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Production and characterization of bioaerosols for model validation in spacecraft environment

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ABSTRACT

This study aimed to evaluate the suitability of two bioaerosol generation systems (dry and wet generation) for the aerosolization of microorganisms isolated from the International Space Station, and to calibrate the produced bioaerosols to fulfill the requirements of computational fluid dynamics model (CFD) validation. Concentration, stability, size distribution, agglomeration of generated bioaerosol and deposition of bioaerosols were analyzed. In addition, the dispersion of non-viable particles in the air was studied. Experiments proved that wet generation from microbial suspensions could be used for the production of well-calibrated and stabile bioaerosols for model validation. For the simulation of the natural release of fungal spores, a dry generation method should be used. This study showed that the used CFD model simulated the spread of non-viable particles fairly well. The mathematical deposition model by Lai and Nazaroff could be used to estimate the deposition velocities of bioaerosols on surfaces, although it somewhat underestimated the measured deposition velocities.

Introduction

Bioaerosols are recognized as important contributors to impaired indoor air quality. They are defined as artificially generated or naturally released particles of biological origin (*e.g.*, bacteria, fungal spores and fragments, viruses *etc.* as well as other parts or products of organisms *i.e.*, endotoxins, lipopolysaccharides or fungal mycotoxins) suspended in the air (Gorny et al., 1999; Kulkarni et al., 2011). Airborne biological particles can exist as single cells and spores or as agglomerates of microorganisms. Many indoor bioaerosols originate outdoors but specific bioaerosol sources may develop because of microbial growth in building materials, communication devices or heating, ventilation and air-conditioning (HVAC) systems (Agranovski and Grinshpun, 2010). Humans are important sources of certain bacteria and viruses (Nazaroff, 2014) and human occupancy and activities affect indoor microbiology (Hospodsky et al., 2012). Also for fungi, human activities play also an important role, for example, in shedding particulate matter from our clothing that can contain materials of fungal origin (Nazaroff, 2014).

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In order to grow, microorganisms must have water and numerous chemical elements, *i.e.*, nutrients, to fulfill the chemical growth requirements. Physical growth requirements are for instance temperature, pH and osmotic pressure. For instance, most fungal species are not able to grow unless the air humidity is at least 20% or the equilibrium relative humidity of a material exceeds 65% (Pasanen et al., 2000). Nevertheless, accumulations of dirt and dust can serve as nutrients and may allow fungi to grow at a lower relative humidity level (Viitanen, 1994; Pasanen et al., 2000; Viitanen et al., 2010). In contrast, bacteria can grow under very diverse conditions, which is why they are found nearly everywhere on Earth.

Microbes are also found in spacecraft, which can be seen as an exceptional environment because of microgravity, vacuum, UV rays, fluctuating temperatures and humidity, space and cosmic radiations. The presence of microbes in space habitats can cause health risks for crewmembers and expose instrumentation to biodegradation.

Crewmembers are a major source of microorganisms onboard spacecraft, with most of the released microbes being generally harmless, in addition to some opportunistic pathogens (Checinska et al., 2015; Pierson, 2001). Most of the detected bacteria from the International Space Station (ISS) belong to the human microbiota (Ichijo et al., 2016). In combination with exceptional working and living conditions, astronauts are vulnerable to microbial exposures, which may cause adverse health effects including infections and allergies (Vesper et al., 2008). Moreover, microbes have caused biodegradation of materials on board spacecraft (Van Houdt et al., 2012; Vesper et al., 2008). These risks will be more significant during long-duration spaceflight missions.

Airborne bacterial and fungal contamination levels were monitored at ISS (1986–2001) and Mir space station (1998–2005) during the occupation. In general, bacterial concentrations were less than 500 colony forming units CFU/m^3 in the indoor air, although occasional increases were noticed because of human exercise (Novikova et al., 2006; Novikova, 2004). The concentration of fungi ranged up to 10,000 CFU/m^3 of air, and the fungal contamination of surfaces onboard Mir and ISS was high, with levels ranging up to 10^7 CFU/100 cm^2. The contamination levels on surfaces and in air varied strongly even over the threshold limits but were in most cases low and below the implemented threshold limits (Novikova et al., 2006), which are 1000 CFU/m^3 for bacteria and 100 CFU/m^3 for fungi in the indoor air of ISS (ISS MORD SSP 50260). On surfaces, limits are 10,000 CFU/100 cm^2 for bacteria and 100 CFU/100 cm^2 for fungi. The most commonly isolated bacteria and fungi from the air of spacecraft are *Staphylococcus* and *Bacillus* species, and *Penicillium* and *Aspergillus* species, respectively (Novikova et al., 2006; Vesper et al., 2008). Ichijo et al. (2016) showed that members of the *Actinobacteria*, *Firmicutes* and *Proteobacteria* were frequently detected on the surfaces of the ISS KIBO module. In particular, *Staphylococcaceae* belonging to the *Firmicutes*, and *Enterobacteriaceae* and *Neisseriaceae* belonging to the *Proteobacteria* were dominant on the equipment surfaces.

In order to monitor and control biocontamination, it is important to increase the understanding of bioaerosol dispersions. The latter can be described and predicted by mathematical models, which require well-calibrated bioaerosols for their calibration and validation. Bioaerosols are used to verify that biological particles behave similarly to those generated from non-biological origin. The mathematical model can be used to pinpoint critical locations in a certain habitat design or operation and to steer the development of adequate prevention and monitoring procedures. Numerical simulations with validated models will help to identify the risk areas where microbial growth may occur and to direct control measures appropriately.

In the BIOSMHARS project, a 2-year (2011–2013, EC FP7) joint EU-Russia research effort, a computational fluid dynamics model (CFD) was developed to describe and predict the airborne microbial contamination in confined space habitats taking into account the specific ventilation characteristics in such habitats.

1. Materials and methods

1.1. Microorganisms

Three microbial strains, which were previously isolated from ISS, were selected for bioaerosol production studies. These selected isolates belong to species that were reported to be the dominant species causing microbial contamination in MIR, ISS and analogues (Ichijo et al., 2016; Novikova et al., 2006; Vesper et al., 2008). The isolates were identified as belonging to the species *Penicillium expansum, Staphylococcus epidermidis* and *Bacillus aerius/licheniformis*. Fungal identification was based on morphological characteristics. Bacterial identification was based on partial 16S rRNA gene amplification (with universal primer set 8F/1492R), sequencing and comparison. Based on the obtained 16S rRNA gene fragment, discrimination between *B. aerius* and *B. licheniformis* was not possible because of too high sequence homology.

1.2. Dry generation

1.2.1. Penicillium expansum

A sporulating fungal culture of *P. expansum* was prepared with the glass beads method adopted from Schmechel et al. (2003) and Salmela et al. (2017). Briefly, *P. expansum* was inoculated with a loop on Czapek Dox agar (Sigma-Aldrich Co., St. Luis, MO, US) and incubated for 7–14 day at 26 ± 2°C. Fungal spores were harvested from matured well-sporulating cultures by dry, sterilized acid-washed glass beads by adding 1 g of glass beads to the agar plate and rolling the beads over the fungus. Next, the beads were transferred into a sterile tube containing 15 mL sterile deionized water with 0.05% Tween80. The spores were suspended and separated from the beads by briefly shaking the tube and decanting the spore suspension into a new tube. An aliquot of the *P. expansum* suspension (10^6 spores/mL) was used to inoculate fresh Czapek Dox agar with Chloramphenicol (10.1 g 0.01 L^{-1} EtOH) (plate size of 12 × 12 cm), which were incubated for 7–21 days at 26 ± 2°C, to obtain fungal cultures for aerosolization with a Fungal Spore Source Strength Tester (FSSST) (SKC, PA, US) at airflow rate of 12.5 L/min.

1.3. Wet generation

1.3.1. Penicillium expansum

P. expansum suspension was prepared for wet generation experiments as described in Section 1.2.1, except that the

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