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Feasibility of Generating Peaks of Bioaerosols for Laboratory Experiments

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ABSTRACT

Bioaerosol concentration peaks are frequently encountered in real-life atmospheres (indoor, outdoor, workplace, etc.), where they can be caused by several factors. However, evolution over time and variability of microbiological pollutant concentrations remain under-documented. The contribution of such peaks in the onset or the worsening of respiratory symptoms – in particular immuno-allergic reactions – has yet to be extensively studied. Although experimental bioaerosol generators are increasingly used, the intentional and controlled production of concentration peaks of biological agents has not yet been utilized in laboratory experiments. The main objective of this study was to show that it is possible to produce experimental bioaerosol concentration peaks with defined characteristics. Experiments were performed with a 'Liquid Sparging Aerosoliser'-type generator. With this system, peaks can be created by increasing the bubbling airflow through a film of bacterial (*Escherichia coli*) or fungal (*Penicillium brevicompactum*) liquid culture. The higher the set point value of the bubbling airflow, the greater the maximum bioaerosol concentration during the generated peak. Similarly, longer-lived peaks can be created by maintaining the increased airflow for a longer period of time. For both studied species, the relative size distribution was constant over time, regardless of modifications to the bubbling flow rate. The operator can monitor and control peak formation with this system thanks to real-time measurement of the number concentrations, targeting the appropriate particle size classes corresponding to diameters of the aerosolised microorganisms. This generator, characterized by gentle aerosolisation and a capacity to produce bioaerosol peaks, may contribute to enrich laboratory experiments for numerous applications.

Keywords: Bubbling generator; Bacteria; Fungi; *Escherichia coli*; *Penicillium brevicompactum.*

INTRODUCTION

Bioaerosol concentration peaks are encountered in many atmospheres (indoor, outdoor, workplace, office, etc.). To date, the relationship between these concentration peaks and effects on human health are not well known for any atmosphere. However, sharp peaks of airborne biological and allergenic particles may be an important characteristic of exposure (Burge, 1995a; Elms *et al.*, 2001). Among the different determinants of individual reactivity to antigen, the duration, intensity and frequency of exposure affect response and health effects (Burge, 1995b; Müller *et al.*, 2006). Human biologic responses may intensify with high peaks of concentrations that overwhelm lung defense mechanisms. It therefore appears necessary to study exposure to bioaerosols with concentration peaks, and to determine the effects of this exposure on the onset or the worsening of respiratory symptoms, particularly immuno-allergic symptoms.

This type of peak can be produced in a real atmosphere by many events: working tasks (Agranovski *et al.*, 2004; Gillum and Levetin, 2008; Duquenne *et al.*, 2012; O'Shaughnessy *et al.*, 2012), ventilation systems (Law *et al.*, 2001), various processes (manufacturing, treatment, spreading, machining /metalworking fluids, etc.), climatic or seasonal events (e.g., pollen), accidental situations, cleaning operations (including the use of compressed air guns contrary to recommendations), etc. Problems related to concentration peaks have been studied for some pollutants, including the following non-exhaustive list: silica (Checkoway and Rice, 1992), synthetic fibres (Daroowalla *et al.*, 2005), particulate air pollution (Delfino *et al.*, 2002), wood dust (Edman *et al.*, 2003), seed dust (Smit *et al.*, 2006), sodium borate (Eisen *et al.*, 1991), metalworking fluid aerosols (O'Brien *et al.*, 2001; Oudyk *et al.*, 2003; Lillienberg *et al.*, 2008), flour dust/aeroallergens (Nieuwenhuijsen *et al.*, 1995; Meijster *et al.*, 2008), organic solvents (Preller *et al.*, 2004). In addition, video exposure was used to monitor exposure to gases and dust (Rosen *et al.*, 2005). But in the case of bioaerosols, the evolution over time of concentrations remains under-documented and have yet to be studied.

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Laboratory bioaerosol generators are increasingly used in studies measuring the performance of bioaerosol samplers and real-time monitoring instruments, developing sampling and analysis methods, evaluating new protective equipment, investigating inhalation toxicology or studying transport, ageing and deposition of airborne microorganisms (Thorne, 2000; Reponen *et al.*, 2001; Agranovski *et al.*, 2002, 2003; Seo *et al.*, 2007; Huang *et al.*, 2008; Kanaani *et al.*, 2008a, b; Agranovski *et al.*, 2010; Grinshpun *et al.*, 2010; Hwang *et al.*, 2010; Xu *et al.*, 2011).

In the best case, tests were performed with a stable bioaerosol in terms of concentration, size distribution, etc. However, experimental bioaerosols can be significantly affected by fluctuations linked to the technique used to generate them (see Wong (2007) for the example of inhalation exposure systems). For example, the physical and biological properties of a bioaerosol generated with a nebuliser change progressively during generation (Qian *et al.*, 1995; Terzieva *et al.*, 1996; Reponen *et al.*, 1997; Mainelis *et al.*, 2005). Similarly, blow-type dry generators often release most of the biological particles in a short time, creating a primary particle puff at the outlet of the delivery system. Because of the rapid decrease in the particle number concentration, such dry generation methods cannot generate a stable and uniform bioaerosol concentration over extended periods (Jung *et al.*, 2009; Kanaani *et al.*, 2009). The operator generally has to manage these unexpected changes in the composition of the bioaerosol generated, indeed in most tests they are not sought or controlled.

To our knowledge, intentional, controlled generation of bioaerosols with concentration peaks has never been used in a laboratory study. It could, nevertheless, be a useful, or even necessary, tool for novel research and also to complete some previous works. Different examples can be cited:

- Link between acute health effects and exposure to isolated or repeated peaks of biological agents/Inhalation toxicology studies: As many sources release bioaerosols as "concentration bursts", how does this type of short-lived strong exposure affect the onset or exacerbation of respiratory diseases and allergy symptoms? Feasibility to deliver the same inhaled or deposited dose of an aerosolised biological agent either as a constant bioaerosol or as an aerosol with controlled peaks during inhalation toxicology studies (notion of inhaled dose vs. inhaled dose rate)?
- Biocollector efficiency and definition of sampling approaches reflecting exposure to real bioaerosols: What are the performances and limits of long-term (2 to 8 hours) bioaerosol samplers when faced with a very unstable biological aerosol consisting of peaks of variable duration and intensity? When peaks of exposure are expected, is it better to perform a single long-term sampling or several successive short-term samplings (where cost, time-consumption, consistency with the analysis' limit of quantification, sampling durations, etc. have to be defined)?
- Real-time detection of aerosols containing microorganisms (military and non-military applications): What are the performance (e.g., fluorescence sensitivity) and limits (e.g., saturation) of a selected real-time system (e.g., UVAPS) for detection of sudden increases in the airborne biological particle concentration above a typical background level? How should fluorescence data be interpreted when the system encounters many bioaerosol peaks containing several different microorganisms?
- Performances of personal or collective protective equipment (capture devices, filtration, ultra-violet purification, thermal inactivation, etc.) against bioaerosol: Is a given protective equipment reliable when faced with Dirac pulses and/or brief peaks of biological airborne contaminants? Performance of protective systems may deteriorate with time, is this deterioration exacerbated when the system has to deal with peaks of pollution?

A 'Liquid Sparging Aerosoliser'-type generator was considered as a promising choice to produce peaks of bioaerosol. The selection of this liquid-based generation was mainly guided by 1) its gentle bubbling aerosolisation, which minimizes stress and damage to microorganisms and 2) its flexibility, allowing the operator to adjust several operating conditions to produce a bioaerosol with specific expected properties (concentration, size distribution, composition, etc.). *Escherichia coli* and *Penicillium brevicompactum* were chosen as the model microorganisms.

The main objective of this work was to show the ability to produce bioaerosol concentration peaks with defined characteristics in an experimental setting.

MATERIALS AND METHODS

Description of the Setup for Bioaerosol Generation and Characterisation

The test rig includes a bubbling-type generator and a chamber to condition and sample bioaerosols (Fig. 1). This setup and all the materials used have been previously described (Simon *et al.*, 2011, 2013). Briefly, a peristaltic pump feeds the liquid culture onto the upper surface of a 2-mm thick stainless steel porous disc (Stemm, 30 mm in diameter, 1 µm pore diameter), which settles as a liquid film. A patented dispersion cell is used to produce a constant, reproducible liquid film height (H_{liq} = 8 mm) throughout a test and between tests. Microorganisms are dispersed by bubbling compressed air (flow rate $0.1 \leq Q_G \leq 5$ L/min) through the film of liquid culture. Transport of the aerosolised particles towards the generator outlet is improved by an upward injection of entraining air (flow rate Q_E = 20 L/min), which is composed of a mixture of dry and humid air in adjustable proportions to ensure a constant relative humidity (RH = 50 ± 2% - thermo-hygrometer Rotronic®, HygroPalm2) throughout the assay. The compressed air was first dried and filtered in a treatment unit (TSI™, model 3074B); airflow rates were then adjusted using regulated thermal mass flow metres (Brooks®, models 5850S and 5851S). During experiments, values of bubbling airflow Q_G were modified using a mass flow metres' read-out and control unit (Brooks®, model 0154–0.1 L/min accuracy).

The test rig is completed by a 12-L sampling chamber where the bioaerosol is conditioned and sampled. This chamber is equipped with six 20 cm long, 10 mm i.d. sampling probes, for simultaneous connection of devices (real-time instruments, samplers, etc.).

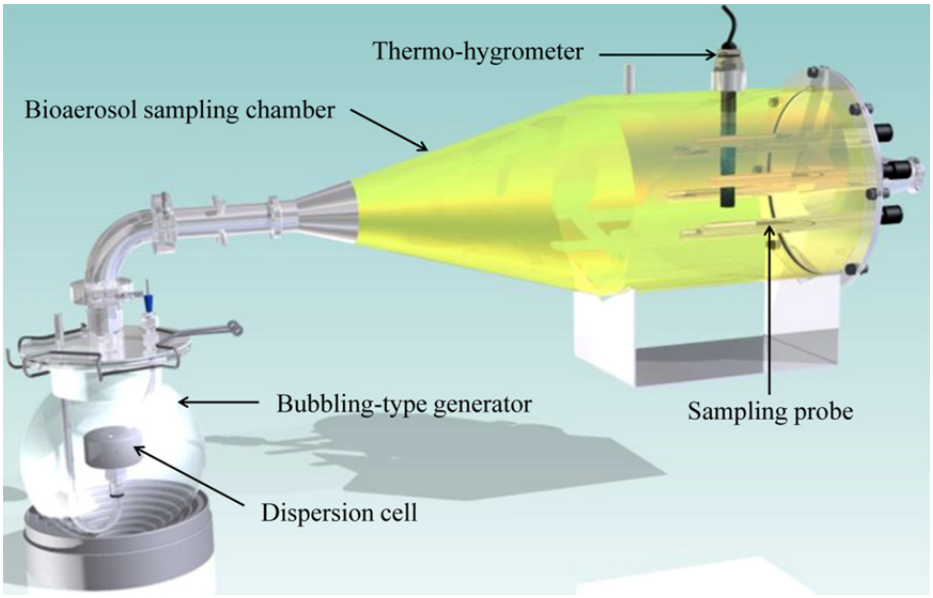

Fig. 1. Diagram of the test rig used to generate and sample the experimental bioaerosol.

Number Concentration and Size Distribution of Airborne Particles

An Optical Particle Counter (OPC-Grimm®, G1109) and a Laser Aerosol Spectrometer (LAS-TSI™, model 3340) were used for real-time monitoring of the particle number concentration and the size distribution of the particles generated. The OPC-Grimm® works at a sampling airflow of 1.2 L/min and classes particles according to their size ("optical" diameter, d_{opt}) between 0.25 and 32 µm, across 31 channels. Particle counting with this instrument was integrated over 6 s. The LAS is an optical-scattering laser-based spectrometer which detects particles in the 0.09 to 7.5 µm size range. Counted particles are binned into up to 100 user-defined particle size channels anywhere within its size range. Sample flow can be adjusted from 5 to 100 sccm and sheath flow is typically 650 sccm. Particle counting with the LAS was integrated over 12 s.

The size distribution of the particles generated was also assessed with an Aerodynamic Particle Sizer® (APS-TSI™, model 3321). The APS spectrometer measures the aerodynamic diameter (d_{ae}) in real-time for particles from 0.5 to 20 µm, using 52 channels and a sampling flow rate of 5 L/min.

Each averaged number size distribution was used to adjust a log-normal distribution (least squares optimization) and to determine the median optical diameter ($\overline{d_{opt}}$) or median aerodynamic diameter ($\overline{d_{ae}}$), and the associated geometric standard deviation (σ_g).

Microbial Strains

The model organisms *E. coli* (Institut Pasteur CIP 53.126) and *P. brevicompactum* (UMIP 2338.96) were used for the tests in this study. They were selected as representative of sensitive bacteria and fungi. Both these microorganisms are frequently used in laboratory-based bioaerosol assays; they are also widely present in indoor air or occupational environments.

Preparation of Microbial Liquid Cultures

The preparation of liquid cultures was completed on the day of bioaerosol generation.

E. coli was first sub-cultured on trypicase soya agar medium (AES Chemunex) for 24 h at 37°C. Freshly grown cells were then scraped from the agar surface and used to inoculate a sterile tryptone-salt solution (AES Chemunex) at a final optical density at 600 nm (OD_{600}) close to 0.5 (ThermoSpectronic, Spectrophotomètre UV/visible Helios Gamma). A 2 mL aliquot of this preparation was added to 25 mL sterile lactose broth (AES Chemunex), and immediately incubated for 24 h, at 37°C and 300 rpm in a shaking incubator (Infors HT, Minitron). Cells were then harvested by washing the liquid culture three times in sterile ultra-pure water. Each washing cycle consisted in centrifuging the culture at 5100 g for 7 min (Sigma®, 3–18 K) and eliminating the supernatant. After washing, the cell pellet was suspended in sterile ultra-pure water to produce a cell suspension with an $OD_{600} = 0.30 \pm 0.03$. The corresponding culturable bacteria concentration in the liquid suspension was 2×10^8 CFU/mL. This suspension was subsequently used to produce experimental bioaerosols.

P. brevicompactum was first sub-cultured on malt extract agar medium (BD Difco™ Malt Agar) for 7 days at 25°C. Freshly grown spores and other fungal entities were scraped from the agar surface and transferred into 10 mL sterile water. This preparation was filtered on a glass frit (Duran®) of porosity 2 (40–100 µm). The frit was then rinsed with 5 mL sterile water. The filtrate volume was topped up to 25 mL with sterile water. Spores were harvested by washing twice the liquid culture in sterile ultra-pure water. Each washing cycle consisted in centrifuging the culture at 5100 g for 7 min and eliminating the supernatant. After washing, the fungal pellets were resuspended in 25 mL water to produce a suspension with an optical density at 600 nm, $1 \leq OD_{600} \leq 1.3$. The corresponding culturable fungi concentration in the liquid suspension was 1×10^6 CFU/mL. Microscopic

examination of this suspension revealed that it contained mostly spores and few mycelium fragments/strands.

Experimental Procedure for Bioaerosol Generation

The first test generated a bioaerosol from a liquid *E. coli* culture for around 160 min. The bioaerosol was initially generated at a low bubbling airflow, $Q_{G,baseline}$ = 0.1 L/min. The bubbling airflow was then increased to a higher value, $Q_{G,peak}$. The $Q_{G,peak}$ airflow was maintained for a Δt period (which was either short ≈ 3 min, or long ≈ 8 min) before returning to $Q_{G,baseline}$. Various $Q_{G,peak}$ values were set: 1.0, 1.8, 2.5 and 5.0 L/min. *E. coli* generation was monitored using both APS and Grimm® G1109.

The second test generated a bioaerosol from a liquid *P. brevicompactum* culture for around 90 min. The bioaerosol was first generated at a low bubbling airflow, $Q_{G,baseline}$ = 0.5 L/min. The bubbling airflow was then increased to different $Q_{G,peak}$ values: 2.0, 3.5, 4.0, 4.6, 4.8 and 5.0 L/min. The high $Q_{G,peak}$ values were maintained either for a short (Δt ≈ 3 min) or a long (Δt ≈ 10 min) period. *P. brevicompactum* generation was monitored using the APS and LAS systems.

RESULTS

Evolution of Particle Number Concentration - Escherichia Coli Experiment

Fig. 2 shows the evolution of the particle number concentration measured with the Grimm® G1109 for d_{opt} > 0.3 µm over 160 min continuous bioaerosol generation.

The first four events on this profile correspond to plateau peaks produced by setting the $Q_{G,peak}$ value to 5, 2.5, 1.8 and 1 L/min, respectively from left to right. Plateau peaks emerged when the $Q_{G,peak}$ was maintained for Δt ≈ 8 min.

Between 60 and 125 min of generation, two comparable series of short-lived peaks (Δt ≈ 3 min) were created. Each of these series shows four peaks, the amplitude is related to the four $Q_{G,peak}$ values studied. Finally, after 130 min, a more complex event was created with a plateau ($Q_{G,peak}$ = 1 L/min) onto which a first transient peak ($Q_{G,peak}$ = 5 L/min), then a second ($Q_{G,peak}$ = 2.5 L/min) were added. This is followed by a final peak ($Q_{G,peak}$ = 1.8 L/min).

A longer Δt produces a longer-lived peak, or plateau peak; a shorter Δt produces a temporary peak, or transient peak. The higher the $Q_{G,peak}$ airflow set by the operator, the higher the maximal particle number concentration ($C_{N,max}(d_{opt}$ > 0.3 µm)) measured for the peak. Thus, $Q_{G,peak}$ values of 5 L/min supply the highest concentrations, at > 350,000 #/L, while $Q_{G,peak}$ values of 1 L/min provide lower concentrations, between 30,000 and 40,000 #/L depending on the peak generated. The two other $Q_{G,peak}$ values, 2.5 and 1.8 L/min, provide intermediate concentrations. Thus, for the $1 \leq Q_{G,peak} \leq 5$ L/min range, the relationship between the maximal particle number concentration reached during a peak, $C_{N,max}$ (d_{opt} > 0.3 µm) and the airflow $Q_{G,peak}$ is statistically significant: $C_{N,max}(d_{opt} > 0.3\ \mu m) = -39173 + 86118 \times Q_{G,peak}$ (n = 16, r = 0.987, P = 0.000, StatGraphics Centurion XV version 15.2.00).

Size Distribution of the Particles Generated - Escherichia Coli Experiment

An example of the size distribution of airborne particles as a function of their optical diameter, measured using a Grimm® G1109 is shown in Fig. 3(a).

It characterizes the bioaerosol generated as a plateau peak, produced with a bubbling airflow rate, $Q_{G,peak}$, of 2.5 L/min (Fig. 2). The main population has a median optical diameter, d_{opt} , of 0.66 µm, with a geometric standard deviation, σ_g, of 1.20. Airborne bacterial particles presented

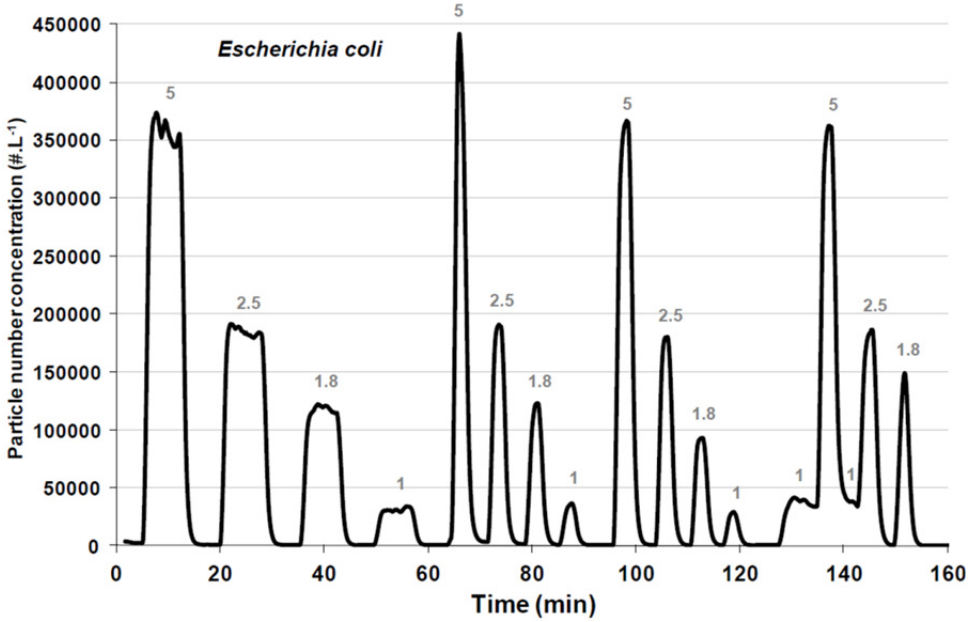

Fig. 2. Number concentration (Grimm® G1109, d_{opt} > 0.3 µm) over 160 min bioaerosol generation as a function of Q_G. Grey number above each peak indicates the corresponding value of $Q_{G,peak}$ airflow rate (L/min). $Q_{G,baseline}$ = 0.1 L/min; $1 < Q_{G,peak} < 5$ L/min; Q_E = 20 L/min; RH = 50 ± 2%; H_{liq} = 8 mm.

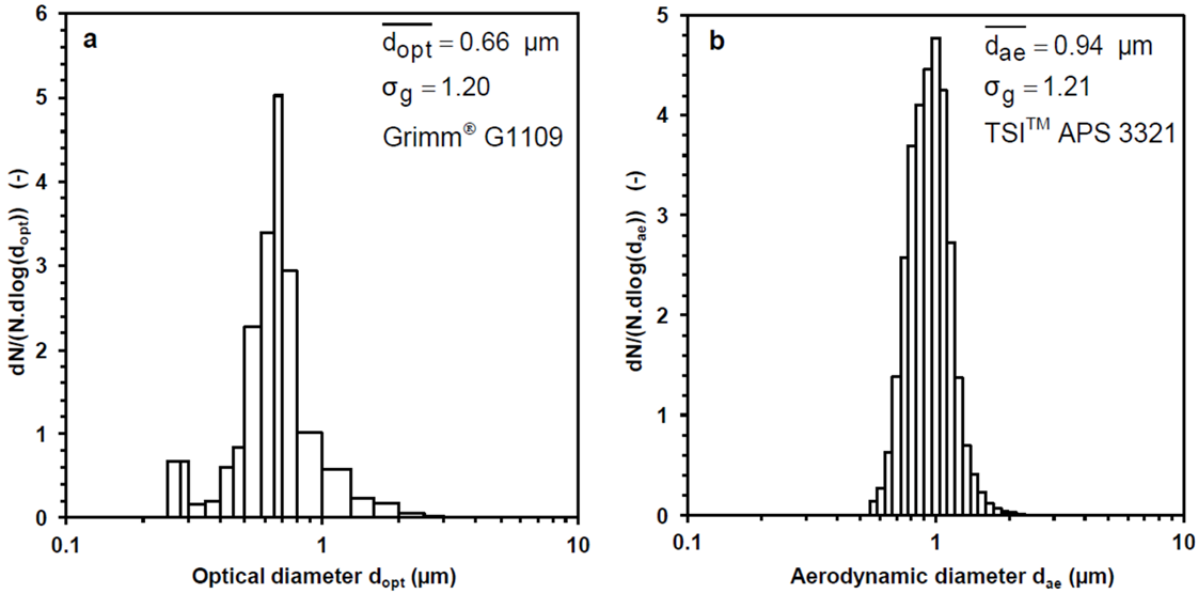

Fig. 3. Size distribution for particles generated during a representative plateau-type peak. *E. coli*; $Q_{G,peak}$ = 2.5 L/min; Q_E = 20 L/min; RH = 50 ± 2%; H_{liq} = 8 mm.

optical diameters between 0.3 and 3 µm. The corresponding size distribution, measured using an APS-TSI™, presents a median aerodynamic diameter, $\overline{d_{ae}}$, of 0.94 µm and a geometric standard deviation of 1.21 (Fig. 3(b)).

Similar values were found across all the peaks generated for both short-lived and plateau-type peaks: between 0.65 and 0.67 for the median optical diameter; and between 1.19 and 1.21 for the geometric standard deviation.

Evolution of Particle Number Concentration - Penicillium brevicompactum Experiment

Fig. 4 shows how the particle number concentration, measured with the LAS-TSI™ for d_{opt} > 1.5 µm, evolved over 90 min continuous bioaerosol generation. A first series of six short-lived peaks ($\Delta t \approx 3$ min) is apparent. The intensities of these peaks correlated with the $Q_{G,peak}$ value: 5, 4.8, 4.6, 4, 3.5 and 2 L/min, respectively from left to right. Between 45 and 60 min of generation, short-lived peaks were generated with $Q_{G,peak}$ values of 4 and 5 L/min. Finally, two plateau peaks were created by adjusting the generation flow to 5, then 4.8 L/min for 10 min. The maximal concentrations reached at the apex of peaks are between 9 500 #/L for the highest $Q_{G,peak}$ studied (5 L/min), and 1 200 #/L for the lowest (2 L/min). Over the $2 \leq Q_{G,peak} \leq 5$ L/min range, the relationship between the maximal particle number concentration reached during a peak, $C_{N,max}(d_{opt} > 1.5$ µm) and the airflow $Q_{G,peak}$ is statistically significant: $C_{N,max}(d_{opt} > 1.5$ µm) = 352.3 × exp(0.635 × $Q_{G,peak}$) (n = 10, r = 0.989, P = 0.000, StatGraphics Centurion XV version 15.2.00).

Size Distribution of the Particles Generated - Penicillium brevicompactum Experiment

An example of the size distribution of airborne fungal particles as a function of their optical diameter, measured using the LAS-TSI™ is shown in Fig. 5(a).

This distribution characterises the bioaerosol generated throughout the duration of the plateau peak produced with a bubbling airflow rate, $Q_{G,peak}$, of 5 L/min (Fig. 4). The population presents a median optical diameter of 1.91 µm with a geometric standard deviation of 1.10. Airborne fungal particles presented optical diameters between 1.5 and 4 µm. The corresponding size distribution, measured using the APS-TSI™, has a median aerodynamic diameter of 3.31 µm with a geometric standard deviation of 1.09 (Fig. 5(b)). Similar values were found for both short-lived and plateau-type peaks: between 1.89 and 1.91 for the median optical diameter; and between 1.09 and 1.11 for the geometric standard deviation across all the peaks generated.

DISCUSSION

Feasibility of Generating Peaks of Bioaerosol

The bubbling generator studied is clearly capable to produce concentration peaks (Figs. 2 and 4). Changing the value of bubbling airflow, Q_G, created peaks of different intensity. This intensity can be maintained as long as Q_G remained constant.

The system is very sensitive: an increase in Q_G value results in instantaneous rise of the particle number concentration for the diameters of interest. A higher $Q_{G,peak}$ value results in a higher maximal concentration (crest value). Similarly, maintaining the $Q_{G,peak}$ value for several minutes produces a longer-lived peak, while short-lived modifications to the bubbling airflow rate produces narrow, short-lived peaks. The intensity of peaks is determined by the $Q_{G,peak}$ value.

Our results clearly show that the peaks generated can readily be distinguished from each other when Q_G returns to its baseline value between two $Q_{G,peak}$ values. If necessary for specific laboratory experiments, peaks can be generated with a higher frequency, or even combined as shown by the event in Fig. 2 produced after 130 min of generation.

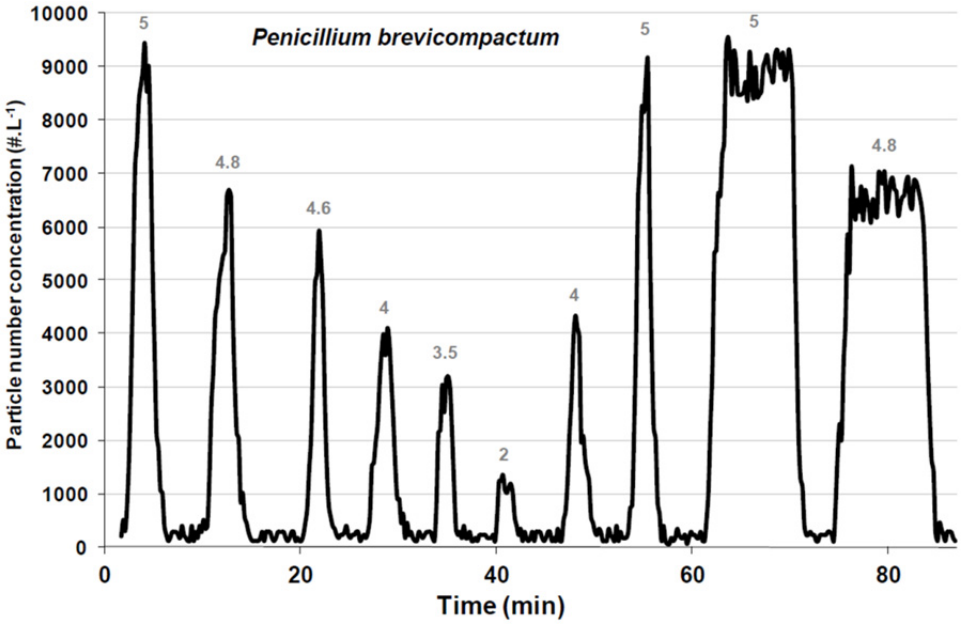

Fig. 4. Number concentration (LAS-TSI™, $d_{opt} > 1.5$ µm) over 90 min bioaerosol generation as a function of Q_G. Grey number above each peak indicates the corresponding value of bubbling airflow rate (L/min). $Q_{G,baseline} = 0.5$ L/min; $2 < Q_{G,peak} < 5$ L/min; $Q_E = 20$ L/min; RH = 50 ± 2%; $H_{liq} = 8$ mm.

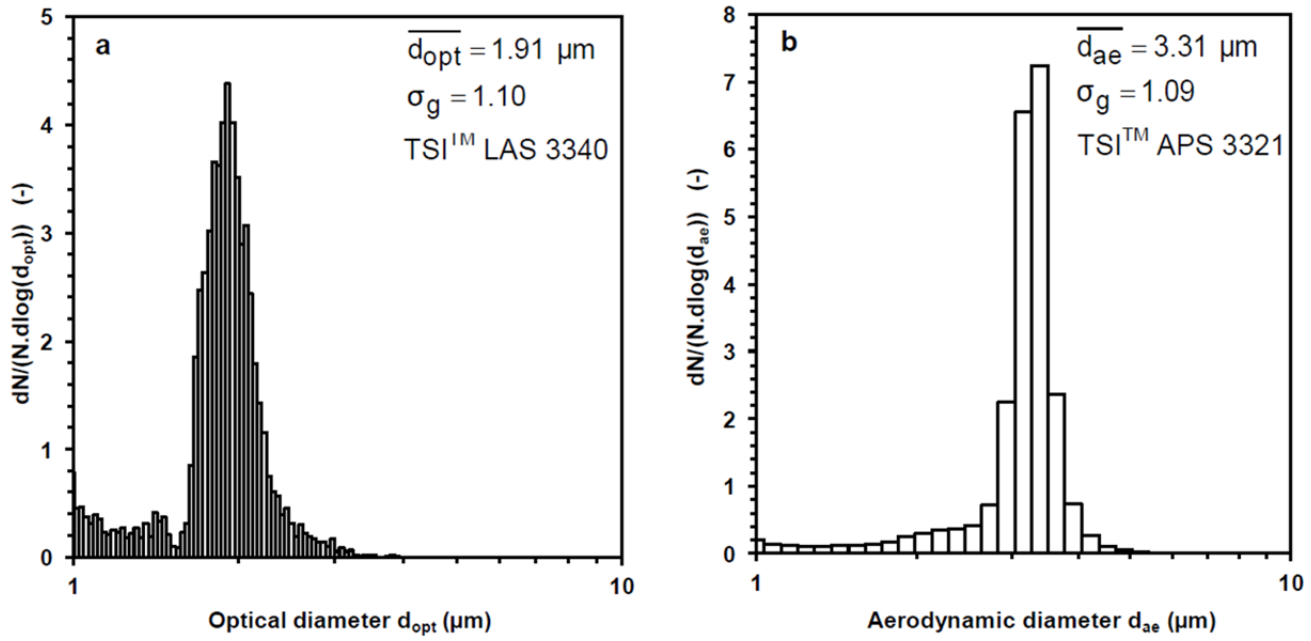

Fig. 5. Size distribution for particles generated during a representative plateau-type peak. *P. brevicompactum*; $Q_{G,peak} = 5$ L/min; $Q_E = 20$ L/min; RH = 50 ± 2%; $H_{liq} = 8$ mm.

Nature of the Particles Generated

The size distributions for the *E. coli* and *P. brevicompactum* particles generated had a median aerodynamic diameter close to 0.94 µm (Fig. 3(b)) and 3.31 µm (Fig. 5(b)), respectively. The median optical diameter was close to 0.66 µm (Fig. 3(a)) and 1.91 µm (Fig. 5(a)), respectively. This difference between the optical and aerodynamic diameters was previously reported for airborne microorganisms (Terzieva *et al.*, 1996; Simon *et al.*, 2011) and may be due to differences between the measurement methods. The values reported here differ somewhat from previous results, but this could be explained by differences between generation methods, measuring instruments, operating conditions (e.g. relative humidity) or composition of the bioaerosol (e.g. proportion of mycelium) (Reponen *et al.*, 1996; Lee *et al.*, 2002).

E. coli is a rod-shaped gram-negative bacterium, ranges in size from 0.3 to 1.0 µm in diameter and from 1.0 to 6.0 µm in length (Brenner, 1984). The mean aerodynamic diameter

of an *E. coli* aerosol has been reported to be in the range of 0.8 to 0.9 μm, with a geometric standard deviation of 1.19 (Ding and Wang, 2001). The population of optical diameters between 0.3 and 3 μm corresponds to vegetative *E. coli* cells and is quite discernible from another population of smaller ($0.3 < d_{opt}$) undesired residual particles (Simon *et al.*, 2011).

P. brevicompactum spores are spherical or slightly ellipsoidal and range from 3 to 4.5 μm in physical diameter (Samson *et al.*, 1995). Previous studies reported a mean aerodynamic diameter for aerosolised *P. brevicompactum* spores of 1.8–2.4 μm ($1.11 < \sigma_g < 1.17$) (Reponen *et al.*, 1996) or 2.56 ($\sigma_g < 1.15$) (Jankowska *et al.*, 2000). The population of particles with optical diameters greater than 1.5 μm described in Fig. 5(a) is mainly composed of *P. brevicompactum* spores. However, microscopic observation also reveals the presence of some mycelium strands but in small proportion. These larger particles explain the right asymmetry of the size distribution presented in Fig. 5.

Stability of the Size Distribution of the Bioaerosol Generated

A change in the bubbling airflow rate, Q_G, to induce peak formation increases the particle number concentration generated but does not affect the median diameter or the geometric standard deviation of the population of interest. For the two species studied, the descriptive parameters of the size distributions vary very little during tests (Sections 3.3 and 3.4). We therefore chose to use the particle number concentration for particles with an optical diameter greater than 0.3 μm ($C_N(d_{opt} > 0.3$ μm) - Grimm® G1109) to control the *E. coli* population generated. Similarly, the particle number concentration for particles with an optical diameter greater than 1.5 μm ($C_N(d_{opt} > 1.5$ μm) - LAS-TSI™) was used to control the *P. brevicompactum* population generated.

During peak formation, the operator can view real-time data concerning the measurement of the number concentrations for the appropriate size classes for the microorganism aerosolised. This provides excellent control over peak formation. Peak formation was tracked over time (Figs. 2 and 4) for the $C_N(d_{opt} > 0.3$ μm) indicator for *E. coli* and the $C_N(d_{opt} > 1.5$ μm) indicator for *P. brevicompactum*. Only these targeted concentrations make sense to represent the fluctuations of the bioaerosol generated. Notion of real-time indicator and its use to control and better reproduce bioaerosols between experiments were described previously (Simon *et al.*, 2011).

The methods used to prepare liquid cultures for generation (Section 2.2) are adapted to the needs of aerosol generation with our bubbling system. The populations of interest of both *E. coli* and *P. brevicompactum* bioaerosols could be tracked by optical counter without being affected by undesired residual particles (Figs. 3(a) and 5(a)). Residual particles can be due to the presence of lactose broth residues, cellular debris, salt or other impurities in the liquid culture which were not removed by the washing steps included in the preparation protocols. The use of real-time indicators such as those we have defined above is only possible because of the non-overlapping optical diameters of the biological particle population with another unknown and uncontrolled particle population.

Relations between Particle Number Concentration and Biological Parameters

The OPC-Grimm® G1109 detects and counts all individual airborne particles, whether biological particle or not. Thus, the counting data correspond to both live and dead microorganisms, fragments, agglomerates, mycelium strands and may also include inert particles. In a previous study (Simon *et al.*, 2011), 15 independent generation assays were performed with *E. coli*, and the correlation between average particle number concentrations ($\overline{C_N}(d_{opt} > 0.3$ μm)), average total bacteria concentrations ($\overline{C_T}$) and average culturable bacteria concentrations ($\overline{C_B}$) were investigated. The concentration ranges studied were: $3.7 \times 10^5 \leq \overline{C_N}(d_{opt} > 0.3$ μm$) \leq 5.7 \times 10^8$ ≠/m^3 (OPC-Grimm® G1109), $3.5 \times 10^5 \leq \overline{C_T} \leq 1.1 \times 10^9$ Cell/m^3 (fluorescence microscopy) and $2.6 \times 10^4 \leq \overline{C_B} \leq 2.2 \times 10^7$ CFU/m^3 (culture on TSA, 37°C, 24 h). Statistical analysis reveal strong correlations between these values: $\log(\overline{C_T}) = 1.03 \times \log(\overline{C_N}(d_{opt} > 0.3$ μm$)) - r^2 = 0.98$; $\log(\overline{C_B}) = 0.83 \times \log(\overline{C_N}(d_{opt} > 0.3$ μm$)) - r^2 = 0.95$. Thus, from OPC data, we can extrapolate the total and culturable bacteria concentrations obtained when generating *E. coli* peaks (Fig. 2). The total and culturable bacteria concentrations would therefore have shifted from around 8×10^5 Cell/m^3 and around 6×10^4 CFU/m^3, respectively, at baseline levels ($\overline{C_N}(d_{opt} > 0.3$ μm$) \approx 5.3 \times 10^5$ ≠/m^3) to over 6×10^8 Cell/m^3 and 1×10^7 CFU/m^3, respectively, at $Q_{G,peak} = 5$ L/min ($\overline{C_N}(d_{opt} > 0.3$ μm$)) > 3.5 \times 10^8$ ≠/m^3).

The relationship between particle number concentration and biological parameters for *P. brevicompactum* was not investigated. However, we have established that stable generation from a liquid *P. brevicompactum* culture at a bubbling flow rate, Q_G, of 5 L/min ($\overline{C_N}(d_{opt} > 1.5$ μm$) \approx$ 9000 #/L) corresponds to a culturable fungi concentration of 8.0×10^6 CFU/m^3 (sampling with a 37-mm closed-face cassette for 50 min and analysis by culture on malt extract agar, 25°C, 5 days).

Repeatability/Reproducibility of Concentration Peaks

No detailed analysis of the repeatability and reproducibility of the concentration peaks produced was performed in this study. However, some $Q_{G,peak}$ values were repeatedly applied during generation tests for the two species. These repetitions indicate comparable peak intensity (Figs. 2 and 4). The statistically significant relationships between the $C_{N,max}$ and $Q_{G,peak}$ parameters (Sections 3.1 and 3.3) also indicate good performance of the system. Nevertheless, the true capacity of the generator can only be assessed by a full and complete study.

Results from our previous work (Simon *et al.*, 2011) also indicate that the concentrations obtained using independently prepared liquid cultures generally had coefficients of variation below 20%, for all Q_G bubbling rates tested. This results make us self confident in the capacity of the bubbling generator to reproduce similar peak concentrations between different runs.

This system offers several advantages for the operator, including flexibility and the possibility to track the particle populations of interest using the above defined real-time indicators ($C_N(d_{opt} > 0.3$ μm) for *E. coli* and $C_N(d_{opt} > 1.5$ μm)

for *P. brevicompactum*). These features significantly improve the generator's performance in terms of test reproducibility. However, if bioaerosol peaks must be perfectly calibrated and repeatable (e.g., for inhalation toxicology applications), the generator's performance should be studied more extensively. For other tests, for which the requirements in terms of repeatability/reproducibility are less stringent, the bioaerosol peaks can be characterised after generation using the measurements recorded.

CONCLUSIONS

This work has shown that it is possible to produce concentration peaks of bacterial (*E. coli*) or fungal (*P. brevicompactum*) particles in a laboratory setting. The concentration peaks are created by modifying the bubbling airflow that passes through the liquid culture film in a wet generation system. The maximal concentration reached during the peak (crest value) is directly correlated to the rate of bubbling airflow. Similarly, longer-lived peaks can be created by maintaining the increased airflow for a longer period. The system reacts very quickly, it allows the creation of individual peak or the creation of more complex events, during which several short-lived or plateau-type peaks can be combined. The operator can track the populations of interest, using real-time measurement devices, to check peak formation during generation. This feature also makes it possible to adapt the operating conditions to ensure that the peaks conform to the needs of the tests. Such a monitoring can be made because the descriptive parameters of size distributions of the two species studied (median diameter and geometric standard deviation) remain constant during tests, whatever the modification of the bubbling airflow. This generator and its capacity to produce bioaerosol peaks in a laboratory setting can therefore be used for many applications. Laboratory experiments concerning inhalation toxicology, test of new protective equipments, evaluation of the performances of bioaerosol samplers and real-time monitoring instruments could all be enhanced by the possibilities offered by this tool. Depending on the objectives of experiments and the microbial strain chosen, the generator performances as regards to repeatability and reproducibility should have to be specified.

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