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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Atmospheric chemistry of carboxylic acids: microbial implication versus photochemistry" *by* M. Vaïtilingom et al.

M. Vaïtilingom et al.

mickael.vaitilingom@univ-bpclermont.fr

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"General comments:"

"This manuscript describes biodegradation experiments of mono- and dicarboxylic acids with bacterial strains isolated from cloud water. The rates of degradation are compared to experimentally and theoretically derived photodegradation rates. The authors conclude that in low OH regimes, microbial degradation can compete with or even exceed photochemical degradation. This is surely an interesting result and within the scope of the journal. However, the manuscript needs major revisions according to the comments below to be suitable for publication in ACP."

We thank the reviewer for her/his contribution to this work. We address our response

point by point below.

"Specific comments:"

"The authors state that in their previous paper Vaitilingom 2010 they already measured the biodegradation of formate and acetate. Why then measure again in this work? And how does the data presented here compare to the older data? Or is it the same? This is confusing and needs clarification."

The two last paragraphs of the introduction have been rewritten to clearly the differences between the two papers and the objectives of the present work. The paragraphs are now the following:

To test this hypothesis, in previous work, our strategy was first to screen the ability of a large number of microbial strains (60) isolated from clouds to degrade organic compounds (formate, acetate, lactate (D & L), succinate, formaldehyde and methanol) under optimal conditions (27°C) (Amato et al., (2005; 2007a)). We have shown that enzymes responsible for these transformations were present in these microorganisms. To go further, we studied the response of one single strain (Pseudomonas graminis) under more realistic atmospheric conditions (Vaitilingom et al., 2010). We used a microcosm mimicking cloud chemical composition typical from continental influence at two different temperatures representative of warm clouds (5°C and 17°C). Biodegradation rates of formate, succinate and acetate were measured and compared to radical chemistry. For this, the reactions of the followed organic compounds with the two major free radicals OH and NO3 were considered. We calculated theoretical photodegradation rates from radical concentrations simulated by cloud chemistry models (Ervens et al., 2003b; Herrmann, 2003). This comparison showed that microorganisms would dominate the degradation of certain organic compounds in clouds during the night.

The objective of this work was to generalize our conclusions previously obtained on a single strain. For this, we selected 17 strains representative of the viable community existing in clouds and worked on two distinct artificial cloud media that reproduce

marine and continental cloud chemical composition. Biodegradation rates of acetate, formate, oxalate and succinate were measured for all the strains on both media at 5°C and 17°C. The goal was to investigate the effect of environmental parameters on the biodegradation efficiency of microorganisms. Biodegradation rates in a natural cloud water sampled at the puy de Dôme station (France) with its own endogenous microflora and chemical composition was also investigated. The objective was to compare artificial microcosm and natural clouds. In addition, photodegradation experiments with hydrogen peroxide (H2O2) as a source of hydroxyl radicals were performed under the same microcosm conditions to compare directly photochemistry to biodegradation process. This is the first report of such comparison using experimental data. In the discussion, theoretical calculations based on various scenarios of the OH reactivity were presented.

"Throughout all the manuscript the term "reactivity" is often used in a somewhat strange context. For example in the abstract the authors state that they "investigated to which extent the active biomass" represents an alternative route to the chemical reactivity of carboxylic acids." Although I can vaguely guess what the authors intend to say (alternative way of degradation?), I suggest to phrase such sentences more accurately."

We agree with the reviewer. In the manuscript, the term "reactivity" is sometimes badly used. We changed it when necessary.

"The structure and content of section 3 has to be improved. In section 3.4 for example, the main result is that the biodegradation rates in the natural cloud water are very similar to the ones measured in the artificial cloud water. Most of the paragraph, however, consists of experimental descriptions. The authors might want to think about combining the results of biodegradation (both in natural and artificial cloud water) into one section and they should present them in a clearer way."

Yes, we agree with your comment. In the final version, we have combined biodegradation rates in natural cloud and artificial cloud media (see section 3.1 on biodegradation).

Figure 3 and Table 6 in the original manuscript have been deleted and only biodegradation rates are indicated in the text. Table 5 describing the composition of the natural cloud has been also integrated in Table 1.

"Next, section 4 (Discussion and conclusion) has to be completely re-written. At present stage it is very poor. Instead of discussing the (sometimes surprising and striking) experimental results, it rather justifies how and why the study has been conducted in a very lengthy and confusing way. It is furthermore spiked with statements that are not justified neither by the experimental data nor by any reference. For example on p4895, l.21-22 "photodegradation processes are less sensitive to temperature": The photodegradation experiments were done at one temperature only in this work. Or on p.4896, l.21-22 "this work . . . showed that microbial activity could be much more important than NO3 reactivity on the carboxylic acid degradation and that it could be considered as a relevant sink for organic compounds during night": I cannot see in which part of the manuscript this has been shown or discussed."

We agree. We modified the structure of the results and discussion for more clarity. The discussion on radical concentrations estimated by models is now in the discussion part. A new Table 4 describing our theoretical calculations (at 5°C and 17°C) that are compared with the experimental biodegradation rates is added in the revised manuscript. The Figure 2 is also modified and now only present experimental comparisons of photochemical rates (S1 and S2) and biodegradation rates at 17°C. A new Figure 3 is now introduced in the paper that presents relative contributions (at 5°C and 17°C) of photochemical (OH) and biological degradations of carboxylic acids based on Table 4.

"p.4882, l.1-2: dissolved organic compounds, dominated by carboxylic acids. I doubt this is true. Usually the carbon fraction of cloud water TOC that can be attributed to identify carboxylic acids is in the range of a few percent. Often even the water soluble organic carbonyl compounds make up a larger fraction, irrespective of all the yet unidentified material."

This sentence about the dominant fraction of carboxylic acids among organic compounds is not justified. After checking, the percentage of 36% was wrong. Actually, the corrected carboxylic acids fraction at the puy de Dôme station represent between 5 and 10% of the dissolved organic content (DOC) (Charbouillot et al., submitted paper, 2011). This is confirmed by other field measurements (Saxena and Hildemann, 1996; Hadi et al., 1995).

"p.4882, l.3-4: Is solar radiation really a "catalyzer" of the reactivity"? I think I know what is meant here, but it should be phrased more accurately."

Based on referee 2 comment, the abstract has been completely rewritten and shortened because it was too complex and confusing. In the new one, this point has been changed.

"p.4882, l. 10: "viable community of clouds" does not make sense"

The point has been changed.

"p.4882, l.13-14: "major carboxylic acids existing in cloud water": Among the DCAs, malonic and malic acid often show higher concentrations than succinic acid. Is there any reason why succinic acid was selected over the other two?"

Yes, we agree with the reviewer. The malonic acid is detected at various sites such as the puy de Dôme mountain and is in the same range of order than succinic acid. However, we selected succinic acid since we are sure that microbes can use it in their metabolic pathways. The previous study from Marinoni et al., ACP, 2004 at the puy de Dôme station studied the 12 major carboxylic acids in cloud water. They didn't detect malic acid in their samples and consequently, we decided not to follow it.

"p.4883, l.1-2: "aqueous reactivity": see comment above"

This point has been modified.

"p.4883, l.11-12: "through reactions between the gaseous and the aqueous phase":

Do you mean: through reactions within the aqueous phase?"

The sentence was unclear and is not modified.

"p.4883, l.15: The value of 36% is certainly not universally true. Also, it seems rather high compared to the carbon fractions of carboxylic acids reported by others. In fact, Marinoni et al. 2004, seem to have calculated the fraction of total carboxylic acid mass to dissolved organic carbon, which is not correct. It has to be the carbon fraction, which will be much lower. Please include other/more references here."

We agree. See comments above about the relative contribution of carboxylic acids in the DOC.

"p.4883, l.20: "aqueous phase reactivity". See comment above."

Changed.

"p.4883, l.21: Are these really the best references for aqueous phase sources of carboxylic acids?"

This part of the introduction was rewritten and new references have been added to better represent the various sources of carboxylic acids in cloud water.

"p.4884, l.11: Do you mean: "influence the budget"?"

Yes. We follow your suggestion.

"p.4884, l.22: "reactivity of certain compounds". See comment above."

Changed.

"p.4884, l.26: How is the new setup different from the previous one? The authors should not expect the reader to be aware of the technical development of their laboratory setups."

This text has been changed as explained above.

“p.4884, l.29: see comment above, this is not generally true”

This text has been changed as explained above. This statement has been suppressed.

“p.4885, l.9: Which model is meant here?”

This text has been changed as explained above.

“p.4885, l.12: In their previous work, the authors studied 60 bacterial strains. Why only 17 in this work? Do they represent the most frequent ones? Or are they– as stated – among the most frequent ones? Then, what about the other frequent ones?”

In the work from Amato et al. (2007a) where they studied 60 strains, no precise degradation rates were determined. Only the percentage of degradation after 24h of incubation was given. In this work, we determined biodegradation rates (mol cell-1 s-1) for 17 strains that are representative of the cloud microflora. For this, we used 272 plots to determine biodegradation rates (17 strains, 2 different temperatures, 2 media, 4 carboxylic acids).

“p.4886: In line 6 it is stated “artificial cloud water solution (see Table 1 for composition)”, in line 14 it is stated “the cloud water solutions were prepared 10 times more concentrated than indicated in Table 1”. It is not fully clear to me to which (presumably different) solutions it is referred here.”

Yes, this sentence was unclear. Wed rephrased it as following: “. . .the artificial cloud media were prepared 10 times more concentrated than indicated in Table 1.”

“p.4886, l.24-26: Why not use the average bacterial concentration, rather than a 10 times more concentrated one?”

We wanted to respect a constant ratio “cell concentration/degraded chemical compound concentration”. Vaitilingom et al., AEM, (2010) showed that in the range of concentrations investigated, biodegradation rates are independent of the absolute cell and chemical concentrations if the ratio “cell concentration/degraded chemical compound

concentration” is constant.

“p.4886, l.26: What does OD575nm mean?”

We agree with the reviewer, this acronym was not detailed. OD575nm is the optical depth at 575nm. We have changed the text accordingly.

“p.4886, l.27-28: I am not sure whether ACP readers can be assumed to be familiar with CFU counts. I am not and I would appreciate some more (brief) explanations and maybe a reference here.”

CFU refers to “Colony Forming Units”. We counted the number of colonies on a petri dish composed of a R2A medium. Each colony corresponds to one single cell that has grown on this medium. We changed the text accordingly.

“p.4887, l.14: A brief explanation of how different light spectra influence the photodegradation would help here to understand why two different photochemical setups were used.”

The S2 setup was used because it offers the possibility to run various experiments in parallel and to perform replicates. However, the S2 setup is less efficient than S1 to produce radicals. Therefore, we decided to keep the two systems. For future combined photo-biodegradation experiments, the S2 setup will be more convenient for microorganisms since the oxygenation is improved compared to the S1 setup.

“p.4888, l.17: Why not every hour from 0 to 8 h, as done in the artificial biodegradation experiments?”

This first experiment on real cloud water was performed to evaluate if the endogen microflora can lead efficient biodegradation. We didn’t expect that the biodegradation was so efficient. This is why we designed an experiment over a long time (120h). Since the volume of the cloud water was limited, we could take only a few samples for the analysis. However, the biodegradation slope was linear up to 60h for all carboxylic compounds.

“p.4888, l. 19-22: Please give more details of the method or at least a reference. What are the detection limits etc.?”

The IC analyses are described initially in Jaffrezo et al., Atmos. Environ. (1998) and also detailed in Marinoni et al., ACP, (2004). These two references are now indicated in the revised manuscript. Detection limits are fully detailed in Marinoni et al., ACP, 2004.

“p.4888-4889, section 2.6: As also stated by the other referees, it is not fully clear how the rates were determined. Please explain in more detail and include a Figure.”

Yes, this section has been improved. The section 2.6 was totally rewritten for more clarity and we added an example of calculation in the Supplementary Material. The explanations are now the following:

To calculate the bio- and photodegradation rates, time evolution of each carboxylic acids concentration was plotted (Figure S1 for illustration). Then the pseudo-first order decay “k” (s-1) is determined by the linear regression of:

ln([C]/[C]0) = f(t) = -k . t (see Figure S2 in the SM).

With [C]0 (mol L-1) the initial concentrations of selected carboxylic acid C. In biodegradation experiments, the pseudo-first order decays was determined over the 6 and the 8 first hours of incubation at 17°C and 5°C, respectively. In photo-degradation experiments, the pseudo-first order decays was determined over the 4 and the 8 first hours of irradiation in the photochemical setups S1 and S2, respectively; at this incubation time, H2O2 was available since it was totally consumed after 6h in S1 and 48h in S2. The measured degradation rates of the compound C (vc) have been determined as follows:

- for biodegradation rates per cell: vc = (k . [C]0) / Ncells in [mol cell-1 s-1] (1)

- for photodegradation rates : vc = k . [C]0 in [M s-1] (2)

With Ncells (cells L-1) the concentration of cells participating to the biodegradation. The estimated photodegradation rates resulting from the reactivity with free radicals

from data of the literature: vc = K . [OH] . [C]0 in [M s-1] (3)

With [C]0 the initial concentrations of selected carboxylic acid C (mol L-1), [OH] is the concentration of free radical OH from literature and K (M-1 s-1) is the degradation rate constant of the carboxylic acid C by OH.

“p.4889, l.13-15: This has been mentioned in the Experimental section already.”

Yes, we understand your comment but we prefer to repeat this sentence to help the reader.

“p.4889, l.16-22 and p.4890, l.1-5: This could be moved to the experimental section.”

We think that explaining the differences between the two artificial media need to be indicated in this section because these data are necessary to explain and understand the results.

“p.4891, l.14: “organic photoproduct” sounds odd to me. I guess “oxidation product” is meant here.”

Yes, we agree. We replaced the wrong term.

“p.4891, l.18: “Acetate and succinate were not degraded by photolysis in all experiments.” This is a surprising result to me. How can this be explained? The authors don’t even discuss it, which should definitely be done here.”

In our experimental conditions, it was not possible to determine the photodegradation rates of acetate and succinate in both systems (S1 and S2) because their degradations were too slow. The photodegradation of formate is followed due to the photolysis of H2O2 that produces OH radicals. The formate degradation is more efficient because the oxidation rate constant of formate with OH radicals is higher than the ones for acetate and succinate. As underlined by the reviewer, we were also surprised by these results. This is why we decided to use simulated OH values from models to confront the potential oxidation of acetate and succinate by radicals with the potential biodegra-

dation process. For the OH concentration of 10-14M, the main conclusions concerning the relative contribution of microbial and photochemical degradation are consistent with our experimental results. The major problem is the absence of measurements of OH radical concentrations in real clouds. The absence of data avoids the design of perfectly accurate experimental setups for photodegradation and of realistic calculation. The discussion part refers to this statement.

“p.4891, l.24- p.4892, l.10: This might be better suited in a discussions section.”

To our opinion, this paragraph only discusses the differences between the two systems (S1 and S2) and between the two irradiated media. We prefer to keep it in this section that is descriptive.

“p.4893, l.4-5: This obviously is a result of the non-existent photodegradation of acetate and succinate in S1 and S2, but how realistic is this in real clouds?”

As explained above, it is difficult to extrapolate our results about the photodegradation of acetate and succinate to real clouds. Until now, the radical concentrations are uncertain in real clouds. In addition, organic compounds are inter-connected (for example, acetic acid leads to oxalic acid) and the measured rates results from various processes since the artificial media contain several organic compounds. Note that this remark also stands for biological processes in these media. Finally, the real cloud is a multiphasic system with transfers of chemical compounds between the gas and the aqueous phase. In this frame, only models will be able to quantify the photochemical and biological contributions in the degradation of organic compounds. However, we have to start with simplified conditions to get experimental data to complete models.

“p.4893, l.18-28 and p.4894, l.1: This should all be moved to the experimental section, some information is also redundant.”

We agree with the referee; it has been changed in the new version of the manuscript.

“p.4904: What are global experimental uncertainties and how were they determined?”

The global experimental uncertainty is the result from two additive errors: 1) the one from the analysis with the ionic chromatography (less than 10%); 2) the one from the biological variability that was calculated from five replicates on 2 strains (between 20 and 25%).

“p.4908: What does the asterisk after “Microbial activity” stand for?”

The Tables have been modified and this problem is solved. Table 6 has been deleted.

“p.4908: How were the measurement uncertainties determined? Is the uncertainty the standard deviation of a mean value?”

Yes, the uncertainties correspond to the standard deviation of the mean biodegradation rate value. This was determined from 3 replicates.

“p.4911: Fig. 3 is pretty much the same as the corresponding part of Fig. 2 because of the high similarity between the rates in artificial and natural cloud water. It might therefore be redundant.”

We followed your comment and Figure 3 has been deleted because this was redundant.

“Technical corrections:”

“p.4882, l.1: multiphase atmospheric systems”

The abstract has been rewritten.

“p.4883, l.17: monocarboxylic”

Done.

“p.4884, l.7: carbon sources”

To our point of view, chemical composition refers to chemicals that can be used as carbon sources for microbes but also can interact with them (for instance, oxidants that can stress them).

“p.4884, l.22: dominate the degradation: (if this is what is meant here)”

Yes, we changed reactivity by degradation.

“p.4885, l.4: were studied”

Done.

“p.4886, l.1 and l.4: consisted of”

Done.

“p.4886, l.9: NH4NO3”

Done.

“p.4887, l.24 and elsewhere: rpm”

Done.

“p.4891, l.3: and vice versa”

Done.

“p.4892, l.16: comma instead of semicolon.”

Done.

“p. 4910: Fig.2: Increase font in Figure 2.”

This was considered in the new manuscript.

“p.4903: Table 1 caption: delete “similar but”.”

This sentence has been deleted.

Interactive comment on Atmos. Chem. Phys. Discuss., 11, 4881, 2011.