Co-Editor Decision: Publish subject to minor revisions (review by editor) (23 Oct 2017) by Alex Huffman
Comments to the Author:
Authors,

After reading through the referee comments and your responses, I am confident that the manuscript will soon be acceptable for final publication. There are a few areas that I would like you to improve upon somewhat more before final acceptance, and I've listed these below. Hopefully these comments will be relatively efficient for you to process edits for. There are also still some areas that require minor English language edits, but these will likely be corrected during the copy-editing process by the Copernicus staff.

Alex Huffman

General comments:
Comment 1: Section 2.1, Description of the microcosms – I think the addition of this section was important, but I'm still a bit confused by how these microcosms were produced in your experiments. I suggest adding a sentence after the first in the section to say something like: "Microcosms were developed by …" and then follow with an few-sentence, explicit overview of how you got droplets of cloud-like water. It wasn't clear to me whether the water was collected from clouds and processed in some way or synthetically produced. Make this very clear at the beginning of this section before you move into the details of the radiation that was supplied to the microcosms.

Answer: We understand that this section was not clear enough and confusing. We have completely changed this section as follows. We moved the description of the cells and their growth conditions in section 2.1 (initially section 2.2) and merged "the description of the microcosm" (initially section 2.1) and "the biodegradation assays" (initially section 2.3) in the same section (now section 2.2 "Incubations in microcosms"). We also added new sentences.

2.1 Bacterial strains and growth conditions

Pseudomonas graminis, 13b-3, DQ512786; *Pseudomonas syringae*, 13b-2, DQ512785, *Sphingomonas sp.*, 14b-5, DQ512789 were grown in 10 mL of R2A medium (Reasoner and Geldreich, 1985) under stirring (200 r.p.m) at 17°C for approximately 17 h, 24 h or 48 h, depending on the strain. The three selected bacterial strains belonging to the Gamma-Proteobacteria (*Pseudomonas*) and Alpha- Proteobacteria classes (*Sphingomonas*) were isolated from cloud water and are representative of the genera most frequently found in cloud water samples (Vaïtilingom et al., 2012) collected at the PUY site.

Cells in the exponential growth phase were collected by centrifugation for 3 min at around 10000 *g*. The supernatant was removed and the bacterial pellet was suspended and washed twice with an artificial cloud solution and incubated in microcosms to perform biodegradation experiments (see section 2.2). The bacterial cell concentration was estimated by optical density at 575 nm to obtain a concentration close to 10^6 cell mL^{-1}. Finally, the concentration of cells was precisely determined by flow cytometry analysis (BD Facscalibur Becton-Dickinson; λ_{exc}= 488 nm; λ_{em} = 530 nm) using a method based on the addition of a fluorochrome (SYBR-green) for their counting (Marie et al., 1999).

2.2. Incubations in microcosms

Microcosms were designed to simulate as much as possible the water phase of cloud waters. They provide the opportunity to work under artificial solar light condition and also in the presence of microorganisms. The experiments were performed under bulk conditions as cloud droplets cannot be reproduced in these bioreactors (Infors HT Multitron II).

For irradiation condition the bioreactor was equipped with lamps that emit UV-radiation (Sylvania Reptistar; 15 W; 6500 K) to mimic solar light measured directly in clouds at the PUY station (Fig. SM1). The incubation flasks were Pyrex crystallizers covered with a Pyrex filter and equipped with Teflon tubes of 8 mm Ø plugged with sterile cotton, letting air and light pass (see Vaïtilingom et al. 2013) while for dark conditions they were amber Erlenmeyer flasks.

All incubation flasks contained 100 mL of artificial cloud solution under agitation (130 rpm), its composition was first described in Vaïtilingom et al. (2011). This solution was mimicking cloud chemical composition from cloud samples classified as "marine" following the work from Deguillaume et al. (2014) at the PUY station The major part of the collected cloud samples were classified as marine (52%) supporting our choice for the artificial cloud composition. Stock solutions of this artificial cloud medium were prepared with the following concentrations: 200 µM for acetic acid (CH_3COOH; Acros organics), 145 µM for formic acid (HCOOH; Fluka), 30 µM for oxalic acid ($H_2C_2O_4$;Fluka), 15 µM for succinic acid ($H_6C_4O_4$; Fluka), 800 µM for ammonium nitrate ($H_4N_2O_3$; Fluka), 100 µM for magnesium chloride hexahydrate ($MgCl_2$, $6H_2O$; Sigma-Aldrich), 50 µM for potassium sulfate (K_2SO_4; Fluka), 400 µM for calcium chloride dihydrate ($CaCl_2$, $2H_2O$; Sigma-Aldrich), 2000 µM for sodium chloride (NaCl; Sigma-Aldrich), 1100 µM for sodium hydroxide (NaOH; Merck), 315 µM for sulfuric acid (H_2SO_4; Sigma-Aldrich). Finally, the obtained solution was adjusted to pH 6 as necessary with a few drops of the solutions of NaOH or H_2SO_4 used for the preparation of the marine artificial cloud water solution and sterilized by filtration (Polyethersulfone membrane, 0.20 µm; Fisher Scientific) before use.

For biotic conditions, the flasks were inoculated at 10^6 bacterial cells per mL. The artificial cloud water solution was ten times more concentrated than a real cloud water solution in order to stabilize the pH. This was also the case for bacteria concentration because the bacteria/substrate ratio should be kept identical to that of real cloud. Indeed, it has been demonstrated that if this ratio is maintained, the degradation rate remains constant (Vaïtilingom et al., 2010). The equipment was sterilized by autoclaving at 121°C for 20 minutes and all manipulations were performed under sterile conditions.

Depending on the conditions, hydrogen peroxide and iron complex (Fe-[EDDS]) were added or not to the solution in the incubators. These two compounds are present in marine cloud water collected at the PUY station at average concentrations of 7.5 µM (with a dispersion of mean values ranging from 0.1 – 20.8 µM) for H_2O_2 and 0.5 µM (with a dispersion of mean values ranging from BDL. – 4.9) for Fe(III) (Deguillaume et al., 2014). In the cloud aqueous phase, Fe(III) may be complexed by organic compounds. Recently, it has been hypothesized than iron can be chelated by other organic ligands of biological origin (Herckes et al., 2013; Herrmann et al., 2015), and in particular by siderophores (Vinatier et al., 2016) that are ligands characterized by high complexing constants ($K>10^{20}$). Fe-[EDDS] was chosen as an iron(III) complex model because this ligand has a complexing constant for iron very close to the values for siderophores. Moreover, it is known to be stable at the working pH of 6.0 and because its chemistry has been studied in details by Li et al. (2010).

Hydrogen peroxide solution was prepared from a commercial solution (H_2O_2, 30%; not stabilized Fluka Analytical). 1:1 stoichiometry iron complex solution was prepared from iron (III) chloride hexahydrate ($FeCl_3$, $6H_2O$; Sigma-Aldrich) and from (S,S)- ethylenediamine-N,N'-disuccinic acid trisodium salt (EDDS, 35% in water). The hydrogen peroxide solution and the iron complex solution were freshly prepared before each experiment and the final working concentrations were fixed at 20 µM and 4 µM respectively, in agreement with the real concentrations detected in samples collected at the PUY station multiplied by a factor ten when median values measured in marine cloud waters are considered (Deguillaume et al., 2014).

In addition, the working temperature was fixed at 17°C which is the average temperature of cloud samples in summer. For all the incubation conditions, samples were taken at regular intervals, and stored at -20 °C before analysis.

Comment 2: For Figures 1,2, and 4, I think it would benefit the reader to consider using color as a part of the traces/markers. Since color figures can be reproduced in the final version at no additional cost, I think this edit would improve readability, especially in the relatively complicated Figure 1. You might consider coloring in such a way as to make one theme of colors to be biotic and another theme to be abiotic, etc.

Answer: thank you for this remark, all the figures are in color now.

Connected to this comment, e.g. at L222 where Figure 1 is discussed, the interpretation of the figure takes some time for the reader. I suggest specifically referring to which trace you discuss by the color that you change it to in the final form, i.e. "the degradation of hydrogen peroxide is clearly effective … (Fig. 1, blue trace)"

Answer: we took into account this remark (lines 222 and 231).

Comment 3: Reviewer #2 suggested major manuscript revision, including several specific areas of improvement. My feeling is that some of these suggestions for improvement are reasonable, but can be handled with only mild additional discussion. One area relates to their first major comment about the differences between the laboratory setup and the 'real' cloud water environment. I agree with your response that a full analysis of this is beyond the scope of the manuscript. However, I suggest taking some of your response to the referee's question and including these major ideas somewhere in the manuscript – probably the final discussion, Section 4.

Comment 4: The same response is true for the second comment from Referee #2 ("In the studies described here …"). I think it would be worthwhile to add an additional sentence or two of discussion regarding how an atmospheric chemist might treat or use these data. In this context it is fine to say what would need to be done before it could be modeled or scaled, and how it might be important or complicated. I encourage you to use thoughts you have already formulated and put into the response document. Take the most important of these and add a few ideas to the discussion.

Answer to comments 3 and 4: In order to take into account some of Referee #2 comments ,we added this paragraph to the discussion section (lines 327-337):

"…induced by H_2O_2, then ATP is depleted, and finally all the metabolic pathways involving these compounds are impacted and a complete change in the metabolome can be expected.

The measurements preformed in microcosms do not reproduce what is really occurring in cloud droplets. First incubations were performed with artificial cloud water and model strains, nevertheless the obtained results were consistent with those obtained with real cloud water samples. Second the potential growth of microorganisms during a cloud event could also modify transformation rates, this is only realistic for long cloud lifetimes (> 24 hours). Finally experiments were performed under bulk conditions and not with individual cloud droplets, only models can take into account the complexity of cloud conditions, in particular the multiphase aspect of cloud chemistry. To go further and integrate biodegradation rates in atmospheric chemistry models, complementary experiments should be performed and biodegradation rates should be expressed as $mol^{-1}.cell^{-1}.h^{-1}$.

However the most important result of this work was to show the correlation between H_2O_2 concentrations and ATP concentrations. This result obtained under our microcosm conditions was confirmed using data measured in real cloud samples that experienced multiphase and real cloud conditions. Indeed, we have shown, thanks to statistical analyses, that there was also a high correlation between H_2O_2 and ATP concentrations in real cloud…"

Technical comments and typos:

L61: Typo – remove "have been"

Done

L106: New sentence – I would suggest moving this sentence a bit one sentence higher into the paragraph so that the sentence beginning "This work will …" can be the last sentence of the introduction.

Done

L114: I was a bit confused by how to interpret the "up to 30%" comment about the UV radiation. 30% of what, of the light energy emitted from the chosen source? Please clarify this.

Answer: This information is not important (%); we have changed the text to " Sylvania Reptistar; 15 W; 6500 K " (line 114)

Figure 1 caption: I suggest changing to: "Where error bars are not visible they are smaller than the symbol." Also, the two sentence previous to this are almost exactly redundant. Keep only one of those two sentences.

Answer: We agree this is redundant. We have changed the legend by your proposition "Where error bars are not visible they are smaller than the symbol"

Figures 1, 2, and 4 are produced at low resolution. After acceptance, please make sure to submit higher resolution versions of these figures.

Answer: The image format of these figures has been changed (Export), the resolution is quite correct now.

Table 1: Is there a reason why no standard errors are reported for section (a), abiotic degradation? It would be better if these were included.

Answer : it was a mistake, we have added the standard errors.