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CRITICAL REVIEW

Detection and analysis of airborne particles of biological origin: present and future

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Detection and identification of bioaerosols in the environment presents a unique analytical challenge. The complexity and variation of the analyte, coupled with the disparity of the end users required information has led to the establishment of a huge number of approaches for detection. In general these approaches may be divided into two elements; sampling, describing the physical process used to capture the bioaerosols and analysis, the method by which the bioaerosols are counted and identified. There are a large number of methodologies for both these elements, mainly due to the diversity of applications, and a very unhealthy absence of consensus on standardisation for these approaches. This is an analytical application where 'one size does not fit all'; nevertheless standardisation is still essential. The focus of this review will clarify the challenge, by discussing the many different bioaerosols to be measured and the required user output, also to give a critique of the various analytical approaches that exist to date, including other promising methodologies that could be applied.

1 Introduction

Potential threats to human health of airborne biological agents, or bioaerosols is becoming a growing international issue, from bioterrorism to emerging respiratory diseases.[1,2] The nature of bioaerosols can be numerous and range from whole bacterial cells and spores to viruses and pollen, depending on the environment. The need to monitor these particulates is mainly driven by their release by human activity and health concerns from human exposure to bioaerosols, which may lead to a range of effects from mild allergic response or in more serious cases, fatal infection.[3,4–10] Despite the necessity of measuring these particles; there are currently no recognised standardised protocols for the assessment and detection of airborne biological particles. Much of the problems arise with the sampling methodology and the breadth of different biological particles that exist in the air.[11]

The term bioaerosol refers to the condensed phase particle of biological origin and the gas medium as a system, in this review bioaerosol will only refer only to the particle. The scope of this review is to summarise and describe the different classes of bioaerosols that may be found in different environments, specifically posing a threat to human health, and present the current ways of measuring and identifying these particles. The intention is to expose these analytical applications beyond microbiologists and aerosol scientists, to the wider analytical community, and to emphasise the need for innovation for this unique analytical challenge. Historically the analytical approach to measure these particles has been based on capture and culture, in combination with knowledge of the biological particle, *i.e.* to capture on agar plates and incubate. The most rudimentary approach is to use a settle dish where microbial colony forming units (CFU) can fall on an agar dish and grow.[12] This culture method may be suitable to measure the number of viable bacterial cells, endospores and some fungal spores, but not for an airborne toxin or a pollen grain, which will not grow to form colonies, but can still present a threat. Nevertheless, capture and culture methods still remain very important in many instances, as it provides a good

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assessment of airborne viable biological bacteria. It would be fair to say that different analytes and applications will require a slightly different approach. The criteria for the detection of specific bioaerosol, in conjunction with the individual needs for each application will be highlighted and discussed in this review. More recently other approaches have been developed that consider the particles not necessarily as living organisms, but as analytes that exhibit different physical characteristics such as composition, size and density.[13] Identification of bioaerosols based on physical differences rather than biological, will also be described in this review.

Currently there is precise legislation covering human exposure of key pollutants in the atmosphere such as NO_x, SO_2, O_3, *etc.* and for particles of size 2.5 and 10 μm issued but the Environmental Protection Agency (the clean air act) and the European Commission.[14,15] Currently, there is no similar legislation to cover the release of bioaerosols and their presence in the built environment. Although, as the prevalence of bioaerosols is becoming increasingly realised, it is likely that measures to control and monitor their presence will soon be introduced. This is particularly important in environments where human activity results in the release of biological material in the air, such as livestock houses and composting sites. Currently standard operating rules issued by the Environment Agency covering composting biodegradable waste mention the release of bioaerosols in contained systems, but not for open systems.[16] At present only guidelines are available covering the monitoring of bioaerosols at and around composting sites.[17,18]

Due to the absence of recognised standard approaches for measurement and identification of bioaerosols, setting safe levels in any environment (whether in the work place or in residential areas) is very difficult.[19] The absence of wide consensus for bioaerosol detection, particularly for collection, has led to numerous papers in the literature describing various approaches.[1,20] The principal problems encountered here are the breadth of the type of analyte (pollen, endospores, spores, live or dead bacteria, viruses), the specific species (either harmful or not) the variation in analyte due to ambient conditions (*e.g.* relative humidity) and the number density, as shown in Table 1.

2 The analyte

Biological material in the air is ubiquitous in the atmosphere, from ground level to the upper atmosphere and is a natural phenomenon in the most part. Some plants, such as flowering grasses and trees, release pollen into the atmosphere to enable pollination which is an important step in exchange of genetic material and ecological diversity. Other organisms release similar particles such as fungi and lichen. Very often these releases are seasonal and linked to life cycles of these organisms.[33] Bacteria can also exist as airborne particles, as viable cells or as endospores which are dormant states normally triggered by particular conditions. Generally biological particles that are produced by plants or animals and wilfully released into the atmosphere normally exist as very monodispersed uniform particles. The size reflects how far the particle is required to travel. Pollen need only travel short distances (<1 km) so is normally comparatively large, between 9–40 μm in diameter, and is carried by wind currents close to the ground. Endospores that have been produced due to harsh conditions will be required to travel longer distance to increase chances of survival by landing in more life-conducive environments. These are normally smaller 0.5 to 2 μm, and as a consequence can be found at higher altitudes.[34,35]

Bioaerosols that are released through other processes *e.g.* spray formation (from sea, rain) and some human activity can exist as clumps of biological cells, *e.g.* algal cells, or as accretions on liquid or (inorganic or organic) solid mater. These are hard to characterise as they are not monodispersed, but still make up an important fraction of the bioaerosol content. Unclassified bioaerosols that may be parts of cells or biological material (*e.g.* human skin cells, natural textiles) make up a large proportion of other aerosols, again these are very difficult to characterise. These are of biological origin but they are generally benign, unless at high quantities when they can exacerbate some existing

Table 1 Showing classes and number density of bioaerosols in specific applications and environments. PFU plaque forming unit; CFU, colony forming unit

Application	Principal Bioaerosols	Concentration range	Environment	References
Security Bioterrorism	Endospores/Viruses/Bacteria	1000 CFU m^{-3} spores/1–100 PFU m^{-3} viruses/1–500 CFU m^{-3} bacteria, all depending on species	Urban environment/war zone	21 and 22
Work place	Fungal spores/Bacteria	100–10000 CFU m^{-3} highly dependent on work place activity	Urban environment (e.g, composting sites, poultry breading, *etc.*)	23, 24 and 25
Atmospheric	Fungal Spores/Cells/bacterial fragments	1–3000 cells m^{-3}	Cloud forming regions	25, 26 and 27
Dwelling	Fungal Spores/Pollen	<300 CFU m^{-3} for viable non-toxic or non-pathogenic bacteria,<50 CFU m^{-3} for viable pathogen	Buildings	28, 29 and 30
Infection control	Bacterial/Viruses/Endospores	100–500 CFU m^{-3} total viable bacterial	Hospitals	31 and 32

breathing conditions, such as asthma, but are not believed to be the cause of the condition.[36] The focus of this review will be the smaller biological aerosols, which are either living or can be of direct concern to human health. The inhalable fractions of particles that are < 10 μm in diameter reach the deeper parts of the respiratory tract and pose the highest risk.[37,38] Most bioaerosols may be classified according to typical biological descriptions, each will be taken in turn. Where seasonal occurrences of common bioaerosols occur, some specific genera or species will be given.

Viruses

Viruses are very small with dimensions in the range of hundreds of nm.[39] They are rarely found as isolated airborne entities, but will normally be part of an agglomerate either within a living cell infected by the virus or in a liquid aerosol droplet. The stability outside a host system can vary from a few minutes to several days.[37] They are normally very simple structurally, composed of a protein rich outer envelope with a core containing some genetic material, either DNA or RNA. The outer surface of most viruses contain unique protein structures which may be used as identifiers for particular classes of virus, but the DNA or RNA sequence is the modern way of identifying and classifying the virus.[40] There is no machinery required for protein synthesis or respiration so there is a complete absence of ATP and NADH which are molecules associated with the energy currency of any living organism.

Cellular

In general these are small (< 2 μm diameter) and will exist as single entities, agglomerates on solid particles or in liquid aerosol droplets. The most common type of cellular bioaerosols are bacterial cells, which exist as unicellular organisms. Bacteria can be robust due to a thick cell wall and can survive in the air for a reasonable amount of time, depending on the ambient humidity and temperature. Studies on aerosolised bacteria in laboratory tests show that survivability can be quite good; some bacteria can remain viable for hours to days.[1,37] In general they are composed of polysaccharide outer cell wall, lipid and protein membranes and an aqueous core containing proteins salts and an array of small molecules required to sustain and propagate life such as NADH, ATP, DNA and RNA. Proteins in some bacterial cells can be made up of amino acids which are of the opposite chiral structure to human proteins.

Historically bacteria were classified by the exterior make-up of the cell wall (propensity to absorb crystal violet stain, the so called Gram stain), shape (spherical, cocci, or rod shaped, bacilli), life cycle (spore forming or non-spore forming bacteria) and motility (flagellum). Modern bacterial identification approaches rely on molecular systematics, using genetic techniques to identify marker sequences unique for a specific species or to determine the guanine/cytosine ratio. There is a seemingly endless list of bacterial species; all bacteria are named according to the International Committee on Systematic Prokaryotes (ICSP) which maintains international rules for the naming of bacteria and taxonomic categories and for the ranking of them in the International Code of Nomenclature of Bacteria.[41]

Spores

Spores are produced by some green plants, mosses and fungi as part of their reproductive cycle in most cases function much like seeds. Their classification is based on the physiological mechanism of production (asexual or sexual), anatomy and the mode of dispersal. Some spores are actively mobile such as zoospores, others are immobile and dispersed by wind. Wind dispersal is passive and in order to fulfil their biological function spores are normally released in great numbers and adapted for survival over long periods in harsh conditions.[42] They are normally between 1 and 10 μm in diameter, and are able germinate and grow if conditions are favourable. They are small and simple in structure, composed of a tough outer layer protecting a DNA core.[43] Airborne fungal spores occur throughout the year and across the inhabited world, they occur with strong seasonal dependences and their spectrum depends on the type of climate. Generally speaking most fungal spores can induce an allergic response in humans, but the fungal species which are pathogenic and/or release mycotoxin into the environment are of greatest concern.[44] It is difficult to generalise, which spores occur most frequently and at greatest concentrations in air since no detailed investigation is available except for a few studies.[45] Many authors claim that spores of the genera *Alternaria* and *Cladosporium* are most numerous both side and outside environments with spores from green plants making up a smaller proportion of the spore population.[46] Other genera of fungal spores with common species, particularly in indoor envrionments are *Cladosporium* (*C. herbarum*, *C. cladosporoides*), *Mucor*, *Alternaria* (*A. alternata*), *Penicillium* (*P. glaucum*, *P. notatum*), *Aspergillus* (*A. glaucus*, *A. niger*, *A. vesicolor*), and *Rhizopus*.[45]

Endospores

Some commonly occurring bacterial species produce spores, called endospores are produced as a response to harsh conditions in a dormant state as a survival strategy. These are not the same as spores from fungi or some green plants as they are not part of their bacterial life cycle. They are small extremely tough (withstanding, severe desiccation, to 10–30% of water content and temperatures above 70 °C for prolonged periods), small typically 0.5 to 2 μm and in many cases designed to be dispersed by release in the air.[47,48] They do not contain adenosine triphosphate (ATP) one of the indicator molecules for living cells. The occurrence of endospores in the environment is not as numerous as fungal spores, but can still present a health concern, with effects ranging from incidence of allergic alveolitis and other more severe or fatal infection. The greatest threat and classic endospore forming bacteria are those produced by *Bacillus spp*. in particular *B. anthracis* which is historically associated with warfare and now with bioterrorism. But other genera are often found in indoor environments and occupational environments, such as agriculture and waste composting facilities.[49]

Pollen

Pollen are well known air borne biological particles which are not toxic directly, but can stimulate an allergic response in about 15% of the human population.[50,51] A single pollen grain contains vegetative (non-reproductive) cell(s) (only a single cell in most

flowering plants but several in other seed plants) and a generative (reproductive) cell containing two nuclei.[52] The group of cells is surrounded by a cellulose-rich cell wall called the intine, and a resistant outer wall composed largely of sporopollenin called the exine. These two outer walls provide incredibly tough protection against chemical and physical degradation and can survive in the gas phase for several weeks. Only wind pollinating plants release pollen in great quantities in the air and are specifically made to fly as single particles. They are typically 9 to 40 μm in diameter, and their occurrence is very seasonal. The size and occurrence is also dependent on the meteorological conditions.[53]

Not all pollen grains stimulate an allergic response, and in fact the allergic response is triggered by a water soluble protein or glycoprotein found on the outside of the pollen which is 10–70 kDa.[53] The most common pollen allergens are Hazel (*Corylus spp.*), Alder (*Alnus spp.*), Birch (*Betula spp.*), Grasses (*Poaceae*), Sorrel (*Rumex spp.*), Mugwort (*Artemisia spp.*), Plantain (*Plantago spp.*), Ragweed (*Ambrosia spp.*), Pinaceae (*Pinaceae*).[51]

3 Bioaerosol monitoring

Monitoring bioaerosols in target environments may vary from only the need to detect the presence not necessarily to quantify or identify (normally because the identity of the specific bioaerosol release would be known), to the ability to measure and identify continuously with high sensitivity (right down to 1–10 units m^{-3}). This is the source of ambiguity with bioaerosol monitoring with regards to standardising monitoring strategies. Standardisation of such varied analytical application requires the establishment of recognised monitoring methodologies which can be calibrated with a common standardised method. As stated above there are many ways of quantification of bioaerosols which have evolved to suit the required end user information. Comparison between collection and detection efficiencies is very difficult in absence of any standardised protocols;[1,54,55] this causes grave problems from a fundamental analytical perspective. In turn, and perhaps more importantly, introducing legislation to control monitoring of release of bioaerosols from high risk work environments, such as composting sites, is very difficult. The greatest area of variation is sampling and shall be discussed below.

3.1 Sampling

Sampling is perhaps the most difficult issue in this analytical application. Variation of the physical properties of the analyte has led to the development of a variety of approaches each tuned to a slightly different application.[56] Of course, as a consequence a comparison of data from different sampling methods is difficult, as they provide variation in the concentrations of microorganisms. A number of very good reviews are now available that have provided guidance on best practice. Most notable is the Environment Agency Science report SC040021/SR3 2009 which is specifically for compositing sites, but can be more widely applicable.[17] The types of sampling were reviewed and recommendations made on the methods currently available to collect and quantify the numbers of microorganisms.[57] Sampling in any application needs to be carefully considered from a point of view of knowledge of the analyte(s) and the required end user information. In some instances only bioaerosols that are culturable need to be determined. For other applications total amount of aerosols is needed where the risk to human health is based on the direct toxic or allergic effects of the particle.

The 'holy grail' for sampling is to be able to capture *all* bioaerosols in the sample with high efficiency and retain the same viability or state as in the original sample environment. Furthermore, this ideal approach needs to assay a reasonably large sample at a high rate, > 10 l min^{-1}, capable of measuring from 1 to 1 × 10^4 particles m^{-3}, be reliable (stable and precise), can be deployed remotely, is resistant to bioaerosol contamination and low cost. In the absence of such an ideal instrument, Table 2 shows some characteristics of a number of alternatives approaches that have been assessed under laboratory conditions. The current methods for sampling are discussed below.

Impact samplers. There are several types of impact samplers which can vary in function, but as a general rule the bioaerosol particles are impinged onto a solid surface. Jets of sample air directed onto a solid surface where the inertial forces of the particles in a jet lead to impact onto a surface and are collected. There are two very well established collectors of this type, the Andersen sampler and the Burkard sampler. The Andersen volumetric sampler is a multistage impactor that is able to fraction the particles on the basis of size (or inertia) of the particles.[58] The collection in this case is normally directly onto an agar plate which is then incubated. The Burkard volumetric spore trap collects the airborne particles on an adhesive strip which is on a rotating drum providing an indication of the concentration of airborne particles over a 24 h or up to a 7 day period period.[59] This technique is normally applied to non-culturable bioaerosols, such as pollen. The identification is by microscopy.[60]

It would be fair to say that impactor samplers are the leading method of choice owing to low cost and portability, and the availability of a number of commercial systems. It lends itself very well to capture and culture methods, particularly in the Andersen sampler configuration where the collection surface is an agar plate. The plate is simply incubated, each colony is the result of a single viable bacteria or colony forming unit. There are many variants of this technique and many of them are listed in Table 2. All rely on a jet of air impinging on the solid surface, the shape and size of the jet and distance from the surface can be critical in this case.[61] There are many commercially available impactors and as a result they are very widely used. The efficiency of these devices is dependent on the adhesion of the particle to the surface,[62] and in the case of bioaerosols the stress imparted on the particle.[63]

Liquid collection. This is another form of capture sampling (also referred to as impingement) using sterilised buffered aqueous solutions as the collection medium, although other liquids have been used.[64] There is some evidence that this method is not suitable for some fungi spores and endospores due to a hydrophobic outer coating, preventing them from being retained in the liquid.[64] Also prolonged use results in the evaporation of the liquid making them useful only for short periods only. The capture of viruses using this technique has shown that using distilled water as a collection fluid results in the decay of

Table 2 Summary of laboratory studies of sampling devices for bioaerosols published since 2004, grouped by analyte. Abbreviations: BWWC-EC, Batch wetted wall cyclone evaporation compensation; BWWC-NC, Batch wetted wall cyclone no evaporation compensation; CCI, Compact cascade impactor; AGI-30 and MAS-100, are commercially available types of impinger and impactor respectively; RCS, Reuter Centrifugal sampler; qPCR, Quantitative Polymerase chain reaction; ELISA, Enzyme linked immunosorbent assay; RH, relative humidity and VOAG, vibrating orifice aerosol generator

Analyte and aerosolisation method	Sampler Type	Air sampler rate and duration	Sampling efficiency	Analytical method	Comments	Reference
Viruses						
Virus Nucleotides and *B. subtilis* vegetative cells, collison nebulizer	BioSampler and Button Aerosol Sampler with gelatin filter	4–12.5 l min^{-1} for 1–5 mins	Gelatin filters showed 1–2 times higher nucleotides and *B. subtilis* retention than BioSampler	qPCR and ELISA	BioSampler more efficient at collecting dust mite allergen than Gelatin filters.	77
Influenza Virus, 6-jet collison nebulizer	BioSampler, CCI, Teflon Filters and gelatin filters	One pass aerosol chamber	Efficiency for viable virus capture 75–110% for BioSampler 16–29% for CCI, 15–43% for Teflon filters and 11–19% for gelatin filters. Total virus capture was similar for BioSampler, Teflon filters and gelatin filters, but lower for CCI.	Molecular (for total virus) and infectivity assays (for viable virus).	New sampler for viruses required design parameters and criteria discussed	78
MS2 Bacteriophage or Polystyrene 30 μm, placed in the impingers	AGI-30 and BioSampler	N/A	N/A	Condensation unit	Investigated the reaerosolisation from impingers.	79
Coronavirus (transmissible gastroenteritis virus) different RH, collison nebulizer	AGI-30 and Biosampler impingers	12 l min^{-1} for 10 mins	Biosamplers collected more airborne virus than AGI-30 at all RH	Filtration	TGEV and possibly other coronaviruses remain viable longer in an airborne state and are sampled more efficiently at low rather than high RH	80
Influenza virus	Two stage size selective Cyclone		Quantification of H1N1 and H3N2 virus strains.	qPCR	Detection of virus particles was proportional to collection time	81
Four different bacteriophage containing aerosols, 3-jet collison nebulizer	Anderson impactor, AGI-30 impinger, gelatin and nucleopore filters.	28 l min^{-1}	Huge discrepancies between samplers due to differences in physical properties of virus particles (mainly hydrodynamic radius).	Culture to determine PFU	Hydrophilic virus particles where more efficiently collected than hydrophobic viruses.	82
0.1 and 2 μm particles simulating virus carrying aerosols, TSI nebuliser	Greenburg-Smith 500ml	Collection for 60 mins	Low bioaerosol collection efficiency for particles <0.7 μm	LAS-X (Particle Measurement Systems)	Comparison of G-S 18/9 and 28/15 impingers.	83
Bacteria, Fungi and viruses, collison nebuliser	Porous diffuser impinge	4 l min^{-1}	Collection efficiencies between 95 and 100% for bacteria, lower of viral particles	PCR	New design of sampler, no comparison with other samplers.	40, 84 and 85

Table 2 (*Contd.*)

Analyte and aerosolisation method	Sampler Type	Air sampler rate and duration	Sampling efficiency	Analytical method	Comments	Reference
Bacterial						
E. Coli L. pneumophila, Collison three-jet nebulizer	MAS-100, Anderson 1-STG, AGI-30 and BioSampler	1–60 mins	Collection efficiency was consistently better for culturable *L. Pneumophila* than *E. coli.* Was dependent on sampler type and better with pure water than with Tween mixture.	qPCR and Culture	Recommends replenishing of water in impingers.	86
Three Bacterial and fungal species, six-jet collison nebulizer	Seven portable impactors compared to Anderson-type impactor and Button aerosol Sampler	4 l min^{-1} for 5 mins	Collection performance varied from model to model	Culture	For bacterial species collection efficiencies were correlated with samplers' jet velocity and jet-to-plate distance. Fungal collection was different	87
Polystyrene spheres (1–10 μm), *Pantoea agglomerans* (vegetative cells) and *Bacillus atrophaeus* spores, 24-jet collison nebulizer and VOAG	AGI-30, BioSampler, BWWC-EC and BWWC-NC	Various depending on sampler	For Polystyrene 4–67% AGI-30 34–105% BioSampler, 5–65% BWWC-EC 55–88% BWWC-NC	Fluorescence microscopy and culture	Significant performance differences between units. *Pantoea agglomerans* loose culturability and show lower retention efficiencies for impingers	88
Pantoea agglomerans (vegetative) Biowarfare simulant	BioSampler and AGI-30 with three different liquid media	Two generating systems; collison and bubble nebulisers	Bubble nebuliser 15% more efficient at creating aerosolised viable bacteria. Both samplers showed similar collection efficiencies	Culture	Relative humidity showed to effect the viability in the gas phase, (below limit of detection at 15% RH rising exponentially to 75% RH)	89
Chlamydophila psittaci (Class B Biowarfare agent, Cirrus™ nebulizer	Dry filtration, AGI-30, BioSampler, MAS-100	For personal monitors 2 l min^{-1} for static 100 l min^{-1}		PCR and culture	MAS-100 was most suitable for stationary monitoring, for personal monitoring gelatin filters.	90
Pseudomonas fluorescens & *B. subtilis*, Collison nebulizer	Electrostatic sampler		Collection efficiency of 75%	Microscopy and qPCR	Very low detection levels	91
Pseudomonas Fluorescens and *B. Subtilis*, nebuliser	BioSampler		*Pseudomonas Fluorescens* 69% and *B. Subtilis* 54%	ATP bioluminescence based assay and microscopy	ATP assay specifically developed to assess sampling efficiencies	92
Microorganism sampling in poultry stables	Filtration and impingement (AGI-30)	27 l min^{-1} for 20 mins	Impingement gave 40% lower than filtration	Fluorescence microscopy	No distinction between viable and non viable cells. Impingement gave consistently lower capture rates that filtration.	93
Polystyrene, oleic acid/ Fluorescein and *B. atrophaeus* (spores), collison nebulizers and VOAG	BWWC	400 l min^{-1}	50–60% for polystyrene <50 for *B. atrophaeus* 1.5% for 10 μm Oleic acid drops	Microscopy and PCR		94

Table 2 (*Contd.*)

Analyte and aerosolisation method	Sampler Type	Air sampler rate and duration	Sampling efficiency	Analytical method	Comments	Reference
Bacterial Endospores and Fungal Spores						
Two species of fungal spores, Dry aerosol delivery	Cyclone, silt impactor (Air-*O*-cell) and single stage (N6) multiple hole impactor	Various times from 10 to 320 mins	Cyclone equalled or underestimated the other samplers (mean ratio 0.75 to 1.04)	Optical particle counter, microscopy, culture, PCR	Cyclones are suitable for sampling	70 and 71
Total thermotolerant fungi and specifically *A. fumigatus*	Sartorius MD8 airport and Merck MAS-100		Both samplers gave a very similar capture efficiency.	culture	Comparable collection efficiencies for thermotolerant fungi	95
NaCl aerosol and Fungal spores, *Cladosporium cladosporioides*, *Aspergillus versicolor*, and *Penicillium melinii*	Single stage, single nozzle, slit impactors, Allergenco-D and Air-*O*-cell		Laboratory and field studies, self-consistent	Microscopic enumeration	Design of the jet and jet size critical for the collection of spores	96
Fungal and Bacteria species ranging from 0.61 to 3.14 μm	Impactors: BioStage, SMA MircoPortrable, BioCulture, Microflow, Microbiological Air Sampler (MAS-100), Millipore Air Tester, SAS Super 180, and RCS High Flow portable microbial samplers	100 l min^{-1} for portable impactors or 28.3 l min^{-1} BioStage	Indoor and outdoor samples	Culture	BioStage gave consistently higher collection than All but RCS High flow and MAS-100. In general very specific collection efficiencies for different organisms	97 and 98
26 different species, mainly fungal	Andersen N6 and RCS	Incremental duration 1–15 mins, sampling at 28.3 l min^{-1}	Xerotolerant and normotolerant moulds as well as *Aspegilllus* and *Cladosporium* collection was higher for the RCS	Culture	Significant species specific differences between the different collectors.	99
Fluorescent microspheres 0.5 to 6.1 μm diameter and fungal spores, 3-jet collison nebulizer	Two stage cyclone	2 or 3.5 l min^{-1}	*P. chrysogenum*, 78 + 14% for first and second stages respectively and *A. versicolor* 84 + 9% for first and second stages respectively	PCR or immunoassay	For size 3.1 μm 98% efficient, less efficient for larger spheres. At 3.5 l min^{-1} 92% of aerosolised spores were collected	100
Microspheres 0.5 to 16 μm, to emulate fungal spores (*Candida famata* and *Penicillium citrinum* spores) and *E. coli* and endospores of *B. subtilis*, 3 jet collison nebulizer	Cyclone samplers	12.5 l min^{-1} with a sampling time of 45 min	At 4 l min^{-1}. Collection efficiencies decrease as sphere size decreased.	Culture and flow cytometry with fluorochrome	Assessment of flow rate and relative humidity.	101

Table 3 Bioaerosols or mimic of bioaerosols suggested as model systems as simple comparison and calibrant for sampling and detection of bioaerosols

Class of analyte	Biological particle	Method of Aerosolisation
Virus	Polystyrene spheres (0.1–0.5 μm). Any bacteriophage, *e.g.* T4 or MS2. These are most suitable for liquid droplet containing virus agents. MS2 for RNA containing virus and T4 for a DNA containing virus both infect *E. coli*	Suspended in deionised water and nebulized.
Bacteria	Polystyrene spheres (2–5 μm). *E. coli* K12 or *Pantoea agglomerans* (formerly known as *Erwinia herbicola*)	Suspended in deionised water and nebulized.
Endospores	Polystyrene spheres (0.5–2 μm), endospores of *B. subtilis* simulant of anthrax-causing *B. anthracis*	Nebulized wet or dry
Spores	Polystyrene spheres (1–10 μm), Any spores from *Cladosporium* spp. For example *Cladosporium herbarum* or *Cladosporium cladosporioides.*	Nebulized wet or dry
Pollen	Polystyrene spheres (6–30 μm) Bermuda grass pollen (*Cynodon dactylon*) and black walnut pollen (*Juglans nigra*)	Dry

viable virus particles when bubbled for extended periods.[65,66] Nevertheless, this is a very well established technique, again as with the impactors, impingers are cheap and several different designs are commercially available. These devices are particularly suitable in instances where the airborne particle count is high.

Filtration systems. There are many commercial filtration samplers mainly developed for aerosol measurement, and adapted for bioaerosol collection. Their application to quantify bioaerosols can be problematic. Whilst cheap and easy to run, bioaerosols are prone to desiccation on filters making quantification very difficult. However, they can still be very suitable if used in conjunction with DNA sequencing techniques where conserving the viability (or not) of the bioaerosols is not so important. This technique is often disregarded as a sampling method for bioaerosols but can still play an important part in this application, particularly in instances where a chronological record of bioaerosols is required.

Cyclones. Cyclones are gas phase sorting devices that can sort airborne particles on the basis of size. They are capable of collecting only a small range of particle sizes.[67,68] The collection efficiencies and the survival rate of bacteria sampled by cyclones seem to be very positive. There are reports that when cyclones are used for collection in liquids the evaporation rate appears to be more pronounced than liquid collectors (impingers).[68,69] However, cyclones hold promise when used in conjunction with direct counting.[70] They have the capacity of sampling a large volume of gas. They are also small, suitable for use as mobile samplers or even personal samplers.[71]

Electrostatic precipitation. In this method the particles are charged as they pass through an electric field and are then collected, normally on a solid surface (one being the electrode to generate the electric field) or in liquids.[72–74] This is a technique developed for measuring solid particles and is very useful for the collection of ultrafine particles (nanometre sized). In general there are not many studies in the literature to assess the applicability of this collection method to bioaerosols.[75]

Condensation collection. Condensation of water onto aerosols to increase the effective radius can lead to easier capture and/or detection. This is a relatively new strategy for the collection of bioaerosols, as well as other matierals such as inorganic particles. This can be very effective for the detection of small particles such as viruses (or ultrafine particles) as described by Oh *et al.*[76] The approach can also preserve the viability of the bioaerosol particle and can be used in conjunction with impinger samplers, which are not very successful at retention of small particles.

Table 2 shows a good collection of studies presenting a cross section of the literature on the assessment of samplers. The many analytes tested and the variation of collection efficiencies is very different, as is the breadth of sampling methodology. On the one hand, each of the techniques above can be faulted, on the other hand each one of these methods may be well suited to specific applications. An overlooked aspect to enable achieving this goal is to identify a method of calibration or even to select a list of model samples, see below. This is an analytical challenge; any development of an analytical tool needs calibration and assessment by a model traceable standard analyte(s). Producing a protocol to create an airborne 'standard' bioaerosol(s) will not only serve as a calibration technique, but minimise variation such as reaerosolisation and bounce effects, which are species specific and sampling technique specific. However, the significance of these effects are probably over-estimated as they can be easily accounted for in a transfer coefficient, a simple multiplier to convert the observed number density to the actual number density. Furthermore, standards will also enable accelerated development of new (perhaps novel) and potentially useful methodologies for direct bioaerosol detection, quantification and identification.

3.2 Aerosolisation of sample

The common feature with most of the literature presented in Table 2 is that almost all the studies are as a comparison between two or more different samplers. So the actual concentration of analyte is not known, unless aerosolisation is monitored by a physical counting technique. Generation of bioaerosols is challenging and often can reduce the viability of bacterial cell and viruses. Even very tough fungal spores can be produced as whole spores or fragments depending on the conditions of aerosolisation.[102]

Producing high outputs of monodispersed bioaerosols from liquid suspensions is often done using glass atomisers and

nebulisers. Rule *et al.* reported that bubble nebulisers are more efficient at creating air borne viable vegetative bacteria (*Pantoea agglomerans* used as simulant for biological warfare agent) than collison nebulisers.[89] The effect of aerosolisation on the viability is likely to be very species specific and some bacterial cells will be more or less sensitive to the aerosolisation process. It is very difficult to try and predict this without detailed knowledge of perhaps related bacterial strains. The best approach is to select a small number of simulants and understand how these behave, under different RH and temperatures at known points in their life cycle.

Dry powders may also be aerosolised, but can be prone to agglomeration and electrostatic charging.[71] Pollen and some spores and endospores can be used dry quite successfully as long as the sample is fresh. Where charging is a problem there are protocols to neutralise the charge using low intensity alpha emitters such as Polonium 210.

Choosing the correct simulant for the particular target analyte (or application) is very important; to be able to compare different devices and develop new approaches for bioaerosol detection as well as producing a system that can be used as a calibrant. Several different classes of model bioaerosol are suggested in Table 3. There are no 'perfect' simulant for each class of analyte, however using polystyrene spheres with a close size match to the analyte as mimics can be a very stable form of comparison, as seen in Table 2. Although the drawback here is that it would only work for methods of sampling which do not involve cell culturing. The mimics listed in Table 3 have been chosen due to their availability and non-hazardous to human health. In the case of fungal spores, the species chosen is prevalent in indoor environments. The disadvantage of creating simulants is the can sometimes be time consuming and requires some standard biological cell culturing equipment.

It is very easy to concentrate on the sampling methodology. The analysis is just as important to perfect; but it is fair to say that development or refinement of analytical techniques applied to this application needs more consideration. In the next section an assessment of the methods available to quantify and identify bioaerosols with particular focus on the emerging methods for direct analysis.

3.3 Analysis

There are two approaches to the identification of bioaerosols. The first relies on the physical capture of the biological particle in liquid or agar and analysis usually based on culturing, molecular analysis, microscopy or spectroscopy. The second is gas phase analysis using a physical based technique such as spectrometry or spectroscopy. The former of the two approaches is better established simply because the technique has developed through modification of existing laboratory techniques. Physical based techniques are preferable as the need for capturing the sample is eliminated and potentially can give an immediate response, but are more challenging and requires a significant amount of method development. A brief over view of different methods will be discussed.

Cell culture. Perhaps the best known and established is the culturing method where viable bacterial are collected on agar plates where they can grow into colonies and the number of colonies on an agar plate are counted and identified. The same can be done for viruses but plagues are produced on a special agar plate covered with a bacterial film. This is very suitable because it reflects the air borne infectious agents in the air, however it is slow and requires incubation for at least 48 h.

Molecular based analysis or recognition. There are several unique identifiers of biological particles such as, the DNA and the proteins.[103] Both can be used as a handle for the unambiguous identification of biological material. DNA amplification and sequencing techniques are very accessible, and libraries of unique marker sequences exist for most of the microorganisms present in the air. These are techniques which have migrated from being exclusively laboratory bound techniques to the field with some significant intervention for making the test kits small and fast.[104,105] Techniques such as quantitative polymerase chain reaction (qPCR) can be sensitive enough to analyse a single copy of a bacteria.[103,106,107] Still, the approach depends on expensive and perishable biological components and requires the bioaerosols to be captured and pre-treated. Furthermore, there are issues with regards to recovering the entire DNA from a collected sample. Once the DNA is isolated there are many efficient ways to interrogate the DNA and analyse the sequence using Surface Enhanced Raman, DNA Chips and fluorescence detection.[108,109]

Protein based analysis which picks up the presence of a unique protein or component on the outside of the particles, are equally as sensitive and also relies on biological materials for the analysis. Enzyme linked immunoassays (ELISA) have been proven in the field to be extraordinarily sensitive and often require minimal pre-treatment. However, these sorts of tests are specific for particles so some knowledge of what is or could be present in the air is needed. Although with miniaturisation specific tests for many particles can be multiplexed. A good example of this is cantilever technology where the binding event imparts stress in a thin strip of silicon and the deflection is measured.[110,111]

In general molecular analysis is far more expensive than the more established culturing methods. Also the integration into an autonomous device which could be placed in the field is not yet a reality, but it is probably a question of further development and refinement to achieve this. In critical threat situations the cost of unambiguous identification can be justified so this approach will be good for certain non-routine situations.

Physical detection. By design, biological materials are replicated quite precisely and in the most part exist as monodispersed particles if they are produced to be air borne; the classic example is pollen.[112] Of course, there may be some fluctuations depending on ambient conditions such as humidity and temperature, where clumping of these particles can occur. But in the most part biological material that is naturally made to be airborne is extremely tough. This property provides a handle on their identification through physical techniques. Perhaps the most primitive approach is microscopy. Biological cells can be stained and identified reasonably well and this is the method of choice to analyse material captured with the Burkard volumetric spore trap.[60] There are initiatives to use image analysis to automate the technique.[113] This is still very commonly and successfully used to monitor pollen.

The best established technique to measure particles in the atmosphere is light scattering.[114,115] This is routinely used for monitoring airborne particle and measuring size.[116] However, it is very nonspecific a carbon particle can be mistaken for a bacterial endospore of the same size as the light will interact in the same way. There has been significant progress in improving this technique in two main ways. Firstly the algorithms for the analysis of the scattered light have been improved to determine information on the shape as well as size. Secondly, additional spectroscopic signatures from fluorescence are used to differentiate between biological and non-biological particles.[117–119] The fluorescence from tryptophan contained in most proteins and NADH present in cells is detected. This approach has proven very successful in the field and is used in specialist applications where real time analysis is important.[120–122] The drawback of this technique is the inability to differentiate between biological species or to tell if the biological agents are viable. Sensitivity with this technique can be an issue as the fluorescence intensity can be very low and is a challenge when the number density is low. On the other hand fluorescence based techniques using very specific fluorescent probes, can be used to determine the viability of bacterial and fungal cells in the liquid phase.[123] Flow cytometry using fluorescent dyes specifically developed for microbial cells have shown real promise to distinguish between classes of bioaerosols and provide information on the integrity of cellular membranes as an indication of viability.[124,125]

Mass spectrometry has also been applied for analysis of biological material.[126–129] This is done by using fragmentation strategies to breakdown individual particles either in the gas phase or on a surface.[130,131] The mass spectrometer then provides the detail of the fragmentation products based on mass and charge. This is a very powerful technique and provides excellent sensitivity,[132] but the technique is very expensive and requires significant technical expertise, and would be difficult to put into the field.

A slightly related approach which relies on the fragmentation of airborne particles is plasma assisted fragmentation followed by plasma electrochemical detection.[50,133,134] Here the advantage is that the fragmentation and the detection are performed in unison. The detection is based on the measurement of a zero current potential between two or more electrodes to electrochemically image the plume of fragmentation products from single particle combustion events. The differentiation is based on the interpretation of the fragmentation pattern which is dependent on the biological particle and the propensity to fragment. The sensitivity with this technique is down to single copy level.

4 Concluding remarks

This review has focused on the analytical aspects of bioaerosol detection. There are other considerations in bioaerosol detection which have not been discussed as they fall outside the remit of this review. These include position of sampling is important, aeroallergens are generally monitored at roof level, however, there is fair amount of debate on the ideal height of sampling.[135,136] Also the issue of static and mobile monitors is also a point to address.[3,137,138] Again these questions are application specific.

In the long term it is probable that qPCR or a related molecular based analytical system that will be used to routinely identify bioaerosols in instances where identification is an issue. It is likely that capture and culture is here to stay; for many applications this approach fits well. In the short term it is likely that a physical based method will be used with reasonable capability to differentiate between bioaerosols (*e.g.*, between some species of fungal spores). This is especially true in instances where real time monitoring is essential, *e.g.*, homeland security and some areas of healthcare. With most physical analyses they are very adept at providing immediate analysis of bioaerosols, in general, at the expense of specificity.

Of course there are a number of other considerations namely cost, usability, portability, specific application, *etc.* which supports the statement of "one size does not fit all". It is important that consensus on standard sample and method of aerosolisation is reached, even before the sampling and detection methodologies are agreed for various applications. The availability of a recognised standard sample will enable more developments of analytical strategies, which is certainly required of this very unique analytical challenge.

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