated by multi-physics simulation software, “COMSOL”. No data were obtained inside the beaker.
in Figs. 4 and 5. The small chamber test was conducted for checking the fungal spores generating characteristics, and a full scale chamber test was conducted for obtaining the size distribution of the generated fungal spores.

### 2.2.1 Small chamber test

High intensity ultrasound was irradiated to the fungal disk in the chamber for 5 min., and it was left unattended afterwards for 15 min.

According to Abe, the most readily spore-dispersing fungi were the *Penicillium* H15 and *Aspergillus flavus* IFO 6343 strains isolated from an air conditioner. In this study, *Penicillium* H15 was used as shown in Fig. 4 (A).

To disperse fungal spores using ultrasonic waves, disks with a diameter of 7 mm on which *Penicillium* H15 spores were cultured were prepared. The disk was placed in the center of acrylic box as shown in Fig. 4 (B). The capacity was 70 L. Ultrasonic wave was applied to the fungal disk for 5 min. in the acrylic box and then left standing for 15 min. This means that the fungal spores were scattered in a non-contact.

To confirm the fungal spores distribution caused by ultrasonic waves, 5 agar plates (DG18) were lined on the bottom face of box.

Samples were taken of naturally falling mold spores on five plates (one in the center, others in each corner of the chamber). This method of confirmation was taken because in the case of mold spores being dispersed uniformly in the air, similar colonies would be formed after cultivation on all the plates.

All agar plates were collected after the ultrasonic fungal spores dispersion test. *Penicillium* H15 was cultured at 25 °C for 14 d, and the course of fungal growth was observed. The number of *Penicillium* H15 colonies was homogeneous among the plates, showing no difference associated with the position in the acrylic box.

### 2.2.2 Full scale chamber test

To confirm the ultrasonic wave induced fungal spores dispersion, a simple clean booth made of unwoven nylon cloth was used as shown in Fig. 5.

At the time of a high air purifying rate within the chamber aerial ultrasonic waves were emitted. A negative and positive pressure switchable filter fan unit (Amenity Technology Co.) was connected to the acrylic box through a 50 mm diameter tube. For monitoring the presence of fungal spores in the simple clean room, time-course changes in the particle were measured by particle size using a laser particle counter, MOD-
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EL3886 (KANOMAX Japan Inc.).

The *Penicillium* sp. spores on the six DG18 agar plates are shown in Fig.6. The results indicated that a large quantity of mold spores were generated using the ultrasonic device and that these spores were distributed almost uniformly in the acrylic box. To confirm the pattern of fungal spores dispersion, plates (DG18 agar) were placed on the bottom face of the simple clean booth at the 4 corners (A, B, C, and D) and center (E and F), and *Penicillium*H15 that fell were collected from all plates. Similar colony formation was observed on all plates A-F.

For the measurement of airborne fungal spores, time-course changes in the number of airborne fungal spores were measured using a slit-type airborne bacteria autosampler (Autosampler BAS-1 model 1) as shown in Fig.7.

The sampling equipment was set in the center of the chamber. After the examination the whole equipment was moved out of the chamber and the plates were collected.

*Penicillium*H15 was continuously collected on one plate (DG18) exchanged every 5 min. as shown in Fig.8.

On the axis of abscissa in the graph, beyond 10-minute-sound wave irradiation is ‘0 minute,’ because the setting condition of Bio Auto Sampler is that after 5-minute-collection should be 5-minute-interval, with 10 minutes’ passage necessary before the next collection. 0th minute on the axis of abscissa means the sampling starts along with sound wave irradiation. The plot on 5th minute shows the result obtained by a five-minute-collection of airborne mold. Thus, plots are placed at 10-minute intervals. The plots on the axis of ordinates refer to the number of mold colonies in a cubic meter of air, which were obtained by counting the number of mold colonies bred in 28.3 liters of air for five minutes before conversion.

The collected airborne fungi were quantified by counting colonies after culturing *Penicillium*H15. Regarding *Penicillium*H15 collected using the slittype airborne bacteria autosampler, there were too many colonies to accurately count.

In the current experiment windless state was maintained within the chamber after the inside was completely cleaned. After that mold spores were dispersed, and the concentration changes of the mold spores within the chamber were examined. The more mold spores fall naturally, the fewer mold spores will stay in the air. This tendency is affected by the particle diameter of mold spores. Of the mold spores used in this study, floating spores decreased in number after the dispersion, after which the spores were considered to remain floating for approximately one hour. It was considered that the spores with relatively large diameters immediately after the dispersion fell down, whereas the spores with relatively small diameters remained in the air.

![Fig.8 Sedimentation of airborne *Penicillium*H15 after 10 min. of ultrasound radiated in the chamber.](image)

![Fig.9 Sedimentation of airborne particles without ultrasound.](image)

![Fig.10 Sedimentation of airborne particles after 5 min. of ultrasound radiated in the chamber.](image)
3. Results

The results of time-course changes in the particle count by particle size simultaneously measured using the laser particle counter are shown in Figs. 9 and 10.

The condition in the simple clean booth without ultrasonic wave induced fungal spores dispersion is shown in Fig. 9. Particles with a 0.3 μm particle size were cleared within about 20 min. when the pressure in the simple clean booth was switched to positive.

The number of airborne particles in the simple clean booth started to increase immediately after emitting ultrasonic waves, and the number of 0.3 μm particles continuously increased at 1 h as shown in Fig. 10. Constant changes were noted in particles with 0.5, 1.0, and 3.0 particle sizes. The number of 5.0 μm particles slightly decreased with time.

For the current experiment an equipment with HEPA filter-applied air purifying function was used in order to exclude as many fine particles within the chamber as possible. The highest rate of air purifying was shown 10 minutes after starting operation of this equipment. (At 10 minutes on the axis of abscissas operation of the air-purifying equipment starts.) At the time of a high air purifying rate within the chamber aerial ultrasonic waves were emitted.

Fig. 11 shows the distribution of counted colony forming unit plotted on the logarithmic diameter scale.

The size of the generated mold spores was distributed between 1.1 and 7 μm, and showed a lognormal distribution with a median diameter of approximately 4 μm. The size distribution of mold spores suspended in indoor environments has been reported to exhibit a lognormal distribution with a median diameter of approximately 4 μm.

4. Discussion

The purpose of the present study was to generate fungal spores for evaluating the performance of air cleaners to remove such particles; thus, polydisperse fungal spores were required. There are various methods for generating polydisperse aerosols, such as nebulization and the dispersion of powders, but these were developed for the purpose of generating aerosols and not necessarily bio-aerosols.

These methods are not suitable for generating a very small quantity of spores. For example, the concentration of output from dry dispersion generators ranges from milligrams per cubic meter to greater than 100 g/cm³, and the output of a nebulizer ranges from 1 to 1000 mg/min. [11, 12].

The results from the present study clearly indicated that the developed ultrasonic device was effective in generating not only aerosols (Figs. 1 and 2) but also fungal spores (Fig. 8). Moreover, experimental results using this ultrasonic generator in a full-scale chamber showed that the generated mold spores exhibit a lognormal distribution with a median diameter of approximately 4 μm as shown in Fig. 11.

Because the size distribution is in agreement with the size distribution of fungal spores present in general indoor environments, our method for developing and generating fungal spores is suitable for evaluating air cleaner performance.

5. Conclusion

In the present study, the method for generating fungal spores using dry dispersion and the ultrasonic device, capable of generating polydisperse fungal spores with a size distribution that approximates that of fungal spores in an indoor environment was discussed. Improved evaluation of air cleaner performance was possible using fungal spores generated by the ultrasonic device we developed.

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超音波による胞子分散法の検討

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概 要

室内環境に浮遊する微生物の除去を目的とした空気清浄装置および防止機器の除去性能評価の場合、空気中に微生物を均一に分散させる方法として細菌培養液をネブライザーで噴霧する方法が一般的な方法であった。また室内環境に浮遊するカビの胞子は細菌よりも粒子径が大きく自然落下しやすいため、再現性を得ることが困難であった。

本研究では空中超音波を応用し、飛散し易い真菌としてPenicillium属の菌株を使用し、一定量のカビの乾燥胞子を均一に飛散させる方法を検討した。実験方法は、カビ胞子の分散状態を確認するため簡易クリーンブース内に空中超音波を一定時間間発信させて、カビ胞子を導入し、簡易クリーンブース内のカビ胞子の粒度別粉塵数の経時的な変動をレーザーパーティクルカウンターを用い測定した。空中浮遊したカビ胞子の経時的変動の測定には、スリット式空中浮遊菌オートサンプラーや装置にてカビ胞子をDG18培地に捕集し、培養後のコロニー数を計数した。

さらに簡易クリーンブース内の四隅（A, B, C, D）および中央（E, F）の床面にプレート（DG18培地）を起き落下菌を採取し、直ちに培養し、カビ生育の経過を観察した。その結果、曝露チャンバーにおいてカビ胞子は、均一に分散していることを確認したことから、空気清浄装置および防止機器の除去性能評価法として有用であることが分かった。
Biographical Sketches of the Authors

Tetsuro Otsuka was born in Saitama, Japan on Jan. 26th, 1951 and received his B.S. and M.S. degrees in Electrical Engineering from the Department of Electrical Engineering, College of Industrial Technology, Nihon University, Japan, and Doctor degree from Nihon University.

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