



## D4.13.1: Release of a dataset on bioaerosol characterization [B8]



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## LIST OF ACRONYMS

**ACTRIS:** Aerosols, Clouds, and Trace gas Research InfraStructure

**ASC:** Atmospheric Simulation Chamber

**CFU:** Colony Forming Unit

**ChAMBRé:** Chamber for Aerosol Modelling and Bio-aerosol Research

**ERIC:** European Research Infrastructure Consortium

**INFN:** Istituto Nazionale di Fisica Nucleare

**NF:** National Facilities

**OD:** Optical Density

**RI:** Research Infrastructure

**RPM:** Revolutions Per Minute

**SCADA:** Supervisory Control And Data Acquisition

**SLAG:** Sparging Liquid Aerosol Generator

**TSB:** Tryptic Soy Broth

**WIBS:** Waveband-Integrated Bioaerosol Sensor

**WP:** Work Package

## 1. INTRODUCTION

This deliverable is prepared in the context of the ITINERIS project, within the WP4 that deals with the integration of Research infrastructures working in the atmospheric domain through synergistic approaches and cross boundaries developments. This deliverable reports on the on-going implementation of the task 4.13. The objective of the activity is two-fold: the set-up of experimental procedures to characterize the time evolution of the formation, composition, modification and deposition of different aerosol species inside the ChAMBRe atmospheric simulation chamber; and the production of a dataset of compositional and optical features of specific aerosol types. In this report, we focus on bioaerosol. The instrumental equipment to reach the goal is composed of specific items to manage, inject and collect viable bioaerosol species at ChAMBRe and to measure their aerodynamic behaviour and evolution. Besides the dataset on aerosol properties, the procedures to study different aerosol species and mixtures will be categorized, standardized, and offered as services both to the scientific and social (i.e. industries, environmental agencies, etc.) communities in the frame of the ACTRIS RI.

The document is structured in five different chapters, and references are reported at the end of the document.

## 2. ChAMBRe

ChAMBRe (Fig.1; Massabò et al., 2018) is an atmospheric simulation chamber (ASC). ASCs are exploratory platforms which allow to study atmospheric processes under realistic but controlled conditions. Inside an ASC, atmospheric conditions (i.e., both chemical and physical parameters) can be maintained and monitored in real-time for periods long enough to reproduce realistic environments and to study interactions among their constituents. ChAMBRe is installed at the Physics Department of the University of Genoa, in collaboration with INFN. It is built in stainless steel; it has a cylindrical shape with total volume of about 2.2 m<sup>3</sup>; the body is made up of two domed cylinders connected by a central ring. Scattered all over the main body, there are ISO-K flanges, with different diameter, which permit access to the inner volume. Connected to ChAMBRe, several instruments and online monitors complete the facility (Danelli et al., 2021; Vernocchi et al., 2022 and 2023). The whole set-up is managed by a custom NI LabVIEW SCADA.

In this frame, the participation to PNRR-ITINERIS gives to ChAMBRe the possibility to develop new experimental procedures, used to produce dataset of characterized aerosol.



Figure 1: ChAMBRé, the atmospheric simulation chamber.

### 3. SCIENTIFIC OBJECTIVES

Bioaerosol are a subset of atmospheric aerosol, that influence climate, air quality, and health via several mechanisms which often are poorly understood. Among all the different bioaerosol microorganisms, bacteria are considered to play a significant role in the composition and dynamics of bioaerosols (Gong et al., 2020). The interactions between bacteria and their living environment, as well as the atmospheric conditions, play crucial roles in determining their behavior and impacts on climate (Deguillaume et al., 2008) and, consequently, on health. In particular, the quantitative study of possible relationships between bacteria viability and air quality or meteorological conditions is an open and relevant issue. The difficulty of retrieving such possible correlations by analyses of data collected during infield campaigns can benefit from targeted experiments conducted in well-controlled conditions inside atmospheric simulation chambers. In this context, the main objectives are:

- 1) the development of an experimental procedure to perform quantitative studies on the impact of different pollutants on bacteria viability using an ASC.
- 2) the exploitation of the experimental procedure to investigate the interaction between different bacteria strains and air quality conditions inside ChAMBRé.

In the following, the assessed experimental procedure will be described, and the first data, obtained with *Escherichia coli*, shown. Efforts are planned to repeat the observation with other bacteria strains (i.e., *Bacillus subtilis*, *Pseudomonas fluorescens*) and pollutants.

## 4. EXPERIMENTAL PROCEDURE AT ChAMBR<sub>e</sub>

One of the objectives here described is the assessment of a multi-step and well-controlled protocol to perform experiments on the impact of air quality on bacteria viability using an atmospheric simulation chamber. Here, a short description of the experimental procedure is given.

The bacteria strain selected for the experiment is firstly characterized for the growth curve by recording the OD at  $\lambda = 600$  nm ( $OD_{600\text{ nm}}$ ) at specific time intervals and measuring bacteria total concentration by the QUANTOM Tx™ microbial cell counter (Logos Biosystems) and bacteria viable concentration by standard dilution plating.

To prepare the inoculum of *E. coli* for the chamber experiments, the bacterium is grown, in 30 ml of fresh TSB (Tryptic Soy Broth) nonselective medium in a shaking incubator at 37 °C and 200 rpm, until the mid-exponential phase, when  $OD_{600\text{ nm}}$  0.5. Then, 20 ml of this liquid preparation is centrifuged at 3000 rpm for 10 min and the bacteria pellet is resuspended in 20 ml of sterile physiological solution (NaCl 0.9% w/v). If needed, the suspension is properly diluted. The concentration of the solution to be injected inside ChAMBR<sub>e</sub> is controlled in terms of total cells ( $\text{ml}^{-1}$ ) by QUANTOM Tx™ and viable concentration by standard dilution plating. The bacteria suspension, properly diluted, is injected into the chamber volume by using the SLAG (CH Technologies). The injection phase lasts 5 minutes and both airflow and duration are automatically controlled: 2 ml of the bacterial suspension are nebulized inside ChAMBR<sub>e</sub>.

The concentration of total bacteria inside chamber volume is monitored by the WIBS-NEO (Droplet Measurement Technologies®). Active sampling via the Andersen impactor is performed increasing sampling time progressively after the injection to collect a suitable number of CFUs. After the experiments in the simulation chamber, the sampled plates are incubated at 37 °C for 24 h. The CFUs are then counted, and the  $\text{CFU cm}^{-3}$  calculated.

The possible correlation between bacteria viability and air quality can be investigated in terms of change in bacteria viability due to the exposure to atmospheric pollutants. Effects on bacteria viability are compared in relation to “baseline experiments”, in which the viability of airborne bacteria is measured at atmospheric pressure, with temperatures around 20 °C and with relative humidity around 60 %. The baseline was assessed both in dark and light (using solar simulator by Sciencetech Inc.™) conditions. During light conditions, the solar simulator was used with the AM1.5 filter mounted. The baseline assessment was followed by a set of exploratory experiments in which bacteria are exposed to selected pollutants: different concentrations of NO and NO<sub>2</sub>, kept constant by a feedback control system.

A time window of 2 h after the bacteria injection was considered to observe the bacteria viability behavior under different atmospheric conditions, and it was possible to quantify the lifetime.

More details on the procedure are available in Vernocchi et al., 2023.

## 5. TESTS AND RESULTS

Results of the first set of experiments on *Escherichia coli* are published in (Vernocchi et al., 2023) and here summarized. Experiments with other bacteria strains are in progress while tests with different pollutants are planned for the future. These results will be presented in future report and papers.

- Baseline – dark conditions

*E. coli* behavior in baseline dark conditions was determined with a set of eight replicated experiments. The average total concentration and standard deviation of *E. coli* inside the chamber at  $t_0$  (i.e., 3 min after the conclusion of the injection to allow proper mixing or homogenization inside the ChAMBR<sub>e</sub> volume) was  $(0.34 \pm 0.08)$  cells  $\text{cm}^{-3}$ , as measured by the WIBS; the average viable concentration and standard deviation, determined by the Andersen impactor sampling at  $t_0$ , was

$(0.04 \pm 0.02)$  cells  $\text{cm}^{-3}$ . The average ratio and standard deviation of viable:total (V:T in the following) bacteria concentration inside ChAMBRe at  $t_0$  turned out to be  $V:T = (0.13 \pm 0.07)$ .

Bacteria lifetime in ChAMBRe can be calculated by fitting the data of time trends of the averaged total and viable concentration with an exponential function. The total *E. coli* average lifetime is about 153 min, the viable *E. coli* average lifetime is about 32 min, lower than the aerodynamic lifetime, thus indicating the difficulty this microorganism has in surviving in the atmospheric medium. In Figure 2, time trend of *E. coli* average bacteria total and viable concentration inside ChAMBRe are shown. *E. coli* average lifetime in baseline experiments, calculated on the V:T ratio, turned out to be about 40 min.

The experimental sensitivity, in terms of coefficient of variation is 13%, which corresponds to the sensitivity to changes in *E. coli* viability due to exposure to pollutants.

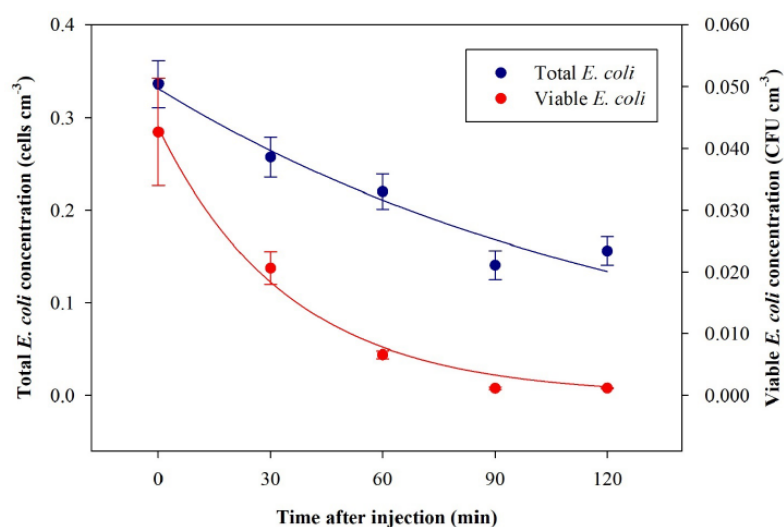


Figure 2: Time trend of *E. coli* average bacteria total (blue) and viable (red) concentration inside ChAMBRe obtained by eight repetitions of baseline experiments.

- $\text{NO}_x$  – dark conditions

Bacteria were exposed to  $\text{NO}_2$  and  $\text{NO}$  concentrations, with 900 and 1200 ppb for both the pollutants. The exposure of bacteria to such pollutants showed a V:T reduction. The quantitative reduction in the *E. coli* lifetime, due to the exposure to pollutants, can be evaluated considering the V:T ratio and fitting the data with an exponential curve. In Figure 3, the comparison of V:T ratio time trends obtained in baseline and polluted experiments are shown. The exposure of *E. coli* to 900 and 1200 ppb of  $\text{NO}_2$  reduced the lifetime to approximately 25 and 11 min, respectively. The exposure to 900 and 1200 ppb of  $\text{NO}$  decreased bacteria lifetime to 26 and 25 min, respectively, and the values are similar to the value obtained with the lowest  $\text{NO}_2$  concentration. The increase in the  $\text{NO}$  concentration did not correspond to a decrease in the *E. coli* viability, as observed instead with  $\text{NO}_2$ ; these results suggest a greater toxic effect of  $\text{NO}_2$  than of  $\text{NO}$  on *E. coli*.

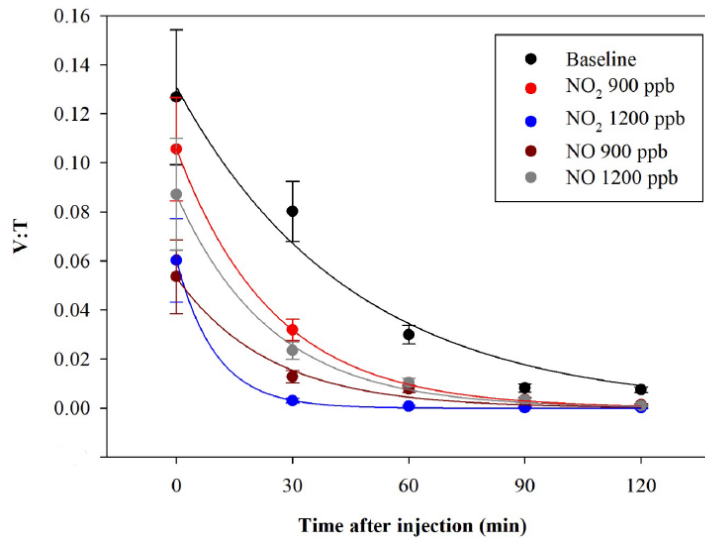


Figure 3: Time trend of the V:T ratio for *E. coli* in the baseline (black) and in the experiments with ChAMBRé maintained at a constant concentration of NO<sub>2</sub> (900 ppb red and 1200 ppb blue) and NO (900 ppb in dark red and 1200 ppb in grey).

*E. coli* behavior when exposed to light was determined in a set of dedicated experiments. After the injection, the average total concentration of bacteria inside the chamber was  $(0.30 \pm 0.03)$  cells  $\text{cm}^{-3}$ , which is compatible with the dark baseline, while the average viable concentration was  $(0.019 \pm 0.005)$  cells  $\text{cm}^{-3}$  and lower than what was obtained in dark experiments. The resulting V:T ratio was  $(0.06 \pm 0.02)$ . The viable concentration collapses quickly, reaching zero after 30 min. These results indicate a significant decrease in bacteria viability due to their exposure to solar radiation. The comparison between dark and light baseline experiments, in terms of V:T, is shown in Figure 4.

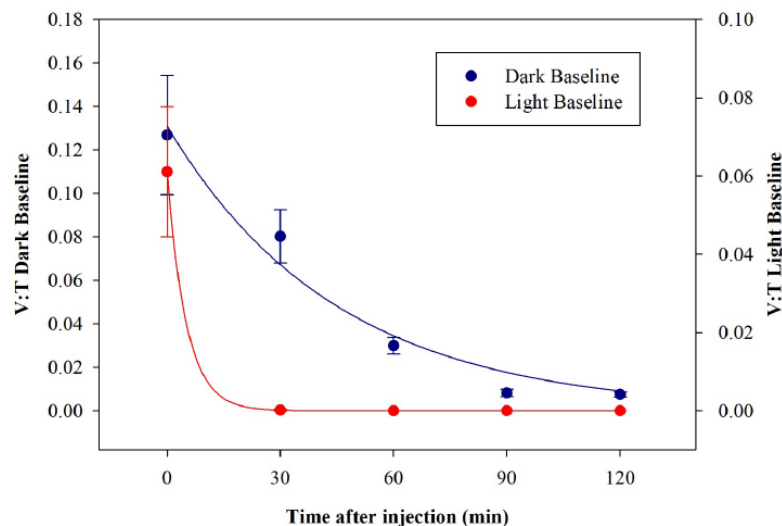


Figure 4: Time trend of the V:T ratio for *E. coli* in the dark baseline (dark blue) and light baseline (red) experiments.

## 6. REFERENCES

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