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Point-by-point response to the reviews, list of all relevant changes made in the manuscript and a marked-up manuscript version.

Anonymous Referee #1
Received and published: 16 July 2018
Review of the manuscript amt-2018-147 with title: "ChAMBRe: a new atmospheric simulation Chamber for Aerosol Modelling and Bio-aerosol Research" by Massabò et al.

General comments:

This manuscript describes a new atmospheric simulation chamber and its potential use to simulate the interaction of trace gases and biological aerosol particles like bacteria. Therefore, it fits well in the scope of the journal of atmospheric measurement techniques and focusses on an interesting scientific topic which is rarely addressed in other existing simulation chambers. The manuscript aims to describe the chamber, its equipment, its instrumentation, and to characterize the aerosol particle behavior (lifetimes), trace gas wall losses, and background levels of particles and trace gases. Furthermore, specific procedures for handling, aerosolizing, and sampling of bacteria are described and results of first test experiments on the viability of bacterial aerosol in the chamber are presented. Due to the focus on chamber characterization and first tests the scientific results are only of limited significance. Rather poor are the overall presentation quality, the English, and the scientific quality. While standard methods are described in great detail the applications or connection to the new simulation chamber are not given in a sufficient manner. Furthermore, several aspects of the tests and experiments are described insufficiently. Hence this manuscript should only be accepted for publication after major improvements.

We thank the Reviewer for the extremely useful suggestions/criticisms and comments. We have carefully taken into account each point to improve the quality of the paper.

Specific comments

Page 1 line 1: It isn't evident why and how the new simulation chamber facility can potentially contribute to aerosol modelling. Therefore the title is misleading. Either change the title or add a detailed explanation of the potential aerosol modelling link to the chamber.

The Referee is right. Actually, the title contains the explanation of the ChAMBRe acronym and we fear it could be difficult to change. We can however include the following explanation at the end of sec. 1.2:

While ChAMBRe, as other ASCs, is a multi-purpose facility, the outcomes of the correlation between bacteria viability and atmospheric condition/composition will provide the input for developing ad-hoc modules to be then implemented in chemical transport models. This can be done following a scheme often used for the chemical mechanisms parameterization (see for example the smog chamber experiments used for the evaluation of Carbon Bond mechanisms in Parikh H. M. et al., "Evaluation of aromatic oxidation reactions in seven chemical mechanisms with an outdoor chamber" Environ. Chem. 2013, 10, 245–259). Such software tools, are widely used both in scientific research and in air quality evaluations, to predict the fate (i.e. transport, deposition and chemical changes) of the atmospheric pollutants and, at the moment, they do not include any biological patch.

Page 2 line 43: Explain specific what you mean. How can bacteria be chemically active in the atmosphere?

It has been proposed that bio-aerosols have a potential role in the chemistry of organic compounds in the troposphere via microbiological degradation and hence inducing changes in the IN or CCN ability of organics in atmosphere (Ariya and Amyot, 2004).

This statement will be included in the revised manuscript in sec. 1.1

Page 2 line 44: Explain what you mean. How can bacteria favor the formation of condensation nuclei?

Bauer et al. (2003) suggested that the chemical composition, structure and hydrophilicity of the surface layer of bacteria could play important roles in CCN activity.

This statement will be included in the revised manuscript in sec. 1.1.

Page 2 line 54: Specify how many or which types of organisms can survive and what you mean with a long airborne transport. Give typical atmospheric transport or lifetimes.

Airborne bacterial communities are highly diverse, and variations in their species diversity are quite complex. The bacterial composition in air is strongly dependent on many factors such as seasonality, meteorological factors, anthropogenic influence, variability of bacterial sources and many other variables. Still, the general trend from the literature is that bacteria found in the air often belong to groups that are also common in the soil (e.g. Firmicutes, Proteobacteria, Actinobacteria) (Després et al., 2012). Due to their small size, bacteria have a relatively long atmospheric residence time (on the order of several days or more) compared to larger particles and can be transported over long distances (up to thousands of kilometers). Measurements show that mean concentrations in ambient air can be greater than 1x10^4 cells m^-3 over land, whereas concentrations over the sea may be lower by a factor of 100-1000 (Burrows et al., 2009a, Burrows et al., 2009b).

This statement will be included in the revised manuscript in sec. 1.1.

Page 2 line 63-64: Explain which biogeochemical issue you mean.

We simply mean the fact, reported and described in the quoted literature and in other works, that particulate matter transported from desert areas to the parts of the world, is made not only by dust but it contains several biological "particles" and bacteria in particular. As a matter of fact, bacteria can stick the dust particles and can be more efficiently (i.e. remaining viable) transported through long distances. We just quoted the references in the text but we can easily add a few words to make the point more clear.

Page 2 line 64-65: Explain what you mean with complex ecosystem.

Actually, we tried to wrap-up the previous points just underlying the fact that bacteria are influenced by the atmospheric conditions but they also contribute to modify the atmosphere. An ecosystem is a biological community of interacting organisms and their physical environment, such as air, water, and mineral soil. Ecosystems include interactions among organisms, and between organisms and their environment, through very complex mechanisms. However, we agree that the statement is too generic and we propose to delete it. The information of sec. 1.1 does not change.

Page 3 line 73: You should skip "mainly" in this sentence.

This will be done in the revised manuscript.

Page 3 line 74-75: The cited work is related to aerosol-cloud interaction but not to cloud chemistry.

We propose to modify the text as follows:

ASCs have been used to study chemical and photochemical processes that occur in the atmosphere, such as ozone formation (Carter et al., 2005 and references therein) and cloud chemistry (Wagner et al., 2006) or aerosol-cloud interaction (Benz et al., 2005), etc.

Page 3 line 79-80: Be more precise what you mean.

W*e propose to modify the text as follows:*

Since the interplay of bio-aerosol and atmospheric conditions is still poorly known, suitable facilities are needed, where transdisciplinary studies gathering atmospheric physics-chemistry and biology issues are possible.

Page 3 line 84-86: You should be more specific about the subjects of previous studies.

We have reported these studies just to cite the principal topic related to atmospheric chambers i.e. ice nucleation and cloud condensation.

We propose to modify the sentence in the revised manuscript as follows:

The use of atmospheric simulation chambers has been much more limited and focussed on the interaction of bacteria with atmospheric parameters, regarding bio-aerosols release effects (Jones and Harrison, 2004), and on ice nucleation and cloud condensation (Möhler et al., 2008; Bundke et al., 2010; Chou, 2011).

Page 3 line 93-96: Do you mean that ASCs with realistic simulation capabilities should be combined with biological facilities for adequate handling and characterization of bio aerosols? You should reformulate this sentence.

We agree that our manuscript was not clear enough on this point. We could change the statement in this way:

Such experimental evidence made clear that the effects of atmospheric pollution on bacteria viability could be studied in atmospheric chambers. In order to perform systematic studies to resolve and describe the physical and chemical mechanisms ruling these interactions, dedicated facilities with a microbiology laboratory linked to the ASC for the handling and characterization of bio-aerosol are needed.

Page 3 line 104: Mention the modelling tools in this section.

Actually, the ChAMBRe task in EUROCHAMP is double-fold: the development of a protocol to perform experiments in ASCs with bacteria and, more in general, bio aerosol and the collection of data (through a set of experiments) to correlate bacteria viability and atmospheric conditions. The latter should results, in two year from now, in the assessment and implementation of specific routines/patches to be inserted in chemical transport models (that should/could so evolve to BCTM). This is a very ambitious program, likely longer than the EUROCHAMP time frame.

We agree that the statement in the manuscript is too sharp and, to not be too long and to remain in the aims of the present work, we could modify the statement just in:

…is one of the nodes of the EUROCHAMP-2020 network with specific tasks on bio-aerosol studies.

Page 3 line 108: In Figure 1 the central ring has a height of 60 cm. Please be consistent.

The right number is 60, the text will be changed accordingly.

Page 3 line 110: Figure 1 shows 4 flanges of 10 cm and 2 of 40 cm diameter. Please be consistent between text and figures.

Numbers in the text were wrong and will be corrected (2 x 40 and 4 x 10).

Page 4 line 115: What do you mean with an ad-hoc metallic structure?

The lower dome is hold by a metallic support to maintain the entire structure in vertical position.

This statement will be included in the revised manuscript.

Page 4 line 121: Explain carefully if there are other means to retain pump oil to diffuse into the chamber. Explain why a two-step process to refill the chamber is needed.

The statement refers to the "safety" equipment. In the normal operation, the chamber is also equipped with a manual valve (positioned between the chamber and the gate valve). Before quitting the pumps such manual valve is closed so ensuring that no oil can diffuse in the chamber during the slowing-down phase. We do not considered to add this detail in the text but we can of course add a proper statement.

The two-step process comes from a long expertise with vacuum systems: in the very first phase of the venting, the use of nitrogen from a cylinder guarantees (or anyway help) that no water vapor (or others) possibly entering the chamber can penetrate in the walls. When the walls are "coated" with nitrogen, ambient air is used through the HEPA filter to bring back the chamber to atm. pressure.

Page 4 line 129: Give the fan speed in terms of revolutions per minute.

This will be done in the revised text.

Page 4 line 131: Give the manufacturers of all components you mention in this section.

This piece of information will be included in the revised text.

Page 4 line 138-139: Give positions and sensitivities for these sensors.

This piece of information will be included in the revised text.

How do these sensors interact with reactive trace gases like ozone?

The models selected for ChAMBRe are, according to the data sheets, resistant to reactive gases.

Page 4 line 142: Are both lamps permanently installed or can the second one be installed on purpose?

The second one can be installed when needed.

Give type and manufacturer for both lamps.

This piece of information will be add at the revised manuscript.

Page 5 line 160: It seems not necessary to me to describe an SMPS instrument in such detail.

We could shorten a little bit the length in the revised manuscript, however we believe that the given information could help the reader not familiar with such instrument.

Page 5 line 163: Reformulate this sentence.

The sentence could be reshaped in this way.

The DMA is available with two different columns, working alternatively in the size range 5.5-350.4 nm (MDMA), and 11.1-1083.3 nm (LDMA), and classifying particles in 50 dimensional classes.

Page 5 line 163: Note that a pre-impactor is required for a correct SMPS measurement to prevent false sizing due to multiple charged particles.

The pre-impactor is routinely used, we did not include this detail in the text but we can better specify if needed.

Page 5 line 170: I suppose the CPC is sensitive to particles larger than 4.5 nm.

Yes of course, the right wording is "larger than 4.5 nm"

Page 5 line 173: It is quite important to describe the design of the sampling lines and potential losses that could occur in them. E.g. sedimentation losses of larger aerosol particles in horizontal tubes.

The SMPS has been connected to ChAMBRe through a smoothly bended pipe in a way to have a horizontal length of about 10 cm followed by a vertical part of about 30 cm. The OPC, which counts larger particles is connected to ChAMBRe by an ad-hoc set-up with the inlet directly sucking from one of the large flanges: no horizontal tubes (actually no tubes at all).

These detail will be added to the revised text.

Page 5 line 175: Explain how you calibrate your instruments and how you ensure their traceability.

The OPC is sent back to the factory for re-calibration at regular period: it has been calibrated just before the experiments described in the manuscripts.

Page 5 line 185-188: Explain how you distinguish between NO2 and NOx.

The chemiluminescence principle is used for automatic monitoring of NO, NO_x and NO_2 in ambient air. The reaction between NO and O_3, which is the basis for the CLD (chemiluminescence detector), emits photons that are detected by a cooled photomultiplier tube (PMT).

$NO + O_3 \rightarrow NO_2^* + O_2$
$NO_2^* \rightarrow NO_2 + h\upsilon$

The CLD output voltage is proportional to the NO concentration. In order to measure the NO_2 concentration, the sample must first be reduced into NO, and this is achieved with a heated molybdenum NO_x converter:

$Mo + 3NO_2 \rightarrow MoO_3 + 3NO$

NO cycle: The sample moves directly into the reaction chamber (of the instrument) where NO oxidation by ozone takes place. The photomultiplier tube signal, minus the black signal, is proportional to NO molecule number within the sample.
For the NOx cycle the sample passes through the converter oven which reduces NO_2 to NO, then it is mixed with ozone in the reaction chamber. The photomultiplier tube signal, minus the black signal, is proportional to the sum of NO and NO_2 molecule (reduced to NO in the converter) contained in the sample.

A shorter explanation will be included in the revised manuscript.

Page 5 line 190: I suppose you mean aerosol particle lifetime here. You must be precise with your language here since aerosol means a mixture of gas and particles.

Yes, we'll use "particle aerosol lifetime" as section 3.1 title.

Page 5 line 191: Some processes proceed on time scales of seconds.

The mistake will be corrected in the revised manuscript

Page 5 line 192: The manuscript describes the lifetime of NaCl particles within the simulation chamber for different particle sizes. Indeed an important characteristic for aerosol simulation chambers. However, the manuscript doesn't explain sufficiently how these lifetimes are defined, why the lifetimes for the different particle sizer are different, and the relevance of the lifetimes for experiments with typical bio aerosols. Several questions in this respect remain open. An important aspect is for example also the role of mixing in the chamber. How are the mixing times determined and how does the mixing fan influence the particle or trace gas lifetimes in the chamber? How is the mixing and particle lifetime influenced by injecting the sliding shelf? How broad is the particle lifetime distribution e.g. for bacterial aerosol particles ranging between 2.5 - 6.5 μm in length? The discussion of the possible time scales for studying typical bio aerosols in the new chamber compared to typical atmospheric residence or aging times is missing. Regarding Figure 3: You may combine the particle size measurements done by the SMPS (mobility size) and OPC (optical size) instruments to obtain the geometrical particle size. Explain the very high particle lifetimes obtained only from analysis on the OPC data for the size range _300-500 nm. Are these data realistic? Explain how you calculate the uncertainties for size and lifetime and show them.

We try to answer point-to-point:

"How lifetimes are defined?"

Particles concentration in the chamber decreases with time due to effects: dilution and wall deposition. With a sucking flow rate (i.e. the sum of the working flows of all the equipment connected to the chamber) constant in time (Φ), the particle concentration trend can be described by a simple equation:

$C(t) = C(0)e^{-kt}$, with $k = \beta + \Phi/V$ (V = chamber volume). The term β, which summarizes the wall losses effects, is the inverse of the particle life-time. We did not include this definition in the text since we considered this point as been clarified by previous literature studies but we can easily add a few lines in the revised manuscript. Moreover, the procedure to measure "lifetimes" have been described in previous literature paper and it has also been assessed within the EUROCHAMP2020 consortium, basically as shown in Fig. 1.

Experiment to determine physical wall loss of particles

The physical wall loss of particles in close vessels such as chambers is a key parameters that vary with the size of the considered particles. The wall loss rate as a function of the size is depending on 1/ the chamber shape, 2/ the mixing regime (especially for small particles), 3/ the density of the considered particles, 4/ the electrostatic state of the wall. It has been very well studied (McMurry and Grosjean, 1985; McMurry and Rader, 1985) and efficient parameterization are available (Crump et al., 1983; Crump and Seinfeld, 1981; K. Lai and Nazaroff, 2000)

A rather simple experiments provide straightforward procedure. The principle of these characterization experiments is to generate a polydisperse aerosol sufficiently diluted to neglect coagulation and sufficient inert to neglect condensation or evaporation. This can be done by the nebulisation of a saline solution (eg. NaCl or ammonium sulfate) or by the chemical conversion of gases (ozonolysis of small quantity of pinene, photolysis of SO2/ozone/water…) for sub-micronic particles or by mechanic generation for super-micronic particles. The total number concentration must be below 10^4 #/cc to minimize the collision probability and so the coagulation process. When this is achieved (and when the aerosol formation is negligible for chemical generation), one just have to follow the number size-distribution as a function of time (with a SMPS or a APS for sub-micronic particles, with Grimm™, Welas® or other OPC for super-micronic particles). Then one have to fit for each size-bins the decay with a first order law. This hypothesis is generally working well as bouncing or re-emission from the wall are often not too significant.

Being a basic knowledge about a chamber, it is recommend that such an experiment (for sub- and/or super-micronic depending on the use) should be done at least once for rigid non-electrostatic chamber and regularly for Teflon chamber

Crump, J.G., Flagan, R.C. and Seinfeld, J.H., 1983. Particle Wall Loss Rates in Vessels. Aerosol Science and Technology, 2(3): 303 - 309.

Crump, J.G. and Seinfeld, J.H., 1981. Turbulent deposition and gravitational sedimentation of an aerosol in a vessel of arbitrary shape. Journal of Aerosol Science, 12(5): 405-415.

K. Lai, A.C. and Nazaroff, W.W., 2000. Modelling indoor particle deposition from turbulent flow onto smooth surfaces. Journal of Aerosol Science, 31(4): 463-476.

McMurry, P.H. and Grosjean, D., 1985. Gas and aerosol wall losses in Teflon film smog chambers. Environmental Science & Technology, 19(12): 1176-1182.

McMurry, P.H. and Rader, D.J., 1985. Aerosol Wall Losses in Electrically Charged Chambers. Aerosol Science and Technology, 4(3): 249-268.

"why the lifetimes for the different particle sizer are different ?"

We do not have a completely firm explanation. We want emphasize that the uncertainties given in Figure 3 contain the statistical part only. The region where the two sizers overlap is quite narrow and the discrepancy is concentrated between 300 and 600 nm i.e. the particle size corresponding to the longer life-times we could appreciate. In such interval, the statistics in the SMPS data is very low, as can be inferred by the large error bars plotted in figure 3. We cannot completely exclude that other contributes to the error budget could come, for instance, from background subtraction/fluctuation. On the contrary, the OPC counts are pretty high and no significant statistics uncertainty affects those results (but the error bars are plotted…see also below our answer to your comment on Fig. 3). However, even if in this case the geometric diameter differ from less than 10% from the optical one, it is known that the first bins of the Grimm OPC could suffer of systematic effects (Santi et al., 2010. Real-time aerosol photometer and optical particle counter comparison. Nuovo Cimento B 125(8):969 -981).
Putting all these things together, we must actually admit that the results in the named size range could be more uncertain than as shown in the plot and that the manuscript did not make this point clear enough. We did not further investigate this issue since we were focused in assessing the typical values of "bacteria lifetime" (particles > 2 microns) in ChAMBRe.

We propose to modify the text in the manuscript as follow:

Particles lifetime in ChAMBRe varies from few hours to about 1 day depending on particle size. The uncertainty on particles life-time plotted in Figure 3 has been evaluated on a pure statistical basis. Actually, in the size region between 300 and 600 nm, both the SMPS and OPC data could be particularly sensitive to other effects (e.g. background fluctuation for the SMPS, systematic artifacts in the first OPC bins) which have not been fully investigated in this work and that do not change the typical feature depicted in Figure 3.

The caption of Figure 3 will be updated adding the sentence:

Error bars include statistical uncertainties only.

"what's the relevance of the lifetimes for experiments with typical bio aerosols ?"

while the life-time global picture is part of a more general characterization of the chamber performance, we consider the values in the range 1 – 3 μm as indicative of the possible bacteria life-time. We mean that bacteria are subjected not only to wall deposition but the number of the viable units would decrease with time for other reasons. So, the life-time of viable bacteria cannot be simply inferred from the data in Figure 3 which could, in a certain way, be seen as "upper limit" of the bacteria life-time and hence of the effective time interval to run any experiment. Actually, as reported in sec.4 we decided, for the very first tests reported in the manuscript, to limit the exposure time of the petri dishes to a maximum of 5 hours i.e. a life-time of particles slightly smaller than 1 μm.

We could add a proper sentence in the text even if we would prefer to keep separate the discussion on the general performance/characterization from the issues directly linked to bacteria.

How are the mixing times determined and how does the mixing fan influence the particle or trace gas lifetimes in the chamber?

See in the following our answer to the comment at page 6, line 202.

How is the mixing and particle lifetime influenced by injecting the sliding shelf?

Aerosol particle life-time reported in fig. 3 have been measured without inserting the sliding shelf. This way, they represents the general feature of the chamber.
When the shelf is inserted to collect bacteria, it certainly produces an effect (likely a life-time reduction) that we are going to assess through a complete fluid dynamic calculation (in progress but it will take a few months). However, when we use the shelf we must be sure to maintain it in the chamber for a time long enough to collect all the viable bacteria and the 5-hour upper limit quote above goes in this direction.

"How broad is the particle lifetime distribution e.g. for bacterial aerosol particles ranging between 2.5 - 6.5 μm in length?"

According to the data reported in Fig. 3, the life-time of particles in the quoted range varies (roughly) from 1 and 3 hours. So far, we cannot collect any direct information on bacteria lifetime (we are working in this direction introducing further time-sensitive collection methods) and, again, this is part of the arguments that brought us to select a 5-hour exposure time for the Petri dishes during the experiments with bacteria. We have to add that, after each experiment, a new set of Petri had been inserted in the chamber looking for residual bacteria but we never observed any sizeable signal. So, (at the moment) we can conclude that the life-time of viable bacteria (both the strains) is lower than 5 hour.

The discussion of the possible time scales for studying typical bio aerosols in the new chamber compared to typical atmospheric residence or aging times is missing.

We have considered this point above (Referee comment on **line 54, pag 2***). We are aware that the life-time in the chamber is much shorter than the typical (or possible) residence time in the atmosphere however our aim is to have time enough to study the impact on the bacteria viability of specific pollution levels. This seems to be a realistic goal considering the results of the pilot experiment performed at CESAM (where life-times are very similar to those detected in ChAMBRe) by Brotto et al., 2015. The program of systematic experiment we are going to start will assess this point, furthermore the fluid dynamic calculation that we are performing (actually, some Colleagues from the Engineering Dept.) we'll hopefully highlight solutions to increase the life-time.*

Regarding Figure 3: You may combine the particle size measurements done by the SMPS (mobility size) and OPC (optical size) instruments to obtain the geometrical particle size. Explain the very high particle lifetimes obtained only from analysis on the OPC data for the size range _300-500 nm. Are these data realistic? Explain how you calculate the uncertainties for size and lifetime and show them.

Uncertainties are calculated during the fitting (weighted on the statistic uncertainty of each value) of the exponential decay trend of particle concentration measured by SMPS/OPC. They are correctly reported in Fig. 3 and in some cases, the error bar length is comparable with the dimension of the points in the plot (and therefore are difficult to see but they can appreciated when zooming Fig. 3). On the horizontal axis, points are positioned at the center of each size bin. The OPC data in the range 300-500 nm have very low statistical uncertainty (at least when compared with the corresponding SMPS values) and therefore much smaller error bars. However, it is known that the first channels of the Grimm-OPC can suffer of systematics uncertainty which could affect the results. We have to say that a similar study performed at CESAM resulted in life-time in the quoted interval of about 3 days (in that case OPC data were available from 400-500 nm and, more in general, with number very similar to those here reported, see https://www.eurochamp.org/Facilities/SimulationChambers/CESAM.aspx). We agree that this part of the manuscript is too sharp and that in the revised manuscript we should comment that the OPC data at diameters lower than 500 nm are not completely firm and that, conservatively, we consider maximum life-time in ChAMBRe of about 1 day for particles around 300 nm.

Page 6 line 197: Give type and manufacturer for the BLAM nebulizer.

This piece of information will be added in the revised manuscript.

Consider adding this information to section 2.

We could add this information in sec. 2 even if we'd prefer to maintain separate the discussion on bio-aerosol equipment.

Page 6 line 199: Explain what you mean with "a full range of particle dimension."

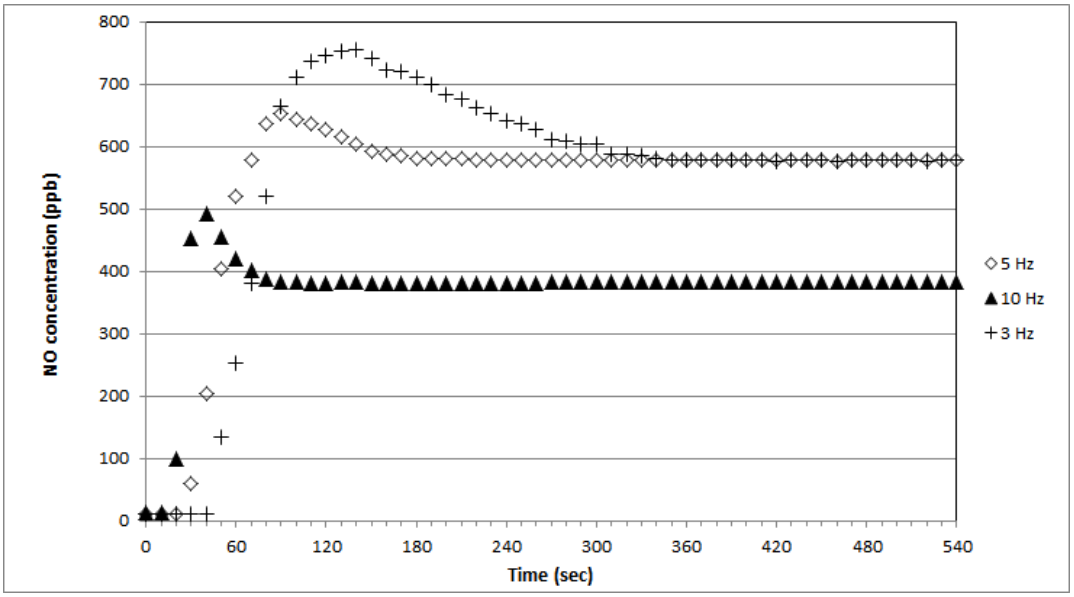
We agree the statement is too generic. The BLAM can generate poly-disperse aerosol (or it can be used to nebulize particles dispersed in a liquid solution) up to the micrometric range. We could actually detected injected particles and/or bacteria up to 6 μm.

We could modify the sentence in the text in "up to the micrometric range"

Page 6 line 202: Is the rotation speed of 5 Hz 5 revolutions per second? Explain how you determined the mixing time of 1 minute.

Yes, 5 Hz = 5 rev/sec = 300 rpm. We'll modify the units in r.p.m. in the revised manuscript.

We performed several experiments to determine gas phase mixing time at different fan speed. To do so, we used nitrogen monoxide as a tracer. Firstly, we checked that nitrogen monoxide is sufficiently inert inside our chamber, to perform this kind of studies. It was injected into the chamber at atmospheric pressure and 25 °C and monitored by using the AC32e monitor installed at ChAMBRe with a sampling flow rate of 0.66 L min^{-1}. The inlet line was mounted on a lateral port in the upper dome of chamber. The Fig. 2 shows that the tracer injected in the chamber can be considered as well-mixed in less than 60 s at the maximum of fan speed (600 rpm). This mixing time is relatively short comparing to the experiment durations (which may last for several hours). Nevertheless, when designing experiments, one will have to take into account this information.
In the manuscript we did a material mistake quoting at 5Hz=300 rpm the mixing time actually measured at 10 Hz = 600 rpm. We'll change the sentence in the revised manuscript as "in a mixing time of about 2 minute".



Page 6 line 204: How did you determine the mass decay curves?

See above our answer to Referee question on line 192, page 5.

Page 6 line 210: Replace aerosol lifetime by particle lifetime.

This correction will be done in the revised manuscript.

According to figure 3 the NaCl particle lifetime ranges from about 1 h for particle diameters of 20 nm and 3 µm to about 10-15 h for particle diameters of 300 nm. Hence the 4 days are not justified.

This is true and the "4-day" is due to a material mistake. The revised manuscript will report a consistent lifetime value with what is shown in figure 3 (i.e. max. around 1 day).

Which lifetimes did the two different bacteria strains have in your chamber? You should add this to figure 3 for comparison.

As a result of a preliminary test of bacteria time-segregated collection, bacteria life time in the chamber is expected to be lower than 5 hours, according with the particles lifetime plot. At the moment we do not have any on-line monitor of bacteria concentration (a WIBS unit is on the shopping list and a budget request has been submitted including this item). We inject (sec. 4) bacteria in a NaCl solution, hence during the experiments reported in the manuscript the particle sizers signal was completely dominated by salt particles. As reported above, we controlled that after a 5-hour exposure, all the viable bacteria get collected on the Petri dishes but at the moment we are not in the condition to answer precisely to the Referee's question.

What was the relative humidity for the lifetime studies with NaCl particles?

It was about 47%, we'll include this piece of information in the revised manuscript.

Page 6 line 214: No all trace gases are lost to the chamber walls.

We'll correct the sentence in "as the gaseous species can be lost to the chamber walls"

Page 6 line 220: Typically, ozone wall losses also depend strongly on its concentration showing a bi-exponential behavior with much faster loss rates for the first few ppb.

We fitted the O_3 concentration decay curves cutting-off the first part of the trend (i.e. the first 15 min) to avoid possible artefacts and actually we did not observe a double-exponential decay, at least in the range (of initial concentration) 300-1000 ppb quoted in the manuscript. We could maybe see a faster decrease in the first minutes = first 10 -15 ppb but we have basically neglected this part.

Page 6 line 224: You should replace aerosol by particles here.

This will be corrected in the revised manuscript.

How many particles (number & mass) are generated when you add ozone to the chamber and how does this change for subsequent experiments or after several cleaning cycles with high ozone concentrations.

As we wrote in the manuscript, we used ozone just to sterilize the chamber after each experiment with bacteria and in between two vacuum cycles. Actually, we monitored the particle background level after the cleaning procedure and at the beginning of each experiment and till now it remained in the range indicated below (see answer to comment at **Page 6 line 228)**. *By the way, we cannot observe any particle formation in the OPC range while producing O_3 (so far, we preferred to have the SMPS not working during the cleaning procedure with O_3).*

Page 6 line 227: Reformulate this sentence better explaining the cleaning procedure.

We'll reformulate the sentence in this way:

After each experiment, the chamber is cleaned by a multi-step procedure: the UV lamp (see sec. 2.1) is first switched on for10 min, the chamber is then evacuated and vented to atmospheric pressure through an HEPA filter (section 2.1). Afterwards, a high ozone concentration (>500 ppb) is produced to be sure to sterilize any part of the set-up possibly not reached before by the UV rays. Finally, the chamber is evacuated and vented again.

Page 6 line 228: Reformulate this sentence and give the detection limit of your particle measurements to define what no significant particle presence means. Didn0t you count the particles directly with a CPC?

The statement will be reformulated as follow:

Background level measurements performed subsequently to chamber cleaning showed no significant particles presence (i.e. about 2 and 0.5 particle cm^-3, respectively in the SMPS-LDMA and OPC range).

Page 6 line 228: Filling the simulation chamber with air from the laboratory through a HEPA filter can lead to changing amounts of trace gases in the chamber e.g. VOC which may impact the experiments and their reproducibility. An additional filter with an activated surface could improve this. Determination of the air quality in the chamber as well as controlling it constituents should be described in detail. Please note that it is not necessary to describe how a gas monitor works if you give type and maker but you should explain how you generate, dose and control the different gases including water vapour. The relative humidity is of special importance for many bioaerosols. Please note that the Humicap sensors typically suffer from exposure to higher ozone concentrations. Did you double check the humidity calibration after experiments with high ozone concentrations?

Actually, as reported at the end of sec. 2.1, a zeolite trap is mounted upstream the HEPA filter. We do not have at the moment a VOC monitor even if we are aware of its importance (as the WIBS, it is on the shopping list for next future). This, in our opinion, does not impact on the first results reported in the manuscript in sec. 4, where we simply show that the procedure to grow, inject and collect viable bacteria is well under control. It's clear however that VOC as well other parameters will be important for the next phases of our program. The Humicap sensor has been selected (see comment above in answer to your comment on **Page 4 line 138-139).** *We checked the Humicap by comparing its output with another unit (used in the lab surrounding ChAMBRe) never exposed to ozone.*

Page 7 line 233: Handling of bacteria is described in detail but it is not clear where this handling can be done and e.g. how quickly they can be transferred from the biological laboratory to the simulation chamber. It could be a unique strength of this simulation chamber facility e.g. if the handling would be possible in a nearby biological laboratory. Therefore, this aspect should be described in detail.

We propose to add at the beginning of sec. 4.2 the following text:

Several techniques for bacteria and bio-aerosol characterization are available on site. In the same building that hosts the atmospheric simulation chamber there is a basic microbiology lab equipment allowing for culture analysis in vitro (isolation, identification, growth) and biochemical tests (e.g. catalase and oxidase): autoclave (Asal mod.760), vortex, centrifuge and micro-centrifuge (Eppendorf centrifuge 5417R), water purification system

Milli-Q (Millipore-Elix), incubator for temperature control Ecocell and Friocell MMM Group, Steril-VBH Compact "microbiological safety" cabinet, Thermo electron corporation steri-cycle HEPA Class 100 incubator; optical microscope (Nikon Eclipse TE300) for bacterial detection and live/dead discrimination by epifluorescence with specific dyes and for immunoassay fluorescence to label antigenic bacterial target, fluorescent molecule or enzyme. The transfer of bacteria from the biological laboratory to the simulation chamber takes only a few minutes, ensuring a quickly execution of the chamber experiments, once the desired phase of bacteria growth is reached, and then a quick treatment of the samples collected after the experiments in the chamber.

Page 7 line 246-247: Give a reference for this statement.

We'll add a reference to Earl et al., 2008

Page 7 line 270: How good could you estimate the number of cells.

Data, obtained from spectrophotometric measurements (OD600 nm), were used to estimate when the mid-exponential phase (corresponding an OD_{600nm} of 0.5) is reached, not to determine the cells concentration. The number of cultivable cells is determinate as Colony Forming Units (CFU), by standard dilution plating. The uncertainties for the CFU numbers are reported in tables.
We understand however that the text in the manuscript was not clear enough and we propose to modify the text as follow:

The optical density of the bacterial solution, measured at a wavelength of 600 nm, is a common method for estimating the concentration of bacterial cells in a liquid. The amount of the light scattered by the microorganisms suspension is an indication of the biomass contents (Sutton, S. 2011). Data, obtained from spectrophotometric measurements (OD_{600nm}), were used to estimate when the mid-exponential phase (corresponding an OD_{600nm} of 0.5) is reached. Actually, the number of cultivable cells was counted as Colony Forming Units (CFU), by standard dilution plating etc. etc.

Page 7 line 273: Explain OD600nm at first occurrence.

See the answer to the previous comment.

Page 8 line 278: Give the parameters in the equation.

The parameters are: (B. subtilis curve, a is 1.10 ± 0.01, b is 38 ± 2; E. coli curve, a is 0.83 ± 0.01 and b is 41 ± 1). This information will be added in the revised manuscript.

and compare them to literature values.

We did not find any literature value to be compared with these numbers. Actually, according to the opinion of the microbiologists in the group, this is a piece of information usually neglected.

Page 8 line 287: Give proper uncertainties for the CFU numbers and OD600nm values.

Always use the same abbreviation throughout the text e.g. OD600nm not OD600.

The abbreviation will be harmonized in the revised text. Uncertainty on CFU/ml in each single experiment are fully reported in Tables 2 and 4. The sentence here aims simply to clarify the order of magnitude of the working conditions

What was the OD600nm for E.coli.?

Values and uncertainties of OD600nm for E. coli is given in Table 4. The text will be modified as follow:

In particular, for E. coli, to obtain the final concentration of 10^6 CFU mL^{-1}, the initial cells suspension with an OD_{600nm} around 0.6 (single values are reported in Table 4) was diluted etc. etc.

Page 8 line 289: Give the uncertainty for the "OD600 around 0.6" e.g. OD600nm of (0.6±0.3).

The expression "OD600nm around 0.6" indicates that in each experiment we tried to reach the same OD value, to obtain bacteria cells indicatively at the same growth phase. The OD values for each experiment are shown in Table 4 where we'll add, in the revised manuscript, the corresponding uncertainties. Typical values for uncertainties are 0.03.

Page 8 line 290: What do you mean with excessive bacterial concentration?

We actually try to avoid an excessive bacterial concentration of colonies counted on each petri dishes as explained in the paragraph 5.2. We propose to reformulate the sentence as follow:

to avoid an excessive bacterial concentration on the Petri dishes exposed inside the Chamber (see the paragraph 5.2).

Page 8 line 292-305: The technical details e.g. of the BLAM should be described in section 2 including the dimensions of the tubing.

As reported above we'd prefer to have the BLAM description in sec. 4.2 but we can easily move this part of the text to sec.2. In any case the sentence on the tubing will be modified as follow:

…connected to the chamber with a curved stainless-steel tube (length = 50 cm, diameter = 1.5 cm).

Page 8 line 305: Define the nebulizing efficiency.

The nebulization efficiency is defined as the ratio between the mass of the produced aerosol to the mass of the solute or of the material suspended in the liquid inserted in the BLAM.

We propose to modify the text as follow:

with a nebulization efficiency (i.e. mass ratio between the mass of the produced aerosol to the mass of the solute or of the material suspended in the liquid inserted in the BLAM) between 1 % and 8 %.

Page 8 line 307: Replace "tiny droplets" by a proper description of the droplet size distribution.

We give this information in the answer to the next comment.

Page 8 line 309-310: Give the size distribution (e.g. mean diameter and standard deviation) generated and define what you consider respirable range. Explain why the respirable range is of interest here.

We propose to change the text as follow:

"The accelerated air jet breaks up the liquid into droplets. The aerosol generated by this process is sprayed downwards inside the jar where the larger droplets are collected on the liquid surface due to impaction as they cannot make the U-turn while the finest droplets are forced up through the outlet tube on top of the BLAM lid. The result is a very fine mist, well within the respirable range (i.e. with diameter smaller than 10 μm) and with narrow size distribution. The size distribution, immediately after the injection of physiological solution (with or without bacteria) in ChAMBRe, shows a mean value of 0.45 μm with a standard deviation of 0.25μm".

This information, however, is just a typical figure since the actual size depend on the solution we nebulize according to the type and concentration of the solute. The interest in the respirable range is triggered by health issues. We'd consider this point quite clear but we can easily add a sentence in the text may be in the introduction.

Page 9 line 321-329: How do you avoid contamination of the Petri dishes, as they seem to be exposed to laboratory air?

See page 10 point e) The sterilizing UV lamp (ozone free, see section 2.2) is switched on for 15 minutes before injection to guarantee the Petri dishes sterilization.

Page 9 line 335: Explain the gravitational settling method. What is the settling time distribution for the bacteria you studied? Compare the average settling times with the typical atmospheric residence times of those bacteria.

It is assumed that the living microorganisms present in the aerosol are deposited on the petri dishes by gravity without undergoing any stress, from those related to the permanence in the experimental setup atmospheric conditions. In this way, it can be assumed that the number of units forming colonies counted on a Petri dish is proportional to the number of aerosolized and suspended living microorganisms within the chamber and also to the concentration value of viable bacteria in the aerosol.
Lee et al., 2002 suggest that the average aerodynamic diameters of generated E. coli and B. subtilis aerosols were 0.63 and 0.75 µm respectively. If compare these data with data obtained with NaCl solution to determine particles life time in chamber, the bacteria life time is aspect to be around five hours. The mean global residence time calculated by Burrows et al., 2009b, lie between 2 and 15 days for bacteria traces.

This explanation will be included in the revised manuscript.

Page 9 line 336: Define in which respect you consider this method as efficient.

We understand the statement is too crude. Actually, during the pilot experiment described in Brotto et al., several methods to collect viable bacteria had been preliminary testes (including) filtration and impaction but the "gravimetric" collection on petri dishes was the best to keep bacteria alive and count the formed colonies. This was the background of the term "efficient". We propose to change the sentence in this way:

…proven to be a very suitable way to collect and count viable bacteria colonies (Brotto et al., 2015).

Page 9 line 338: Describe how the filter samples are collected.

The sampling was performed by exposing filters to the stream of aerosol coming out of the nebulizer, through a secondary flange connected at the chamber.

This sentence will be included in the revised manuscript.

Page 9 line 340: What do you mean with "tendency to aggregation"? Do you refer to sampling artifacts or to coagulation?

It is referred to bacteria tendency to form aggregates and biofilms in response to stress conditions.

We propose to change the sentence in the following way:

…ideal to study the morphology of cells and possible bacteria aggregates (e.g. biofilm formation) by scanning electron microscopy (Capannelli et al., 2011).

Page 9 line 348: Explain what kind of filter unit you used.

This is a very simple tool, quite common in chem. labs. (Fig. 3-4). Should we describe it in detail?

Page 10 line 369: Give an uncertainty for the estimated living bacteria concentration in the chamber e.g.: (10ˆ5 ± ??) CFU mˆ-3 = 0.1 CFU cmˆ-3. Compare the number concentration of living and dead bacteria. Compare the number and size (mass) concentrations of the aerosol particles measured with the dead and living bacteria concentrations.

At the moment our equipment cannot discriminate between the number of live and dead bacteria, because our sampling method on solid petri dish allows us to evaluate only the cultivable fraction of aerosolized bacteria in the chamber. The 10^5 values is given just to inform on the OoM of the concentration of injected bacteria. Actually, we refer and anchor our experiments to the CFU/ml in the BLAM solution which are known with the accuracy reported in Tables 2 and 4.

Page 10 line 373: What do you mean with "statistically compatible", significant?

We considered, in each petri dish, the number of counted colonies and its sqrt. value (i.e. the SD according to Poisson distribution) and we could observe that, within the interval delimited by the SD values, the counts in the four petri dishes were in agreement (i.e, statistically compatible) in each experiment.

We propose to change the sentence in:

turned out to be statistically compatible (i.e. within the interval delimited by the statistical uncertainty, the counts in the four petri dishes were in agreement).

Page 10 line 377: Do you mean no significant effect related to RH? Would you expect a significant effect related to the variations of relative humidity?

Yes, because for Escherichia coli a R.H at least 70% was essential for the success of the experiments (see section 5.2). We chose gram negative E. coli and gram positive B. subtilis because of the difference in their cell wall structure. Gram positive B. subtilis has a single, relatively thick, and hardy cell wall, while gram negative E. coli has double layers and a soft cell wall (Madigan, Martinko, & Parker, 2000, Chapter 3. Brock biology of microorganisms (9th ed.)). From this difference in cell walls, we suppose a difference in the aerosolized behavior, and the results seems to confirm this prediction (FESEM micrographs).

How could this be related to the residence time or drying time of the bacteria?

Lee et al., 2002 suggest the changes of aerodynamic diameters of the aerosols as a function of the relative humidity. E. coli and B. subtilis aerosols grow significantly above 85% relative humidity and E. coli aerosols grow more than B. subtilis (Fig. 5-6). Our range of R.H. was always under this percentage, so we do not aspect a difference in the residence time between the experiments.

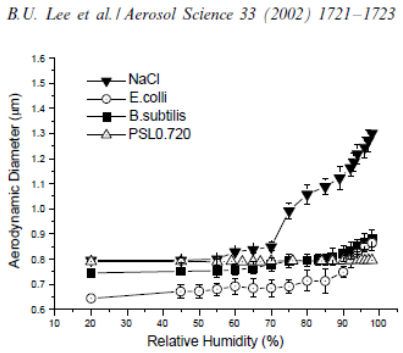


Fig. 1. The changes of aerodynamic diameters of aerosols as a function of the relative humidity.

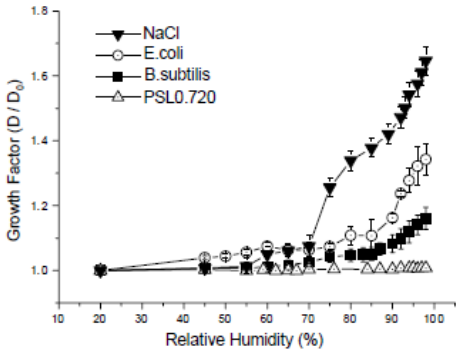


Fig. 2. Hygroscopic growth factors as a function of the relative humidity.

Page 10 line 382: Explain how the relative humidity in the chamber was increased and what you mean with the environmental value.

The relative humidity inside the chamber was controlled by changing the working condition of the humidifier. "registered environmental value" means the relative humidity value recorded that day in the laboratory surrounding the simulation chamber.

We propose to change the sentence in:

the environmental value recorded in the laboratory, by changing the working condition of the humidifier.

Page 11 line 395: Indicate if the uncertainty in the slope of the correlation (±5 %?) includes the uncertainties of the individual measurement values in the plot. Replace "about 5 %" and "around 10 %" with well-defined values.

T*he slope uncertainty is determined including those of single points. We'll correct the text with values better defined (i.e.: 7% for B. subtilis and 4% for E. coli).*

Page 11 line 399: Explain how the use of the optical density measurements influences the uncertainty of the cell quantification.

We agree the sentence is not clear enough. Actually, our aim here was simply to underline that the results reported in the manuscript are encouraging in this direction too (that's why se said: seems therefore sufficiently adequate) but the possibility to firmly related OD to collected CFU remains to be verified. We propose to change the sentence as follows:

For E. coli suspension, the evaluation of the microbial concentration through the fast and simpler control of the optical density, seems possibly be accurate enough to perform controlled experiments, provided an adequate calibration of the whole procedure is carried out.

Page 11 line 404-408: Double check these observations by comparison with the particle measurements in the chamber.

Actually, this check cannot be performed at the moment since the particle sizers counts during the experiments are totally dominated by salt particles.

Page 11 line 410: The conclusions should be reformulated and extended including a comparison of typical atmospheric residence times for bacteria with those that can be achieved within ChAMBRe.

We propose to add in the conclusions the following statement:

Residence times of viable bacteria in ChAMBre are less than 5 hours, much shorter than the generic residence time in the open atmosphere. However, previous literature studies (Brotto et al., 2015) suggest that such time window is long enough to observe the effects (i.e. viability change) of bacteria exposure to air pollutants. The assessment of such effects is objective of the fore coming studies at ChAMBRe.

Table 1: Explain the range of relative humidities.

The range shows the minimum and the maximum value of relative humidity, measured inside the chamber during the experiment.

Tabel 3: For which times during the experiments did you determine the relative humidities and temperatures?

Actually, these parameters are measured in continuous.

Tabel 4: Can you estimate the ratio of CFU vs. non-CFU deposited on the Petri dishes e.g. based on the bacteria concentrations, sedimentation rate and area of the Petri dishes?

No, we can't. Actually, we could say that the ratio could arrive to be around 10 but this figure would be based on too much speculative arguments and we prefer to postpone this kind of consideration to further experiments performed with other collection methods (presently in the set-up phase).

Figure 1: Explain the different parts of the chamber in the caption. Is there any air guiding tube surrounding the fan? Is the fan efficiently mixing the upper and typically warmer part of the chamber? What is the typical vertical temperature gradient? Would it be possible to heat the chamber to 37_C?

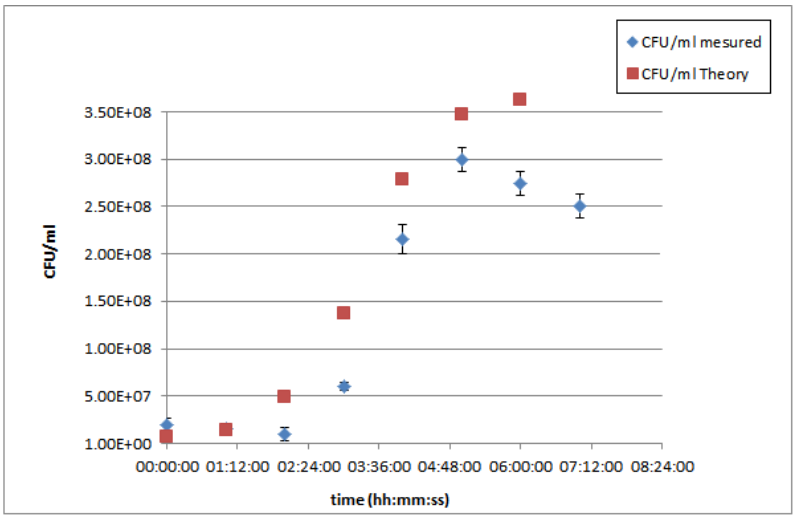
*There is no guiding tube. The mixing time has been measured connecting the analyzer in the upper part of the Chamber (see above our answer to question on **Page 6 line 202).** We have at the moment just one T sensor and we cannot measure the T gradient however, considered that the stainless chamber is relatively small and it is inside a climatic room we do not expect large variations. It is certainly possible to heat the chamber and we are working to implement a stable and reliable system.*

Figure 3: Indicate RH and temperature for the experiment in the caption.

These values will be added in the caption: 47%, 21 °C.

Figure 4: Include uncertainties for the individual data points. Compare the optical density measurement to the CFU data and/or literature data.

Uncertainties are at the 5% level, they will be indicated in the revised figure. The relationship between OD and CFU is quite standard (at least in micro-biology), an example for E. Coli is reported in Fig. 7 (red: theory, blue: measured).We'd not consider to add this kind of information (well known in literature) in the text but we could obviously add a Figure.



Figures 5&6: Indicate if the uncertainties for the individual data points are included in uncertainties of the slopes.

*Please, see our answer to comment at **Page 11 line 395***

Technical corrections:

Technical corrections will be considered and added to the revised manuscript.

Page 1 line 16: . . .processes at realistic but controlled conditions.
Done.
Page 1 line 21: . . .is made of stainless steel. . .
Done.
Page 1 line 22: . . .10 to 2 hours.
Done.
Page 1 line 24: . . . have impact on several levels as: . . .
Done.
Page 1 line 25: . . ., and geochemistry.
Done.
Page 2 line 40: . . .and maintain their pathogenic potential, . . .
Done.
Page 2 line 41-42: check wording
Page 2 line 44: . . .chemical, and biological properties. . .
Done.
Page 2 line 61-63: Reformulate sentence without brackets.
Done.
Page 3 line 109: . . .height.
Done.
Page 4 line 114: . . .designed to move specific samples inside the chamber as described. . .
Done.
Page 4 line 120: . . .failure it automatically closes in less than one ms, . . .
Done.
Page 4 line 127: . . .with four metallic arms of 25 cm length each. . .
Done.
Page 4 line 131: . . .and an accuracy of ±10% of its reading. . .
Done.
Page 4 line 133: The pressure transducer contains. . .
Done.
Page 5 line 185: . . .concentrations are monitored. . .
Done.
Page 7 line 236-238: Reformulate the sentence in correct English.

The sentence will be modified as follows:

In this section we describe the standard methodology developed for the bio-aerosol experiments (injection, collection and storage) and the related experimental conditions, that should be representative of the typical environmental ones.

Page 7 line 255: Reformulate the sentence in correct English.

The sentence will be modified as follows:

The same culture preparation technique was applied at both the bacterial strains, in order to minimize experimental variations.

Page 7 line 257: . . .prior to the injection.
Done.
Page 7 line 268: In both cases,. . .
Done.
Page 8 line 278: The cultivable cell concentration. . .
Done.
Page 8 line 288: . . .was prepared for nebulization. . .
Done.
Page 8 line 290: . . .was diluted (. . .

Done.
Page 8 line 306: . . .with a cavity depth and a cone diameter of . . .
Done.
Page 8 line 313-314: . . .completely separate the cylinder, which can be connected to the main chamber or. . ..
Done.
Page 9 line 316: This volume can be evacuated through a by-pass to the. . .
Done.
Page 9 line 325: Valve V2 is closed and the volume inside the pipe is flushed with clean air from the chamber.
Done.
Page 9 line 332: . . .bacteria have been injected. . .
Done.
Page 9 line 33: After exposure to the chamber atmosphere, . . .
Done.
Page 9 line 345: . . ..were not done in this case as the study. . .
Done
Page 10 line 371: . . ..contributions.
Done.
Page 10 line 374: . . .appears to be adequate. . .
Done.
Page 11 line 402: . . .second set of experiments providing the Gram-negative microorganisms a more. . .
Done.

Anonymous Referee #2

Received and published: 24 August 2018

The manuscript provides a characterisation of the new ChAMBRe atmospheric simulation chamber at Genova. The importance of the quantification of chamber-specific influences and interferences is not routinely recognised and the authors are to be commended on their attempts to provide such a characterisation. This manuscript provides the first part of "a user manual" for such a chamber and a demonstration of its fitness for purpose. As such, it is highly suitable for publication in AMT.

The range of important questions that can be addressed in ChAMBRe is succinctly and concisely summarised in the introduction, which thereby highlights the need for such a facility.

Key characteristics: Size dependent particle lifetime; Preservation of bioaerosol viability through injection and extraction; Ability to explore bioaerosol viability under variable controllable and measurable changes in atmospheric composition; Repeatability under clean conditions demonstrated for 2 bacterial strains; one each of gram positive and gram negative groups, showing that it is possible to investigate viability under changing environmental conditions.

The chamber and instrument description section is sufficiently detailed to provide the reader with the information to evaluate the suitability of the facility. It appears that the facility is very appropriately designed and well-appointed.

The chamber characterisation is one of the main foci of the paper and has been carried out and described well - certainly adequately for a reader to evaluate the characteristics of the facility.

The protocol for preparation and execution of the bacterial experiments has been well developed and is described appropriately. As a non-specialist, I am not qualified to comment on the appropriateness of the biological handling protocols or e.g. choice of bacteria; however, for the purposes of the manuscript, the details provided are sufficient to reproduce the protocol.

The description of the first experiments was informative and provided a good illustration of the suitability for purpose of the facility. The repeatability within Poisson statistical expectation was convincing evidence for this. The fact that an empirically-determined dilution was required for E. coli (and that CFU was extremely RH dependent) is interesting and these experiments demonstrated the great care that will need to be employed in future investigations using the facility.

Generally, I find the publication very suitable for publication in AMT. However, I think there is one very important omission that can be addressed with modest discussion.This relates to the closeness to ambient conditions that is achievable within the facility

A key challenge in investigating PBAP in general and airborne bacteria in particular is their extremely low ambient abundance (from 0.01 to 1 cells/cc). Such low concentrations present difficulties with particle sizing and counting instrumentation relating to the counting statistics, often physically limited by the instrument sample volume. I believe it is important that the authors discuss such limitations and the implications of necessarily studying under higher than ambient concentrations within ChAMBRe (such as differences in amount of reactant per cell). In this regard, it should be noted that the facility description indicates a maximum particle number measurement threshold

of 10^7 /cc, but now lower detection limit and sensitivity. Indeed, the only mention of the instrument sensitivity for the lower limit is on line 229 where the value is still not provided. A general discussion of the accessible ranges of concentrations, the challenges with instrumentation and the comparison to ambient conditions should be provided. One final point on this subject - one might have expected that a key instrument for PBAP experiments would be one of the recent online fluorescence instruments (e.g. WIBS, UV-APS or more recent developments). Could the authors discuss whether these factor in their plans. It would appear to provide an ideal opportunity for comparison of online and offline techniques and perhaps address some of the concentration concerns I have raised above.

We thank the Reviewer for the extremely useful suggestions/criticisms and comments. We have carefully taken into account each point to improve the quality of the paper. In the text, we have added some information on typical bacteria residence time and concentration in the atmosphere as well as about minimum detection levels and sensitivity of used instrumentation. During our experiments, the typical bacteria concentration in the chamber is about 10^5 cells/m^3 –see lines 369 of the original manuscript- (since 10^7 is the concentration before the aerosolization process). This value is actually very close to the values of 0.01-1 cells/cc reported in literature (and by the reviewer) of typical ambient abundance. As the reviewer correctly notes, recent florescence instruments can help a lot in our experimental activity. In fact, the installation of a WIBS unit is expected in the next 6 months.

line 98 and elsewhere: Genova or Genoa? Consistency should be ensured throughout the document.

The format will be consistent in the revised manuscript.

line 108 and 118: I presume this should be 5 * 10^{-2} mbar, not 510^{-2} (presumably for AMT, SI units should be used - the editor can advise). All subsequent pressure values also suffer from this notation and it should be corrected.

The corrected notation will be used in the revised manuscript.

line 121: I presume the 2 step procedure is to ensure the HEPA and zeolite efficiency is not challenged by operating from 5 * 10^{-2} mbar to 5 mbar. Is this correct? If so, it would be useful to state it.

Actually, the two-step process comes from a long expertise with vacuum systems: in the very first phase of the venting, the use of nitrogen from a cylinder guarantees that no water vapor (or others) enter the chamber and penetrate in the walls. When the walls are "coated" with nitrogen, ambient air is used through the HEPA filter to bring back the chamber to atm. Pressure.

line 142 - 147: I presume the 253 nm lamp is to allow sterilisation without ozone generation. If so, it would be useful to state it for the reader's benefit.

Exactly, a clarification was added to the text.

line 174 - 179: It is interesting to note the dehumidification system in the OPC. This will be very useful to avoid droplet ingress into the instrument and prevent too much hygroscopic growth. It will also ensure a good chance that non-spherical "solid" particles do not assume spherical geometry by water uptake. What is the implication of this for OPC sizing of e.g. rod-shaped or other non-spherical bacteria or dust? Don't OPCs rely on refractive index and shape assumptions?

The "artifacts" in the OPC response related to particle shape are known and need proper correction (see for instance Caponi et al. (2017). Spectral- and size-resolved mass absorption efficiency of mineral dust aerosols in the shortwave spectrum: a simulation chamber study. ATMOSPHERIC CHEMISTRY AND PHYSICS, vol. 17, p. 7175) however, in our experiments we do not try to measure the bacteria size-distribution by OPC (since the counts are totally dominated by the salt particles of the physiological solution) and the OPC is just used to check the global quantity of the injected material.

line 191: I'd suggest the upper limit to typical reaction times should be days rather than hours (e.g. the gas phase oxidation lifetime of SO2, OH oxidation lifetime of methane, ageing of organic aerosol and increase in O:C ratio etc...)

The statement will be corrected in the revised manuscript. .

line 198: I think the reference should be to 4.2 not 4.3

Done.

line 205: It is stated that "Aerosol dilution due to the air flow through the two counters (in total: 1.6 L min-1) was taken into account and properly corrected". Does this mean that pressure is held constant and the same amount of clean scrubbed air is supplied to the chamber? This should be stated.

The chamber is designed to ensure that the pressure is kept constant: the same amount of clean air is introduced into the chamber through the input from the HEPA filter.

This statement will be included in the revised manuscript.

line 208 - 209: "nicely reproduced" - please provide a more scientific description – a goodness of fit metric ideally.

The Lai & Nazarov model can of course predict the life-times in a approximative way (but we also must underline that what it is really important to know is the typical lifetime values for particles in a given dimensional range). As a matter of fact, "nicely" here corresponds to a mean discrepancy between calculated and measured lifetimes of about 50%. This figure will be included in the revised text changing the sentence in:

"…nicely reproduced (i.e. the mean discrepancy between measured and calculated values is around 50%)".

Trivial: I believe the Pasteur reference was originally from Annales des sciences naturelles, Zoologie, 4th series (1861), followed by its publication in Annales de chimie et de physique, 3rd series, 64 (1862), not 1890

Citation will be corrected in the revised manuscript.

ChAMBRe: a new atmospheric simulation Chamber for Aerosol Modelling and Bio-aerosol Research

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Abstract. Atmospheric simulation chambers are exploratory platforms used to study various atmospheric processes at realistic but controlled conditions. We describe here a new facility specifically designed for the research on atmospheric bio-aerosol as well as the protocols to produce, inject, expose and collect bio-aerosols. ChAMBRe (Chamber for Aerosol Modelling and Bio-aerosol Research) is installed at the Physics Department of the University of Genova, Italy, and it is a node of the EUROCHAMP-2020 consortium. The chamber is made of stainless steel with a total volume of about 2.2 m^3. The lifetime of aerosol with dimension from a few hundreds of nanometres to a few microns varies from about 10 to 2 hours. Characteristic parts of the facility are the equipment and the procedures to grow, inject and extract bacterial strains in the chamber volume while preserving their viability. Bacteria are part of the atmospheric ecosystem and have impact on several levels as: health related issues, cloud formation, and geochemistry. ChAMBRe will host experiments to study the bacterial viability versus the air quality level, i.e. the atmospheric concentration of gaseous and aerosol pollutants. In this article, we report the results of the characterization tests as well as of the first experiments performed on two bacterial strains belonging to the Gram positive and Gram negative groups. A reproducibility at the 10% level has been obtained in repeated injections and collection runs with a clean atmosphere, assessing this way the chamber sensitivity for systematic studies on bacterial viability vs. environmental conditions.

1. Introduction

1.1 The problem of bio-aerosol and bacterial strains

The biological component of atmospheric aerosol (bio-aerosol) is a relevant subject of both atmospheric science and biology. From the pioneer investigations at the end of the nineteenth century (Pasteur, 1862), the study of primary biological aerosol particles (PBAP) has definitively become a multidisciplinary field of research, which requires expertise in physics, chemistry, biology and medical sciences (Desprès et al., 2012). Among PBAP, bacteria have a crucial role (Bowers et al., 2010). They show atmospheric concentrations from 10^4 to 10^6 cells m^{-3} (Ligthart, 1997, 2000) with a wide range of diversity (Amato et al., 2007; Burrows et al., 2009; Gandolfi et al., 2013; Maki et al., 2013). Bacterial viability, including the capability of pathogens to survive in aerosol and maintain their pathogenic potential, depends on the interaction between bacteria and the other organic and inorganic constituents in the atmospheric medium: such interplay is still far from a satisfactory knowledge and understanding (Jones and Harrison 2004; Kellogg and Griffin 2006; Deguillaume et al., 2008; Tang, 2009; Bowers et al., 2010). On the other side, bacteria and PBAP dispersed in the atmosphere can be chemically active (Ariya et al., 2002) and favour the formation of ice and cloud condensation nuclei (Ariya et al., 2009; Hoose et al., 2010; Möhler et al., 2008). Primary biological aerosol particles are generally assumed to be efficient CCN, provided that their surfaces are wettable (Després et al., 2012). Bauer et al. (2003) suggested that the chemical composition, structure and hydrophilicity of the surface layer of bacteria could play important roles in CCN activity. Ariya and Amyot (2004) proposed that bio-aerosols have a potential role in the chemistry of organic compounds in the troposphere via microbiological degradation and hence inducing changes in the IN or CCN ability of organics in atmosphere.

So far, PBAP have been studied in-field through a variety of sampling and analysis techniques and addressing their physical, chemical, and biological properties (Reponen et al., 1995; Li and Lin, 1999; Brodie et al., 2007; Georgakopoulos et al., 2009; Fahlgren et al., 2010; Lee et al., 2010; Urbano et al., 2011). The connection between PBAP and dust dispersion and transport over very long distances (Goudie and Middleton, 2006) deserves a particular mention. Dust clouds may contain high concentrations of microbiota, e.g. fungal spores, plant pollen, algae and bacteria. Bio-aerosols associated with dust events can spread pathogens over long distances (Prospero e al., 2005; Griffin, 2007; Nava et al., 2012; Van Leuken et al., 2016) and can impact ecosystem equilibria, human health and yield of agricultural products. For many microorganisms long-range and high-altitude transport in the free atmosphere can be very stressful due to strong ultraviolet radiation, low humidity (inducing desiccation), too low or too high temperatures, and complex atmospheric chemistry (e.g. presence of radicals or other reactive species) (Després et al., 2012; Zhao et al., 2014). Only very resistant organisms are able to survive, so the composition of microbiota can change during the long airborne transport prior deposition (Meola et al., 2015).

Airborne bacterial communities are highly diverse, and variations in their species diversity are quite complex. The bacterial composition in air is strongly dependent on many factors such as seasonality, meteorological factors, anthropogenic influence, variability of bacterial sources and many other variables. Still, the general trend from available reports is that bacteria found in the air often belong to groups that are also common soil bacteria (e.g. Firmicutes, Proteobacteria, Actinobacteria) (Després et al., 2012). Due to their small size, bacteria have a relatively long atmospheric residence time (on the order of several days or more) compared to larger particles and can be transported over long distances (up to thousands of kilometres). Measurements show that mean concentrations in ambient air can be greater than 1 x 10^4 cells m^{-3} over land, whereas concentrations over the sea may be lower by a factor of 100-1000 (Burrows et al., 2009a, Burrows et al., 2009b).

Bio-aerosols also seem to play an important role in the reactivity of particulate matter. They can induce Reactive Oxygen Species (ROS) production and modify particulate matter (PM) toxicity due to their ability to modulate the oxidative potential (OP) of toxic chemicals present in PM (Samake et al., 2017).

Therefore, within the bacterial survival studies there are four interconnected topics. One is related to health issues: exposure to bio-aerosols has been linked to various health effects (disease spreading e.g. Meningitis and bioaero-contamination, like

Legionella and refrigerating towers. Pearson et al, 2015; Ghosh et al, 2015; Sala Ferré et al, 2009). Another topic is connected to climate and CCN/IN impact, where viability and proliferation of airborne bacteria are the significant investigation subjects (Bauer e al., 2003; Deguillaume et al., 2008; Amato et al., 2015). A biogeochemical issue is related to the long range transport of bacteria and dust events (Meola et al., 2015; Nava et al., 2012; Van Leuken et al., 2016). ~~Finally, the role of bacteria in making the atmosphere a complex ecosystem has still to be assessed.~~

1.2 Atmospheric simulation chambers and bacteria

The study of relevant processes taking place in the Earth atmosphere is usually pursued through a wide range of field observations where complicate, unexpected and interconnected effects are often difficult to disentangle. The possibility of planning and performing experiments in controlled conditions is therefore highly desirable. This need triggered the concept and the development of the atmospheric simulation chambers (ASCs in the following), i.e., small- to large-scale facilities (with volumes ranging between a few to hundreds cubic meters), where atmospheric conditions can be maintained and monitored in real time for periods long enough to mimic the realistic environments and to study interactions among their constituents (Finlayson-Pitts and Pitts, 2000). ASCs have been used to study chemical and photochemical processes that occur in the atmosphere, such as ozone formation (Carter et al., 2005 and references therein) and cloud chemistry (Wagner et al., 2006) or aerosol-cloud interaction (Benz et al., 2005), but the high versatility of these facilities allows for a wider application covering all fields of atmospheric aerosol science. A full list and review of the approach and of the main facilities around the world can be found in Becker (2006). In Europe, there are several ASCs organized through the network EUROCHAMP-2020 (see all the details at the link www.eurochamp.org).

Since the interplay of bio-aerosol and atmospheric conditions is still poorly known, suitable facilities are needed, where transdisciplinary studies gathering atmospheric physics-chemistry and biology issues are possible.

Experiments conducted inside confined artificial environments where physical and chemical conditions/compositions can be controlled, can provide information on bacterial viability, biofilm and spore formation and endotoxin production. Currently, the literature reports several examples of studies performed in small reactors (Levin et al.,1997; Griffiths et al., 2001; Ho et al., 2001; Ribeiro et al., 2013; Sousa et al., 2012). The use of atmospheric simulation chambers has been much more limited and focussed on the interaction of bacteria with atmospheric parameters, regarding bio-aerosols release effects (Jones and Harrison, 2004), and on ice nucleation and cloud condensation (Möhler et al., 2008; Bundke et al., 2010; Chou, 2011).

In 2014, some of the co-Authors of the present work, designed and performed an exploratory experiment (Brotto et al., 2015) at the CESAM (French acronym for: Experimental Multiphasic Atmospheric Simulation Chamber) atmospheric chamber (Wang et al., 2011). On colonies of *Bacillus subtilis* injected, then extracted from CESAM on Petri dishes, they could observe a clear increase of bacterial viability when concentrations of NO/NO_2 and CO_2 were contemporarily maintained inside the simulation chamber at a level of about 65/630 ppb and 400 ppm, respectively. *Bacillus subtilis* is a well-known Gram positive bacterial strain (Burrows et al., 2009; Gandolfi et al., 2013) and the viability increase observed in the two experiments was by a factor 35 and 10, respectively (Brotto et al., 2015). Such experimental evidence made clear that the effects of atmospheric pollution on bacteria viability could be studied in atmospheric chambers. In order to perform systematic studies to resolve and describe the physical and chemical mechanisms ruling these interactions, dedicated facilities with a microbiology laboratory linked to the ASC for the handling and characterization of bio-aerosol are needed.

Prompted by the outcomes of pilot experiments (Amato et al., 2015; Brotto et al., 2015), a new dedicated atmospheric chamber, ChAMBRe (Chamber for Aerosol Modelling and Bio-aerosol Research), has been designed and installed in Genova (IT). While ChAMBRe, as other ASCs, is a multi-purpose facility, the outcomes of the correlation between bacteria viability and atmospheric condition/composition will provide the input for developing ad-hoc modules to be then implemented in

chemical transport models. This can be done following a scheme often used for the chemical mechanisms parameterization (see for example the smog chamber experiments used for the evaluation of Carbon Bond mechanisms in Parikh et al., 2013). Such software tools, are widely used both in scientific research and in air quality evaluations, to predict the fate (i.e. transport, deposition and chemical changes) of the atmospheric pollutants and, at the moment, they do not include any biological patch.

2. Description of the facility

2.1 ChAMBRe main structure

ChAMBRe is installed at the ground floor of the building hosting the Department of Physics of the University of Genova, where it is jointly managed by the Italian National Institute of Nuclear Physics (INFN) and the Physics Department (www.labfisa.ge.infn.it). Since the beginning of 2017, ChAMBRe is one of the nodes of the EUROCHAMP-2020 network with specific tasks on bio-aerosol studies ~~and modelling~~.

CHAMBRe has a cylindrical shape with domed bases (Figure 1). It has maximum height and diameter of 2.9 m and 1 m, respectively and a total volume of 2.23 m^3. The latter includes all the secondary volumes connected to the main body and has been determined measuring the volume of air needed to bring the chamber at atmospheric pressure after an evacuation down to 5 x 10^{-2} mbar. The main body is divided into three parts: two domed cylinders (see Figure 1) connected by a central ring 60 cm height. The lower dome has a bottom aperture with a pass through for the shaft of a fan and two lateral ISO-K250 flanges. The central ring allocates symmetrically six flanges (two with a diameter of 40 cm and four with a diameter of 10 cm). Finally, the top cylinder is equipped with two lateral and symmetrical ISO-K100 flanges plus another flanged aperture (ISO-K250) on the dome. The interior of the chamber can be accessed through the two ISO-K400 flanges or removing the top dome by a crane. One of the two flanges in the bottom part is connected through a pneumatic valve to a smaller horizontal cylinder, (length = 1 m) which hosts a movable shelf designed to move specific samples inside the chamber as described in section 4.3. The whole structure is maintained in vertical position by an *ad-hoc* metallic structure, i.e. the lower dome is hold by a metallic support to maintain the entire structure in vertical position (Figure 2).

While ChAMBRe has been designed to operate at atmospheric pressure, the second ISO-K250 flange of the lower cylinder is connected to a composite pumping system (a rotary pump model TRIVAC® D65B, Leybold Vacuum, followed by a root pump model RUVAC WAU 251, Leybold Vacuum) which can evacuate the internal volume to a vacuum level of about 5 x 10^{-2} mbar in about 15 minutes. . A safety valve (Leycon Secuvac DN 63, Oerlikon Leybold Vacuum) is mounted as a gate between the pumping system and ChAMBRE: in the event of a power failure it automatically closes in less than one ms, thus preventing possible backwashes of the pumps oil inside the chamber. The return to atmospheric pressure is a two-step procedure: first pure N_2 from a compressed gas cylinder is flushed in, until a pressure of 5 mbar is reached, and then the ambient air can enter the chamber through an absolute HEPA filter (model: PFIHE842, NW25/40 Inlet/Outlet - 25/55 SCFM, 99.97 % efficient at 0.3 μm) and a zeolite trap (upstream).

2.2 Basic equipment

To favour the mixing of the gas and aerosol species in the reactor a fan is installed in the bottom part of the chamber (Figure 1). It is a standard venting system with four metallic arms of 25 cm length each connected to an external engine through a rotating shaft. A particular pass through has been designed and built at INFN-Genova to ensure the vacuum seal. The fan speed can be regulated by an external controller and varied between 0.0 Hz and 50 Hz in steps of 0.1 Hz (0 to 3000 rpm, in steps of 6 rpm).

A set of two pressure gauges is used to measure the atmospheric pressure inside and outside the chamber. A MKS Instruments 910 DualTrans™ transducer is installed inside with a measuring range of 5 x 10^{-4} to 2 x 10^3 mbar and an accuracy of ±10 % of its reading, in the range of 5 x 10^{-4} to 1 x 10^{-3} mbar, ±5 % of reading in the range of 10^{-3} to 15 mbar and ±0.75 % of reading

in the range of 15 to 1000 mbar. The pressure transducer contains two separate sensor elements: a MicroPirani™ sensor element, based on measurement of thermal conductivity, and a Piezo sensor, based on measurement of the mechanical deflection of a silicon membrane relative to an integrated reference vacuum. The Piezo measures true absolute pressure independent of gas composition and concentration. A Vaisala BAROCAP® Barometer PTB110 is installed outside the chamber with a measuring range of 5 x 10^2 to 1.1 10^3 mbar and accuracy of ±0.3 mbar at 20° C.

Internal temperature and relative humidity are continuously measured by a HMT334 Vaisala® Humicap® humidity and temperature transmitter for high pressure and vacuum application (up to 100 bars). This sensor is mounted in the upper ISO-K100 flange on the top dome. In the operative range (from 15 to 25 °C) the accuracy is ± 1 %RH (0 to 40 %RH) and ± 1.7 %RH (90 to 100 %RH) and ± 0.2°C at 20 °C.

All the atmospheric gauges are connected to a NI Compact-RIO acquisition system (based on the NI cRIO-9064 controller) which also allows the remote monitoring of the ChAMBRe parameters through an Ethernet connection.

Two type of UV lamps are permanently installed inside the chamber. A 90 cm long lamp is inserted through the flange in the top dome (Figure 1): it produces a 85 W UV radiation at λ = 253.7 nm (UV-STYLO-NX, Light Progress srl) which is used to sterilize, without producing ozone, the chamber volume, in particular after any experiment with bio-aerosol. A second type of lamp, producing UV radiation at λ <240 nm, can be inserted through one of the ISO-K100 flanges of the central ring to generate ozone. Two different units of mercury lamps (length = 5 cm, power = 6 W and length = 20 cm, power = 10 W; both of BHK Incorporated, Analamp models), can bring ozone concentration inside ChAMBRe from zero to about 300 ppb in about 30 or 15 minutes, respectively.

2.3 Instruments connected to ChAMBRe

The large number of free flanges in the main structure gives the possibility to connect several external instruments to ChAMBRE. Aerosol samplers and multi-stage cascade impactors can be easily connected through the ISO-K flanges and maintained in operation for times depending on their nominal flow and the needs of the particular experiment (e.g. a typical 10 L min^{-1} device, like the 13-stage rotating NanoMoudi-II™ - Nano-Micro orifice uniform deposit impactor, Model 125B, MSP Corporation; Hwan et al., 2010 - extracts a 10 % of the total chamber volume in about 20 minutes). A similar figure holds for impingers (*Flow Impinger* by Aquaria srl) which can be filled with 20 mL of sterile physiological solution. Such devices must be operated at a constant air flow of 12.5 L min^{-1} (e.g. by a Low Capacity Pump Model LCP5, Copley Scientific).

Particle concentration inside the chamber is measured continuously by two different instruments: a Scanning Mobility Particle Sizer (SMPS, GRIMM Technologies, Inc.) and an Optical Particle Counter (OPC, mod. Envirocheck 1.107, GRIMM Technologies, Inc.).

The SMPS is formed by three components in sequence: a neutralizer (i.e. a bipolar diffusion charger) supplied by Eckert & Ziegler Cesio (Prague), a differential mobility analyzer (DMA, model 55-U) and a condensation particle counter (CPC, model 5403), both from Grimm GmbH (Ainring, Germany). The neutralizer is based on a radioactive source of ^{241}Am with an activity of 3.7 MBq. The DMA is available with two different columns, working alternatively in the size range 5.5-350.4 nm (MDMA), and 11.1-1083.3 nm (LDMA), and classifying particles in 50 dimensional classes. Scanning the voltage through the entire electrical particle mobility range requires about 5 min with MDMA and about 10 min with LDMA. If necessary (relative humidity >80 %), the system is equipped with a dedicated air dryer to be inserted upstream of the DMA. A pre-impactor can be also used to remove particles larger than a fixed upper size limit. In the CPC, downstream of the DMA, the particle size is increased by n-butanol condensation on their surface and then the particles are optically counted. The CPC can also be operated as a standalone unit to measure the total particle concentration, with a response time of 4 s and a sensitivity to particle size larger than 4.5 nm. The maximum measurable concentration can reach 10^7 particles cm^{-3}. Both the CPC and the SMPS are operated at an air flow of 0.3 L min^{-1} at atmospheric pressure. To prevent possible damages, the inlet is connected to ChAMBRe through a gate valve which is closed before any evacuation procedure. The SMPS has been connected to

ChAMBRe through a smoothly bended pipe in a way to have an horizontal length of about 10 cm followed by a vertical part of about 30 cm. The OPC, which counts larger particles is connected to ChAMBRe by an ad-hoc set-up with the inlet directly sucking from one of the large flanges: no horizontal tubes (actually no tubes at all).

The OPC is a Grimm 1.107 - Envirocheck version, which operates in 31 size intervals with diameters in the 0.25-32 µm size range with a 6-sec time resolution. The Grimm OPC uses a dehumidification system which operates when ambient relative humidity is higher than 70 %. This optical particle counter has a patented light scattering technique based on an advanced low water sensitive laser source (λ=675 nm). The OPC is factory calibrated via monodisperse Latex particles for size classification. The reproducibility of the OPC in particle counting is ± 2 % (Putaud et al., 2004). The OPC working flow is 1.2 L min^{-1} and it is connected to ChAMBRe through a gate valve which is closed before emptying the chamber volume.

The ozone concentration is monitored by a M400A Ozone Analyzer from API (Advanced Pollution Instrumentation, Inc.). The M400A uses a system based on the Lambert-Beer law for measuring ozone in ambient air. A 254 nm UV light signal is passed through the sample cell where it is absorbed in proportion to the amount of the ozone present. Periodically, a switching valve alternates measurement between the sample stream and a sample that has been scrubbed of ozone. The instrument has a sampling rate of 0.8 L min^{-1}, a response time of 6 seconds and a detection limit of 0.6 ppb (update UV Photometric Ozone Analyzer, model O342e from Environnement SA).

The nitrogen oxides (NO and NO_2) concentrations are monitored by an AC32e, from Environnement SA. The AC32e utilizes the principle of chemiluminescence, which is the standard method for the measurement of NO and NO_2 concentration (EN 1421), for automatically analyzing the NO - NOx and NO_2 concentration within a gaseous sample. The analyzer measures the photons emitted after the reaction between NO and O_3. The analyzer initially measure the NO concentration in the sample, through NO ozone oxidation. Subsequently, the sample passes through the heated molybdenum converter which reduces NO_2 to NO and is then mixed with ozone in the reaction chamber and the resulting NO concentration is determined. in this way, the signal is proportional to the sum of the molecule NO and NO_2 (reduced to NO in the converter) in the sample. With a sampling rate of 0.66 L min^{-1} this instrument reaches a detection limit of 0.2 ppb with a response time of 40 s.

3. Characterization

3.1 Aerosol particles lifetime

Depending on kinetics, processes in the atmosphere have typical reaction times ranging from a few seconds ~~minutes~~ up to several ~~hours~~ days. For this reason, in the case of simulation chambers, the evaluation of aerosol lifetime is of primary importance: it is necessary to keep in suspension enough aerosol for a sufficient time, in order to allow chemical or biological transformations of particles. Aerosol lifetime in chambers depends on many factors e.g. wall losses caused by adsorption/deposition, diffusion and mixing processes, gravitational settling, electrostatic drawing, all of them depending of course on particle properties (i.e. density, dimensions, shape and vapour pressure).

For the characterization of aerosol lifetimes in ChAMBRe, a Blaumstein Atomizer (BLAM, single-jet model, CH Technologies) was used. This nebulizer, specifically developed for generating bio-aerosol from bacteria suspended in water solutions (see a detailed description in section 4.2) can produce particles up to the micrometric range. By feeding the BLAM with saline solutions (NaCl and $(NH_4)_2SO_4$) with different concentration (up to very concentrated solutions, about 10 g L^{-1}), it is possible to generate polydispersed particles with continuous size distributions from few nm up to about 5 µm. During these experiments, the mixing fan was kept on at a constant rotation speed of 5 Hz, this resulting in a mixing time of about 2 min. Thanks to the combined SMPS-OPC measurements, the aerosol lifetime was measured as a function of particle size (Figure 3). For each size bin of the two instruments, particle lifetime has been determined by fitting the mass decay curve with a simple

first order exponential. Relative Humidity in ChAMBRe during the measurements was around R.H.= 47%. Aerosol dilution due to the air flow through the two counters (in total: 1.6 L min^{-1}) was taken into account and properly corrected; the chamber is designed to ensure that the pressure is kept constant: the same amount of clean air is introduced into the chamber through the input from the HEPA filter. The first time interval after each injection, when coagulation could take place, was excluded in the analysis, considering this way the concentration values smaller than 10^4 particle cm^{-3} only. Results are reasonable and very close to literature values (Lai and Nazaroff, 2000; Cocker et al. 2001; Wang et al., 2011); in particular experimental data are nicely reproduced (i.e. the mean discrepancy between measured and calculated values is around 50%) by the wall deposition model described in Lai and Nazaroff, (2000) treating ChAMBRe as a rectangular cavity with a friction velocity of ca 6 cm s^{-1} (Figure 3). Particles lifetime in ChAMBRe varies from few hours to about ~~4 days~~ 1 day depending on particle size. The uncertainty on particles life-time plotted in Figure 3 has been evaluated on a pure statistical basis. Actually, in the size region between 300 and 600 nm, both the SMPS and OPC data could be particularly sensitive to other effects (e.g. background fluctuation for the SMPS, systematic artifacts in the first OPC bins) which have not been fully investigated in this work and that do not change the typical feature depicted in Figure 3.

3.2 Ozone and wall reactivity

The presence of walls obviously influences the chemical and physical dynamics of the experiments carried out inside simulation chambers, as the gaseous species can be lost to the chamber walls. To describe the behavior of the walls of our chamber, we considered the dark reactivity of ozone, due to its chemical reactivity towards surfaces, its relevance to chamber experiments (as reactant or as sterilization agent) and as atmospheric oxidant.

A series of five experiments have been done with initial concentration ranging from 300 to 1000 ppbv. The ozone concentration in the chamber was monitored as a function of time. The pseudo-first order rate for loss processes is equal to $(3.04 \pm 0.40) \times 10^{-5}$ s^{-1} and it is in good agreement with what reported in the literature for other similar facilities (Wang et al., 2011). This parameter is highly dependent on the chamber wall material, on its history, related to the cleaning protocol and the operating conditions such as temperature or relative humidity (Wang et al., 2011). As a consequence, the quantification must be carried on regularly and before each set of experiments for any type of study.

3.3 Background levels (PM, O_3, NOx)

The background level of particles inside the chamber was measured by SMPS and OPC. The coupling of the two counters provides a comprehensive picture of the particles inside the chamber ranging from few nm up to 31 microns (for more information, see section 2.3). After each experiment, the chamber is cleaned by a multi-step procedure: the UV lamp (see sec. 2.1) is first switched on for 10 min, the chamber is then evacuated and vented to atmospheric pressure through an HEPA filter (section 2.1). Afterwards, a high ozone concentration (>500 ppb) is produced to be sure to sterilize any part of the set-up possibly not reached before by the UV rays. Finally, the chamber is evacuated and vented again.

Background level measurements performed subsequently to chamber cleaning showed no significant particles presence (i.e. about 2 and 0.5 particle cm^{-3}, respectively in the SMPS-LDMA and OPC range).

Background concentrations of O_3 and NO_x, could be introduced in the chamber during the venting after an evacuation, since both the gases can be present in the room air: concentration values measured periodically in the chamber along 4 months turned out to be smaller than 1-2 ppb i.e. close to the analyser sensitivity (see section 2.3).

4. Protocols to prepare, inject, expose and collect bacteria

The usefulness of ASCs in providing new possibilities for the study of bacteria and other biological particles in air critically depends on the associated protocols, which are essential to understand how the bacteria survive and if they are in able to grow and reproduce in the atmospheric conditions of the simulation chamber. In this section we describe the standard methodology developed for the bio-aerosol experiments (injection, collection and storage) and the related experimental conditions, that should be representative of the typical environmental ones.

4.1 Bacterial strains

Experimental procedures involved two strains consisting of *Bacillus subtilis* (ATCC® 6633™) and *Escherichia coli* (ATCC® 25922™). These microorganisms are extensively used as model organisms in microbiology and molecular biology fundamental and applied studies (Lee et al., 2002).

Bacillus subtilis is a Gram-positive, rod-shaped bacterium with length ranging between 2.5 and 6.5 µm. It is commonly found in soils but has been also observed in other environmental matrices such as water and air (Earl et al., 2008). It has a wide commercial use as it is nonpathogenic. *B. subtilis* serves as a model organism and is considered a reference for cell differentiation and adaptation. This model status makes it one of the most extensively studied organisms in nature given its ability to survive and even thrive in a wide range of harsh environments (Earl et al., 2008).

Escherichia coli is a Gram-negative, rod-shaped, enterobacter, is about 1–2 µm long and about 0.25 µm in diameter. It is a common inhabitant of the gastrointestinal tract of warm-blooded animals, including humans, but recent studies have reported that some specific strains of *E. coli* can also survive for long periods of time, and potentially reproduce, in extra-intestinal environments. *Escherichia coli* is among one of the most studied model organism. Its fast-growth characteristics under optimal conditions make it suitable as host organism for many gene manipulation systems, producing countless enzymes and other industrial products, and to study the evolution of microorganisms (Jang et al., 2017).

4.2 Preparation of bacterial suspension and injection in ChAMBRe

Several techniques for bacteria and bio-aerosol characterization are available on site. In the same building that hosts the atmospheric simulation chamber there is a basic microbiology lab equipment allowing for culture analysis in vitro (isolation, identification, growth) and biochemical tests (e.g. catalase and oxidase): autoclave (Asal mod.760), vortex, centrifuge and microcentrifuge (Eppendorf centrifuge 5417R), water purification system Milli-Q (Millipore-Elix), incubator for temperature control Ecocell and Friocell MMM Group, Steril-VBH Compact "microbiological safety" cabinet, Thermo electron corporation steri-cycle HEPA Class 100 incubator; optical microscope (Nikon Eclipse TE300) for bacterial detection and live/dead discrimination by epifluorescence with specific dyes and for immunoassay fluorescence to label antigenic bacterial target, fluorescent molecule or enzyme. The transfer of bacteria from the biological laboratory to the simulation chamber takes only a few minutes, ensuring a quickly execution of the chamber experiments, once the desired phase of bacteria growth is reached, and then a quick treatment of the samples collected after the experiments in the room.

The same culture preparation technique was applied at both the bacterial strains, in order to minimize experimental variations. Firstly, it is important to ensure the maximum bacteria cells viability prior to the injection. Typically, to understand and define the growth of a particular microbial isolate, cells are placed in a culture medium in which the nutrients and environmental conditions are controlled. If the medium provides all nutrients required for growth and environmental parameters are optimal, a growth curve can be obtained by measuring the increase in bacterial number or mass as a function of time. Different distinct growth phases can be observed within a growth curve: these include the lag phase, the log phase, the stationary phase, and the death phase. Each of these phases represents a distinct period of growth that is associated with typical physiological changes in the cell culture. Therefore, the growth curve for both of bacterial strains was obtained quantifying the rate of change in the number of cells in a culture per unit time thus identifying the mid-exponential phase (log phase), where the maximum viability

of the cells is ensured and the number of dead microorganisms is minimum. *B. subtilis* was purchased as water soluble freeze-dried Selectrol discs. The discs were dissolved in sterile Tryptic Soy Broth (TSB), also known as soybean-casein digest medium (SCDM), incubated at 37° C for 1 day and then rejuvenated; *E. coli* cells were scrapped off agar medium using sterile plastic loops and suspended in sterile culture broth medium. In both cases, the growth curve was then followed, once every hour, with a spectrophotometer V-530 UV-vis (Jasco International Co. Ltd, Hachioji, Japan), where the number of cells per mL of culture was estimated from the turbidity of the culture. The optical density of the bacterial solution, measured at a wavelength of 600 nm, is a common method for estimating the concentration of bacterial cells in a liquid. The amount of the light scattered by the microorganisms suspension is an indication of the biomass contents (Sutton, S. 2011). Data, obtained from spectrophotometric measurements (OD_{600nm}), were used to estimate when the mid-exponential phase (corresponding an OD_{600nm} of 0.5) is reached. Actually, the number of cultivable cells was counted as Colony Forming Units (CFU), by standard dilution plating: 100 µL of six fold serial dilutions of the solution was spread on an agar non-selective culture medium, and incubated at 37° C for 24 h before counting the formed colonies. Data, obtained ~~from spectrophotometric measurements (OD_{600nm}) and~~ from CFU counting on Petri dishes, were averaged and used to estimate the uncertainty range of the bacterial concentration in the solution. The growth curves for the two strains are reported in Figure 4. The measured OD_{600nm} values were fitted with a three-parameter sigmoidal curve (Eq. 1), where Abs is the absorbance, or optical density, measured at 600 nm, *a* and *b* are constants (B. subtilis curve, a is 1.1 ± 0.01, b is 38 ± 2; E. coli curve, a is 0.83 ± 0.01 and b is 41 ± 1).

$$Abs = \frac{a}{1+e^{-((t-t_0)/b)}} \quad (1)$$

Before each injection we followed the bacterial growth up to the mid-exponential phase, reached in about 4 h, thus allowing the bacteria to enter the exponential phase of growth.

Spectrophotometer measurements were used to achieve the correct dilution and also to provide the first evaluation of bacterial concentration in the solution which has to be nebulized, as explained below. The suspension was then centrifuged at 3000 rpm for 10 min, the supernatant was discarded and the pellet was evenly vortexed for 1 min in physiological solution (NaCl 0.9 %) before the injection. The cultivable cell concentration was determined following the above-mentioned procedure. The average on CFU counting is used to estimate the uncertainty range of the bacterial concentration in the nebulized solution.

In each experiment, a volume of 10 mL of the cells suspension, with a concentration of approximately 10^7 CFU mL^{-1} for *B. subtilis* (OD_{600nm} around 0.5, single values are reported in Table 2) and 10^6 CFU mL^{-1} for *E. coli*, was prepared for nebulization and placed into a syringe. In particular, for *E. coli*, to obtain the final concentration of 10^6 CFU mL^{-1}, the initial cells suspension with an OD_{600nm} around 0.6 (single values are reported in Table 4) was diluted (1:10, 1:15, 1:20, 1:40) before the injection, to avoid an excessive bacterial concentration on the Petri dishes exposed inside the Chamber (see the paragraph 5.2).

A volume of about 2 or 3 mL of the cells suspension was sprayed into the simulation chamber using a Blaumstein Atomizer (BLAM, single-jet model, CH Technologies), connected to the chamber with a curved stainless-steel tube (length = 50 cm, diameter = 1.5 cm). The single jet BLAM is specifically designed to provide bio-aerosols with the enhanced viability of microorganisms for aerobiology research (Zhen et al., 2014) with respect to the Collison nebulizer, employed in the pilot test performed by Brotto et al. (2015). The BLAM's viability is essentially due to its efficiency in that it utilizes minimal energy to properly aerosolize a liquid. The single-jet BLAM is used in one-pass mode, where the liquid medium is subjected to the sonic air jet only one time. The atomizing head is composed of two main parts: Nozzle Body and Expansion Plate. The atomization occurs when the pressurized air (air flow 2 lpm, pressure 3.8 bar) pushes at sonic velocity through a precisely laser cut ruby crystal (fixed size 0.010" diameter) pressed into the Nozzle Body, while the liquid with particles is carried into a cavity between the Nozzle Body and Expansion Plate at a desired flow rate (liquid feed = 0.4 mL min^{-1}) using precision pump (NE-300 Just Infusion™ Syringe Pump, New Era Pump Systems, Inc.). The properties of the aerosol generated by the single-jet BLAM are a function of the jet hole size, depth of the liquid cavity and expansion cone size. The atomizer features a modular design, composed of five interchangeable plates which enable it to accommodate liquids of varying properties to

produce aerosols in specific size ranges and output concentration, with a nebulization efficiency (i.e. mass ratio between the mass of the produced aerosol to the mass of the solute or of the material suspended in the liquid inserted in the BLAM) between 1 % and 8 %.

For these experiments, the expansion plate with a cavity depth and a cone diameter of 0.001 and 0.020 inch, respectively, has been used. The accelerated air jet breaks up the liquid into droplets. The aerosol generated by this process is sprayed downwards inside the jar where the larger droplets are collected on the liquid surface due to impaction as they cannot make the U-turn while the finest droplets are forced up through the outlet tube on top of the BLAM lid. The result is a very fine mist, well within the respirable range (i.e. with diameter smaller than 10 μm) and with narrow size distribution. The size distribution, immediately after the injection of physiological solution (with or without bacteria) in ChAMBRe, shows a mean value of 0.45 μm with a standard deviation of 0.25μm.

4.3 Collection and extraction methods

The main body of ChAMBRe is connected through a ISO-KF250 pneumatic valve to a cylindrical horizontal volume which is accessible from a second ISO-KF250 gate valve (see Figures 1 and 2). The two gate valves completely separate the cylinder, which can be connected to the main chamber or alternatively opened without perturbing the ChAMBRe atmosphere. This home-made device has been specifically developed to ensure the insertion and extraction of bio-aerosol samplers, in order to minimize the risk of contamination. This volume can be evacuated through a by-pass to the ChAMBRe main pumping system and can be then refilled to atmospheric pressure both with particle free dry air or through a pipe connected to the ChAMBRe main body. Inside the cylinder, there is a sliding tray which can be inserted in ChAMBRe by a home-made external manual control (Figure 2) The tray can host up to six Petri dishes (diameter 10 cm, each) which can be inserted in ChAMBRe to collect bacteria (or in general BPAP) directly by deposition onto a proper culture medium. The procedure to insert the Petri dishes in ChAMBRe is organized in consecutive steps (reference to Figure 1 for the valves names):

a) With V1 closed, the V2 valve is opened to allow the positioning of the Petri dishes (pre-filled with a suitable amount of culture medium) on the sliding tray
b) Valve V2 is closed and the volume inside the pipe is flushed with clean air coming from the chamber.
c) The atmospheric pressure inside the pipe is recovered by opening the connection to ChAMBRe
d) V1 is opened and the sliding tray is completely inserted in ChAMBRe
e) The sterilizing UV lamp (ozone free, see section 2.2) is switched on for 15 minutes to guarantee the Petri dishes sterilization
f) The UV lamp is switched off and ChAMBRe is ready for injection of bacteria.

The chamber sterility before the injection of bacteria was tested through a blank experiment by injecting only sterile physiological solution: no bacterial contamination was observed in the four Petri dishes positioned on the sliding tray.

In a standard experiment, once the bacteria have been injected into ChAMBRe, the Petri dishes remain exposed for the desired time and then the sliding tray can be moved back to the pipe. The ventilation system is on during the exposure period, to maintain a homogeneous distribution of particles inside the chamber volume. Closing V1 and opening V2 the Petri dishes can be removed without perturbing the conditions inside the main chamber. The gravitational settling method has been developed to minimize microbial damage, and has been previously proven to be a very suitable way to collect and count viable bacteria colonies (Brotto et al., 2015). After exposure to the chamber atmosphere, Petri dishes are incubated for 24 h at 37° C, after which the number of formed colonies can be counted. It is assumed that the living microorganisms present in the aerosol are deposited on the petri dishes by gravity without undergoing any stress, from those related to the permanence in the experimental setup atmospheric conditions. In this way, it can be assumed that the number of units forming colonies counted on a Petri dish

is proportional to the number of aerosolized and suspended living microorganisms within the chamber and also to the concentration value of viable bacteria in the aerosol.

Lee et al., 2002 suggest that the average aerodynamic diameters of generated *E. coli* and *B. subtilis* aerosols were 0.63 and 0.75 µm respectively. If compare these data with data obtained with NaCl solution to determine particles life time in chamber, the bacteria life time is aspect to be around five hours. The mean global residence time calculated by Burrows et al., 2009b, lie between 2 and 15 days for bacteria traces.

Bacteria from the original liquid suspensions, both in broth and in physiological solution (Section 4.2), were also collected on polycarbonate filters (Isopore membrane track-etched filters, pore size 0.05 µm) with a smooth surface, ideal to study the morphology of cells and possible bacteria aggregates (e.g. biofilm formation) by scanning electron microscopy (Capannelli et al., 2011). The sampling was performed by exposing filters to the stream of aerosols coming out of the nebulizer, through a secondary port connected to the chamber. For electron microscopy observation the simple protocol adopted here is the following. Bacterial suspensions (1 mL) were dehydrated and diluted progressively in a graded series of ethanol bathes (30, 50, 70 and 90 %). This protocol was established by simplifying the standard method named "air drying" (Robinson et al., 1987; Janecek and Kral, 2016), as it was ascertained that the structures of the cells were preserved without requiring the fixation step. Other final treatments (e.g. with tetramethylsilane) were also suppressed as the study of cell ultrastructures were not done in this case as the study ~~goes beyond the scope of this work~~. Compared with the original suspensions the final dilution is 1:1000, in order to reach on the filter an optimal surface density, able to maintain the biological particles well separated. Following this step the diluted liquid samples were passed through polycarbonate filters held inside a dedicated filter unit. For each sample, 150 µL were loaded with a micropipette onto the filter in the unit, then a syringe was attached to the upper part of the filter holder, in order to filter the sample by pushing gently the plunger. Then the filter was removed and allowed to dry for 3 hours. Dry filters were cut in half, mounted on Aluminum stubs and sputter coated with carbon before observation by a Field Emission Scanning Electron Microscope (FESEM) Zeiss Supra 40 VP. The selected conditions were: voltage 10 kV, signal in-lens, magnifications ranging from 5000 to 200000×.

5. First Experiments

Experiments to study the correlation between bacterial viability and the atmospheric composition/conditions in ChAMBRe rely on an assessed protocol to inject and extract bacteria from the chamber. A first set of experiments was therefore devoted to measuring the reproducibility of the whole process with a clean atmosphere (i.e. with the background levels given in section 3.3) inside ChAMBRe.

5.1 Experiments with B. subtilis

Five different experiments were performed in the period from July and November 2017. The protocol described in section 4 was followed for the bacteria growth, the injection in the chamber and the bacteria collection by four Petri dishes inserted by the sliding tray (section 4.3). Values of the atmospheric parameters in ChAMBRe during each experiment are reported in Table 1. The bacteria concentrations measured in the aerosolized solution and the average number of colonies counted on the Petri dishes after the exposure in ChAMBRe are reported in Table 2. The volume of the bacterial suspension injected through the BLAM atomizer was equal to 2 mL, except during the fourth experiment where the volume was increased to 3 mL (Table 2). This ensured that the concentration of viable bacteria injected in the chamber was comparable to the values typical of the real atmosphere (Bauer et al., 2003; Burrows et al., 2009). Taking into account the BLAM nebulization efficiency (section 4.2), the initial aerosol concentration of living microorganisms in ChAMBRe after the injection, was estimated to be around 10^5 CFU m^{-3}. In Table 2, the uncertainties quoted on both injected and collected bacteria are just those deriving from the Poisson fluctuation (i.e. the square root of the number of colonies counted in the Petri dishes) and they do not include any other

systematic or statistical contributions. In particular, for the collected CFU, the values reported in Table 2 are the average of the counts of the four Petri dishes exposed in each experiment and that, in each group of four, turned out to be statistically compatible (i.e. within the interval delimited by the statistical uncertainty, the counts in the four petri dishes were in agreement). Despite these simple assumptions, a good correlation between the number of injected and collected CFU was obtained as shown in Figure 5. Furthermore, the uncertainty on the slope of the correlation curve turned out to be lower than 10 %. This level of reproducibility appears to be adequate to design experiments with different atmospheric conditions (i.e. level of particular pollutants), particularly when compared to the pilot test by Brotto et al. (2015), when much larger variations in the bacteria viability had been observed (see section 1.3). No sizeable effect related to the R.H. in ChAMBRe was observed (crf. Results of Exp. 4 and 5 in Table 2).

5.2 Experiments with E. coli

Five different experiments were performed in the period from January and March 2018, following the protocol described in section 4. The values of the atmospheric parameters in ChAMBRe are reported in Table 3. In this set of experiments the relative humidity inside the chamber was increased up to 70 %, compared to the environmental value recorded in the laboratory, by changing the working condition of the humidifier (Benbough, 1967; Cox, 1966; Dunklin and Puck, 1947). *Escherichia coli*, a gram negative bacterium, is more sensitive to the atmospheric conditions inside the chamber than *Bacillus subtilis*, a gram positive strain. As a matter of fact, no CFUs were collected on the petri dishes positioned inside the chamber when the injection of this strain was performed at low relative humidity (RH 35 %, T 20° C). Furthermore, another experiment showed that injecting 2 mL of a cell suspension (concentration of approximately 10^7 CFU mL^{-1} in physiological solution, RH ~ 70 %) resulted in a huge, uncountable amount of CFUs on the petri dishes, and suggested that a dilution before the injection was necessary.

The dilution factor, the bacterial concentrations measured in the aerosolized solutions and the average number of colonies counted on the Petri dishes after the exposure in ChAMBRe are reported in Table 4. It is worth noting that in the experiments discussed in section 5.1, a narrow interval of OD_{600nm} values, around 0.5, was explored, while in the experiments with *E. coli*, depending on the dilution factor, a larger interval of OD_{600nm} values was spanned.

The volume of the bacterial suspension injected through the BLAM atomizer was equal to 2 mL in the first four experiments and was increased to 2.8 mL in the fifth experiment (Table 4). Figure 6 shows the correlation between the number of injected and collected CFU (left panel), indicating that the uncertainty on the slope of the correlation curve (about 4 %) was even better than the same uncertainty related to *B. subtilis* (about 7 %, Figure 5). In Figure 6, the good correlation between the relative optical density of the cell suspensions and the collected CFU (right panel) is also shown For E. coli suspension, the evaluation of the microbial concentration through the fast and simpler control of the optical density, seems possibly be accurate enough to perform controlled experiments, provided an adequate calibration of the whole procedure is carried out.

Although for this bacterial strain a less concentrated solution was injected, more CFUs were collected on the Petri dishes placed inside the chamber. This result could depend on the fact that the humidity in the chamber was generally greater in the second set of experiments providing to Gram-negative microorganisms a more comfortable environment, but also it could depend on the behavior of the two different bacteria strains.

The FESEM micrographs (Figures 7 and 8) of the bacteria contained in the liquid suspensions before injection (see section 4.3) clearly show that the cells of *B. subtilis* tend to aggregate, forming long chains (Figure 7, left panel), while the cells of *E. coli* are mainly present as single individuals (Figure 8, left panel). Therefore, in the first case it is quite possible that the colonies counted on the Petri dishes originated from a group of cells, while in the second case each colony results presumably from a single viable microorganism.

6. Conclusions

A new atmospheric simulation chamber, ChAMBRe, has been installed at INFN-Genova. The facility has been designed to perform experimental studies on primary biological aerosol particles and bacteria in particular. The performance of the new chamber, which may impact on the future experiments on bio-aerosol (i.e. wall reactivity, aerosol lifetime, background levels), has been quantitatively assessed. Furthermore, a protocol to handle the injection and extraction phases has been thoroughly tested both with Gram positive and Gram negative bacterial strains. With a clean atmosphere maintained inside ChAMBRe, the ratio between injected and extracted viable bacteria turned out to be reproducible at a 10 % level. Such result is the first methodologic step in view of a forthcoming systematic study of the correlation between bacterial viability and pollution levels. Resident times of viable bacteria in ChAMBre are less than 5 hours, much shorter than the generic residence time in the open atmosphere. However, previous literature studies (Brotto et al., 2015) suggest that such time window is long enough to observe the effects (i.e. viability change) of bacteria exposure to air pollutants. The assessment of such effects is objective of the fore coming studies at ChAMBRe.

7. Competing interests

The authors declare that they do not have any conflict of interest.

8. Authors contribution

DM, PB, FP and PP designed and built ChAMBRE; DM, SGD and PP ran all the injections with bacteria; SGD, EG, ADC and LV took care of all the biological issues and measurements, AC, CC, LN and MO performed the SMPS measurements and the FESEM analyses; DM, SGD, CC, MO, JFD, PF and PP performed the measurements to assess the aerosol life time in ChAMBRe and the wall reactivity; FF designed and implemented the acquisition software; JFD and PF provided several advises from their longstanding expertise in the field; DM, SGD, CC, EG and PP prepared the article with the contribute of all the other authors.

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FIGURE CAPTIONS

Figure 1: ChAMBRe layout.

Figure 2: Left panel: the main structure of ChAMBRe. Right panel: the cylindrical volume (top) which hosts the sliding tray (bottom) used to introduce up to six Petri dishes (or other objects) inside the main ChAMBRe body

Figure 3: Particle loss coefficient (β) and life time (secondary vertical axis), versus aerosol size measured in ChAMBRe by NaCl salt injection (21 °C, 47 % RH). The curve resulting from the Lai and Nazaroff 2000 model is also shown for reference (see text). Error bars include statistical uncertainties only.

Figure 4: Typical grow curve for *Bacillus Subtilis* (black line, circle) and *Escherichia coli* (red line, triangle): optical density (OD 600 nm) is plotted versus time.

Figure 5: Correlation curve between the number of *B. Subtilis* bacteria injected in ChAMBRe (in units of 10^7 CFU) and the average count on the four Petri dishes exposed in each experiment.

Figure 6: Correlation curve of the average count on the four Petri dishes exposed in each experiment with the number of *E. coli* bacteria injected in ChAMBRe (in units of 10^7 CFU, left panel) and with the optical density (OD 600 nm, right panel).

Figure 7: Detail of *Bacillus subtilis* in physiological solution, magnifications 2000× in the left panel and 100000× in the right panel.

Figure 8: Detail of *Escherichia coli* in physiological solution, magnifications 2000× in the left panel and 100000× in the right panel.

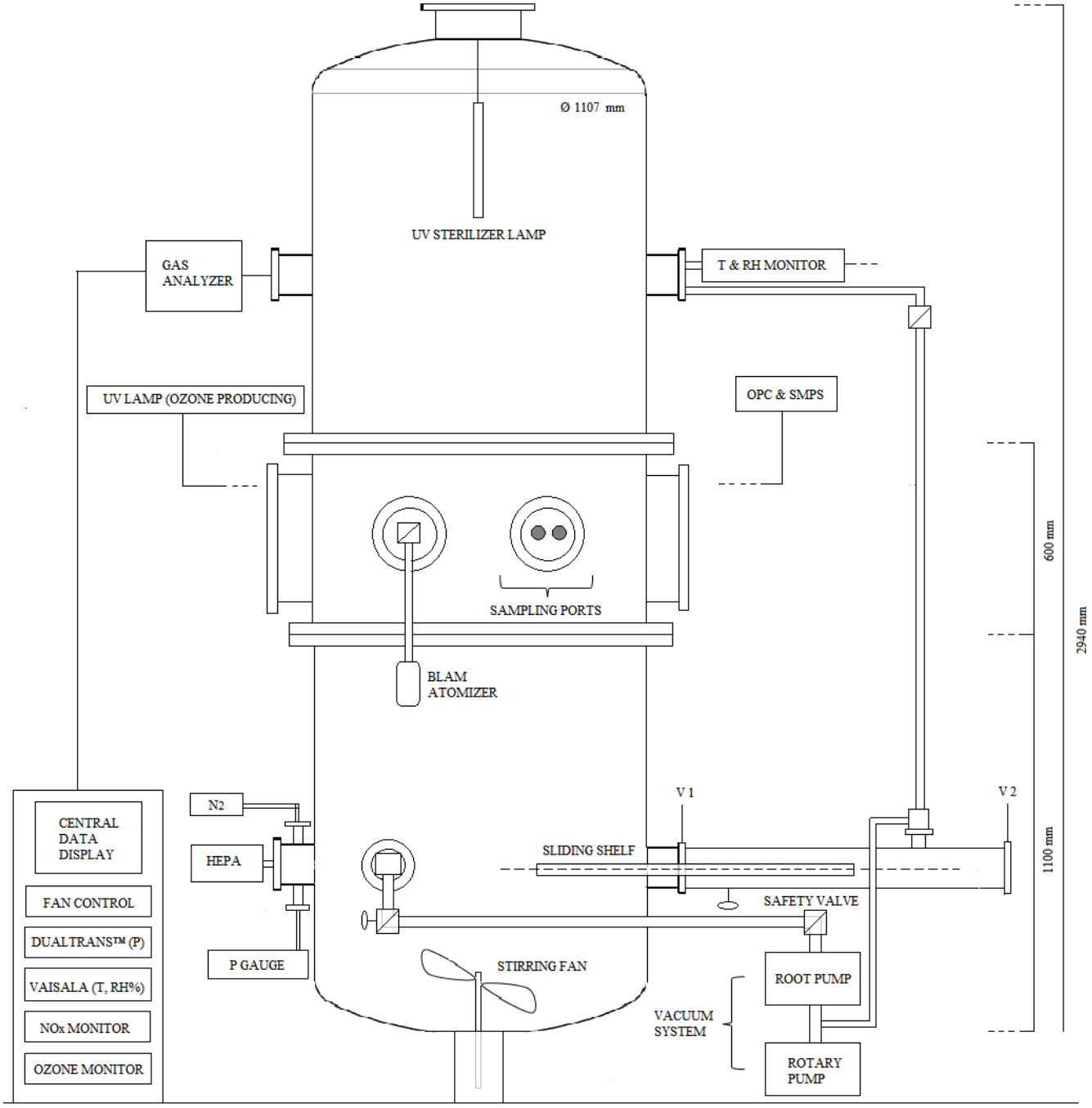


Figure 1

1735

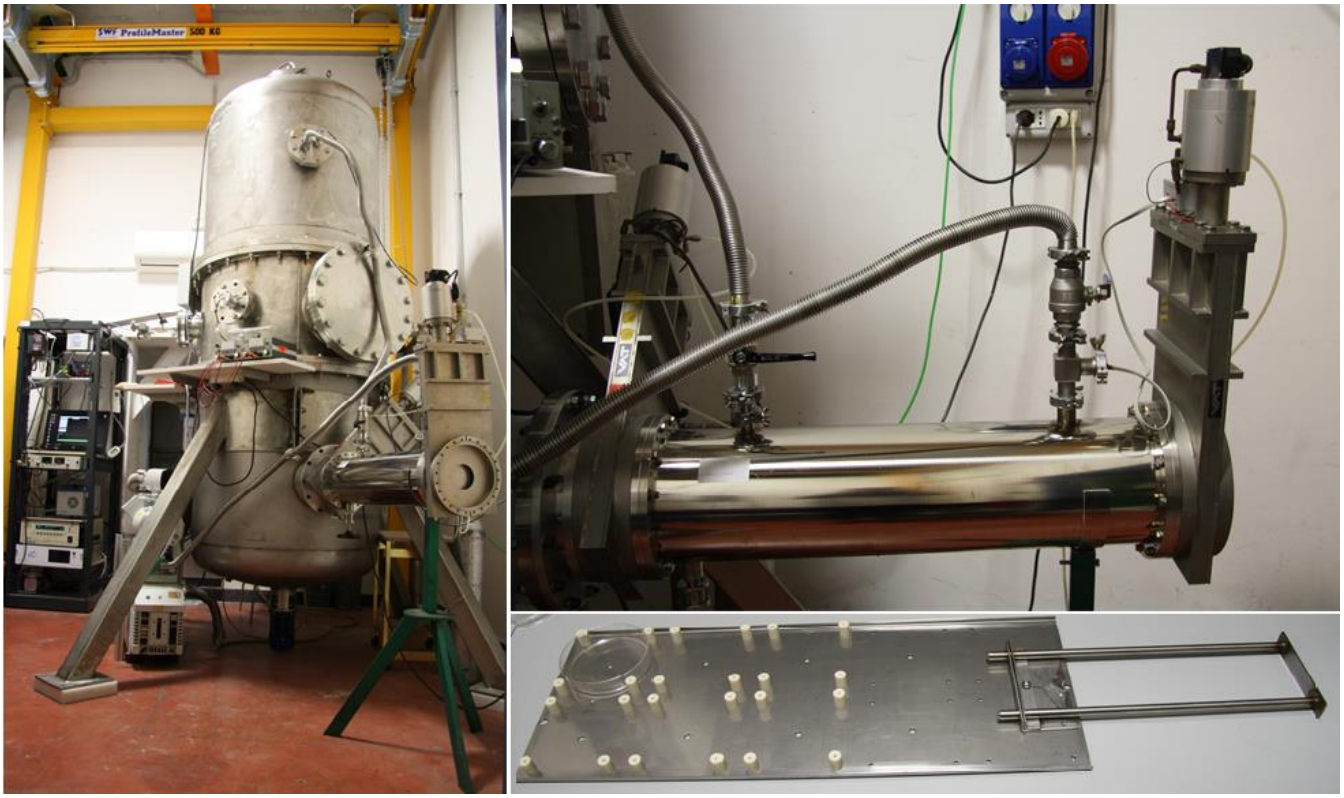


Figure 2

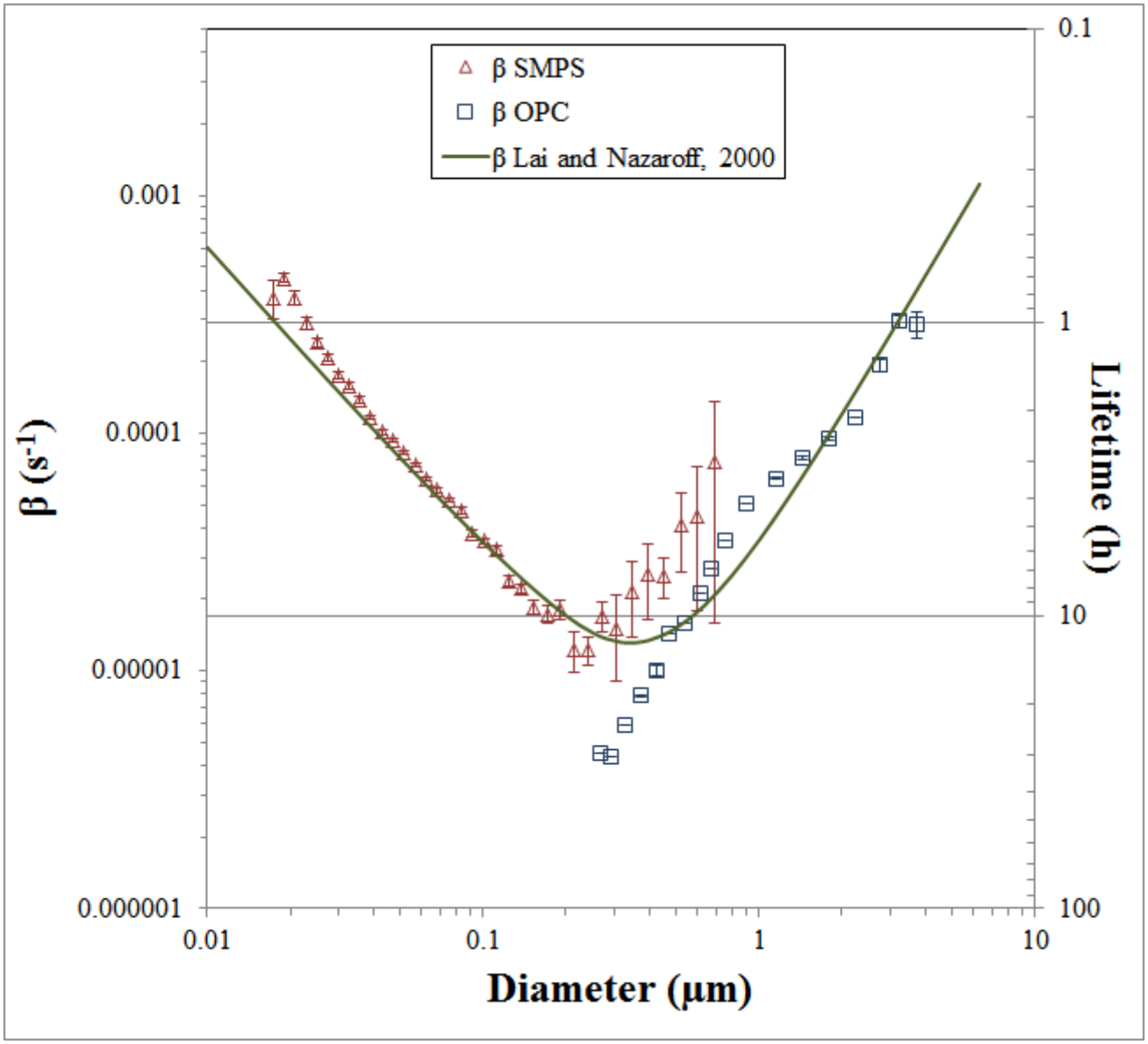

β SMPS
β OPC
β Lai and Nazaroff, 2000
β (s-1)
0.001
0.0001
0.00001
0.000001
0.1
1
10
100
Lifetime (h)
0.01
0.1
1
10
Diameter (µm)

Figure 3

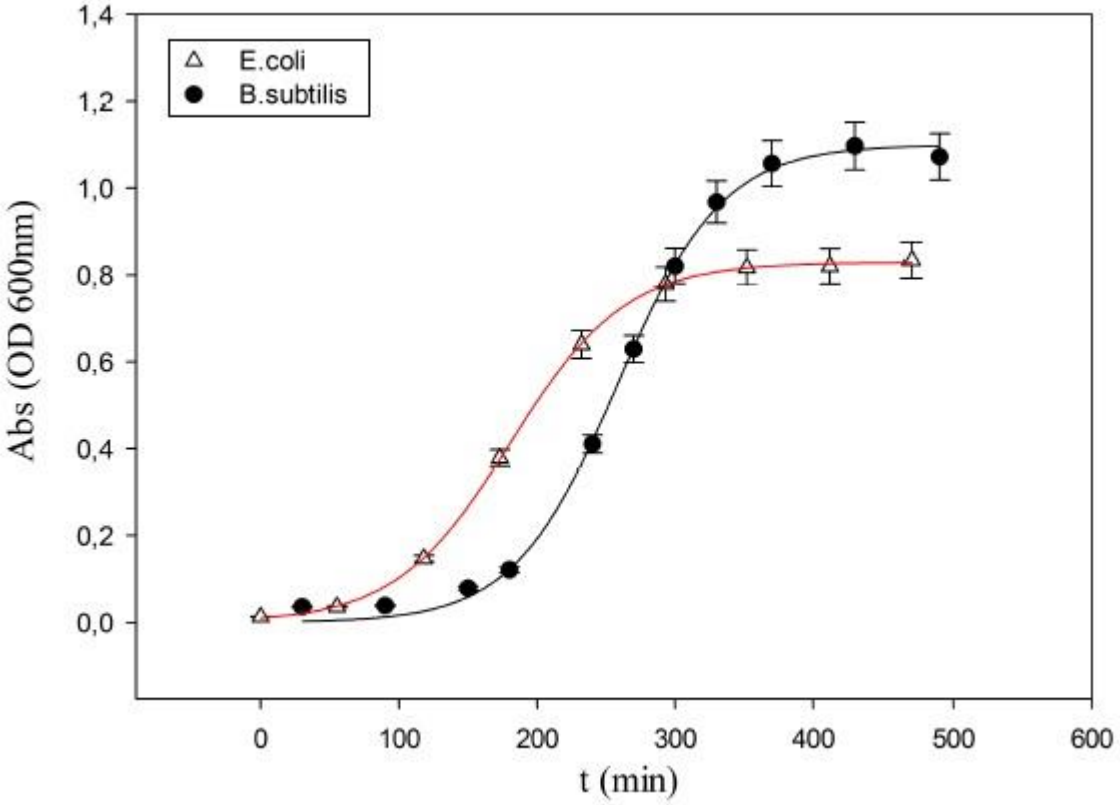
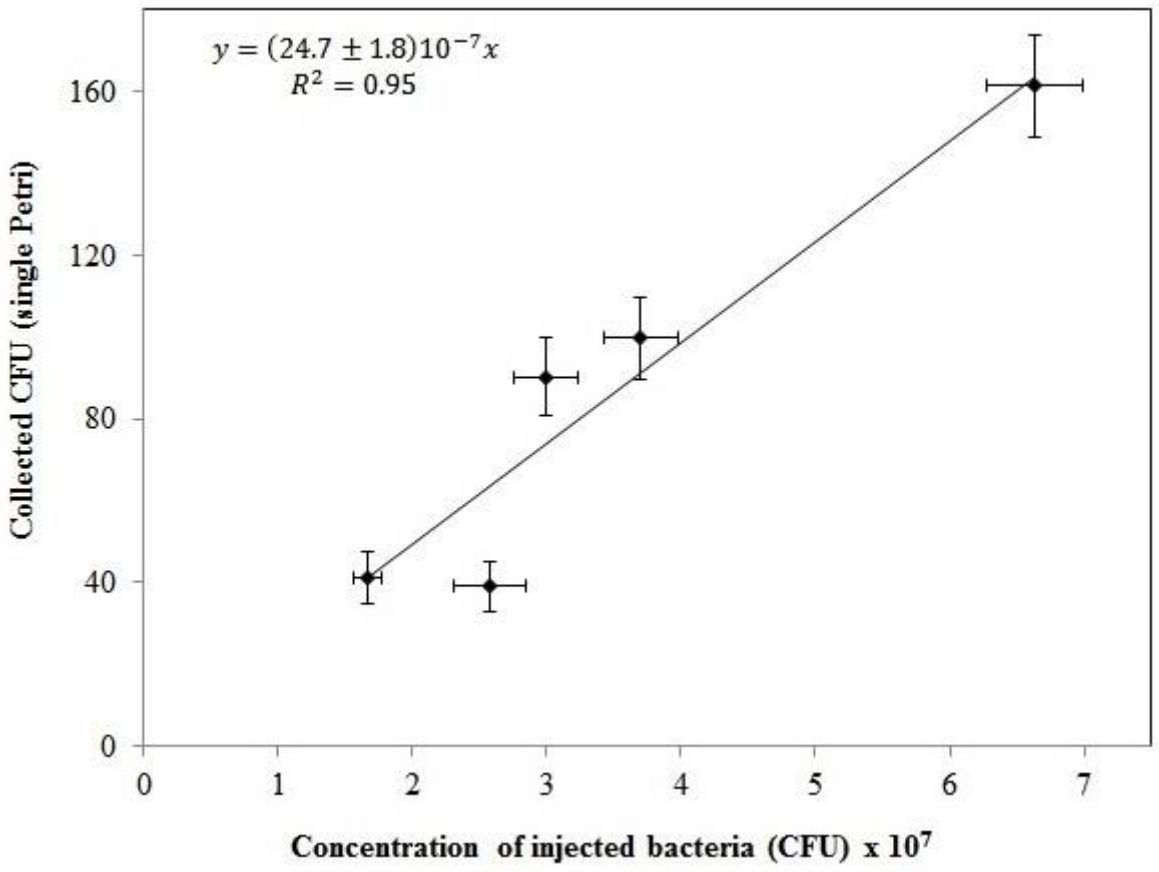

E.coli
B.subtilis
Abs (OD 600nm)
t (min)
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
100
200
300
400
500
600

Figure 4



1770

Figure 5

1775

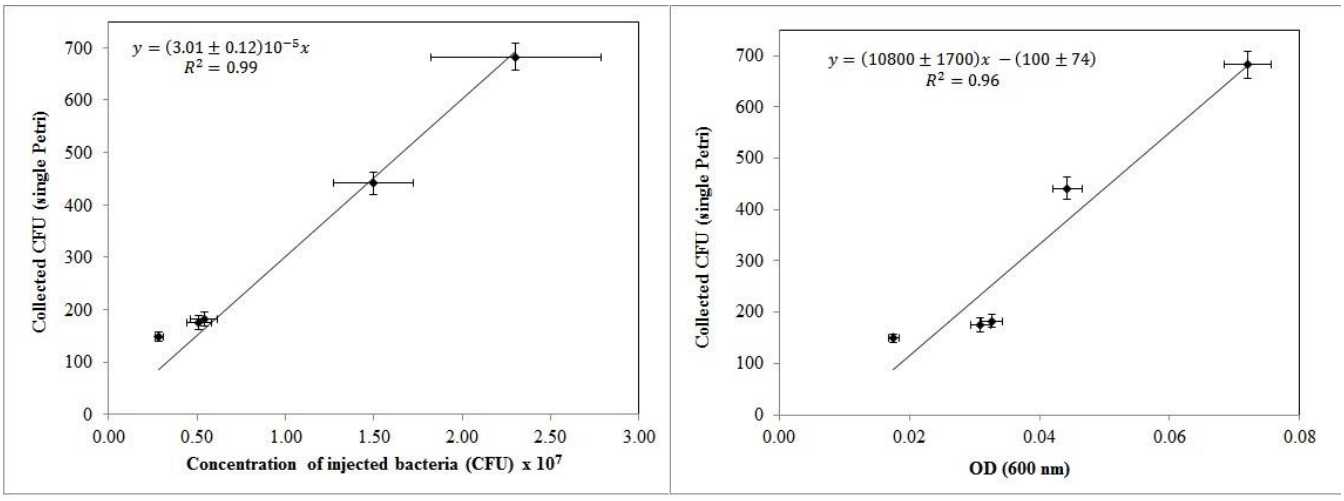


Figure 6

1780

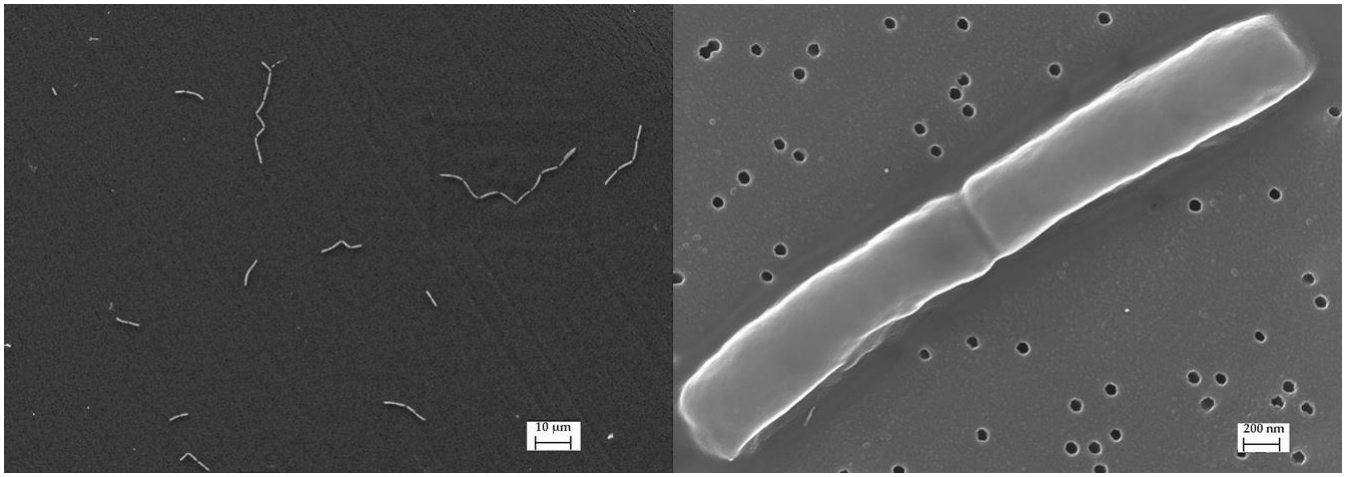


Figure 7

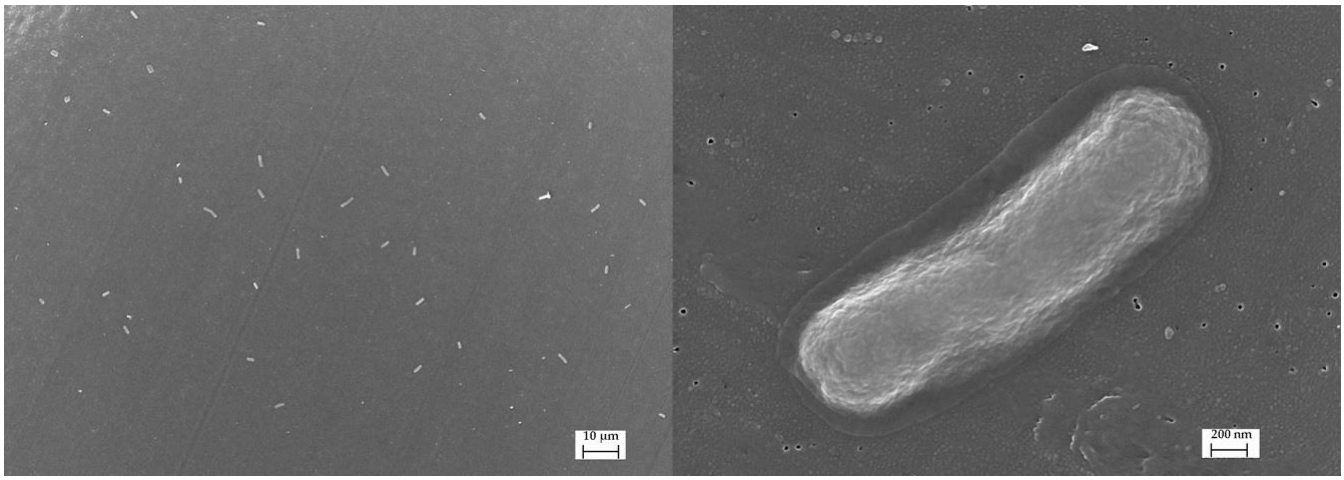


Figure 8

Table 1: Environmental parameters (R.H., T, P) in ChAMBRe during the experiments with *B.subtilis.*

	Relative humidity range (%)	Temperature range (° C)	Pressure range (mbar)	Petri dishes Exposure time (hh:mm)
Exp. 1	55-85	22.0-21.1	1015-1012	05:00
Exp. 2	44-71	23.7-24.5	1010	05:20
Exp. 3	50-43	23.2-21.3	1014-1015	05:15
Exp. 4	44-70	22.0-22.5	1016	05:05
Exp. 5	75-79	20.1-20.8	1005-1007	05:00

Table 2: Bacteria concentration (*B. subtilis*) in the aerosolized solution and average number of colonies counted on one Petri dish.

	OD_{600}	Suspension concentration (CFU mL^{-1}) x 10^7	Bacteria injected CFU x 10^7	Average CFU collected
Exp. 1	0.57 ± 0.03	1.85 ± 0.14	3.70 ± 0.28	100 ± 10
Exp. 2	0.58 ± 0.03	3.32 ± 0.18	6.63 ± 0.36	161 ± 13
Exp. 3	0.58 ± 0.03	1.50 ± 0.12	3.00 ± 0.24	90 ± 10
Exp. 4	0.50 ± 0.03	0.86 ± 0.09	2.58 ± 0.27	39 ± 6
Exp. 5	0.40 ± 0.02	0.83 ± 0.05	1.67 ± 0.10	41± 6

Table 3: Environmental parameters (R.H., T, P) in ChAMBRe during the experiments with *E. coli.*

	Relative humidity range (%)	Temperature range (°C)	Pressure range (mbar)	Petri dishes Exposure time (hh:mm)
Exp. 1	75-77	15.8-18.7	994	05:00
Exp. 2	73-77	23.1-23.6	992-999	05:00
Exp. 3	78-80	19.0-19.3	1010	05:05
Exp. 4	76-83	18.6-19.0	1007-1009	05:00
Exp. 5	72-80	19.8-20.0	1002-1003	06:05

1820

Table 4: Bacteria concentration (*E. coli*) in the aerosolized solution and average number of colonies counted on one Petri dish.

	OD_{600} (before dilution)	Dilution factor	OD_{600} (after dilution)	Suspension concentration (CFU mL^{-1}) x 10^6	Bacteria injected CFU x 10^6	Average CFU collected
Exp. 1	0.57 ± 0.03	1:20	0.031 ± 0.002	2.55 ± 0.36	5.10 ± 0.71	175 ± 13
Exp. 2	0.64 ± 0.03	1:10	0.072 ± 0.004	11.5 ± 2.40	23.0 ± 4.8	682 ± 26
Exp. 3	0.60 ± 0.03	1:20	0.033 ± 0.002	2.70 ± 0.38	5.39 ± 0.76	183 ± 14
Exp. 4	0.65 ± 0.03	1:15	0.044 ± 0.002	7.49 ± 1.12	15.0 ± 2.25	442 ± 21
Exp. 5	0.66 ± 0.03	1:40	0.018 ± 0.001	1.02 ± 0.07	2.85 ± 0.20	149 ± 9