

Comments on “Airborne bacteria viability and air quality: a protocol to quantitatively investigate the possible correlation by an atmospheric simulation chamber” by V. Vernocchi et al.

Microbes circulate in the atmosphere, but their viability as aerosols and the impacts of environmental factors are not yet well characterized. Experiments in atmospheric simulation chambers should enable progress in this direction.

This manuscript describes specific instrumentation and protocols to investigate airborne bacteria viability using the established atmospheric simulation chamber, ChAMBR_e, dedicated to biological aerosols. Results of typical experiments that can be led in the chamber are presented (exposure of *E. coli* to NO_x and solar light).

The protocol involves “baseline” experiments, i.e. experiments led under specific conditions to serve as references to experiments intended to evaluate the impacts of stressors. Bacteria are grown under controlled conditions in order to generate a suspension at known total and viable cell concentration to be injected in the chamber. Samples are collected from the chamber at dedicated times after injection. Instruments connected to the chamber allow monitoring total airborne cell numbers, particle size distribution, gas concentration, T, rH, etc. A sliding tray hosting Petri dishes and placed inside the chamber collects bacteria by sedimentation and allows viable cell counts. Active samplers such as impactors, impingers, or filtration systems can be used as well and allow viable and total cell counts, on a per volume basis. Viability is assessed here as the ratio between viable cells as counted on agar plates and total cells.

The results provide the proofs of concepts that such experimentation as the exposure of airborne bacteria to gas or light can be led in the chamber using the instrumentation and protocols provided, for studies on relevant microorganisms and stressors. The manuscript should be of interest to readers of the journal, and, as a potential user, I personally appreciate the initiative of publishing such manuscript.

The manuscript is well written and sufficiently referenced, although the references used are not always relevant (see specific comments below). It is essentially descriptive, with very few discussion and implications of the findings, which could be developed further as well as perspectives.

I only have minor comments, listed below:

- Abstract: I think that it would be useful to indicate the mean residence/life times estimated for *E. coli* (baseline conditions).
- The manuscript is likely aimed at non-microbiologists, and so very basic protocols for culturing bacteria and characterizing their growth and numbers are given in great detail. I leave these to authors' choice, but these can be found in any handbook of basic microbiology that could eventually be referred to in order to shorten the manuscript. Examples include lines 401-419 regarding bacterial growth (which appeared in addition quite out-of-subject here), or lines 422-438 regarding preparation of cell suspension and estimation of viable cell numbers by dilution-plating. I thought that Figure 6 as well is unnecessary in the context of the study and could be put in supplement. Section 2.3.1 could therefore ultimately be greatly shortened and merged with another section (e.g. 2.3.2).
- Lines 28-29 (abstract): the sentence “This figure quantifies the protocol sensitivity as well to changes in viability when bacteria are exposed in other (e.g., polluted) conditions” is not clear (which figure? Conditions other than what?). In addition, “exposed in” should be “exposed to”.
- Lines 43-45: DMS and other VOCs are given as examples of SBAs, but these are not aerosols! Literally, SBA would correspond to fragments of larger biological particles, material released

from cells (disruption, excretion...), nucleated biogenic gases, or cells “born” in the air from microbial multiplication.

- Lines 46 and 47: the two “can” are not necessary. Moreover, do they really “vary” in size (at the individual scale)? Or do they rather fall within a range of sizes?
- Line 57: It is said that bacteria are at concentration “usually greater than 10^4 cell/m³” over land. The reference Bauer et al 2002 is not appropriate here for such statement. Rather cite the review by (Burrows et al., 2009) where Table 1 summarizes well literature data as a function of land use. In addition, the statement that concentrations in air over sea tend to be lower needs a reference, and it is not fully supported by literature (see for instance (Mayol et al., 2014), Table 1). This whole sentence thus needs revision.
- Lines 61-62: “Bacteria have a relatively long atmospheric residence time, of the order of several days or more, compared to larger particles and can be transported over long distances, up to thousands of km (Després et al., 2012)” is barely a plagiarism of the cited reference, that says: “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).” This may need rephrasing.
- Lines 69-72: Is this statement about the lack of data on abundance and diversity of microbes in the air really relevant here? It would be more helpful to develop a bit on the subject of the paper: viability, and the impacts of environmental conditions, for which even more data is lacking.
- Line 79-80: I would suggest to use more recent references here about the potential impacts on cloud chemistry, such as (Khaled et al., 2021; Jaber et al., 2021, 2020; Fankhauser et al., 2019).
- Lines 86-88: what is “direct deposition”, and how does this differ from precipitation? Do you rather mean “wet or dry deposition”. Also, can you develop what “multifaceted” means here when referring to the mechanisms that govern transport, survival and activity. Eventually cite (Amato et al., 2023) which discusses the fact that biologically- and physically-driven processes are indeed intertwined.
- Line 88-90: what kind of impacts are in question here?
- Line 98-104: In addition of the references cited, I think that a number of other (and early) studies that have investigated the survival and activity of bacteria using simulation chambers should be referenced: (Ehrlich et al., 1970; Krumins et al., 2014; Wright et al., 1969).
- Line 177: provide the reference of the nebulizer inlet please.
- Line 180: I do not see the point of mentioning “(or in the laboratory)”. Please specify (or remove).
- Line 342: quartz filters can be used for offline analysis. Please consider that this is also the case with other sampling methods (impingers and cultures cannot be considered “online” methods).
- Lines 351-352: “The hood is equipped with HEPA filter and UV-light laminar is created inside the cabinet” probably needs rephrasing (what is UV-light laminar?).
- Line 358: specify that the spectrophotometer is dedicated to liquid samples, to avoid confusion.
- Line 364: same as previous comment, regarding the shaker: it might not be obvious for non-microbiologists that these are intended for liquid cultures.
- Line 446: “the results of the logistic fit are only shown” might rather read “the results of the logistic fit only are shown”.
- Line 472: “centrifugated” should be centrifuged.

- Line 468-481: this is quite useless as presented as many details are missing: what is the volume of culture used? And that volume of liquid were cells resuspended into after centrifugation? Dilution step from what (line 480)?
- Line 489 “relative standard deviation” and Lines 500-501 “the ratio between standard deviation and mean”: aren’t these actually coefficients of variation?
- Line 546: nebulizedu
- Line 547: Indicate if these are true independent replicates of experiments (i.e. led from separate cultures? So somehow accounting for biological variability), or just consecutive repeats from same cultures.
- Line 562-565: check language (“the same of”, “this indicating”).
- Line 570: what is a “roughly complete cell mortality”? It is either complete, or incomplete...
- Table 3?
- Lines 588-589: NO is less toxic than NO₂. It would be nice to develop on this (in the discussion section): why could this be? what are the processes of toxicity of these compounds? What does this imply for natural situations? What is known about it?
- Line 598: “30 min”, or less, given the data shown. This is difficult to tell from Figure 9, maybe showing it on log-scale would be more appropriate? (using the 1/10 detection limit to represent “0” for instance).
- Line 600: the reference should be “Figure 9 “here.
- Lines 614 and 621: “next future” probably should read “near future”.
- The perspectives section could be developed based on other sampling possibility. Notably the possibility of using methods other than cultures to evaluate viability would be relevant if this is the case (live/dead staining, RT-qPCR, and others), in particular as non-culturable state is common in viable bacteria (as mentioned line 608). Moreover, neither SMPS data nor their potential use are exploited. It could be mentioned for instance that investigations about the relationship between particle size and survival could be performed, using cascade impactors as samplers for instance.

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