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Design of an environmentally controlled rotating chamber for bioaerosol aging studies

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Abstract

A chamber was designed and built to study the long-term effects of environmental conditions on air-borne microorganisms. The system consists of a 55.5-L cylindrical chamber, which can rotate at variable speeds on its axis. The chamber is placed within an insulated temperature controlled enclosure which can be either cooled or heated with piezoelectric units. A germicidal light located at the chamber center irradiates at a 360° angle. Access ports are located on the stationary sections on both ends of the chamber. Relative humidity (RH) is controlled by passing the aerosol through meshed tubes surrounded by desiccant. Validation assay indicates that the interior temperature is stable with less than 0.5 °C in variation when set between 18 and 30 °C with the UV light having no effect of temperature during operation. RH levels set at 20%, 50% and 80% varied by 2.2%, 3.3% and 3.3%, respectively, over a 14-h period. The remaining fraction of particles after 18 h of suspension was 8.8% at 1 rotation per minute (rpm) and 2.6% at 0 rpm with the mass median aerodynamic diameter (MMAD) changing from $1.21 \pm 0.04 \mu\text{m}$ to $1.30 \pm 0.02 \mu\text{m}$ at 1 rpm and from $1.21 \pm 0.04 \mu\text{m}$ to $0.91 \pm 0.01 \mu\text{m}$ at 0 rpm within the same time period. This chamber can be used to increase the time of particle suspension in an aerosol cloud and control the temperature, RH and UV exposure; the design facilitates stationary sampling to be performed while the chamber is rotating.

Keywords

Aerosol; aging; bioaerosol; germicidal light; relative humidity control; rotating chamber

Introduction

A multitude of natural and human processes can lead to the production of bioaerosols (Kummer et al., 2008), which may include pollens, fungi and other eukaryotic organisms, bacteria, viruses or any of their fragments or by-products. The potential hazards to human, animal and plant health depend on the composition of these bioaerosols which, in turn, depend on the nature of their source. The effects of bioaerosols range from no effect at all to allergic reactions and fatal infections (Caruana, 2011). The risks associated with exposures to bioaerosols also depend on the integrity of the air-borne microorganisms and their capacity to cause disease or to interact with host organisms. This capacity may be altered by the exposure of the microorganisms to destructive environmental conditions. The harsh conditions of the aerosol state could be considered as an extreme environment for biological particles (Gandolfi et al., 2013). Environmental stress factors such as ultraviolet radiations, variations in humidity and temperature or the presence of air-borne contaminants can have a significant impact on the integrity of biological aerosols; the severity of which is dependent on the nature of the stressor, the biological material exposed and the duration of the exposure. Thus, the environmental stress inflicted to air-borne microorganisms may lead to a reduction or a loss of their capacity for biological activity.

In natural environmental conditions, meteorological factors, such as wind and convection currents, can influence the behavior of an aerosol and allow particles that would normally settle rapidly to stay suspended in the air for prolonged periods and potentially travel long distances. Bioaerosols containing infectious particles have been detected at 2.1 km (Corzo et al., 2013) and 9.2 km (Otake et al., 2010) downwind from their sources. Further evidence suggests that infectious aerosols could have caused disease up to 80 km away from the presumed sources and potentially even greater distances (Jones et al., 2004; Verreault et al., 2008). Considering the complexity of the air currents governing the movements of bioaerosols, it is possible that these particles remain air-borne for prolonged periods of time while being exposed to potentially damaging environmental conditions. Specialized laboratory equipment is necessary to simulate naturally occurring environmental conditions as well as the duration of exposure. An environmentally controlled aerosol aging chamber is the key to understanding the potential fate of air-borne microorganisms in a natural setting.

It has been demonstrated that aerosols can retain their air-borne state for prolonged periods in rotating chambers (Goldberg et al., 1958), where particles are suspended in a rotating mass of air. These cylindrical chambers rotate on a horizontal axis with gravity in the vertical direction. In this type of chamber, gravitational forces exerted on air-borne particles are countered by centrifugal forces created by the rotation of the drum. In a static chamber, the trajectory of the particles is downward due to gravitational attraction. When centrifugal forces are added to the equation, air-borne particles adopt a spiral trajectory (Gruel et al., 1987) thereby significantly enhancing the path length to be traveled by each particle before coming into contact with the chamber wall. According to Asgharian & Moss (1992), with the proper adjustment of the speed of rotation in a 1 m diameter drum, particles less than 1 μm can theoretically remain airborne for up to a period of 1 year and particles between 1 and 2 μm , for up to 6 months with only 50% of the particles being lost to the walls.

Controlling the environmental conditions in this type of chamber would allow studying their long-term effects on the aerosols and could eventually be used to study metabolic activity of air-borne microorganisms (Krumins et al., 2008). The present study describes a rotating aerosol chamber designed for temperature and humidity control with the added benefit of an ultraviolet C band (UV-C) radiation control for disinfection studies.

Design and performance evaluation

The chamber, illustrated in Figure 1, is a 55.5-L aluminum cylinder with an interior diameter of 35.6 cm and a wall thickness of 0.48 cm. The two ends of the cylinder are sealed with removable custom-made caps which are made of 0.64 cm thick aluminum discs mounted on 3.3-cm thick double-sealed ball bearings with an outer diameter of 21.0 cm and an interior diameter of 14.0 cm. The interior sections of the bearings, which remain stationary when the chamber is rotating, hold protruding aluminum rods which are supported by the two side panels of the insulated enclosure. Access to the ports on the rods can be attained from outside the insulated enclosure. Both rods have two 5/8" smooth inlets, two half-inch national pipe taper (NPT)-threaded inlets and four 1/4" NPT-threaded inlets located around their middle. All inlets can be sealed with rubber plugs or with ball valves when not in use. The rod from the left cap has a one inch NPT-threaded inlet at the center while the middle of the right rod holds a UV light socket for a 35.6 cm long UV-C lamp emitting at 254 nm (model GCL356T5L/4P, Light Sources, Inc., Orange, CT). The lamp is plugged inside the aerosol chamber at the center of rotation and can emit radiation at a 360° angle. UV-C doses are calculated using a UV sensor (model UV-Air, sglux SolGel Technologies GmbH, Berlin, Germany); doses are measured at a distance of 5 cm from the light source. This UV-C lamp can be used to study the effects of upper-wall germicidal lamps used for air decontamination. The lamp did not affect the temperature inside the rotating chamber when operated for 5 min.

The drum is housed within a 187.5-L polycarbonate enclosure insulated with removable high efficiency closed-cell insulation material composed of one inch thick polyvinyl chloride panels, all of which can be disassembled and reassembled easily for maintenance. Two thermoelectric assemblies (model INB340-24-AA, Watronix Inc., West Hills, CA) equipped with small fans are connected to a dual-action thermoelectric controller and mounted through the top of the posterior wall of the enclosure. A temperature sensor probe located at the bottom of the right-side panel in the front of the enclosure is connected to the temperature control module; temperature can be adjusted and maintained at a range between 8 °C and 45 °C. The temperature and relative humidity (RH) are monitored and recorded in real-time inside the insulated enclosure and within the rotating chamber with two identical RH and temperature probes (model RH-USB, Omega, Farmington, CT). The variation of the temperature within the rotating chamber and insulated enclosure is illustrated in Figure 2. When the temperature is set between 18 °C and 30 °C, variations remain within 0.5 °C over a 48-h period. This is consistent with factory accuracy tolerances of the electronic temperature and RH probes.

The air tightness of the aerosol aging chamber allows maintaining a stable RH within the drum after aerosolization. High humidity can be attained by nebulizing liquid suspensions,

which also contain the particles to be nebulized, with a six-jet Collison (BGI Inc., Waltham, MA) directly into the aerosol chamber. For lower humidity conditions, the aerosol passes through desiccator tubes before entering the chamber. These tubes are made of three inch diameter clear polycarbonate pipes with a three-fourth inch diameter stainless steel mesh tube at their center. The space between the exterior of the mesh tubing and the interior of the polycarbonate tubing is filled with color-indicator desiccant material. The desiccant was heat-regenerated at regular intervals when any color change was visually apparent to avoid saturation. The air passing through the desiccator tubes can be dried to a certain degree depending on various conditions including the time, pressure and flow rate of nebulization, the ambient temperature and RH conditions and the humidity content of the desiccant material. The length of desiccator tubing can be modified in 12 inch increments between 0 and 60 inches in order to reach various levels of RH. The stability of the RH was assessed over 14-h periods (triplicate repeats) with a rotation speed of 1 rpm. Humidity decreased by $2.2 \pm 0.2\%$, $3.3 \pm 0.6\%$, $3.3 \pm 0.4\%$, respectively, at 20, 50 and 80% initial RH.

A sprocket anchored to the outer ring of the ball bearing form the right side cap (Figure 1M) allows chamber rotation by means of a motor driven chain. A DC gear motor, located outside the temperature controlled enclosure, is modulated by a speed controller. A through-wall shaft connects the motor to a nine tooth sprocket which is linked by a roller chain to the 60-tooth sprocket on the cylindrical chamber inside the temperature controlled enclosure. The rotation speed of the chamber can be adjusted between 0.1 and 1 rotation per minute (rpm).

In order to avoid pressure build up in the chamber during nebulization, a ball valve connected to a capsule high-efficiency particulate air (HEPA) filter is opened to release the excess pressure. The same principle is used during sampling; the sampled air is replaced by HEPA filtered air from the surrounding environment. The filters used to equilibrate the pressure during nebulization and sampling are located on the opposite ends of the nebulization inlets and sampling outlets. A differential pressure gauge is integrated through a port of the chamber that allows monitoring pressure variations inside the chamber.

Aerosols were produced within the chamber to measure the variations in particle concentrations and size distributions. A six-jet Collison nebulizer was used at a pressure of 20 psi and a total flow rate of 12-L per minute (L/min) with phage buffer (10 mM Tris-HCl, 100 mM NaCl, 10 mM MgSO_4 , adjusted to pH 7.4) as a nebulization liquid. The nebulizer was operated continuously for 15 min; aerosols were initially partially desiccated by passing through a 24-inch long desiccation tube prior to introduction into the aerosol chamber through one end; a HEPA-filtered outlet opposite the aerosol inlet served as a passive exhaust during nebulization. After the 15-min nebulization period all inlets and exhaust outlets were closed, essentially sealing the chamber. Particle concentration measurements, including size estimations (e.g. mass median aerodynamic diameter (MMAD)) were obtained using an aerodynamic particle sizer (APS, model 3321, TSI Inc., Shoreview, MN) equipped with a diluter (model 3302A setup with capillary 1/100, TSI Inc.) operated at 50 mL/min. The APS extracted air from the chamber for 25 s prior to the initiation of data acquisition which lasted 20 s. Thus, every APS sampling extracted 37.5 mL of air from the chamber representing less than 0.1% of the total chamber volume; this did not measurably

affect the aerosol concentration or the level of RH in the chamber. Initial sampling with the APS was performed 2 min after the completion of the nebulization procedure; a second sample was taken 18 h post-nebulization. This experiment was repeated in triplicate at either 1 rpm or without rotation. The temperature and RH within the aerosol chamber was maintained at 21 °C and 40%, respectively.

An average concentration of $2\text{E} + 05$ particles/cm³ (standard deviation: $1\text{E} + 05$) was initially nebulized into the chamber. The remaining portion of these particles 18 h post-nebulization was $8.8 \pm 0.6\%$ and $2.6 \pm 0.2\%$ at one and zero rotations per minute, respectively. The total mass remaining was $16.2 \pm 0.1\%$ at 1 rpm and $1.8 \pm 0.2\%$ without rotation within 18 h post-nebulization. The MMAD was 1.21 ± 0.04 µm ($n = 6$ measurements) at time 0 and 1.30 ± 0.02 µm ($n = 3$ measurements) after 18 h of suspension with a chamber rotation speed of 1 rpm. The MMAD decreased slightly to 0.91 ± 0.01 µm ($n = 3$ measurements) after 18 h without rotation. The evolution of the aerodynamic profile of the aerosol with and without rotation is illustrated in Figure 3. Statistical analysis (*t*-test) performed with the SigmaPlot software (Systat Software, Inc., Version 11.2, San Jose, CA) indicated no difference between MMAD values at time 0 with and without rotation. The increase of the MMAD at 1 rpm and the decrease at 0 rpm were considered statistically significant ($p < 0.01$). The rotation of the chamber thus allows for higher concentrations of particles in the range of 1 µm to stay suspended for prolonged periods.

Conclusions

The performance evaluation of the apparatus demonstrated the effectiveness of the chamber to maintain 1 µm particles in the air-borne state over longer periods during chamber rotation. The principles of action of these chambers have been well described (Asgharian & Moss, 1992; Gruel et al., 1987) and the test performed in this evaluation confirms that the performance of this particular chamber is consistent with published studies.

Four significant modifications were made to this rotating chamber in order to support bioaerosol studies that require operation over long periods of time. The first modification was the use of stationary access inlet ports used in an earlier study (Verreault et al., 2013) and similar to the configuration used by others (Santarpia et al., 2012). These access ports allow aerosol generation and sampling procedures to take place without necessitating the need to stop rotation of the chamber. Sampling can proceed at different time points with minimal disruption of operation of the chamber. The stationary inlet ports also facilitate installation of a stationary UV light within the chamber as well as the use of electronic sensors, such as RH and temperature probes or photo detectors, all of which are in a stationary position during normal operation (rotation) of the chamber.

The second modification was the use of an insulated polycarbonate enclosure for temperature control. The enclosure was designed to be both the physical support to hold up the rotating chamber and an indirect temperature control for the interior of the chamber. The temperature control evaluation demonstrated the stability of the desired temperature within the chamber with a tolerance of only 0.5 °C over a 48-h period. A wide range of set point

temperatures could be attained (8–45 °C) over the course of an experiment within the enclosure.

A third adaptation was the ability to manipulate and control RH by pretreatment of the aerosols and airstream prior to chamber introduction. Once the chamber is sealed for aerosol aging, a RH probe is used to gather data; however, there is no control of the humidity at this point. The evaluations demonstrated that the humidity is stable within the chamber although minor variations due to diffusion through the sealed bearings and to condensation on the walls of the chamber at lower temperatures. Improvements could be made to the insulation enclosure where the humidity could be controlled in order to minimize losses due to diffusion.

The fourth modification was the addition of a UV-C light placed at the center of the rotating chamber for disinfection experiments. The UV-C can be operated with no short-term effects on the maintained temperature or initial RH. Long-term effects were not evaluated since the bioaerosols which are intended to be studied with this apparatus would be inactivated by prolonged exposures to UV light.

Although it is clear that exact interior and exterior environmental settings cannot be duplicated in this chamber, controlling the temperature, RH and UV-C exposure can provide valuable information on the influence of these parameters on microbial integrity. The simple design and ease of operation of this apparatus makes it an ideal tool to study aerosols of non-pathogenic microorganisms over prolonged periods.

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Declaration of interest

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Figure 1.

Aerosol aging chamber. Left (A) and front view (B). The 55.5-L aluminum aerosol aging chamber (C) is placed inside a temperature controlled enclosure composed of polycarbonate panels (D), which also serves as a support for the chamber. The enclosure is insulated on all sides with one-inch thick closed-cell polyvinyl chloride foam sheets (E). Two thermoelectric cooling/heating assemblies (F) are supported by the back panel of the insulated enclosure; temperature is controlled with a dual-action thermoelectric temperature controller regulated by a temperature probe placed at the bottom front inside the enclosure (not shown). The chamber (C) is sealed on both ends with caps (G; left and H; right) held in place with latches (not shown). The caps are made of aluminum discs (I) mounted on the outer ring of double-sealed ball bearings (J). The interior rings hold aluminum rods (K) through which sampling inlets and electrical connections are installed; these rods remain stationary during chamber rotation. The UV-C lamp (L) is connected to the electrical socket located in the center of the aluminum rod of the left cap (G). The eight other ports around the socket are used for the UV sensor (not shown) and for sampling. The right cap (H) has a one-inch NPT-threaded inlet at its center and eight other ports around it used for nebulization and for temperature and humidity probes (not shown). A sprocket (M) is anchored to the outer ring of the double-sealed ball bearing. Rotation is provided by a speed controlled motor (not shown) placed outside the enclosure and connected by a through-wall shaft to a nine tooth sprocket (not shown) linked with a roller chain to the 60 tooth sprocket (M) on the chamber (C). All sampling inlets can be sealed with threaded caps, rubber plugs or ball valves (not shown).

Figure 2.

Temperature modulation: comparison of the temperature inside the rotating chamber to the temperature inside the temperature-controlled enclosure. The chamber reached parity with the set point temperature in the enclosure in approximately 40 min when enclosure temperature was increased 8 °C to 45 °C.

Figure 3.

Aerodynamic distribution of mass of air-borne particles in the chamber with a rotation speed of 1 rpm (A) and without rotation (B). Sampling was performed immediately after nebulization (0 h) (dotted lines) and + 18 h (solid line) of aging time. The mass median aerodynamic diameter (MMAD) is shown as a vertical line and notated on each graph. The geometric standard deviation is presented within brackets.