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Progress on quantitative assessment methods of biological aerosols in the atmosphere

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Abstract

Biological aerosol is one of the most important pollutants in the air, not only influencing public health, and air quality, but also playing an important role in climate change and both chemical and physical processing regulations in the atmosphere. Therefore, the quantification of biological aerosols in the air needs to be more accurate. This article reviews the progress on quantitative assessment methods of biological aerosols in the atmosphere as well as those related merits that each method used.

1 Introduction

Biological aerosols are ubiquitous in the atmosphere and existing in different sizes. Described by IGAP-workshop in Geneva in June1993 as follows: airborne solid particles (dead or alive) are or were derived from living organism, including microorganisms and fragments of all varieties of living things (Matthias-Maser and Jaenicke, 1995). Biological aerosols span more than intact living organisms (analyzed as CFUs), which also include fragments of both living and dead organisms, plant debris, human and animal epithelial cells, parts of insects, broken hair filaments, and so on (Jaenicke et al., 2007). Biological aerosol is a kind of important pollutants in the air, not only influencing public health, air quality, atmospheric chemistry and physics, but also playing an important role in regulating the atmospheric processes. Acting as ice nuclei (IN) and cloud condensation nuclei (CCN), biological aerosols can enforce the global climate change (Christner et al., 2008; Pöschl et al., 2010).

Since there is no constant source for airborne biological aerosols, plants, soil, water, and animals (including human) all can be sources of biological aerosols. With the development of urban biological treatment technology, the proportion of anthropogenic emission, such as landfill (Huang et al., 2002), wastewater treatment (Karra and Katsivela, 2007), and composting (Pankhurst et al., 2011), has increased significantly. Although most of the biological aerosols transport passively in the atmosphere, many

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studies found that regional and long distance transports of airborne bacteria and fungi are possible (Grant et al., 1994; Otake, et al., 2010). Therefore, biological aerosols research will be more concerned, also in the field of epidemiology (Adhikari et al., 2006).

Some early studies on biological aerosols concerned about species, size distribution, and annual diurnal variation characters of micro-organisms concentrations (Crook et al., 1997), the other studies focused on the effects by exposuring pathogenic and allergenic of microorganisms in either indoor or outdoor air (Schwartz et al., 1995; Gilmour et al., 2006). However, these investigations were based on the fact that micro-organisms could be cultivated. Viable micro-organisms (concentrations expressed as colony forming units CFU m^{-3}) take only a limited fraction of the total microbial spectrum (Griffiths et al., 1994). But biological aerosols contain not only viable organisms, but also non-viable organisms, which are so called "dead biological matter", such as aerosolized pollen, plant debris, animal dander and saliva, insect excreta, and so on (Jaenicke et al., 2007). Although the "dead biological matter" in the air can't form the colony on the culture medium, the specific components of them remain toxic or allergenic (Dillon et al., 1999).

2 Traditional biological aerosols quantitative assessment method – plate count method (concentration expressed as culture forming units, CFUs)

The primitive quantitative assessment method of biological aerosol is determined by plate count method, by which micro-organisms are placed in an appropriate culture medium and raised for a period of time, after that the culture forming units (CFUs) are counted to express the concentration of biological aerosols. However, plate count method, which is a passive sampling method, relies on the gravitational sedimentation of biological aerosols and can be disturbed easily. Due to the low reproducibility and high uncertainty of the result, its application is limited. At present, more and more samplers are invented to collect biological aerosols into liquid or onto agar. The majority of samplers use solid medium, which can quantitate biological aerosols by viable units

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counting, and microbe species identifying. Application of samplers changes biological aerosol sampling method from passive to active, and makes the collection more efficient. But the problem that only limited fraction of micro-organisms could be cultivated still exists. Plate count method relies on the airborne microorganism's viability and cultivability, which are impacted by various factors, such as the characteristics of culture medium, sampling stress, incubation conditions and atmospheric conditions (including temperature, relative humidity, solar radiation, concentration of oxidants, pollutants in air, and so on) (Lin et al., 1997). According to the study of White et al., (1983), there is only 0.1 % ~ 10 % of the microorganism in the air could be cultivated by plate.

3 New biological aerosols quantitative assessment methods

According to the limits of plate count method, the total quantity of biological aerosols in the atmosphere is underestimated. Traditional colony counting method could not truly reflect the total quantity of biological aerosols in the atmosphere, and more time and energy need to be spent. Therefore, researchers have made enormous effort on developing new efficient methods to quantify biological aerosols in the atmosphere, such as staining method, immunoassay method, molecular tracer method, DNA-based method, and so on. These new quantitative assessment methods are much more accurate, and reduce the underestimation of biological aerosols in the atmosphere tremendously.

3.1 Staining method

Staining is a kind of technique used to enhance contrast in the microscopic image. Stains and dyes are often used in biology to highlight biological components for viewing from microscopes. Different stains react with different biological components, and these properties can be used to qualify or quantify the presence of specific compounds, such as proteins, DNA, lipids, carbohydrates, and so on.

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3.1.1 Protein staining method

Protein, the main component of biological aerosol, is one of the most potentially antigenic biological materials. Protein coomassie brilliant blue staining method was employed by Jaenicke (2005) to observe biological aerosols with radius greater than 0.2 µm. He found that the fraction of biological aerosols ranged from 5 % to nearly 50 % in Mainz, Germany, and the common assumption that biological aerosols fraction and concentration low in winter was not confirmed by their studies. According to the study by Matthais-Maser (2000), particulate matters were collected particulate matters on coloured glycerine jelly, and then biological aerosols were distinguished and calculated from a light microscope. And they found the number proportion of biological aerosols to the total airborne particles in Lake Baikal, Mainz, South Atlantic was 19.5 %, 23.7 % and 16.7 %, respectively.

3.1.2 Nucleic acid staining method

Nucleic acids are biological molecules, which abundant in all living things, including DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). DAPI is a fluorescent nuclear stain, excited by ultraviolet light and showing strong blue fluorescence when bound to DNA. It is widely used to determine the number of microorganisms in the atmosphere (Wiedinmyer et al., 2009). As one kind of new nucleic acid stain, SYBRs® dyes, show a large fluorescence enhancement on binding nucleic acids, which could be used to stain DNA in both live and dead microorganisms. Bauer et al. (2008) used SYBRs® gold nucleic acid gel stain to enumerate the spores which appear as bright green or yellowish-green in the fluorescent microscope field of view.

3.1.3 Other staining method

There are many other staining methods used to quantify the airborne biological aerosols. ATP bioluminescence-based method was reported by Seshadri et al. (2009)

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to determine the number of biological aerosols and to evaluate the collection performance of different bio-samplers. Calcofluor White Stains can bind with cellulose and chitin, which are contained in the cell walls of fungi specially. So they are used to distinguish fungi from other microorganisms (Stanley et al., 2004).

Staining methods are used to determine the number of biological aerosols in the atmosphere frequently. However, confined to microscopy technology, they could not be used to detect small microbial (< 1 µm) aerosols.

3.2 Immunoassay method

It has been well established that exposure to the environmental allergens in biological aerosols can cause adverse health effects, immunological reactions, allergic diseases, etc. (Soukup et al., 2001; Monn et al., 1999) Essentially, immunoassay method is a special type of biochemical test that depends on the antigen which is known to undergo a unique immune reaction with its specific antibody. The analytical reagent, including the specific binding antibody, is used to determine the presence and amount of the corresponding antigen. Moreover, besides specificity, the binding partner should be selected and measured accurately.

3.2.1 Endotoxin

Endotoxin is the main component in the Gram-negative bacteria cell wall, which has been confirmed to associate with many health impairments and airway disease significantly (Schwartz et al., 1995). Endotoxin level is usually quantified by a kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay. Yao et al. (2009) reported that endotoxin concentration ranged from 0.8 to 83.7 $ng\,m^{-3}$ in the air, and 7.8 to 14.3 $ng\,mg^{-1}$ in the dust, in all tested surroundings, including home, office and outdoor environment. Menetrez et al. (2009) found that the concentration of endotoxin was higher in PM_{10} than in $PM_{2.5}$, except when near-roadway conditions existed.

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3.2.2 (1,3)-β-D-glucan

Besides endotoxin, (1,3)-β-D-glucan, a major component of fungal cell wall, also can induce inflammation, which is suspected to cause non-allergenic respiratory symptoms and other adverse health effects (Wan et al., 1999). Foto et al. (2004) used LAL assay to determine the total (1,3)-β-D-glucan concentration of different fungal species in outdoor air samples. Chromogenic β-glucan-specific LAL assay is used by Menetrez et al. (2009) to determine (1,3)-β-D-glucan concentration in the indoor and outdoor biological aerosols.

Understanding the concentration and distribution of airborne allergens can help to protect people from the adverse health effects, and monitoring airborne allergens can help to improve public health awareness and to serve as a spatial indicator of climate change (Menetrez et al., 2009).

Although the specificity of immunoassay method is high, this method is more suitable for assessing the activeness of microorganism than indicating the total quantity of biological aerosols in the atmosphere. For example, endotoxin just make up a small portion of the total protein mass, if using endotoxin as indicator, the contribution of airborne biological aerosols will be underestimate. Moreover, application of immunoassay method is restricted to the difficulties of developing antibodies to show the required specificity.

3.3 Molecular tracer method

Molecular tracer can be defined as "Compounds with unique properties that by their pure existence allow for a conclusion about their sources or formation" (Rudich et al., 2007). If the molecule conforms to three conditions: specific emission from one source type, sufficient stability during atmospheric lifetime, availability of sensitive and accurate analytical methods, it will be suitable to be served as a kind of molecular tracer in the atmosphere. Molecular tracer method has been widely used to trace the origin of the process generating particulates in atmospheric aerosols. For instance,

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levoglucosan acted as the molecular tracer for biomass burning (Kehrwald et al., 2010), cholesterol for meat cooking (Dutton et al., 2010), hopanes and steranes for burned fossil fuels (Rogge et al., 1993) odd-numbered (C27–C35) carbon-alkanes for plant wax emission (Rogge et al., 1991), less than C20 homologues of fatty acids for microbial contributions in atmospheric aerosols (Simoneit et al., 1982; Zheng et al., 2000) etc.

The molecular tracer, corresponding uniquely with a particular microbial group or community, can be used to characterize and quantify a specific microbial group or community (Lee et al., 2004). Different molecular tracers can be used to assess different microbial biological groups, such as protein for total bio-particles, peptidoglycan for total bacteria, 3-hydroxy fatty acids for Gram-negative bacteria, ergosterol for fungal biomass, mannitol and arabitol for fungal spores, etc. Although many studies have exemplified that molecular tracer can be used as an alternative approach to assess the quantity of biological aerosol, a lot of studies are still working on developing new specific molecular tracers to characteristic different types of biological aerosol more accurately.

3.3.1 Protein – for total biological aerosols

Protein staining method can express the number of biological aerosols, and there are also many other detection methods to determine the protein mass concentration in atmospheric aerosols. Studies have indicated that a sizable fraction of both coarse and fine PM is of biological origin in the atmosphere. Miguel et al. (1999) found that up to 5 ~ 12 % of the atmospheric total suspended particular matter samples consisted of biological protein allergens in road dust collected on the streets of Southern California. 1 % ~ 4 % of $PM_{2.5-10}$ and 1 % ~ 2 % of $PM_{2.5}$ were made of ambient protein aerosols in a metropolitan area of North Carolina, reported by Menetrez et al. (2007).

3.3.2 Ergosterol and Mannitol/Arabitol—for fungi

Ergosterol is a special component in all fungal cell membrane, largely restricted to fungi, served as an ideal molecule tracer for determining the fungal biomass in a variety of environments, such as soil (Montgomery et al., 2000) sediment (Sridhar et al., 2008) aquatic system (Gessner et al., 1993) and environmental sample (Lindblom et al., 2004) Ergosterol also has been widely used to measure fungal contamination in various types of air samples, such as indoor aerosols (Miller et al., 1997), settled house dust (Saraf et al., 1997), and ambient aerosols (Lau et al., 2006).

Lau et al. (2006) first employed ergosterol as molecular tracer to quantify fungal biomass in atmospheric aerosols, and they found that in Hong Kong, the concentrations of ergosterol in PM_{10} ranged from 30.7 to 407.3 pg m^{-3} and approximately 65–66 % of the ergosterol in PM_{10} was associated with the fine mode particles $PM_{2.5}$.

The conversion factor of ergosterol content for mycelia mass was greatly varied reported. Different conversion factors of ergosterol to dry mycelial mass (pg ng^{-1}) were given by different researches, 2 to 14 in some common airborne fungi (Axelsson et al., 1995) and 2.3 to 11.5 in aquatic environment (Gessner et al., 1993).

Polyols or sugar alcohols, are usually common energy reserve materials in fungi, especially mannitol is high abundant in many fungi, which has been proposed as a suitable molecular tracer for fungi (Solomon, et al., 2007). Recently, arabitol has also been proposed as an another suitable biomarker for fungal spores (Bauer et al., 2008). This method has been widely applied to many environment domains, and the concentrations of arabitol and mannitol in ambient aerosol have been widely reported for forest site (Zhang et al., 2010), rural site (Hu, et al., 2008), and urban site (Suzuki et al., 2001) over the world.

3.3.3 3-hydroxy fatty acids – for gram-negative bacteria

Solvent extractable fatty acids with carbon chain length less than 20 fatty acids were thought to be derived in part from microbial source (Simoneit et al., 1982). This method

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was employed by Zheng et al. (2000), who found the percentage of microbial source in the $PM_{2.5}$ samples in summer was higher than in winter. However, besides microbial activities, kitchen emissions also have been found to be important contributors of < C20 alkanoic acids in urban areas (Rogge et al., 1991). Because of this, solvent extractable fatty acids are not specific enough for the determination of microbial sources. For these limitations, more specific molecular tracers are needed for better understanding on the characteristics and contributions of particular microbial communities in atmospheric aerosols (Lee et al., 2004).

In fact, many researchers are making effort on using diverse analytical technique to obtain characteristic "fingerprints" of the composition of bacterial pyrolyzates by pyrolysis-gas chromatography method (Alexander et al., 1991). Pyrolysis products derive from carbohydrates, lipids and other components, such as 3-hydroxy alkanoic acids, which have been widely utilized for bacterial discrimination(Alexander et al., 1991). Gram-negative bacteria contains a lot of lipopolysaccharide (LPS) biopolymers, which are also called endotoxin, and 3-hydroxy fatty acids (3-OH FAs) traditionally have been used as molecular tracers to determine the presence of LPS (Dworzanski et al., 2005). As unique structural components of the endotoxin, 3-OH Fas are employed by Lee et al. (2004), to estimate the amount of endotoxin and Gram-negative bacteria in atmospheric aerosols in Hong Kong, China.

Laboratory conditions, such as culturing conditions, culturing age, oxygen sufficiency and nutritive status, affect the conversion factor of molecular tracer to biological component. If conversion factors want to be applied in the field ecosystems, identical or at least similar conditions must be established. So the application of such conversion factors based on cultivation in laboratory to field samples should be cautious by individual localities.

3.4 DNA-based detection method

With the development of molecular biology, DNA-based detection methods have huge application prospect in biological aerosol detection, especially in low concentrations

airborne microorganisms, which may be used as an alternative method for air quality monitoring in the future.

Two spores detection level has been achieved by Polymerase Chain Reaction (PCR) based method, reported by Alvarez et al. (1995), who chose *Escherichia coli DH1* as the target organism. Agranovski et al. (2006) investigated that PCR method could speed up the time to detect airborne viruses by a personal biological aerosol sampler combined with a PCR device.

In fact, most of such studies use the laboratorial biological aerosol. But there are many inhibiting factors existing in real air sample, which greatly reduce the sensitivity and feasibility of PCR method. However, a developed fungus-specific PCR assay reacting system was used by Zhou et al. (2000) to detect fungi pollutants in the indoor environment. Nowoisky et al. (2009) reported that they had investigated and characterized the diversity and frequency of occurrence of fungi in the real air particulate matter by DNA extraction and sequence analysis of the internal transcribed spacer region.

Compared with other methods, DNA-based detection method can offer more sensitive and specific detection. With the development of molecular biology, the introduction of DNA sequencing by synthesis technology or "next generation sequencing" must revolutionize molecular biology and show immense potential for aerosol science (Metzker, 2009). The advent of next generation DNA sequencing has started a revolution in life sciences and these new capabilities will further enable our understanding of the atmosphere and the indoor environment (Peccia et al., 2010).

3.5 Real-time biological aerosols detective methods

Conventional off-line biological aerosol detecting methods need to remove particles from the sampler and analyze by microscopic, chemical, and biological assays. So these methods take lots of time to eluted and incubated, and can't provide rapid identification and response (Sengupta et al., 2005). Real-time detection of biological aerosol was first proposed by Hill et al. (1995), which used intrinsic fluorescence measuring method, while the natural fluorescence can't always provide a sufficiently distinct

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spectral fingerprint to identify the biological aerosol (Laucks et al., 2000). Recently, a real-time analytical technique – bio-aerosol mass spectrometry (BAMS) has been confirmed to distinguish a single cell without sample pretreatment or preconcentration (Fergenson et al., 2004).

To improve the performance of the BAMS, aerosol time-of-flight mass spectrometry (ATOFMS) and matrix assisted laser desorption/ionization (MALDI)-mass spectrometry were combined, which could increase the sensitivity greatly (Gregg et al., 2005; Sengupta et al., 2005). Moreover, Sengupta et al. (2005, 2007) had demonstrated that bio-aerosol also could be characterized by the surface-enhanced raman spectra (SERS), with which tree pollen (cottonwood and redwood pollen) and bacterium (*Escherichia coli*) were detected and characterized successfully.

4 Summary

Biological aerosols, which are ubiquity in the atmosphere and take a significant contribution to the atmospheric aerosols, play an important role in adverse health effects and climate change. With the application of improved quantitative assessment methods, biological aerosols have been quantified more and more accurately. However, most of the new detection methods are still on the lab-scale, and their field application should be improved. Moreover, uniform standard quantify methods and the concentration critical value of biological aerosols still be needed to enable different researches in different areas could be comparable.

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