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AIRBORNE BIOLOGICAL CONTAMINATION: MONITORING AND CONTROL

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Abstract
Airborne Biological Contamination includes a vast range of particulate and molecular species that present specific challenges for monitoring and control. In particular, the notion of viability places restrictions on sample collection and analysis. Furthermore, controlling airborne levels of microorganisms requires technologies capable of inactivating and/or chemically eradicating them, as simple capture onto filter media can lead to proliferation and subsequent release. The advent of new assay capabilities using rapid microbiological methods in combination with effective air sampling techniques holds promise for future online monitoring systems, while combinations of different technological bricks can be used to tailor air-treatment systems to remove, inactivate and chemically transform particulate, biological and molecular airborne contamination.

1. Introduction

What is Airborne Biological Contamination (ABC) ? The response to this question depends on the environment and the activities that are to be carried out therein. In a hospital environment disease causing pathogens are of most concern, while in the microelectronics, food processing and pharmaceutical industries a host of different types of particulate matter of biological origin, not necessarily complete organisms (e.g. toxins and biological waste products), may create problems with product reliability, efficacy and toxicity. Taking this into consideration, the reformulated question generally becomes: What is the Airborne Biological Contaminate of Concern ?, and once answered will then dictate how to measure, monitor and treat the air for the presence of the specific target contaminate. In what follows we will focus on complete micro-organisms not their fragments or byproducts.

When considering airborne micro-organisms (e.g. bacteria, viruses, fungi) one of the primary classifications used for charactering them is to determine whether or not they are viable. However, here again questions arise as to what is meant by viable. General definitions of viable include; capable of working, functioning, or developing adequately, sustainable or capable of growing, able to germinate and reproduce. This in turn has fostered a wide range of terms to describe the state of microorganisms, such as, dead, moribund, starved, dormant, resting, quiescent, viabile but non-culturable, injured, sublethally damaged, inhibited, resuscitable, living, active, and vital (1,2). Kell et. al. provide an extensive discussion of these terms and subsequently propose useful operational definitions that assign cells into four major categories as combinations of two alternatives: (i) culturable or nonculturable and (ii) (metabolically) active or inactive, noting that a clear distinction should be made between cells that are not immediately culturable (NIC) from those which are simply nonculturable.

These various notions for characterizing airborne microorganisms necessitate different experimental assays and tools to ascertain their level in the environment. Indeed basing the definition of viability on, that which requires continuing existence of the species to survive, as lead to the ability of cells to reproduce as the benchmark method for determination of viability. For this reason the most common analysis method used is plate counting from appropriately selected culture media. However, over the last decade there has been an increasing interest in the development of rapid microbiological methods (RMM) for the determination of cell viability, such as; Adenosine TriPhosphate (ATP) detection, impedance measurements, CO_2 generation, and auto-fluorescence. In addition to these, a variety of nucleic-acid-amplification methods such as polymerase chain reaction (PCR), which are very sensitive assays for detecting the presence of DNA, have emerged. These rapid microbiological method technologies can provide more sensitive, accurate and reproducible test results when compared with conventional growth-based methods (3). Moreover, they can

be automated and offer the possibility for continuous online monitoring. Adopting these methods as standard routine procedures is currently underway and as their use increases, the current high capital cost of the equipment should go down.
Quantifying biological contamination in air is a two-phase process that requires collection and analysis. First a representative volume of the air in the environment needs to be withdrawn, from which the contaminate is extracted, followed by an appropriate assay to determine its quantity. Indeed the assays used are not specific to airborne contamination, but their selection is strongly dependent on the sample collection method used.

Once ABC concentrations have been determined, special considerations may be needed to subsequently control their levels. Traditionally filtration and adsorption technologies have been used to eliminate airborne contaminates, and they can be effective for removal of particles and chemical pollutants, but microbial growth onto the substrates and subsequently release into the air can turn the systems into potential sources of ABC. To address this issue, so-called active air treatment technologies have been developed. In active systems, chemical and/or physical transformation of the airborne contaminates are achieved. Drawbacks of course are that side products from these transformations can be produced and must be controlled to successfully treat the air.

In what follows we present and discuss the different types of bioaerosol sampling techniques and active air treatment technologies currently used to monitor and abate ABC's.

2. Airborne biological Samplers

The basic components of an aerosol sampling system are an inlet, a size fractionation device that strips unwanted large-sized particles and debris from the distribution, and a concentrator that confines the particles on a surface or a liquid. Sampling for bioaerosol particles employs many of the methods used for non-bioaerosol particles, but the analytical methods impose certain limitations on the sampling process. There are both physical and biological aspects to consider for bioaerosol sampling. The physical aspects are by in large the same for non-bioaerosols while biological aspects may require aseptic handling to prevent contamination and special attention to the viability of the organisms during sample processing and analysis. Viability, which depends on the organism, the environment, and the sample collection and analysis methods used, introduces significant uncertainly into the quantification of airborne concentrations of bioaerosols. Other important factors in bioaerosol sampling are high variability in number concentration, wide particle size range of interest, analytical sensitivity to under- and overloading, and interference by other particles. The efficiency of a bioaerosol sampler is characterized by its physical (inert particles) and biological (viable micro-organisms) collection efficiencies. The following is a brief review of the most common types of microbial air samplers and their use.

2.1 Impactors

The principle of collection by impaction relies on the tendency of a particle to deviate from the airstream due to their inertia and intercept a solid or semi-solid surface - particles of a given size range will impact the surface. Samplers of this type can be subdivided into sieve and stacked samplers, slit samplers and cascade impactors. Typically, airborne particles are forced to impact directly onto selected culture media and after incubation the number of colony forming units (CFU) can be determined, which once normalized by the volume of air extracted provides a concentration measurement expressed in CFU/m^3. The physical mechanisms of this method are very well understood and the extracted particle size range can be easily controlled (4). This is one of the most widely used methods with the advantage of having rather high sampling flow rates (100 l/min), and requiring very little post collection effort to determine the CFU's collected. However, cell damage upon impact, drying of the culture media and saturation are important issues to consider when optimizing the biological collection efficiency. Furthermore, only information concerning the culturablity of the collected microorganisms can be obtained.

2.2 Impingers

Impingers are a special type of impactor for which the airstream is forced onto a liquid instead of a solid surface. As such the basic physical principles of inertial driven collection onto a solid are used, but the airflow streamlines a more difficult to characterize. Nevertheless, physical and biological collection efficiencies can be quite high. Air sampling flow-rates are typically on the order of 12 l/min, and sample recovery into a liquid phase allows for a wide range of different assays, particularly RMM's mentioned earlier. Providing a way to replenish lost liquid due to evaporation can extend the sampling time indefinitely. Care should be taken to use surfactant solutions when targeting hydrophobic contaminates such as fungal spores. Because fluid splashes over the collection chamber and injection nozzle(s), care must be taken to thoroughly clean these components between samples to avoid cross-contamination.

2.3 Wetted-wall cyclones

Another sampler that recovers contaminates in the liquid phase is the wetted-wall cyclone (WWC) sampler. Instead of simply using inertial impact, these devices impose a centrifugal force on the particles allow them to enter the liquid phase with an angular trajectory providing for more gentle collection thus lowering the damage that can occur during the process. In addition, the low pressure drop through these devices permits very high sampling flow-rates (300-600 l/min). Like impingers, liquid-phase collection promotes the use of multiple assay possibilities; however, the theory behind WWC operation is complex and not well described making optimization of thier collection efficiency a trail and error exercise.

2.4 Filters

Filters remove airborne particles through, inertial impact, interception, diffusion and sieving and are the most widely used method for collecting inert airborne particles, such as inorganic matter and fibers. As such, a wide range of sampling devices and collection media, are available that can be adapted for collecting bioaerosols. Indeed, the equipment needed for filtration sampling is fairly simple and inexpensive, compared to many other sampling devices, and different size devices ranging from portable personal samplers to environmental monitoring equipment exist. However, for bioaerosols, filtration poses two disadvantages. First, after collection, dehydration can occur caused by air passing over filter media which can effect the viable recovery of certain microorganisms. Second, subsequent analysis of the collected particles requires their extraction from the filter medium that can be difficult and potentially harmful to the microorganism. Often specific extraction methods are needed to achieve consistent and efficient recovery. Also the choice of the physical and chemical characteristics of the filter media can help optimize recovery.

2.5 Electrostatic enhancement

Although attempts have been made in the past to harness electrostatic forces to increase collection efficiencies in bioaerosol samplers, no commercial devices developed over the years. This is changing and many research groups are actively pursuing the approach to develop new devices (5). The basic idea is to use exiting geometries and devices and simply provide controlled electrical potentials and materials with properties to enhance the collection efficiency. One of the primary goals is to collect concentrated samples in low volumes of liquid in order to better couple the collection and analysis phases for determining ABC levels.

3. Abatement of Airborne Biological Contaminates

As with monitoring, abatement of airborne biological contaminates presents unique challenges not addressed with standard methods for treating particle and chemical pollutants. Filtration is the primary means for removing particulate contaminates from the air, while adsorption is used for molecular species. In as much as ABC's are particulate or chemical in nature these technologies are well suited for air treatment in many situations. However, when viable microorganisms are captured in these processes they can proliferate and grow, resulting in the subsequent release into the air of newly generated microorganisms and their byproducts. In such cases the tools used to clean the air actually become a source for contamination. To prevent this, methods are required to inactivate the organisms and short-circuit the process. Technologies that have been introduced which attempt to achieve this includes, antimicrobial filter

treatments, Ultraviolet germicidal irradiation, UVGI, Photo-catalytic Oxidation, PCO, and non-thermal plasma. While all of these methods can produce germicidal effects under given conditions, their use for effective treatment of ABC's requires sound engineering designs, and in some cases their processes are simply not well adapted to meet the constraints and demands required for realistic air treatment systems.

3.1 Antimicrobial Filter Treatment

Antimicrobial treatments for air filtration products have become a topic of considerable interest. These antimicrobial agents, which are intended to destroy or inhibit the growth of microorganisms, have been used in commercial and industrial surface applications (i.e. walls, carpets, etc.) for several years. Applied to air filter media, the treatments purport to improve indoor air quality by destroying a broad range of microorganisms and preventing the growth of microbes in the dirty filter. Antimicrobial treatments sometimes produce dramatic results under artificial test conditions. However, most studies suggest that these treatments work marginally, if at all, in real world air filter applications (6,7). Direct contact is necessary for the treatment to penetrate and destroy a microorganism. This may occur briefly on clean treated filters. But as soon as dust builds up on the filter surface, direct contact can no longer be achieved-and the treatment is no longer effective.

3.2 Ultraviolet germicidal irradiation, UVGI

Ultraviolet irradiation at 254 nm, so-called germicidal irradiation, UVGI, can inactivate bacteria by inducing the formation of thymine dimers in the DNA. This inhibits the bacteria's ability to replicate and is an effective way to sterilize surfaces. The technique has also been used in attempts to sterilize air. However, unlike surface treatments where exposure times can be rather long, the dose required to be effective is not sufficient in most air streams used for indoor air treatment. Attempts have been made to place UV lamps in rooms for static irradiation but the danger of human exposure severely limits the irradiation zone thus preventing these systems from having a significant effect on lowering the airborne biological levels in the room. Other efforts to implement UVGI include the use of UV lamps placed on the upstream side of mechanical filters to irradiate their collection surfaces. The concept is that the filter will capture biological material from the air stream and provide the time required to receive a fatal UV dose. Proof of concept for this approach has been achieved by placing bacteria on a new filter and exposing it to UVGI in the laboratory. However, light shadowing, continuous buildup of porous dust over the captured species and on the UV lamp itself, prevents the line-of-sight requirement needed for this method to work in realistic air filtration systems. Moreover, the process is sensitive to relative humidity, and above 50% RH a significant reduction in perfromance is witnessed. A recent review by Memarzadeh et al. conclude, that UVGI may be an effective adjunct, but not stand-alone technology (8).

3.3 Photo-Catalytic Oxidation, PCO

Photocatalytic oxidation is the process in which photons from a light source irradiate a catalytic surface and provide energy to initiate oxidation of organic material which is also adsorbed to the surface. The basic process is well known and established with a host of metal oxides being identified as active catalysts. One very popular system being promoted for air treatment is the combination of UV light and Titanium dioxide catalysts. Due to the oxidation reaction involved aggressive marketing claims of reducing room levels of volatile organic compounds, (VOC) have emerged. Often batch laboratory experiments in closed environments that trace the reduction of a target VOC are used to support these claims. However, at best only partial oxidation occurs with rather low single-pass efficiency. This in turn can transform benign VOC's into dangerous smaller compounds such as acetaldehyde or formaldehyde and inevitable increases in these dangerous compounds are witnessed. Moreover, recent studies have found that these systems do not perform as a good germicidal capability for airborne microorganisms (9). In addition to the intrinsic limitation of low efficiency incomplete oxidation, PCO air treatment devices suffer from the same problem as that encountered with antimicrobial filter treatments, direct contact is necessary for treatment (here both the contaminate and the UV photons simultaneously) which may occur briefly on clean treated filters, but as soon as dust builds up on the filter surface, direct contact can no longer be achieved and treatment is no longer effective.

3.4 Non-Thermal Plasma

Plasma consists of a gas phase containing high concentrations of ions, and as such does not obey standard gas laws. For this reason it is considered the fourth state of matter. Non-thermal plasma (NTP) (sometimes referred to as "cold plasma") is a specific case which corresponds to systems having a large difference in temperature between the atomic species and electrons within the plasma. NTP under atmospheric conditions can be created by electrical discharges and a wide variety of techniques exists to achieve this in a controlled manner. The utility of NTP to treat air pollution relies on the large number of different ions and chemical species created in the plasma. These components can provoke both physical and chemical reactions that allow for transformation of particulate, chemical and biological airborne pollutants at room temperature. In particular, gas-phase oxidation of organic material by excited oxygen radicals can destroy the cellular membranes of biological cells. In addition to this, ions adsorbed to the membranes can evoke physical damage and lead to inactivation of their biological function. Thus NTP has emerged as a potential tool for treating airborne biological contaminates. However, commercial implantation of this technology is limited by the high energy requirements and molecular by products produced in the plasma (e.g. ozone and nitrous oxides) which can be harmful if released into the environment.

3.5 Hybrid Technologies

To overcome the intrinsic limitation of individual air-treatment technologies, one solution is to combine different technologies in a synergistic way. This approach as seen the most growth over the last decade and is what we refer to "hybrid technologies". The first and most established hybrid technology for air treatment consist of combing mechanical and molecular filtration. In this case a molecular absorber (e.g. activated carbon, KmN04, etc…) is simply placed in series with a particulate filter. Combined mechanical and molecular filtration systems can prove to be quite efficient at removing both particles and VOC's from an air stream but they have no effect on preventing biological growth.

One more recent attempt to use a hybrid system to resolve the problem of removing multiple types of airborne pollutants is to combine mechanical filtration with UVGI and/or PCO. These systems are designed to capture material by mechanical filtration and subsequently illuminate the surface of the filtration media with UVGI. When PCO is to be used the filtration media is simply coated with the appropriate catalyst material. The concept of combining UV/PCO with mechanical filtration is theoretically appealing, however, due to the line-of-sight requirement for light to reach the surface to be treated, practical use and long-term performance is severely limited. High efficiency mechanical filter are pleated to decrease the pressure-drop through them and as such light is shadowed and doesn't reach the crevasses in the filter pleats. In addition to this build-up of material (inorganic and organic) onto the filter surface over time prevents light from reaching the catalyst and hence short circuits the mechanism used by PCO over time. Placing the UVGI/PCO elements after the filtration does not resolve these issues, because the residence time in the downstream air flow is to short for sufficient doses of UVGI and catalytic supports provide little to no capture efficiency to prevent the release of biological species that have populated the initial filtration stage.

One of the most recent hybrid technologies to emerge is one called HEPA-MD. This technology consists of a three-stage reactor system designed to provide HEPA (high efficiency particle arrestation - 99.97% single-pass removal efficiency for all particles >0.3 mm), with microbial destruction. The system achieves this by combining an upstream NTP stage coupled to an electrical enhanced filtration (EEF) collection unit, followed by a post catalytic converter to transform any undesirable chemical by products. Pollutant are first treated by the NTP and are partially oxidized and highly charged. The charging then allows for highly efficient collection of material in the EEF. Once captured plasma species diffuse through the collection media to completely oxidize any remaining organic matter. Unlike light treatment, the gas-phase mechanism is able to reach all surfaces and is not limited by shadowing. The final stage of the system is designed to adsorb and catalytically convert any gaseous by products using novel blends of room temperature catalysts and adsorption media. The HEPA-MD technology has been exclusively used in hospital applications, and in particular for protecting immune-compromised patents (10, 11).

5. Conclusions

ABC is important to a wide range of, industrial, manufacturing and healthcare sectors. Unlike inert contamination, ABC, can grow, multiple and produce byproducts. These features require special considerations when developing monitoring and abatement control of ABC. By combining well-adapted

sample collection procedures with new RMM, next generation online monitoring devices are on the horizon. Similarly, a strong effort to develop active air treatment systems has seen the development of so-called hybrid systems that combine existing technologies in a synergistic way to remove, particulate, biological and molecular contaminates from the air.

References

1) Kell, D.B., Kaprelyants, A.S., Weichart, D.H., Harwood, C.R., Barer, M.R., "Viability and activity in readily culturable bacteria: a review and discussion of the practical issues", 1998, Antonie van Leeuwenhoek, 73, pg. 169-187.

2) Breeuwer, P., and Abee, T., "Assessment of viability of microorganisms employing fluorescence techniques", International Journal of Food Microbiology, 2000, Vol. 55, Issues 1-3, pg. 193-200

3) Miller, M.J. Microbiology Series. Article 1: The Implementation of Rapid Microbiological Methods European Pharmaceutical Review, 2010, 15(1), pg.39-41.

4) Whyte, W., Green, G., Albisu, A., Collection efficiency and design of microbial air samplers, Aerosol Science 2007, 38, pg. 101 – 114.

5) Han, T.#, An, H.R.# and Mainelis, G.* (2010) Performance of an Electrostatic Precipitator with Superhydrophobic Surface when collecting Airborne Bacteria, Aerosol Science and Technology, 44:339-348.

6) Ohgke, H., Senkpiel, K., Beckert, J., 1993. Experimental evaluation of microbial growth and survival in air filters. Proceedings of the Sixth International Conference on Indoor Air Quality and Climate, Helsinki, Vol. 6, pp. 521–526.

7) Foarde, K.K., Hanley, J.T, Veeck, A.C., "Efficacy of Antimicrobial filter treatments", ASHRAE Journal, 200, Vol. 42,n°12, pg. 52-58.

8) Memarzadeh, F., Olmsted, R., Bartley, JM, "Applications of ultraviolet germicidal irradiation disinfection in health care facilities: Effective adjunct, but not stand-alone technology", *American Journal of Infection Control*, (2010) 38, S13-S24.

9) Lin, CY, Li, CS, "Effectiveness of Titanium Dioxide Photocatalyst Filters for Controlling Bioaerosols", Aerosol Sci. and Technology, 2003, 37, pg. 162-170.

10) Sixt, N., Dalle F., Lafon I., et al. "Reduced fungal contamination of the indoor environment with the plasmair system", Journal of hospital infection, 2007, 65, n°2, pg. 156-162.

11) Sautour, M., Sixt N;, Dalle F., "Prospective survey of indoor fungal contamination in the hospital during a period of building construction", Journal of Hospital Infection, 2007, 67, n°4, pg. 367-373.