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Life Comes from the Air: A Short Review on Bioaerosol Control

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ABSTRACT

Air is filled with numerous tiny organisms, with sizes ranging from 50 nm to 10 μm. These organisms are called airborne biological particles or bioaerosols. In the human history of investigating the origin of life and fighting against contagious diseases, the recognition of bioaerosols and the development of control methods against them have played crucial roles. The pandemic outbreak of flu due to the influenza A H1N1 virus in 2009 and the bio-terror incidents in 2001 have alerted us to the importance of bioaerosol research. Here, control methods against bioaerosols are briefly reviewed, and suggestions are offered for future research on airborne biological particles.

Keywords: Thermal energy; Ultraviolet germicidal irradiation; Silver nanoparticle; Ion; Hybrid filtration.

INTRODUCTION

Bioaerosols are defined as airborne particles of biological origin. Bioaerosols include airborne bacteria, viruses, fungi, and other biological fragments such as airborne DNA fragments. Bioaerosols were thought to exist in only minute concentrations in atmospheric aerosols. However, Jaenicke (2005) showed that the portion of bioaerosols constituting ambient aerosols ranges from 5% to 80%. Ariya and Amyot (2004) suggested that bioaerosols play roles in atmospheric chemistry and physics by altering the chemistry of the atmosphere via microbiological degradation, modifying the chemical composition of other organic compounds upon collision or contact, and driving the chemistry at environmental interfaces such as the air/snow interface.

With this increased awareness of the roles of bioaerosols in atmospheric components, bioaerosols were originally notable due to their effects on health. Bioaerosols have characteristics as biological materials, such as bacteria, fungi, viruses, plant debris, and so on. Therefore, all diseases associated with tiny (the size range: 50 nm to 10 μm) biological materials and microorganisms can also be related to bioaerosols in the cases where such materials are transported through the air. Bioaerosols enter the human respiratory tract via inhalation and contact the skin from air. The three main mechanisms of disease due to bioaerosols are hypersensitivity (allergy), toxic reactions, and infection. Review papers and reports support a relationship between bioaerosols and the occurrence of human diseases (Douwes *et al*., 2003; WHO, 2009). Fungal bioaerosols are known to causes allergies (Bush and Portnoy, 2001), and they are of particular concern to immuno-compromised patients in health-care facilities (Denning *et al*., 1997). The endotoxin of bacterial bioaerosols has been recognized as an important factor in the aetiology of occupational lung diseases including (non-allergic) asthma (Douwes and Heederik, 1997). Bioaerosols were abruptly brought to the public's attention due to the bioterror incidents of 2001. Thousands of Americans were suddenly exposed to airborne *Bacillus anthracis* spores, which raised international concerns about the seriousness of the intentional release of pathogenic bioaerosols. The pandemic outbreak of flu due to the influenza A H1N1 virus also raised awareness of bioaerosols in 2009.

Many methods are being developed to control bioaerosols in air. Each method has advantages and weaknesses regarding their economic requirements and environmental impact. Here, bioaerosol control methods are briefly reviewed, and future technologies for bioaerosol control research are discussed.

FIRST EXPERIMENT ON BIOAEROSOL CONTROL

The first study on bioaerosol control methods coincided with the beginning of modern biology. To prove the so-called 'all-cells-from-cells' hypothesis, which is the great foundation of classical biology, Louis Pasteur (1822–1895) conducted an experiment using a swan-necked flask (a Pasteur flask). In the middle of the 19th century, most biologists believed that organisms arose spontaneously under certain conditions, which was called the hypothesis of spontaneous generation.

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To investigate this, Pasteur placed nutrient broth in the swan-neck flask and then boiled it for sterilization. Even though the nutrient broth was exposed to air, no cell could be found in the swan-neck flask. This experiment proved that the 'all-cells-from-cells' hypothesis was correct (Duclaux, 1920; Madigan and Martinko, 2006). Further, the neck of the swan-neck flask was a good example of a bioaerosol control method. The up-and-down shape of the tube allowed the transmission of gas while simultaneously preventing the passage of aerosol-type microorganisms based on the basic principles of aerosol dynamics, which included impaction and gravitational sedimentation. Thus, Louis Pasteur used bioaerosol control methods to start modern biology which was not "spontaneous generation" but "life comes from the air (in the experiment, all cells came from cells in the air)."

CURRENT REASONS FOR DEVELOPING CONTROL METHODS

To prevent or reduce adverse health effects, bioaerosols must be detected as soon as possible and then controlled immediately thereafter (inactivation, removal, or collection at a specific location). Regarding detection techniques, much research has been conducted on bioaerosol samplers, basically in response to military demands or environmental monitoring (Griffiths and DeCosemo, 1994; Henningson and Ahlberg, 1994; Seshadri *et al*., 2009; Thompson *et al*., 1994). In order to decrease the lag-time of bioaerosol detection, real-time detection methods are under development. One representative method is ultraviolet aerodynamic particle sizer (UVAPS) spectrometry (Hairston *et al*., 1997; Ho *et al*., 1999). Living cells from most organisms exhibit natural autofluorescence due to biochemical fluorophores (e.g., nicotinamide-adenine dinucleotide (NADH), nicotinamide-adenine dinucleotide phosphate (NADPH), and riboflavin). Fluorescent signals from viable bioaerosols are obtained by exciting passing aerosol particles with a UV laser beam at a wavelength of 355 nm in real time, followed by the detection of fluorescence from 420 nm to 575 nm by the UVAPS system. However, the proper response to bioaerosols after their detection is still controversial. In the military field, soldiers can wear individual protective respirators. However, there are still questions regarding the inside of public buildings, if these buildings happen to be filled with pathogenic bioaerosols. Further, concerns remain on how to prevent the airborne transmission of contagious diseases in public facilities such as underground subway systems, which millions of people use everyday. For this purpose, we need to consider bioaerosol control methods.

DIFFERENCE BETWEEN AEROSOL CONTROL AND BIOAEROSOL CONTROL

Bioaerosols, as one type of aerosol particles, are removed whenever aerosol particles as a whole are removed or captured. Therefore, the methodologies of aerosol control also can be used to control bioaerosols. Many aerosol control methods such as filtration, electrostatic precipitation, and impaction had been developed (Hinds, 1999), and these methods are widely used for controlling the air quality in buildings including public facilities and residential premises. However, there are differences between aerosols and bioaerosols. Bioaerosols have biological characteristics, which mean that they can grow and produce offspring even after they are captured by conventional aerosol control methods. Bioaerosols cause secondary problems such as generating rank odors and dispersing pathogenic spores after they are captured; therefore, additional treatment methods are necessary for biological aerosol particles.

THERMAL ENERGY FOR CONTROLLING BIOAEROSOLS

Whereas thermal energy was first used in the experiment on bioaerosol control by Louis Pasteur about 150 years ago, its usage in controlling bioaerosols is currently restricted due to concerns over energy consumption. There are two types of thermal treatment for bioaerosols, moist heat and dry heat. Moist heat utilizes steam under pressure while dry heat is based on high temperature without moisture. While moist heat has been widely used to sterilize experimental devices in an aqueous environment, using moist heat or dry heat to control bioaerosols in a real air environment is very rare. Thermal treatment of bioaerosols with an electric heating coil has advantages in terms of easy installation in the existing systems of buildings and low production of byproducts. As such, several groups of researchers have studied the potential applicability of thermal energy (Grinshpun *et al*., 2010a; Jung *et al*., 2009; Lee and Lee, 2006). In contrast with typical microorganisms in water (that should be exposed to moist heat at 121°C for 15 minutes for inactivation), airborne bacteria are inactivated by sub-second exposure to temperatures of 100–140°C, which may suggest a solution to many of the economic concerns relating to thermal energy usage (Lee and Lee, 2006). Short (~1 second), high-temperature treatment of fungal bioaerosols (Jung *et al*., 2009) has demonstrated that thermal exposure decreases the size of fungal bioaerosols. Further, the process reduced the amount of (1→3)-β-*D*-glucan (a key agent in bioaerosol-induced inflammatory responses), the concentration of fungal bioaerosols, and the number of culturable bioaerosols (Jung *et al*., 2009). In that study, (1→3)-β-*D*-glucan was measured by using the kinetic chromogenic *Limulus Amebocyte* Lysate (LAL) method (Glucatell®, Associates of Cape Cod, East Falmouth, MA, USA), the particle number concentration of fungal bioaerosols was measured by a condensation particle counter (CPC 4330, HCT Inc., South Korea), and the number of culturable bioaerosols was measured by a BioSampler® (SKC Inc., PA, USA) and cultivation technique (Jung *et al*., 2009). Therefore, thermal energy can control the physical, chemical, and biological characteristics of bioaerosols. Short-term thermal treatment of bioaerosols also can be directly related to biodefense/counterterrorism via, for example, the explosion of a reservoir containing hazardous microorganisms. High-temperature exposure at 400°C via simulated combustive explosion was found to inactivate more than 99.99% of *B. subtilis* spore bioaerosols (Grinshpun

et al, 2010a; Grinshpun *et al.*, 2010b). High-temperature exposure is known to cause the denaturation of proteins by breaking the structures of polypeptides and results in damage to microorganisms (Madigan and Martinko, 2006). However, the difference in exposure conditions that results in the same degree of inactivation of both bioaerosols and waterborne microorganisms still is not fully understood, and may be caused by different inactivation mechanisms. Further exploration of the inactivation mechanisms of bioaerosols due to thermal energy is necessary.

ULTRAVIOLET GERMICIDAL IRRADIATION AND PHOTOCATALYSIS

Ultraviolet (UV) irradiation is a widely used method for controlling bioaerosols in indoor environments. The purine and pyrimidine bases of DNA (deoxyribonucleic acid) strongly absorb UV irradiation that is between 220 and 300 nm in wavelength, resulting in modifications or breaks. This sometimes leads to disruption of the genetic function and the death of the exposed organisms (Madigan and Martinko, 2006). UV irradiation was previously found to have a germicidal effect, which has led many researchers to examine the effects of UV irradiation on the viability of bioaerosols via control of the irradiation dose, the air movement patterns, room configurations, and ambient moisture condition (Beggs *et al*., 2006; Kujundzic *et al*., 2006; Lin and Li, 2002; Peccia *et al*., 2004; Xu *et al*., 2003). One study demonstrated a 12-fold reduction in bacterial colony formation in an operating room upon exposure to a high level of UVGI at 290 μW/cm^2 (Lidwell, 1994). For 99% inactivation of bioaerosols, the necessary UVGI dosages were reported as follows: 1,017 to 2,356 μW sec/cm^2 for *E. coli*; 15,949 to 19,345 μW sec/cm^2 for *B. subtilis*; 12,917 to 17,497 μW sec/cm^2 for yeast; and 47,984 to 89,419 μW sec/cm^2 for *P. citrinum* (Lin and Li, 2002). A UVGI dosage of 289 to 860 μW sec/cm^2 was required to produce a 5 log decrease in the concentration of *Legionella pneumophila* bioaerosols (Li *et al*., 2003). The rate of inactivation due to UVGI divided by the average UV fluence rate for *A. versicolor* fungal bioaerosols was $1.2 \pm 0.4 \times 10^{-4}$ cm^2/μJ (Kujundzic *et al*., 2007). This could be interpreted that for a 2 log decrease in the concentration of *A. versicolor* fungal bioaerosols, an estimated UVGI dose of 1.6×10^4 μW sec/cm^2 would be required. Overall, it has been found that higher doses of UVGI are required to inactive fungal than vegetative bacterial bioaerosols. UV irradiation has been found to consume little energy compared with thermal treatment, and can be simply applied by installing and turning on a UV lamp. In the ceilings of surgery rooms of hospitals and health care facilities, UV lamps are often installed and function to inactivate nearby bioaerosols (Kujundzic *et al*., 2006).

UV irradiation is also used with TiO_2 particles and causes photo-catalytic antimicrobial reactions. TiO_2 photocatalysis induced by UV irradiation generates strong hydroxyl radicals ($\cdot OH$), superoxide ions ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2), which are capable of inactivating waterborne and airborne microorganisms (Ireland *et al*., 1993; Lin and Li, 2003; Foster *et al.*, 2011; Markowska-Szczupak *et al.*, 2011). It was reported that the penetration efficiency of *Legionella pneumophila* through photocatalytic filters with UV irradiation was more than four times smaller than that through normal filters (Li *et al*., 2003). In a recent test, three 18 W fluorescent visible white-light lamps with a TiO_2 coating of 5.9 g could reduce 9–84% of culturable bacteria bioaerosols and 3–74% of culturable fungal bioaerosols in a 2 m × 2 m × 2 m chamber within 30–480 min of irradiation (Chuaybamroong *et al*., 2011). The effectiveness of photo-catalysis depends greatly on the concentration of oxidant radicals, which is a function of the reaction range of UV irradiation, the amount of TiO_2 particles, and the operating time. More than 400 publications on photocatalytic reactions regarding microorganisms and biological particles including prions and cancer cells were recently reviewed by two groups (Foster *et al*., 2011; Markowska-Szczupak *et al*., 2011). The groups of researchers concluded that although it is necessary to develop a standard method for testing the antimicrobial efficiency of photocatalytic processes and comparing them, TiO_2 photocatalysis with UV is a powerful tool against pathogenic microorganisms in water, surface, and air.

However, as to UV irradiation, there are concerns over the production of unnecessary pollutant ozone due to UV irradiation (Hwang *et al*., 2009 and 2010). Also, the exposure of human skin and eyes to UV irradiation results in UV-related keratoconjunctivitis and skin erythema, which are other recognized safety concerns regarding the use of UV irradiation (Longstreth *et al*., 1995; Yen *et al.*, 2004; Nardell *et al*., 2008). Besides the side-effects associated with UV irradiation, there are concerns regarding UV-related photocatalysis, with ongoing studies assessing the safety of human exposure and living- organism exposure to TiO_2 nanoparticles, which should be resolved prior to the substantial use of UV-related photocatalytic systems (Liao *et al*., 2009; Stratmeyer *et al*., 2010).

ION EMISSION

An air ion emission technique was developed to remove airborne particles from indoor air environments. The artificial emission of air ions (ion density of 10^5–10^6 $e^{\pm}$ cm^{-3}) for 30 minutes results in the removal of 97% of 0.1 μm particles and 95% of 1 μm particles from indoor air in addition to the natural decay effect (naturally occurring decrease in aerosol concentration due to gravity and diffusion) (Lee *et al*., 2004b). As the physical behavior of biological aerosol particles is similar to that of non-biological aerosol particles, the removal of aerosols by ion emission will result in bioaerosols being transferred from the air to the ground, walls, and ceiling.

In addition to this physical control of bioaerosols, it has been suggested that air ion emission has biocidal effects. Exposure to air ions for 15 minutes results in the inactivation of more than 80% of microorganisms on agar plates (Fletcher *et al*., 2007). Various studies have reported that air ions inhibit the growth of bacterial and fungal species (Shargawi *et al*., 1999; Noyce and Hughes, 2002; Kerr *et al*., 2006). The biocidal mechanisms of air ions still need to be elucidated. Further, there are few data on the effects of air ions on

bioaerosols (not static microorganisms on agar plates, but airborne microorganisms). Fletcher *et al*. (2007) suggested that air ions demonstrate bactericidal effects through the electro-poration of bacteria in addition to ozone exposure. Kim *et al.* (2011) suggested that electro-poration plays a primary role in the antibacterial effects of air ions. Currently, it is estimated that the attachment to and accumulation of air ions on the surfaces of airborne microorganisms induce distortion of the nearby electric fields of cell walls, which would then disrupt the transport of electrons and protons inside microorganisms. More experimental data regarding the effects of air ions on bioaerosols are required and need to be accumulated in order to elucidate the mechanisms. Furthermore, it is necessary to study several side-effects of ion emission. One major side-effect is that the continuous emission of unipolar air ions (unlike bi-polar ions) into an enclosed environment leads to charge accumulation on insulating surfaces, which may cause occasional electrostatic discharges or other "static"-related problems, especially at low humidity levels (Lee *et al*., 2004b). Another major side-effect is that bioaerosols deposited on the ceiling and walls may grow and emit additional bioaerosols into the air. Therefore, for the wide usage of ion emissions, it is necessary to study certain treatments of indoor surfaces where unipolar ions and bioaerosols are deposited.

FILTRATION OF BIOAEROSOLS

Filtration of air is the most widely used method for capturing aerosols in indoor air environments. Airborne particles are physically captured by filters and then removed from the surrounding air environment. However, biological aerosols that are captured by filters remain viable, and some of them even grow on the filters by absorbing air moisture and nutrients in dust. Such bioaerosols residing on filters generate rank odors and could be re-suspended in the air due to reverse airflow caused by a temporary reversal of the surrounding pressure or breakdown and maintenance of the filters. Therefore, there is a demand for the development of new filter materials that are capable of addressing these problems regarding deposited bioaerosols. Filters treated with anti-microbial components such as iodine (Lee *et al*., 2008b; Eninger *et al*., 2008) and membrane-breaking enzymes have been developed and tested as anti-microbial filters. However, when non-biological dust is deposited on the surfaces of filters during operation, the attached anti-microbial materials will be instantly covered by dust and their effects nullified. Therefore, in general, anti-microbial filters have short effective times due to the accumulation of non-biological dust from the air. Therefore, recent developments include hybrid methods that combine several different bioaerosol control mechanisms.

HYBRID FILTRATION AND INTEGRATION OF BIOAEROSOL CONTROL METHODS INTO A SINGLE SYSTEM

As more filtration methods become available, new treatment methods for bioaerosols deposited on filters as well as anti-microbial filtering materials have been developed and tested. Airborne silver nanoparticles have been tested as a new control method for bioaerosols that are deposited on filters (Lee *et al*., 2008a and 2010). The researchers artificially generated and launched airborne silver nanoparticles onto filters where bioaerosols were already deposited. In a 9 minute exposure test, airborne silver nanoparticles were effective in lowering the viability of the deposited bioaerosols. In particular, more than 99% of deposited bacterial bioaerosols were inactivated by the airborne silver nanoparticles (Lee *et al*., 2008a). The death rate due to airborne silver nanoparticles increased under low relative humidity conditions and upon exposure to a high number of silver nanoparticles (Lee *et al*., 2010).

Another developing treatment for aerosols and bioaerosols attached to filters is artificial ion emission. Artificial ion emission treatment of filters was found to increase the filtration efficiency of aerosol particles and decrease bioaerosol activity on filters (Lee *et al*., 2004a; Lee *et al*., 2005; Huang *et al*., 2008; Park *et al*., 2009). However, artificial ion emission can result in unnecessary ozone production as well as accumulation of electric charges and electric shock in nearby environments. Therefore, additional research needs to be conducted on the biocidal effects of ion emission on bioaerosols that are deposited on filters.

Additionally, a filter entangled with tiny wires has been tested for the inactivation of bioaerosols on a filter. As electric current passes through the tiny wire, a high-temperature environment is generated that exposes the nearby bioaerosols to high amounts of thermal energy. Without scientific, numerical approaches such as quantitative bioaerosol inactivation testing, several ideas such as UV irradiation of filter surfaces have been developed and installed in public air ventilation systems such as subway air ventilation systems. More studies using quantitative bioaerosol inactivation testing with aerosol technologies are necessary to further establish these hybrid filtration methods.

There are also approaches for the control of bioaerosols in indoor air environments that rely on integrating different control mechanisms into one facility. Hwang *et al.* (2010) reported the integration of thermal energy and UV irradiation into a single method. In his technique, thermal energy stimuli systems were located inside the passage of air flow while UV irradiation was located outside the passage. Therefore, bioaerosols were simultaneously exposed to thermal energy stimuli and UV irradiation while they passed through the control system. The authors concluded that the hybrid method compensates for the respective weaknesses of the constituent methods, including relatively high power consumption for thermal energy and high production of ozone byproducts under UV irradiation while maintaining the same or even higher effectiveness against bacterial bioaerosols.

There is a great need for the development of this type of technique that integrates individual control methods. Combining together methods such as ion emission, thermal energy, UV irradiation, and hybrid filtration using silver particles is possible, and would increase the effectiveness of controlling bioaerosols while mitigating the drawbacks of individual methods.

APPLICATION OF BIOAEROSOL CONTROL TECHNOLOGY TO BIOLOGY EXPERIMENTS

Bioaerosol research currently focuses mostly on the monitoring and control of ambient bioaerosols. However, bioaerosol control methods have the potential to be applied in basic biology experiments. Thus far, we believe that life originated a long time ago from water, and most of our biological experiments have been conducted in aqueous environments. A relatively limited number of applications of aerosol-based methods are available in biology experiments. If materials become airborne, all their surfaces are exposed to gas. Therefore, regarding gas exchange, aerosol processes have advantages compared to other plate-based or solution-based techniques. Electrospray mass spectrometry is a representative example of how bioaerosol control technology has increased the efficiency of pure biology experiments (Fenn *et al*., 1989). Over the past decades, there have been enormous developments in aerosol control technology and nanoparticle manipulation, such as particle charging, generation, and classification. The accuracy of the control of aerosol particles has become very high compared to the control of liquid-borne particles (hydrosol). Additionally, the time scale for the detection of aerosol particles has almost become real-time. These new developments in aerosol control methods can shed light on exploration to life science. One example is an investigation featuring a combination of an electrospray bioaerosol control method and ultraviolet aerosol particle spectrometry (ES-UVAPS) for the real-time detection of microorganisms in liquid (Jung *et al*., 2010). Aerosol control and analysis for the real-time detection of bioaerosols has been successfully applied to the detection of liquid-borne microorganisms. Another interesting example involved the patterning of many individual bacterial cells onto a semiconductor wafer, at a resolution of 10 μm, in a drop-on-demand manner; at this resolution, a single cell per pattern was obtained using an electrospray bioaerosol control method (Kim *et al*., 2008, 2010). This new method can be applied to investigations using isolated single cells.

CONCLUSIONS

Every day, even right now, we breathe in bioaerosols through our noses. Our respiratory tract controls the entering bioaerosols. As humans, along with all living organisms, we have survived by evolving to achieve effective controls of pathogenic bioaerosols. The future evolutional pathway of experimental methods in life science may follow that evolutional pathway of living organisms via the improvements of bioaerosol control methods.

Bioaerosol control methods have been developed mainly due to environmental health issues, and the technologies will be applied further to various sciences, including pure biological analysis.

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