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Bacterial viability and air quality: Experimental approach and results at the atmospheric simulation chamber ChAMBRe

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Summary. — The effects of bioaerosol, the biological component of atmospheric aerosol, on the environment and health are numerous but still little known, studies on the subject are continually growing. Experiments conducted in Atmospheric Simulation Chambers (ASCs), can provide valuable information on the viability, dispersion and interactions of microorganisms in the aerosols. For this purpose, a multi-step experimental procedure was developed; systematic studies have been carried out in our ASC, to expose bioaerosol to controlled atmospheric conditions. This study reports the results of the experiments conducted on *Escherichia coli* and *Bacillus subtilis*, which highlight how the two bacterial strains react, in terms of viability, to the aerosolization process and to remaining in an aerosolized state in controlled atmospheric conditions.

1. – Introduction

Bioaerosols consist of airborne particles with biological origin, including living and dead microorganisms as bacteria, viruses, fungi, microalgae, and not viable particles as pollen, spores, and animal dander [1]. Bacteria in bioaerosol have concentration greater than $10^4\ \mathrm{m}^{-3}$ over land and can be transported (individually or attached to other particles) for thousands of kilometers [2, 3]. This influences the spread of diseases and allergies and according to some studies, an high concentration of these microorganisms is related to the clouds formation process, atmospheric chemistry and can influence the precipitation processes [4-6].

In Atmospheric simulation chambers (ASCs,) thanks to controlled and monitored conditions, it is possible to study interactions among the constituents of aerosol. By an ASC, different air quality conditions can be set to evaluate the bacteria viability, survival and replications mechanisms in the air. ChAMBRe (Chamber for Aerosol Modelling and

Bio-aerosol Research) is an ASC installed at the National Institute of Nuclear Physics in Genoa (Italy), in collaboration with the Environmental Physics Laboratory at the Physics Department of the University of Genoa. ChAMBRe has been specifically designed for bioaerosol studies.

2. – Materials and instruments

ChAMBRe has a cylindrical shape with domed bases. It has a maximum height of 2.9 m, 1 m diameter and a total volume of about 2.2 m^3 [7]. The main body consists of three parts (two domed cylinders connected by a central ring) with several flanged apertures for different instruments that can be connected to the ASC.

A bioaerosol is injected in the chamber by the SLAG (Sparging Liquid Aerosol Generator, CH Technologies), designed for low-pressure aerosolization of microorganisms: it implements the concept of bursting bubbles to aerosolize particles developed by Mainelis *et al.* [8].

A Waveband-Integrated Bioaerosol Sensor (WIBS-NEO; Droplet Measurement Technologies®) has been integrated in ChAMBRe to measure the bioaerosol concentration inside the chamber as a function of time.

The bacteria are also collected on solid medium in Petri dishes through active sampling by an Andersen impactor (single-stage Andersen Cascade Impactor; Tisch Environmental) in this way the time trend of bacterial viability of the bioaerosol suspended in ChAMBRe can be monitored.

3. – Bacteria cultivation, injection, and experiments in ChAMBRe

Bacteria strains so far used to perform experiments at ChAMBRe are *Escherichia coli* (ATCC® 25922™), Gram-negative and *Bacillus subtilis* (ATCC® 6633™), Gram-positive; both are purchased by Thermo Scientific™ Culti-Loops™. These strains are used as model microorganisms for atmospheric studies.

To prepare the inoculum for the chamber experiments, each strain is grown in 30 mL fresh TSB (tryptic soy broth) non-selective medium in a shaking incubator at 37 °C and 200 rpm (revolution per minute). Bacterial growth is monitored with the spectrophotometer until the optical density (OD600) reaches the value of ≈ 0.5. The bacterial culture is then centrifuged at 3000 rpm for 10 min and the pellet is resuspended in physiological solution (NaCl 0.9% w/v). To retrieve the concentration of viable bacteria, an aliquot of the inoculum is diluted through serial dilutions and plated on petri dishes; the following day the CFU (Colony Forming Units) formed are counted to calculate the number of bacteria in solution. The bacteria suspension is injected into the chamber volume mainly by using the SLAG for 5 min. In this way, 2 mL of the bacterial suspension are nebulized inside ChAMBRe. During the experiments with *E. coli* and *B. subtilis*, active sampling is performed via the Andersen impactor. After the experiments, the plates sampled by Andersen are incubated at 37 °C for 24 h. The following day the CFUs are counted, and CFU (cm^{-3}) are calculated. The viability of airborne bacteria is measured in condition of relative humidity around 60%, atmospheric pressure and around 20 °C.

With *E. coli*, eight replicated experiments were performed (fig. 1(a)), to investigate the possible effects of nebulization on bacteria viability. The average total concentration and standard deviation of *E. coli* was measured at $t = 0$ by WIBS inside the chamber and was TC = (0.33 ± 0.08) cells cm^{-3}. Instead, the average viable concentration at $t = 0$ and standard deviation turned out to be VC = (0.04 ± 0.02) cells cm^{-3}, as determined

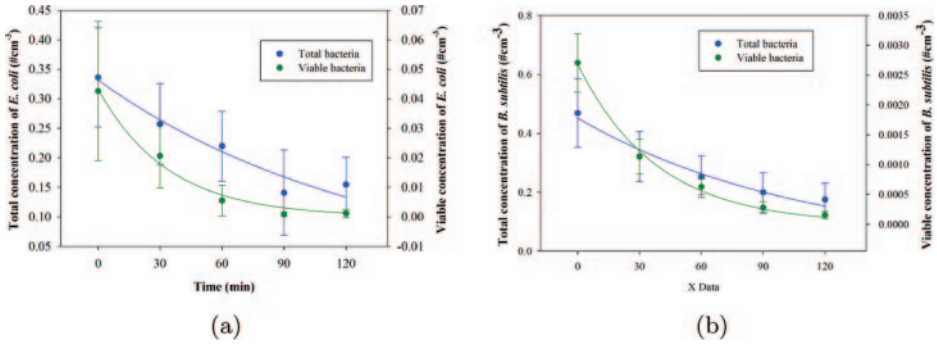

Fig. 1. – Time trend average total bacteria concentration (blue) and viable bacteria concentration (green) inside ChAMBRe of *E. coli* (a) and *B. subtilis* (b) obtained by repetitions of baseline experiments.

by the Andersen impactor sampling. The total *E. coli* average lifetime is $\tau_T \approx 153$ min; the viable average lifetime is $\tau_V \approx 32$ min, lower than the aerodynamic life-time, this indicates the difficulty this microorganism has in surviving in the atmospheric medium. With *B. subtilis*, six replicated experiments were performed (fig. 1(b)). The average total concentration and standard deviation of *B. subtilis* measured by WIBS inside the chamber at $t = 0$ was TC $= (0.47 \pm 0.10)$ cells cm^{-3}. Instead, the average viable concentration and standard deviation determined by the Andersen impactor sampling at $t = 0$ was VC $= (0.0030 \pm 0.0005)$ cells cm^{-3}. The total *B. subtilis* average lifetimes turned out to be $\tau_T \approx 111$ min and $\tau_V \approx 39$ min, also in this case less than the aerodynamic life-time.

Bacteria lifetime in ChAMBRe can be calculated by fitting the data of each experiment with an exponential function as $C(t) = C_0 e^{-t/\tau}$, where C_0 is the total or viable concentration of bacteria just after the injection ($t = 0$), and τ is the total or viable bacteria life-time, respectively. In table I, the average and standard deviation of c_0 and τ for the two strain total and viable concentration of the experiments are reported.

4. – Discussion, conclusion and perspectives

The result presented in this work is the first part of the evaluation of a multi-step protocol to test the impact of air quality on bacterial viability using an atmospheric simulation chamber, which in this case is ChAMBRe. This is just a starting point as the ChAMBRe configuration is constantly evolving and new tools will be implemented in the near future, opening up the possibility of other systematic studies. This set of experi-

TABLE I. – *The average and standard deviation of C_0 and τ for the two strain total and viable concentration.*

Exponential function	Total *E. coli*	Viable *E.coli*	Total *B. subtilis*	Viable *B. subtilis*
C_0 (cells cm^{-3})	(0.33 ± 0.08)	(0.04 ± 0.02)	(0.47 ± 0.1)	(0.003 ± 0.0005)
τ	153 ± 22	32 ± 5	111 ± 19	39 ± 10

ments allowed us to identify the experimental sensitivity to changes in the viability of *E. coli* and *B. subtilis* due to the nebulization process and the permanence in the aerosol state of ChAMBRe. The basic reference will be determined for other bacterial strains, to highlight the natural differences of each bacterium. Tests have been carried out (partly published in [9]) and the effects of air pollutants on viability will be explored in depth thanks to the injection of gas into the chamber simultaneously with the nebulization.

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