Interactive comment on “Biotic and abiotic transformation of amino acids in cloud water: Experimental studies and atmospheric implications” by Saly Jaber et al.

Referee #2

Referee Comment:

In this work the authors conducted microcosm experiments with the aim of differentiating the roles of biotic and abiotic transformation of free amino acids in cloud water. In the experiment, they utilized 19 types of amino acids, four bacterial strains or photo-bioreactors, attempting to mimic ambient cloud conditions. With the kinetic loss/production data of amino acids, they concluded that previous studies may have overestimated the abiotic degradation rates of amino acids, and future modeling efforts should take the biotic and abiotic transformation of amino acids into account. Overall, I think the authors did a solid job in terms of writing and offered an interesting dataset in amino acid dynamics in the field of cloud chemistry. The data seem to be sound. However, I have some concerns the authors need to address before publication.

Authors’ Response:

We thank the referee for their positive evaluation of our manuscript. We address all comments point-by-point below.

Referee Comment:

The authors claimed that they developed a new analytical technique that can analyze amino acids, without the need of any preparation such as derivatization, using UPLCHRMS. This is nice effort, but I am surprised that the incubation medium they used, containing mM of ions such as Na, Ca an K etc. (Table S1), which would directly impact ion source and ionization process, was directly injected to the LC-MS. I don’t think any mass spectrometry expert would be happy if you inject such a solution to the instrument.

Authors’ Response:

As you can see in Table 1 the concentrations are not in mM but in µM, except for Na^+ which is 1mM, therefore we did not find any problem with LC-MS. We are far from concentrations encountered in ocean water samples for instance where concentrations are in the range of 0.1M. As shown in Figure S1 linear plots are obtained for the calibration curves. Also we have calculated the Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates). As you can see in the Table S3 these RSD are rather low, ranging from around 0.5% to 10%, except for Valine and Glycine where it can reach 20%. Finally, we know the initial concentration of the AA (1µM) and we do find this concentration in our measurements. Our conclusion is this that this method is suited for measuring AA concentration in this medium, in addition the obtained values for LOD and LOQ are within the same range of order than those reported in the literature.

We will add the following text and Table S3 into Section 2.2.2:

The obtained values of LOD and LOQ were considered to be fit-for-purpose (Table S2) *and are consistent with data from the literature (*(Helin et al., 2017).

We also have calculated the Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates). As you can see in the Table S3 these RSD are rather low, ranging from around 0.5% to 10%, except for Valine and Glycine where it can reach 20%. It can be noticed that these RSD due to the LC-MS method are much lower than those due to the transformation experiments, especially for biotransformation where there are biological variations (see error bars in Figure 1 and 2).

Table S3: Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates)

	Relative standard deviation (RSD = Standard deviation/mean)		
Amino acid	0.1 µM (n = 3)	0.5 µM (n = 3)	1 µM (n = 3)
ALA		0.71%	3.61%
ARG	0.83%	1.96%	1.56%
ASN	5.23%	4.92%	3.63%
ASP		10.77%	5.96%
GLN	4.19%	4.37%	3.20%
GLU	3.77%	2.89%	3.92%
GLY			21.39%
HIS	0.62%	0.89%	1.22%
ILE	4.48%	0.48%	0.59%
LYS	6.64%	1.96%	1.50%
MET	4.49%	4.35%	6.38%
PHE	4.63%	1.68%	1.02%
PRO	11.67%	5.08%	1.28%
SER	14.34%	3.06%	3.20%
THR	14.15%	3.67%	1.06%
TRP	7.00%	1.67%	1.75%
TYR	0.94%	1.81%	1.15%
VAL	17.94%	2.98%	11.41%

As we have introduced this new Table S3, the previous Tables S3 and S4 will be renamed Tables S4 and S5

***Table S4**: Rate constants for 18 amino acids for the OH, O_3 and $^1O_2$ reactions*

***Table S5:** Selected experimental studies of amino acid oxidation by various oxidants. Note that the experimental conditions were not necessarily atmospherically-relevant. Products are only listed to demonstrate the wide variety of possible reaction pathways and products.*

Referee Comment:

I am wondering what's the inject volume they used and how they can maintain a consistent sensitivity with such high ion strength solution (or how long).

Authors' Response: The injection volume was 5µL which is very low and does not induce any problem.

We shall add this information in the Material and Methods section 2.2.1

The volume of injection was 5µL.

Referee Comment:

In addition, external calibration curves were used to quantify the amino acids in cloud water medium. Did you use the same water medium for the standards? If not, this might be a problem with the matrix effect.

Authors' Response: We used the same medium for the standards.

We shall add this information in the Material and Methods section 2.2.2

In order to quantify the amino acid concentrations, calibration curves were established for each experimental series of LC-HRMS analyses *using the same artificial cloud medium than in the incubations.*

Referee Comment:

It may be also helpful to show the LC-MS chromatograms in the supplementary section. The bottom line is that more info is needed for this new approach you developed.

Authors' Response:

As explained in the 2.2.1 section, the ions were selected using the SIM (Selected Ion Monitoring) mode for each AA so that the raw LC-MS chromatograms are not of great interest (not used for quantification). In addition, as Q-Orbitrap™ was used, the extracted masses are very precise.

Referee Comment:

I like the experimental approach, such as clearly separating abiotic and biotic factors, and using free amino acids and single bacterial strains, which allowed you to tease out the convoluted factors observed in field samples. However, the authors need to realize/justify their experimental conditions which I think are far away from those of the field, thus more discussion is needed. For example, they used 1 uM of 19 types of amino acids, which represent 19uM amino acids or 684 ug C/L (assuming 3 C per amino acid); in contrast, the cloud water only contained 2.4-74.3 ug C/L, cited from the Introduction of the manuscript.

Authors' Response:

Cloud and fog water usually contains several milligrams of carbon per liter, a small fraction of which is composed of amino acids. As cited in the introduction, concentrations of up to 757 µg C L^{-1} amino acids have been identified in cloud water; thus, we do not think that our assumptions are unrealistic. It should be also noted that not all cloud condensation nuclei contain amino acids, while cloud water concentrations are based on the analysis of bulk water samples. Thus, individual cloud droplets might be much more highly concentrated in amino acids than the bulk cloud water. However, since there are no analytical techniques to date that can routinely determine the solute concentrations in single cloud droplets, we can only take average cloud water concentrations as guidance from our experiments.

In addition, if we express the AA concentrations in molarity, the total amino acid in rain varied from 1.1 to 15.5 µM (Mopper and Zika, 1987), from 0.023 to 4.250 µM (Yan et al., 2015), from 1.1 to 10.1 µM (Xu et al., 2019), while in cloud it was from 2.7 to 3.1 µM (Bianco et al., 2016b). Considering that between 13 to 18 AA were measured in general, our total AA concentration in this experiment would be around 19 µM as we have included 19 AAs in the solution. This concentration is consistent with what was reported in rain samples, and about five times higher than the concentrations measured in clouds.

To take this factor of five into account we used an artificial cloud water whose composition was multiplied by 5 compared to what is observed in clouds (Vaïtilingom et al., 2011) and we also used a five-fold concentration for bacteria (Vaïtilingom et al., 2012). So we have respected the concentration ratio of chemical compounds [(main organic and inorganic ions + AA) / number of cells] present in cloud water. In the past we have shown that is the ratio is constant, the rate of biodegradation is constant (Vaïtilingom et al., 2010).

We shall modify the text in section 2.1 to explain and justify better our experimental conditions.

2.1 Experiments in microcosms

The experiments of biotic and abiotic transformation of amino acids were performed in microcosms mimicking cloud conditions at the puy de Dôme station (1465 m). Solar light was fitted to that measured directly under cloudy conditions and the temperature (17°C) was representative of the average temperature in the summer. Rhodococcus enclensis PDD-23b-28, Pseudomonas graminis PDD-13b-3, Pseudomonas syringae PDD-32b-74 and Sphingomonas sp.PDD-32b-11 bacterial strains were chosen because they belong to the most abundant and active bacterial genera in cloud water (Amato et al., 2017; Vaïtilingom et al., 2012). In addition, the complete genome sequences of Rhodococcus enclensis PDD-23b-28, Pseudomonas graminis PDD-13b-3, Pseudomonas syringae PDD-32b-74 have been published recently giving access to their metabolic pathways in more detail (Besaury et al., 2017a, 2017b; Lallement et al., 2017). ~~*Incubations were performed in an artificial cloud water medium containing inorganic ions, carboxylic acids and amino acids within the same range of concentrations as those measured in clouds that were impacted by marine air masses collected at the puy de Dôme station (Table S1, pH = 6.0) (Bianco et al., 2016a; Deguillaume et al., 2014).*~~ *In this work the total AA concentration used for the incubations was 19 µM as we have*

included 19 AAs at a concentration of 1µM each in the solution. This concentration is about five times higher than the concentrations measured in cloud water collected as the puy de Dôme station by Bianco et al. (2016a)(the total AA concentration varied from 2.7 to 3.1 µM). To take this factor of five into account we used an artificial cloud water whose composition in inorganic ions, carboxylic acids and amino acids was multiplied by 5 compared to what is observed in clouds (Vaïtilingom et al., 2011). We also used a 5X concentration for bacteria (~5×10^5 cells mL^{-1}) (Vaïtilingom et al., 2012). So we have respected the concentration ratio of chemical compounds [(main organic and inorganic ions + AA) / number of cells] present in cloud water. In the past we have shown that is the ratio is constant, the rate of biodegradation is constant (Vaïtilingom et al., 2010).

All experiments were performed in triplicates.

Referee Comment:

Your rate calculation is dependent on the concentration, thus the extremely high concentrations you used could have led to a conclusion not relevant to the field (the rate constant could also change depend on how the bacteria take up the substrate).

Authors' Response:

Generally, the rate calculations are only dependent on the bacteria cell and oxidant concentrations, respectively, cf equations 2 – 4. There are several studies that corroborate these cell concentrations in cloud water (Hu et al., 2018; Sattler et al., 2001; Vaïtilingom et al., 2013). Given that bacteria are very efficient cloud condensation nuclei (Zhang et al., 2020), the more abundant measurements of ambient particle concentrations (~10^3 – 10^5 cm^{-3}) can be used to infer similar cell concentrations in cloud water.

OH concentrations in cloud water have been indirectly determined by measurements (Arakaki et al., 2013; Bianco et al., 2015) and by numerous model studies (Ervens et al., 2003; Herrmann et al., 2010; Tilgner et al., 2013). While there are significantly fewer estimates available for singlet oxygen, the few available studies agree that it is about two orders of magnitude higher than OH (Faust and Allen, 1992)(Kaur and Anastasio, 2017). The ozone concentration can be calculated based on its Henry's law constant as cloud water's ionic strength is sufficiently low (< millimolar) to approximate it as an ideal solution. Thus, we are confident that the chemical rate constants and resulting rates are not influenced by the medium as it is also assumed in all cloud chemistry models.

In addition, when bacteria are just harvested from culture medium, in a way they have been pre-trained to take up labile organic matter rapidly, thus the biotic loss rate you obtained could have been overestimated.

Authors' Response:

Usually bacteria have to adapt their metabolism only to new substances which are typically xenobiotics (ex: Phenol) or to compounds which are not part of the central metabolism (ex:

formaldehyde, formate). On the contrary AA are essential substrates for bacteria and are metabolized in the central metabolism, they do not need to adapt their metabolism to these substrates. We have shown in the past that when we incubate real cloud samples, bacteria can grow in this medium showing they do use these substrates (Amato et al., 2007). In our opinion, the only important point is that these rates will depend on the type of bacteria, so on the biodiversity in cloud water that likely varies from one cloud to the other. These rates could also change according to different atmospheric scenarios. More work is indeed needed to have a clear overview of what happens in real clouds, notably biodegradation and photo-degradation rates should be measured with real cloud samples to evaluate the variability of these degradation rates. This is why we wrote in the conclusion *"Our study highlights the need for further mechanistic investigations of the biotic (metabolic) and abiotic (chemical) transformations of amino acids under conditions relevant for the atmospheric aqueous phases (clouds, fogs, aerosols)." (section 4., last paragraph).*

Referee Comment:

Bacteria in the cloud water, on the contrary, may not be that active often due to substrate limitation.

Authors' Response:

In cloud medium the concentration of AA is rather low but the concentration of bacteria is also low (10^5cells.L^{-1}), so in our opinion there is no substrate limitation. This assumption is supported by the following elements: i) in these experiments we have respected the ratio cells/ AA concentrations observed in clouds and have been able to measure rates of biotransformation, ii) we have proven that bacteria can use AA as substrates in incubations with real cloud water containing endogenous bacteria and AAs because they can produce proteins and other cellular component allowing their growth in this medium (Amato et al, ACP, 2007), iii) a recent metatranscriptomic study performed directly in cloud water, showed the presence of transcripts of genes coding for AA biodegradation and synthesis (Amato et al, Sci. Rep. 2019). This is a proof of *in situ* activity of cloud bacteria in clouds.

We shall modify the introduction as follows:

In cloud water, the biodegradation and biosynthesis of AAs is suspected to occur as i) it was shown that bacteria can use AA as substrates in incubations with real cloud water containing endogenous bacteria and AAs because they can produce proteins and other cellular component allowing their growth in this medium (Amato et al., 2007), iii) a recent metatranscriptomic study performed directly in cloud water, showed the presence of transcripts of genes coding for AA biodegradation and synthesis (Amato et al., 2019). This is a proof of in situ activity of bacteria in clouds. However, no data exist about the biotransformation rates ~~and metabolic pathways~~ of AAs in cloud water.

Referee Comment:

Similarly, the abiotic transformation rates would be different when you have an organic matrix present, like cloud water. As mentioned in the Introduction, organic matter in the cloud water is complicated, including many different compounds, which may include quenchers and photosensitizers, the rates you obtained may not represent those of field. I think all these need to be

factored in when you try to argue against previous studies, or apply these data to the field. I would like to see more discussion along these perspectives.

Authors' Response:

We respectfully disagree that cloud water represents an organic medium. The referee is right that it contains many organics with a very complex and variable composition but yet the main solvent is water with dissolved solutes at millimolar or even lower concentrations (e.g., depending on cloud droplet size). This is different in water associated with aerosol particles, i.e. outside of clouds, where indeed ionic strengths of several moles per Liter or more can be present and organic and aqueous phases may be separated due to different solute activities.

The oxidant concentrations used in our estimates are the steady state concentrations. Several studies reported a steady state singlet oxygen concentration in fog and cloud waters on the order of 10^{-14} – 10^{-12} M (Faust and Allen, 1992; Kaur and Anastasio, 2017), similar to concentrations found in surface water (Faust and Allen, 1992), and about two orders of magnitude higher than the OH radical which is considered the main oxidant in the atmospheric multiphase (gas + aqueous) system because of its high reactivity towards many organic and inorganic compounds. The lifetime of singlet oxygen is longer than that of the OH radical in water as it is more selective towards reactants (Kaur and Anastasio, 2017).

These steady-state concentrations are a result of the high production and loss rates of these oxidants (OH and $^1O_2$) from multiple pathways. Such pathways may include processes with quenchers or photosensitizers. However, given that these oxidant concentrations were determined in real cloud, fog and surface waters, the use of the resulting steady-state concentrations to estimate loss rates is common and justified.

The referee is right that there might be other loss processes for amino acids or other organics. However, there is no doubt in the atmospheric chemistry community that the OH radical is the most powerful and most important oxidant both in the gas (Seinfeld and Pandis, 2006) and aqueous phases (Ervens et al., 2003). The overall importance of ozone and singlet oxygen is lower as they are both more selective towards reactants. As they do react with amino acids, however, we consider their loss rates to our estimate in Figure 3.

Referee Comment:

Many places in the Introduction, there are too many references which kind of stops the flow. I would suggest you only choose the key ones.

Authors' Response:

It is rather difficult to choose key references as they are all essential to provide the scientific background for the manuscript's topic. Therefore, we prefer to keep all the cited references.

Minor referee comments

Page 3 line 28: "biotranform"? You meant: ": : :shown to biotransform: : :"

Page 7 line 25: no need to list all these amino acids here.

Page 8 line 3: should be “due to”

Page 8 line 7: should be “bacterial strain”

Page 8 line 9: I think “production” is a better word than “synthesis” here.

Page 9 line 26: delete “in the experiments”, redundant

Page 11 line 5: delete the “: : :”

Page 11 lines 10&17: the equations did not show up right. It might have something to do with the formatting.

Page 12 line 8: delete “It is obvious that”

Page 12 line 30: right, but as I mentioned before, your experimental conditions may not be that relevant, either.

Authors’ Response: Thank you for these corrections, they will be done in the final manuscript.

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