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Design and Application of an Inertial Impactor in Combination with an ATP Bioluminescence Detector for In Situ Rapid Estimation of the Efficacies of Air Controlling Devices on Removal of Bioaerosols

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We proposed a rapid method to estimate the efficacies of air controlling devices in situ using ATP bioluminescence in combination with an inertial impactor. The inertial impactor was designed to have 1 μm of cutoff diameter, and its performance was estimated analytically, numerically, and experimentally. The proposed method was characterized using *Staphylococcus epidermidis*, which was aerosolized with a nebulizer. The bioaerosol concentrations were estimated within 25 min using the proposed method without a culturing process, which requires several days for colony formation. A linear relationship was obtained between the results of the proposed ATP method (RLU/m^3) and the conventional culture-based method (CFU/m^3), with R^2 0.9283. The proposed method was applied to estimate the concentration of indoor bioaerosols, which were identified as a mixture of various microbial species including bacteria, fungi, and actinomycetes, in an occupational indoor environment, controlled by mechanical ventilation and an air cleaner. Consequently, the proposed method showed a linearity with the culture-based method for indoor bioaerosols with R^2 0.8189, even though various kinds of microorganisms existed in the indoor air. The proposed method may be effective in monitoring the changes of relative concentration of indoor bioaerosols and estimating the effectiveness of air control devices in indoor environments.

1. Introduction

The monitoring of bioaerosols is essential for controlling air quality, assessing the exposure in health risk evaluation studies, identifying bioaerosol emission sources, and estimating the performance of air cleaning devices because exposure to bioaerosols can affect human health in variety of ways, including infectious diseases, acute toxic reactions, and allergies (*1*−*4*). Conventionally, a culture-based colony counting method is the most widely used analytical technique for the monitoring of airborne bacteria and fungi in both indoor and outdoor environments (*5*−*7*). However, the conventional culture-based method requires several days for colony formation and this time delay can be one of the most serious defects of the conventional method.

With the increasing concern on biological contamination of indoor environments, the efficacies of air controlling devices on the removal of bioaerosols need to be estimated rapidly in situ. Previous studies have reported the feasibility for rapid quantification or identification of bioaerosols using UVAPS (ultraviolet aerodynamic particle sizer spectrometer), ATOFMS (aerosol time-of-flight mass spectrometry), or Py-GC-IMS (pyrolysis-gas chromatography-ion mobility spectrometry) (*8*−*10*). Although these sophisticated instruments are effective in scientific analyses or for military purposes, they are not affordable for practical purposes, such as monitoring the indoor air quality, estimating performance of bioaerosol control devices, and so on.

Application of adenosine triphosphate (ATP) bioluminescence can be an available and affordable solution for the method for rapidly estimating the efficacy of air controlling devices on bioaerosol removal. An assay using ATP, which plays a central role as an intermediate carrier of chemical energy linking the catabolism and biosynthesis within microbial cells, has long been recognized as "the most convenient and reliable method for estimating the total microbial biomass in most environmental samples" (*11, 12*). ATP bioluminescence is based on a light generating reaction with luciferin and firefly luciferase. Since the light emitted from the reaction is proportional to the ATP concentration contained in the total biological contaminants, hygiene monitoring techniques using the ATP bioluminescence have been used to quickly access and monitor microbial contamination on surfaces, such as in food industries (*13*). Lee et al. (*14*) developed a microfluidic ATP bioluminescence sensor for detection of microbes. Seshadri et al. (*15*) applied the ATP bioluminescence method to characterize the performance of bioaerosol sampling devices. They showed that the collection efficiencies of samplers determined by the ATP method agreed well with direct counting method. They found that a majority of losses occurred in the inlet of the samplers. In most of the previous studies on the ATP bioluminescence, the experiments were performed using specific kinds of test microorganisms in laboratory conditions. Venkateswaran et al. (*16*) used the ATP as a biomarker of viable microorganisms in clean-room facilities, where various kinds of microorganisms coexisted. They concluded that the ATP method was superior to colony counting method due to its speed and its ability to report the presence of viable but noncultivable microorganisms.

In this study, a method to rapidly estimate the efficacy of air controlling devices on removal of bioaerosols was proposed by combining the ATP bioluminescence with an inertial impactor. In this method, the microbial concentrations collected on the impaction plate were measured using ATP device instead of the colony counting following incubation. Since an inertial impactor collects bioaerosols on its impaction plate, it may be possible to estimate the bioaerosol concentration rapidly by measuring the degree of microbiological contamination of an impaction plate using commercialized ATP devices, which have been designed for easy and rapid detection of biological contamination of a target surface. An inertial impactor was designed to have 1 μm of cutoff diameter and its performance was estimated by analytical, numerical, and experimental methods. The con-

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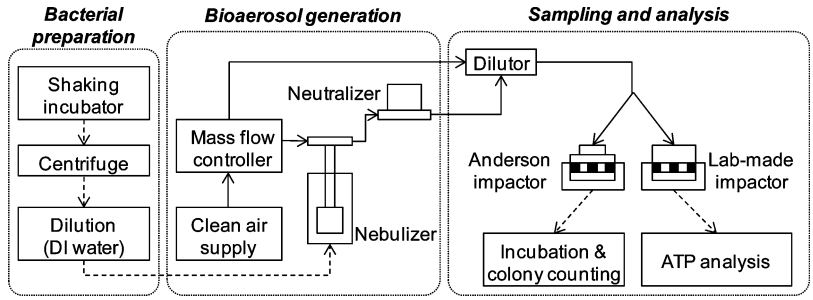

FIGURE 1. Experimental setup for laboratory tests.

centrations of bioaerosols sampled on the impaction plate were measured using a commercialized ATP detector. The cutoff diameter of 1 μm was selected since 1 μm is the lowest size used in the U.S. Government Joint Biological Point Detection System (*17, 18*). The proposed method was then applied to estimate the concentration of indoor bioaerosols, which were identified as a mixture of various microbial species including bacteria, fungi, and actinomycetes. The experiments were carried out in an occupational indoor, controlled by mechanical ventilation and an air cleaner.

2. Materials and Methods

2.1. Preparation of Test Bacterial Suspension. In this study, *Staphylococcus epidermidis*, which has been suggested as a suitable strain for testing the biological efficiency of air samplers according to the ISO 14698-1 (*19*), was used for the performance test. A bacterial suspension of *S. epidermidis* (ATCC 14990) was prepared by culturing 0.1 mL of an overnight culture inoculated in 15 mL of a nutrient broth for 18 h at 37 °C. The nutrient broth was prepared by dissolving 5 g of peptone and 3 g of meat extract in 1000 mL of sterile deionized water. The solution was sterilized in an autoclave. For bioaerosol generation, the prepared bacterial suspension was washed three times with sterile deionized water using a centrifuge (VS-1500N, Vision Scientific, Korea) at 6000 rpm (2697*g*) for 15 min to remove the residual particles, including the components of the nutrient broth.

2.2. Impactor Design and Performance Evaluation. An inertial impactor with a 1 μm of cutoff diameter was designed, fabricated, and its performance for bioaerosol collection was evaluated. The impactor was designed using a step-by-step design procedure (*20*) based on the following equation:

$$d_{50} = \sqrt{\frac{9\mu D \mathrm{Stk}_{50}}{\rho_p V C_c}} \tag{1}$$

where μ is the dynamic viscosity of the air, D is the nozzle diameter, Stk_{50} is the Stokes number at a 50% collection efficiency, ρ_p is the density of a particle, d_{50} is the cutoff diameter, V is the nozzle exit velocity, and C_c is the Cunningham correction factor.

The sampling performance of the designed impactor was estimated using the fractional collection efficiency, $\eta(d_p)$, as follows:

$$\eta(d_p) = 1 - \frac{C_{down}(d_p)}{C_{up}(d_p)} \tag{2}$$

where, C_{up} and C_{down} are the number concentrations of bioaerosols at upstream and downstream of the impactor, respectively. d_p is the equivalent particle diameter. For the experimental evaluation, the prepared bacterial suspension of *S. epidermidis* was dispersed into air using an atomizer (model 9302, TSI, U.S.). An aerodynamic particle sizer spectrometer (APS, model 3321, TSI, U.S.) was used to determine the number concentrations of test bioaerosol particles at upstream and downstream of the impactor. The aerodynamic diameter is the diameter of a standard-density (1 g/cm^3) sphere having the same gravitational settling velocity as the particle being measured.

For the analytical estimation, a simplified model was used as follows:

$$\eta(d_p) = \frac{\pi V}{2r}\left(\frac{\rho_p {d_p}^2 C_c}{18\mu}\right) \tag{3}$$

where r is the radius of curvature of the streamline, and μ is the viscosity of air at room temperature (*21*). r can be assumed to be the same as the radius of the intake nozzle (*22*).

Numerical simulation was also used to evaluate the designed impactor using the commercially available computational fluid dynamics (CFD) software, Fluent (ver. 6.3). After a mesh dependent study, the grid size was selected as 0.02 mm for the entire domain. It was assumed that the air flow was incompressible and steady. The flow field was solved to satisfy the continuity equation

$$\nabla \cdot V = 0 \tag{4}$$

and the Naiver-Stokes equation

$$\nabla \cdot (\rho VV) = -\nabla P + \nabla \cdot (\bar{\tau}) + F_e \tag{5}$$

where V is the gas velocity, ρ is the gas density, P is the static pressure, $\bar{\tau}$ is the stress tensor, and F_e is the external force per unit gas volume. To calculate particle motion in the flow field, the particle force balance was modeled using eq 6,

$$\frac{dV_p}{dt} = \frac{18\mu}{{d_p}^2 \rho_p C_c}(V - V_p) + \frac{g(\rho_p - \rho)}{\rho_p} \tag{6}$$

where V_p is the particle velocity.

2.3. Laboratory Test of Our Method. Figure 1 shows the experimental setup for lab-scale test. The prepared bacterial suspension of *S. epidermidis* was dispersed into air using an atomizer at a flow rate of 2 L/min and introduced into a dilutor. The bioaerosol concentration was controlled by varying the concentration of the bacterial suspension in the atomizer. The dispersed and diluted bioaerosols were sampled simultaneously using the lab-made impactor and an Anderson impactor (TE-10-800, Tisch Environmental, U.S.).

For the lab-made impactor, the impaction plate with area of 7.1 cm^2 was freshly covered with autoclaved aluminum foil to prevent precontamination. The sampling flow rate was 30 L/min and the sampling times were 5, 10, and 20 min. After sampling, the level of biological contamination of the aluminum foil was measured using an ATP detector consisting of a swab stick (LuciPac W, Kikkoman, Japan) and measuring instrument (Lumitester PD10-N, Kikkoman, Japan). The entire surface of the contaminated aluminum foil

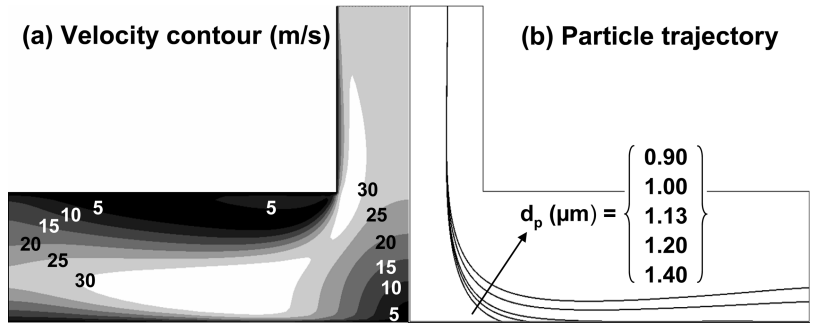

FIGURE 2. Velocity contour and particle trajectory resulting from a numerical estimation.

was wiped with a swab after wetting the surface with 0.1 mL of autoclaved deionized water. The swab was then placed into the swab stick, which contained an ATP releasing reagent and a luminescence reagent for the light-generating reaction. The swab stick was shaken several times and inserted into the measuring instrument. The instrument displayed the intensity of light generated from the reaction between ATP and the luminescence reagent as the relative luminescent units (RLU). The measurable range of the ATP detector was 0−999 999 RLU and 1 RLU corresponded to approximately 10^{-12} mol of ATP. For control check, the surface of autoclaved aluminum foil was measured using ATP detector without sampling of bioaerosols. The result was below 5 RLU and this was negligible.

The fifth stage of an Anderson impactor, with a cutoff diameter of 1 μm was used for the conventional culture-based colony counting method. A nutrient agar plate was used as the impaction plate. The sampling flow rate, sampling time, incubation temperature, and incubation time were 28.3 L/min, 5 min, 30 °C, and 7 days, respectively. After incubation, the colony forming units (CFU) were counted.

2.4. Estimation of the Efficacies of Air Controlling Devices on Removal of Ambient Bioaerosols. The proposed method evaluated by the lab-scale test was applied to estimate the efficacy of mechanical ventilation and an air cleaner for removal of bioaerosols at an occupational office room located in Seoul, Korea. The volume of the test room was approximately 200 m^3. Three different indoor conditions were selected for the model indoor conditions. Under condition (A), no air controlling measures were in operation. The indoor air conditions for (B) and (C) were controlled using a mechanical ventilation system installed in the building. For condition (C), an air cleaner with a high efficiency particulate air (HEPA) filter, which was operated independently from the ventilation system, was operated in addition to condition (B), both before and during the bioaerosol sampling. Three, eight, and five samples were collected from the conditions A, B, and C, respectively. Before each sampling, the air control devices were operated for more than 3 h. The room was occupied by only two persons for experiments during the sampling. As in the laboratory test, the lab-made impactor and the Anderson impactor were used simultaneously. Sampling flow rates and sampling times were same as those of the laboratory test. All samplings were repeated three times.

From the collected microbial samples which were mixtures of various kinds of microorganisms, microbial colonies were first classified according to their shapes and then isolated from the original samples. Trypticase-soy agar, malt extract agar, and half-strength nutrient agar were used for the pure cultures of bacteria, fungi, and actinomycetes, respectively. The isolated microbial samples were identified, all by the Korean Culture Center of Microorganisms. The bacterial isolates were gram-stained and identified using an API kit (bioMérieux Co.) and the BIOLOG Microstation System. The cellular fatty acid compositions of the fungi and actinomycetes were analyzed using a gas chromatography

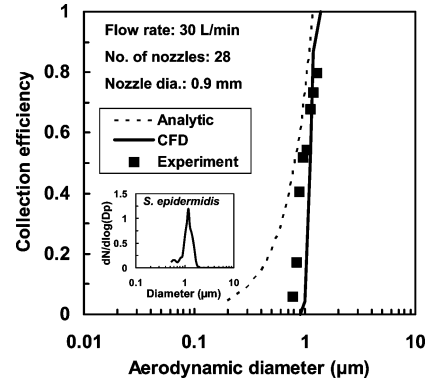

FIGURE 3. Design parameters and collection efficiency curve of the impactor.

(GC, 6890 series, Agilent, U.S.). The retention time of each of the peaks was compared with that of standard samples.

3. Results and Discussion

Figure 2(a) shows the air velocity contour obtained by solving the eqs 4 and 5. In this flow field, the particle motion was governed by eq 6 and the results are shown by Figure 2(b) as a function of particle diameter. Particles larger than 1.13 μm were mainly trapped on the impaction plate whereas smaller ones escaped without impaction.

Figure 3 shows the design parameters and collection efficiency curve of the impactor. The cutoff diameters were 0.82 1.13, and 0.98 μm, resulting from the analytical, numerical and experimental estimations, respectively. The inset of Figure 3 shows the size distribution of the *S. epidermidis* bioaerosols used for the performance test of the impactor. The mode diameter of the *S. epidermidis* bioaerosols was 1.2 μm.

Figure 4(a) shows the RLU values obtained from three sampling events in the performance tests of the proposed method when the concentrations of *S. epidermidis* bioaerosols were 11555, 6944, and 2951 CFU/m^3. The RLU values were shown as a function of the sampling volume, which is the product of the sampling time (5, 10, or 20 min) and sampling flow rate (30 L/min). The RLU values increased linearly with increasing sampling volume. Under the condition of a constant bioaerosol concentration, the number of microorganisms collected was proportional to the sampling volume.

Figure 4(b) shows the correlation between the concentrations of *S. epidermidis* bioaerosol measured using the proposed and culture-based methods in the laboratory test. The bioaerosol concentrations measured using the culture-based method ranged from 2354 to 25300 CFU/m^3. The corresponding concentrations using the proposed method were 41 and 844 RLU/m^3, respectively. Nine data points between CFU/m^3 and RLU/m^3 were obtained and showed a linear relation with R^2 0.9283. The bioaerosol concentration

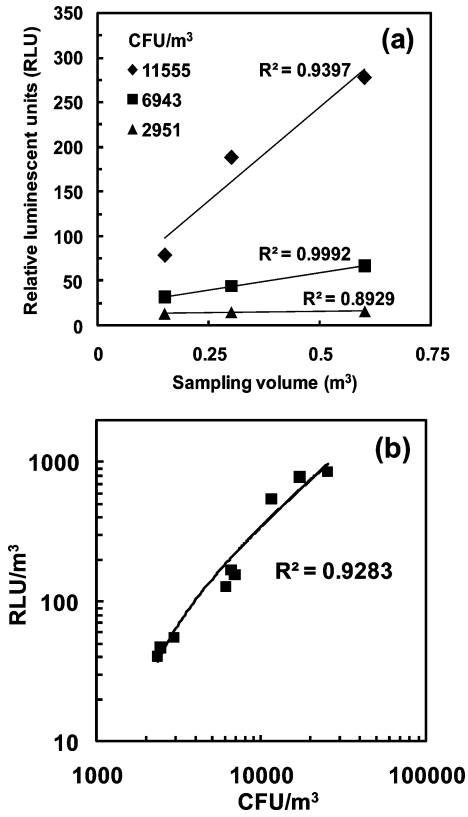

FIGURE 4. (a) Relative luminescent units (RLU) values as a function of sampling volume and (b) correlation between the concentrations of *S. epidermidis* bioaerosol measured using ATP (RLU/m³) and culture-based (CFU/m³) methods.

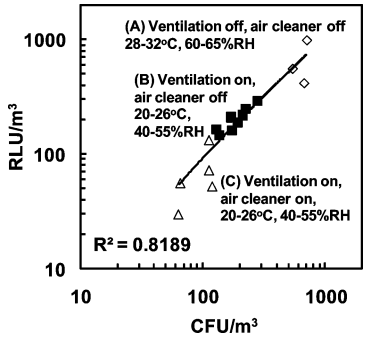

FIGURE 5. Correlation between the concentrations of indoor bioaerosol measured using ATP (RLU/m³) and culture-based (CFU/m³) methods in the indoor environments of (a) ventilation off, air cleaner off, (b) ventilation on, air cleaner off, and (c) ventilation on, air cleaner on.

(in terms of RLU/m³) was calculated by dividing the RLU values by the sampling volumes and averaging them. From the results of our laboratory test, 1 RLU corresponded to 27.5 CFU of aerosolized *S. epidermidis* cells.

Figure 5 shows the correlation between the concentrations of indoor bioaerosols measured using the proposed ATP and conventional culture-based methods under various indoor conditions controlled by mechanical ventilation and an air cleaner. Without any air controlling devices (condition A), the bioaerosol concentrations from the proposed and conventional methods were, on average, 651 RLU/m³ and 637 CFU/m³, respectively. When ventilation was utilized (condition B), the bioaerosol concentrations of both methods were changed to 204 RLU/m³ and 190 CFU/m³ on average, respectively. With the operation of an air-cleaner in addition to the ventilation (condition C), the bioaerosol concentrations were reduced to 68 RLU/m³ and 95 CFU/m³ on average. The

TABLE 1. Microorganisms Identified from the Test Room

	species identified
bacteria	*Corynebacterium striatum, Staphylococcus lugdunensis, Staphylococcus epidermidis, Sphingomonas paucimobilis, Bacillus mycoides, Bacillus pumilus, Rhodococcus* spp., *Micrococcus* spp.
fngi	*Aspergillus niger, Aspergillus fumigatus, Acremonium* spp., *Exophiala jeanselmei, Penicillium* spp.
atinomycetes	*Nocardiopsis dassonvillei, Streptomyces lavendulae, Streptomyces halstedii*

experimental results of condition C showed a little divergence from the linear relationship. In condition C, the relatively clean indoor environment controlled by a ventilator and an air cleaner, colonies formed on a plate were lower than 20 CFU and this low number of colonies could be a reason for this divergence. Consequently, the proposed method showed a linearity with the conventional culture-based method for indoor bioaerosols with R^2 0.8189, even though various microorganisms existed in the test room (Table 1).

The averaged efficacies of air controlling devices estimated by conventional (E_{CFU}) and proposed (E_{RLU}) methods were defined as follows respectively.

$$E_{CFU,(B)or(C)} = 1 - [CFU/m^3at(B)or(C)]/[CFU/m^3at(A)] \quad (7)$$

$$E_{RLU,(B)or(C)} = 1 - [RLU/m^3at(B)or(C)]/[RLU/m^3at(A)] \quad (8)$$

E_{CFU} and E_{RLU} at condition (B), controlled by ventilation, were 0.70 and 0.69, respectively. When the air cleaner was used in addition to the ventilation as in condition (C), E_{CFU} and E_{RLU} were 0.85 and 0.90, on average, respectively. Using the proposed ATP method combined with an inertial impactor, the efficacy of mechanical ventilation and an air cleaner on the removal of bioaerosols was determined within 25 min (including sampling time), whereas several days were required for colony formation using the conventional culture-based method.

The average ratios of the bioaerosol concentrations obtained from the proposed method (RLU/m³) to those obtained from the culture-based method (CFU/m³) were 0.0277 and 0.9547 for the laboratory and field test, respectively. This difference was attributed to the different ATP contents of the various microorganisms. In the case of the field test, there were various kinds of bacteria, and also fungi, which generally contain more ATP. For example, *Staphylococcus aureus* (bacteria, 0.5−1.5 μm) contains 3.1 fM of ATP, whereas *Candida tropicalis* (fungus, 4.0−9.0 μm) contains 90 fM (*23, 24*). Venkateswaran et al. (*16*) reported that fungi contained more ATP than bacteria through a comparison of the intracellular ATP contents and colony counts of 130 microorganisms. They hypothesized that the type of microorganisms that might be present can be indicated from the ratio of the ATP contents (RLU) to the colony counts (CFU). For example, a sample might be bacteria-dominant if it shows a low RLU/CFU. On the other hand, if a sample has a high ratio of RLU/CFU, it may be largely composed of fungi. The other plausible reason for the difference between average ratios of RLU/m³ to CFU/m³ is the existence of VBNC (viable but noncultivatable) microorganisms in indoor air. They can contribute to the RLU data but not the CFU data because they do not form colonies on agar plates even though they have ATP for catabolism and biosynthesis.

In conclusion, we proposed a rapid method to estimate the efficacies of air controlling devices in situ using ATP bioluminescence in combination with an inertial impactor. Even though it was tested with only one microorganism in the laboratory test and one indoor facility, the proposed ATP method showed the possibility for rapid estimation of indoor bioaerosols within 25 min (including sampling time) under various indoor conditions controlled by mechanical ventilation and an air cleaner, showing a linearity with the conventional culture-based method (R^2 0.9283 and 0.8189 for laboratory and field tests, respectively). This method may be effective in monitoring the changes of relative concentration of indoor bioaerosols and comparing the effectiveness of various air control devices in an indoor environment. Even though the current version of our method includes manual works, such as a swabbing process, our further studies are focused on developing an advanced system which eliminates the manual works to apply our method for automatic indoor air control system.

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