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Interactive comment

Interactive comment on “H_2O_2 modulates the energetic metabolism of the cloud microbiome” *by* Nolwenn Wirgot et al.

Nolwenn Wirgot et al.

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Interactive comment on “H2O2 modulates the energetic metabolism of the cloud microbiome” by Nolwenn Wirgot et al.

Anonymous Referee #2 Received and published: 13 September 2017

Comment: The authors present data meant to demonstrate the impact of H2O2 on the metabolism of bacteria in cloud water. The dataset is probably valuable but I ïňĄnd that the data analysis and presentation of the manuscript require major revision before it will be suitable for publication in ACP.

Answer: First of all we would like to thank Referee #2 for all his comments that should help to improve the manuscript.Changes are highlighted in yellow in the revised manuscript (see supplementary file).

Comment: The authors should comment on the important differences that exist between the laboratory setup and the cloud droplet environment, namely due to the much larger volume in the laboratory. How many bacteria can we expect to live in one cloud droplet? How is bacterial population growth in a cloud droplet different from in the laboratory studies discussed here (do we even know the nature of this difference?)?

Answer: Actually nobody really knows the absolute difference between in-lab and droplet conditions for the growth of bacteria. We suspect that one droplet contains one bacterium as bacteria can be considered as CCN and thus form a droplet. If we consider doubling times measured with a few strains isolated from cloud waters (Amato , PhD thesis, 2004) they varied from 5h to 20h at 17°C (average temperature in summer time at the PUY station) and from 16 h to 45 hours at 5°C (average winter temperature). Also during incubation at 17°C of a real cloud sample containing the whole microbiome and chemical composition of cloud water we measured an increase of cell concentration from 105 bacteria /mL to 106 bacteria /mL within 100 hours (Amato et al., Atmos. Chem. Phys, 2007, 5253-5276).

These experiments suggest that, depending on the strains and the temperature, and considering the duration of a cloud for about 2 days, the bacteria could divide from one to ten times.

However we would like to point out that this debate, although it represents still an open question, is out of the scope of this paper. The objective of the experiments presented in Figure 3 was only to demonstrate that bacteria did not die although their ATP content was drastically decreased. Growth measurement is a global proxy to attest the viability of the cells.

Comment: In the studies described here, while bacteria metabolism impacts the concentrations of trace species (and vice versa), the number of bacteria in the sample is also growing (i.e., Figure 3). The different solutions studied showed different growth proïňĄles, as evidenced in Figure 3 - and these growth proïňĄles are no doubt different from what would happen in the much smaller volume of a cloud droplet. Data regarding the kinetic processing of an atmospheric trace species by bacteria in a growing population is not useful, and even misleading, for atmospheric chemists who are the readership of this journal, unless the growth process can be decoupled from the chemical processing rates. One way to do this after the fact would be by normalizing the rate data by the number of bacteria in the sample at each time point. The data should be re-analyzed with this fundamental issue in mind.

Answer: We fully understand the remark of the reviewer; this indicates that we did not clearly define the objective of determining rates of degradation of H2O2. I think that the interpretation of Table 1 might be misleading. We have to clarify different points:

First the biodegradation rates have been calculated from the initial slopes (the first five time points i.e. between 0 and 2 hours) normalized with the concentrations of cells. Looking at Figure 3 it is clear that none of the bacteria are dividing (growing) during that 2 hour period (< 200 min.). Consequently the comparison of the abiotic and biotic degradation rates during that period is not altered by a change in the number of cells.

The purpose of the experiments performed in a microcosm with different conditions (bacteria or not, iron, light. . .) was not to measure degradation rates that will be directly implemented in atmospheric models or to quantify the relative contribution of abiotic versus biotic routes in atmospheric chemistry. In the past we have done it and indeed we have expressed the rates of biodegradation in mol. h-1.cell-1 (Vaitilingom et al. Appl. Environ. Microb., 2010, 76, 23-29 ; Vaitilingom et al. Atmos. Chem. Phys., 2011, 11, 8721-8733 ; Husarova et al. Atmos. Environ., 2011, 45, 6093-6102). If atmospheric chemists want to integrate growth in their model, they have to increment the number of cells at each time step of the calculation in the model. But this is out of the scope of this paper.

The major goal of this paper was to show that H2O2 modulates the ATP concentration of the cloud microbiome. Experiments in laboratory help understanding what the major factor influencing ATP depletion was. The development of the microcosms allowed us to separate the different factors (Fe, H2O2, light, . . .) and to conclude that only H2O2 concentration was important. To raise such a conclusion it was necessary to first validate that the microcosms used could mimic as much as possible cloud conditions. The idea to measure degradation rates in these microcosms was to get values (or rather "orders of magnitude") to be compared with those obtained with more realistic conditions. Our results show that the degradation rates measured are within the same order of magnitude that those obtained with real cloud water samples (Vaitilingom et al, Proc. Natl. Acad. Sci USA, 2013, 110, 559 564) and validate thus these microcosms.

The link between H202 and ATP concentrations observed under laboratory conditions was also validated in real cloud events using statistical analyses.

We hope that these explanations will help reviewer 2 to better understand our purpose. To make the objective of the work clearer and avoid any misleading in interpretation, we have changed the text as follows:

This sentence was added in the Material and Method section 188-190: "The biodegradation rates have been calculated from the initial slopes (the first five time points i.e. between 0 and 2 hours) normalized with the concentrations of cells. During these two hours no cell growth was observed. "

This sentence was deleted line 238: "These results show that artificial light and Fe-[EDDS] and thus HOâŮŔ radicals have no effect on H2O2 biodegradation".

We have modified this section line 238-246: The selected strains all degrade H2O2 within the same order of magnitude (average value for the three strains and for the condition with iron and light 1.76 10- 9 mol L-1 s-1 and with iron without light 1.40 10-9 mol L-1 s-1). In Vaïtilingom et al. (2013), the biodegradation rates of H2O2 were found within the same order of magnitude (average value for two distinct clouds with light 0.98 10-9 mol.L-1 s-1 and without light 0.29 10-9 mol L-1 s-1). The results obtained are within the same order of magnitude of values in real cloud environment thereby validating our microcosm conditions. This demonstrates that under our experimental conditions, the selected strains degrade H2O2 like the microbiome of real clouds. In addition it validates our approach to separately analyse the influence of each parameter (Fe, H2O2, light,. . .) on the microbial energetic state metabolism detailed in the next section.

Comment: The literature review in the Introduction section consists mostly of a discussion of this group's prior work. More of an effort should be made to place this study in the context of the broader scientiïňĄc literature.

Answer: 49 references are cited, from them 16 are from our group.

Among these 16 papers one is a review (Delort et al 2017) citing thus a lot of other references and 9 of them refer to the impact of cloud microorganisms on atmospheric chemistry. Actually, except the group of Ariya (which is cited) no other group works on this specific topic related to the interaction between microorganisms and cloud chemistry.

To extend this aspect to the air, we have added a reference of Krumins, V.; Mainelis G., Kerkhof, L.J.; and Fennell, D.E. Substrate-dependent rRNA production in an airborne bacterium. Environmental Science and Technology Letters, 2014, 9, 376-381.

The other citations of our group concern mainly measurements at the PUY station which are necessary for this work.

Most of the other references are centered on cloud chemistry and have been chosen to focus on hydrogen peroxide as it is the main purpose of this paper. Some of them are reviews (Gunz and Hoffmann 1990, Vione et al 2003) also citing many other papers.

To make the atmospheric chemistry context even wider we have added:

the extensive review of Herrmann H, Schaefer T, Tilgner A, Styler SA, Weller C, Teich M, et al. Tropospheric aqueous-phase chemistry: kinetics, mechanisms, and its coupling to a changing gas phase. Chem Rev. 2015; 115:4259–334.

And theses references:

Li, J., Wang, X., Chen, J., Zhu, C., Li, W., Li, C., Liu, L., Xu, C., Wen, L., Xue, L., Wang, W., Ding, A. and Herrmann, H.: Chemical composition and droplet size distribution of cloud at the summit of Mount Tai, China, Atmospheric Chem. Phys. Discuss., 1–21, doi:10.5194/acp-2016-1175, 2017.

Shen, X., Lee, T., Guo, J., Wang, X., Li, P., Xu, P., Wang, Y., Ren, Y., Wang, W., Wang, T., Li, Y., Carn, S. A., and Collett, J. L.: Aqueous phase sulfate production in clouds in eastern China, Atmospheric Environment, 62, 502-511, https://doi.org/10.1016/j.atmosenv.2012.07.079, 2012.

Arakaki, T.; Anastasio, C.; Kuroki, Y.; Nakajima, H.; Okada, K.; Kotani, Y.; Handa, D.; Azechi, S.; Kimura, T.; Tsuhako, A. A general scavenging rate constant for reaction of hydroxyl radical with organic carbon in atmospheric waters. Environmental Science & Technology ,2013 , 47 (15), 8196-8203.

Hems, R.F.; Hsieh, J.S.; Slodki, M.A.; Shouming, Z. ; Abbatt, J.P.D. Suppression of OH Generation from the Photo-Fenton Reaction in the Presence of α-Pinene Secondary Organic Aerosol Material. Environmental Science and Technology Letters Article ASAP DOI: 10.1021/acs.estlett.7b00381

Wei, M., Xu, C., Chen, J., Zhu, C., Li, J., and Lv, G.: Characteristics of bacterial community in cloud water at Mt Tai: similarity and disparity under polluted and non-polluted cloud episodes, Atmos. Chem. Phys., 17, 5253-5270, https://doi.org/10.5194/acp-17-5253-2017, 2017.

Comment: Finally, the language throughout the manuscript and the abstract needs editing. In many instances the language is too vague or informal for a scientiïňĄc publication. The paper also needs to be edited carefully for English grammar (especially subject-verb disagreement in multiple places in the manuscript).

Answer: We agree with reviewer 2 that the language should be improved. Hopefully reviewer 1 carefully corrected the manuscript and helped us to improve its quality.

Please also note the supplement to this comment:
https://www.atmos-chem-phys-discuss.net/acp-2017-581/acp-2017-581-AC2-supplement.pdf

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