

A letter to reply the comments

Title: Optical properties and oxidative potential of aqueous-phase products from OH and $^3\text{C}^*$ -initiated photolysis of eugenol (acp-2021-895)

Dear Editor

We would like to very much thank you and the reviewers for the comments regarding our manuscript. These comments are important to help us to improve our work. We have carefully revised the manuscript accordingly. A thorough grammar and spelling check of the manuscript were also done. Our point-to-point responses to the reviewers' comments are listed below.

Reviewer #1

This reviewer enjoyed reading this manuscript, which presents a quite systematic investigation of the aqueous-phase photo-oxidation of eugenol. Indeed, these authors conducted a comprehensive analysis of the degradation kinetics of eugenol, along with the chemical-, optical-properties as well as toxicity (oxidative potential) of the products under direct photolysis, OH- and $^3\text{C}^*$ -initiated oxidation in the bulk aqueous-phase. The manuscript is well structured and each subsection comes with a thoughtful discussion. I would therefore recommend its publication subject to minor corrections.

Response:

1. One of the key messages here is that the $^3\text{C}^*$ -initiated oxidation dominates over the OH one. This is interesting and in line with other reports. However, it should be nice to state whether this is only valid for the actual laboratory conditions selected here, or if it can be also extrapolated to atmospheric conditions. In other words, how does the ratio of the $^3\text{C}^*$ to OH radical concentrations compare to realistic atmospheric conditions? Can the authors comment on that and therefore justify their choices of concentrations for the oxidant precursors?

Response: We thank very much the overall positive view on our manuscript. In our previous work (Atmospheric Environment 2020, 54, 117240) about aqueous photochemical reaction of phenolic compounds with hydroxyl radical under the same condition, we detected steady-state concentration of OH radical of about 6×10^{-12} M throughout the course of sunlight OH experiment, which was one order of magnitude higher than that in cloud environment, but close to that in wet aerosol microenvironment. Concentration of DMB as $^3\text{C}^*$ source was determined according to references (Environ. Sci. Technol. 2021, 55, 5199–5211; Phys. Chem. Chem. Phys., 2015, 17, 10227; Atmos. Chem. Phys., 16, 4511–4527, 2016).

2. The use of acronyms for the selected compounds is justified but it does not ease the reading of the manuscript. It would be nice to state the actual of the compounds when firstly mentioned in the result section (and not just in the experimental one).

Response: Thanks for your attention. We have paid attention this issue and revised in this new manuscript.

3. While this manuscript reads well, a final polishing of the English would improve it further (but this is a very minor comment).

Response: In addition, we have made major revisions especially added more discussion for this manuscripts to make it easy to follow. We hope the new version meets similarity requirement.

Reviewer #2

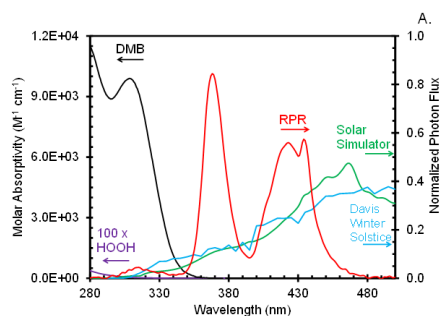
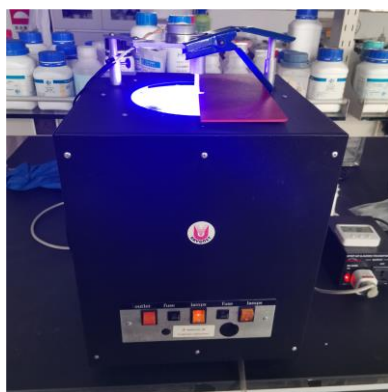
In this study the authors investigated the degradation of eugenol by three ways i.e. photolysis, excited triplet states, and OH radicals. The obtained results suggest that the excited triplet states are most reactive toward eugenol followed by OH radical induced degradation and photodegradation. The fluorescent spectra indicated formation of HULIS. The use of dithiothreitol indicated formation of harmful species during the oxidation process. I think that this topic could be interesting for the readers of ACP and the manuscript should be reconsidered upon major revision. I have some comments that I hope can be helpful for the authors to improve the quality of the manuscript. My main comments are mostly related to the experimental details.

Response: We thank very much the overall positive view of the reviewer on my manuscript. We appreciate very much the useful comments raised by the reviewer below and we have tried our best to address them point-by-point as appended below.

Comments:

1. The authors used mercury lamps with discontinuous emission spectrum at 313 nm, 365nm, 419nm, and 436 nm to irradiate the aqueous solution consisting of eugenol, which can potentially lead to misleading conclusions. For this kind of experiments, it is more appropriate to use Xenon lamp (solar simulator) with continuous emission spectrum from 300 to 700 nm. It will be useful in Figure S1 to compare the spectral irradiance from the mercury lamp with the sunlight spectral irradiance. Figure 5a: How is it caused the photolysis of eugenol that absorbs light at 280 nm with mercury lamp irradiating at 313, 365, 419 and 465 nm?

Response: We are very appreciated for your suggestion. Actually, Rayonet photoreactor (model RPR-200) in this work (See photo below) was frequently used for photochemical reaction and described in detail by several groups (George et al., 2015; Hong et al., 2015; Huang et al., 2018; Jiang et al., 2021; Zhao et al., 2014) to mimic sunlight. The photoreactor and light sources were the same as above mentioned. The normalized distribution of the photon fluxes inside reactor have been reported elsewhere (George et al., 2015, see below Figure). According to their description, the wavelength of photon fluxes was over 280 and 500 nm range. In order to avoid mistaking, we deleted Fig. S1.



Normalized distribution of the photon fluxes inside our RPR-200 illumination system, and, for comparison, the fluxes from our solar simulator and for midday, winter solstice, actinic flux in Davis, CA (George et al., 2015, Atmos. Environ., 100, 230-237)

2. The changes of pH values and the levels of dissolved oxygen before and after the reactions in the aqueous phase are not reported.

Response: We have supplement Section 3.2.4 “Variation of pH value and dissolved oxygen (DO)” in the revised manuscript. In this section, we monitored pH value and DO concentration upon photolysis process, which were shown in Fig.4 and Fig. S5. Additionally, we analyzed the results.

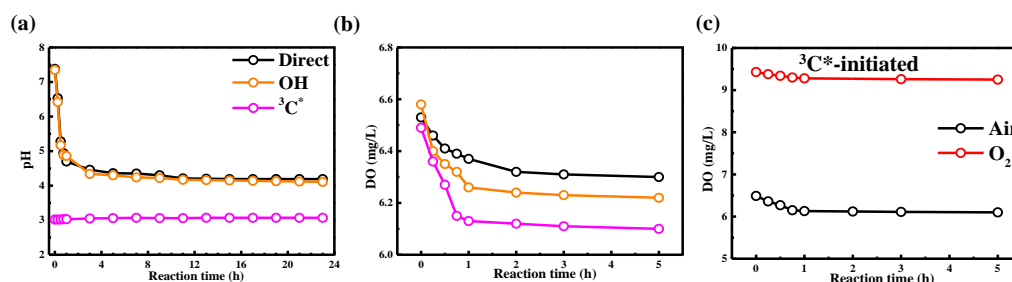


Figure 4. pH values and dissolve oxygen as a function of reaction time for the three systems.

3. Line 88: it should state “several” instead of “sever”. Figure S3: On X axis should be “magnetic field” instead of “magntic field”. Figure 3: It is not correct to state “under OH system”. Please revise it.

Response: Done.

4. Line 251: Which chemical bond?

Response: We have reorganized Section 3.1 “Kinetics of the photo-oxidation” and embellish language in order to express clearly. Chemical bond has been replaced by bond dissociation energies (BDE). So, this sentence “The energies of photons at 313, and 365 nm are 395 kJ/mol, and 338 kJ/mol, which are higher than certain chemical bond energies, for instance, 354 kJ/mol for C-C, and...” has been deleted.

5. Line 255: In a real world environment the photon energy of the sunlight does not have two peaks at 313 nm and 365 nm.

Response: We totally agree with you. Rayonet photoreactor (model RPR-200) in this work was frequently used for photochemical reaction and described in detail by several groups. According to their description, the wavelength of photon fluxes was over 280 and 500 nm range. So, the spectra exhibited a broader range of wavelength. We inferred our spectra determination in the original manuscript has not been corrected.

6. Lines 375-376: To state that the “role of OH is weak” is not appropriate term here. Moreover, the statement is somewhat misleading as the continuous emission spectrum would probably induce different effect.

Response: Thanks. This sentence has been optimized to “As seen in Fig. 5, when adding oxidant H₂O₂, the total variation trend of light absorbance was similar to that without oxidant with some slight difference, for instance at wavelength range of 200-250 nm.”

7. Line 389: “the formation of “brown carbon”. Did authors observed change of the colour of the solution? Did really becomes brownish?

Response: Thanks. We did not observed obvious change of colour of solution. Thus, This sentence has been changed to “The difference plot of UV-Vis spectra between OH and ³C*-initiated photo-oxidation indicated the formation of different products.” to make sense. Furthermore, we added some description in the last paragraph “The increase of light absorbance at 250 nm upon aqueous photo-processing demonstrates the generation of new substances with both the aromatic C=C and carbonyl (C=O) functional groups, while the enhancement at 300-400 nm suggested the probability of HULIS formation, which could be confirmed later.”

8. Please change the title of section 2.3.5. “Products analysis of products” is not right.

Response: Done.

9. Lines 421-422: Please rewrite the sentence starting with “This is likely due.....”. It is not clear at all the meaning of this sentence.

Response: Yes. This sentence has been changed to “This could be attributed to different precursor and aqueous reaction mechanisms (Xie et al. 2016).”

10. My other comments are related to the references. The authors used many self-citations and at first glance the paper looks like that previously not so many groups studied the reactions of phenols and methoxyphenols in the aqueous phase. For example, lines 57-61: Many recent papers related to the photodegradation of phenolic substances are not cited here.

Response: Thanks for your suggestion. We referred to more articles (Gilardoni et al., 2016; He et al., 2019; Jiang et al., 2021; Li et al., 2014; Li et al., 2021; Mabato et al., 2022; Tang et al., 2020; Yang et al., 2021; Yu et al., 2016) published recently and related to reactions of phenolic carbonyl compounds in aqueous-phase.

11. Lines 66-67: There are other more appropriate references related to OH radical concentrations in the atmospheric aqueous phase. See for example: Chem. Rev. 2015, 115, 24, 13051–13092; Chem. Rev. 2003, 103, 4691-4716.

Response: Thanks. We have cited suggested representative paper with regard to OH radical concentration in the atmospheric aqueous phase.

12. Line 93: In which sense these compounds can damage human body? Also reference is here needed about the health implications of quinones and PAHs.

Response: Thanks for your suggestion. We have **rewritten “Section 1: Introduction”**, for example: Earlier report from Chang and Thompson (2010) found fluorescence spectra of reaction products during aqueous reaction of phenolic compounds, with some similarities with aerosol Humic-like substances (HULIS); Tang et al.(2020) also observed light-absorbing products formed in aqueous-phase OH oxidation of vanillic acid and further verified that aqueous reaction was a potential source of HULIS. Recently, Li et al (2021) began to apply EEM technique to characterize formation of light-absorbing compounds in aqueous phase oxidation of syringic acid. Additionally, previous studies (Chang and Thompson, 2010) showed that light-absorbing and fluorescent substances generally have large conjugated moieties (i.e., quinones, HULIS, polycyclic aromatic hydrocarbons (PAHs)), which can damage human body (McWhinney et al., 2013; Dou et al., 2015). HULIS are considered as an important contributor to induce oxidative stress since they can served as electron carriers to catalyze ROS formation. Dithiothreitol (DTT) assay (Alam et al., 2013; Chen et al., 2021;Verma et al., 2015), as a non-cellular method, was widely employed to determine oxidation activity and assess oxidative potential of atmospheric PM via the rate of DTT consumption (Cho et al., 2005; Chen et al., 2019), since oxidative stress was related to adverse health effect. You can see we cited many references. Sentence **“HULIS are considered as an important contributor to induce oxidative stress since they can served as electron carriers to catalyze ROS formation”** can indicate mechanism of damaging human body by HULIS.

13. Lines 251-255: Please give the reference for the reported bond dissociation energies (BDE) which is by the way more appropriate term than chemical bonds energies.

Response: In Section 3.1, we added some description about BDE in detail: The BDEs are 340 kJ/mol for OH, 374 kJ/mol for C-H in -CH₃ group, 345 kJ/mol for C-C in C=C bonds, and 403 kJ/mol for C-H in -OCH₃ group, respectively (Herrmann et al., 2003;He et al., 2019). The lowest BDE was found for the O-H bond and C-C bond. Due to the influence of steric hindrance and intramolecular hydrogen bonding, the H-abstraction reaction from the OH group might have been less favorable. The most favorable H-abstraction reaction might have taken place in the C-C in allyl group. As a result of breakage of C=C into C-C at allyl group site, 2-methoxy-4-propyl-phenol could form (See Section 3.6.1). When photon energy is higher than bond dissociation energy, they can directly break chemical bond of molecules, leading to decomposition of compounds and possibly further mineralization. The energies of photons at 313, and 365 nm in our

light sources are 395 kJ/mol, and 338 kJ/mol, which are higher than the weakest BDEs in eugenol, as a result, eugenol molecule can directly absorb photo energy to decompose.

14. Lines 406-409: There are many previous studies that have shown the formation of fluorescent compounds associated with HULIS at wavelengths 400-500nm. For example: <http://dx.doi.org/10.1016/j.atmosenv.2013.09.036>, [doi.org/10.1016/j.atmosenv.2019.03.005](http://dx.doi.org/10.1016/j.atmosenv.2019.03.005))

Response: Thanks. We have cited references (Laurentiis et al., 2013; Vione et al., 2019; Wu et al., 2021) to illustrate the formation of fluorescent of HULIS at 400-500nm.

Reviewer #3

The manuscript presents an interesting investigation on the aqueous phase photooxidation of eugenol, and its impacts on light-absorption and oxidative potential. However, the entire manuscript is presented as a report, very far from the standard requests of a scientific publication. It lacks organization and a logical follow up : instead of clearly providing UV-vis spectra of eugenol and comparing it to the solar actinic fluxes and to their lamp's actinic fluxes to show the potential importance of the photolysis reaction, the authors directly start with a complex comparison between the kinetics of photolysis vs OH-oxidation vs reactivity towards $^3\text{C}^*$, with no appropriate discussion (see below).

Response: We have reorganized the whole paper. We have made major revisions especially add more discussion for this manuscripts to make it easy to follow.

1.The use of SI is not appropriate: the most interesting figures are provided in SI, and redundant figures are provided in the text with less information (see for example Figure 2 compared to Figure S2)

Response: Thanks for your suggestion. We removed some interesting figures from supplementary materials to the text (i.e., Figure 2 and Figure 3). We added Table 1 to make rate constant under different quenchers more clear. Furthermore, some figures with less important information were placed in Supplementary material. Additionally, we added e changes of pH values and the levels of dissolved oxygen with reaction time.

Table 1. The reaction rate constants of eugenol in the presence of scavengers. The experimental conditions were as follows: 0.3 mM eugenol, molar ratios of eugenol to quencher TBA, NaN_3 , TMP and SOD, of 1.5, 0.15, 0.075 and 2.5 respectively; mole ratio of eugenol to quencher *p*-BQ and TBA of 0.8 and 0.75 respectively.

$^3\text{C}^*$ -initiated quenching			
quenchers	ROS	reaction rate constant k (s^{-1})	R^2
no quencher	-	5.75×10^{-4}	0.996
TBA	OH	2.65×10^{-4}	0.999
SOD	O_2^-	2.22×10^{-4}	0.995
NaN_3	$^1\text{O}_2$	1.12×10^{-4}	0.999
TMP	$^3\text{C}^*$	0.82×10^{-4}	0.999
$\bullet\text{OH}$ -initiated quenching			
quenchers	ROS	reaction rate constant k (s^{-1})	R^2
No quencher	-	2.73×10^{-4}	0.995
TBA	OH	2.22×10^{-4}	0.998
<i>p</i> -BQ	O_2^-	1.20×10^{-4}	0.995

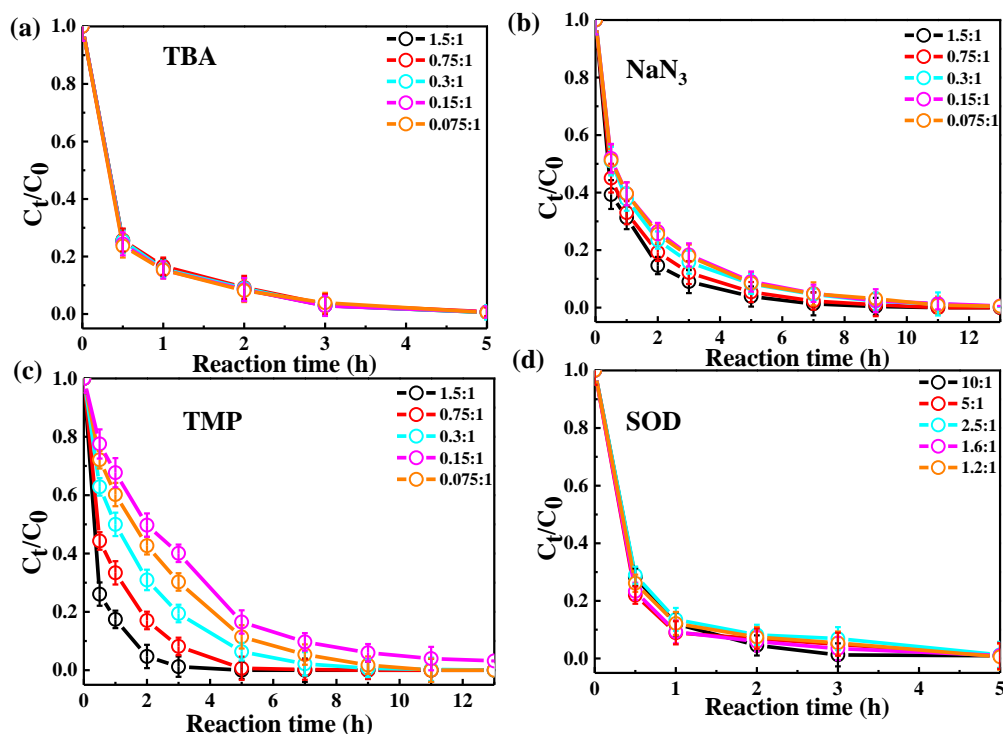


Figure 2. Ratio of residue concentration to initial concentration (C_t/C_0) at different mole ratios as a function of reaction time with (a) TBA quencher, (b) NaN_3 quencher, (c) TMP quencher and (d) SOD quencher. Legend represented mole ratios of eugenol to quenchers.

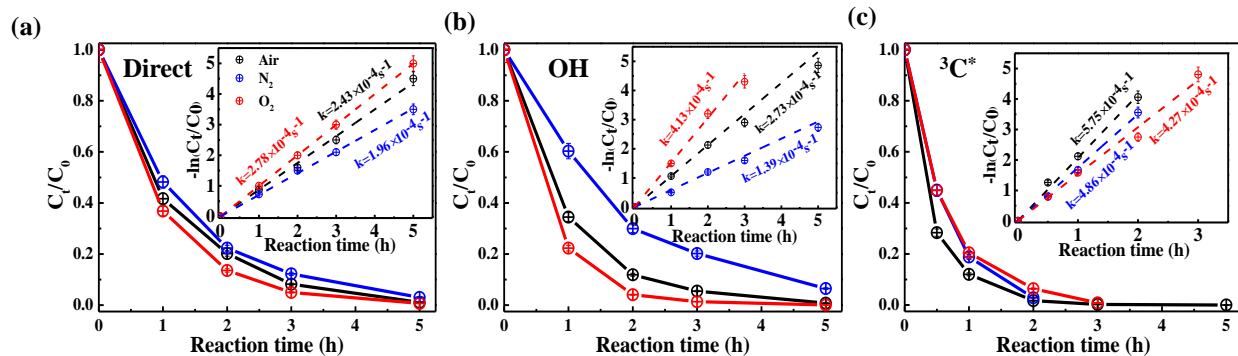


Figure 3. Ratio of remaining concentration to initial concentration (C_t/C_0) as a function of reaction time at different saturated gases under (a) direct photolysis (b) OH-initiated and (c) $^3\text{C}^*$ -initiated oxidation. Insert plots: Plots of eugenol consumption versus reaction time under different saturated gases: (a) direct photolysis (b) OH-initiated and (c) $^3\text{C}^*$ -initiated systems.

2. The authors provide a systematic and very convincing study of quenching reactions during the photolysis of eugenol, but they forget to discuss on their analytical uncertainties when comparing the influence of the various quenchers. This is particularly critical for the discussions on Fig S4 and S5a.

Response: We agree with you. Firstly, we have rewritten **Section 3.2** “Relative importance of ROS to photo-oxidation” and added more detailed description. For each

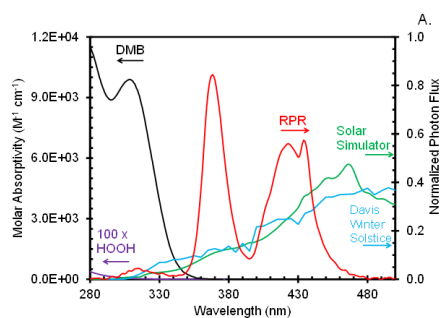
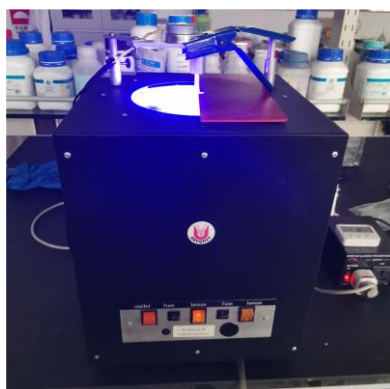
scavenger, we conducted several gradient experiments with varying molar ratios of eugenol to quenchers. Taking C3-initiated reaction for example. The ratios were set as 0.075:1, 0.15:1, 0.3:1, 0.75:1, 1.5:1 for quenchers of NaN₃, TMP and TBA, and 1.2:1, 1.6:1, 2.5:1, 5:1, 10:1 for SOD, which were all within the typical range of molar ratios to quench ROS reported previously (Zhou et al., 2018). Above concentrations of the added quencher have been repeatedly adjusted to ensure the complete reactions between radicals and scavengers. The optimum molar ratios of eugenol to quenchers were selected when the inhibition degree of eugenol degradation unchanged with the increase of added quencher mass (Wang et al., 2021). For example, upon decreasing molar ratios of eugenol to NaN₃ from 1.5:1 to 0.075:1, the inhibitory degree of eugenol degradation was unchanged at ratio of 0.15:1 and 0.075:1, indicating that ¹O₂ has been absolutely quenched at ratio of 0.15:1, so, we finally selected molar ratios of 0.15:1 for NaN₃, since excess scavenger may produce other products that can change the existing reaction. Finally, the molar ratios of eugenol to quencher TBA, NaN₃, TMP and SOD, of 1.5, 0.15, 0.075 and 2.5, were selected, respectively. So, the uncertainties when comparing the influence of the various quenchers can be reduced. Additionally, for most series of experiments, solution was saturated by air and all experiments presented were conducted in triplicate unless otherwise stated. The results were shown in respect of average plus/minus standard deviation. Moreover, in Section 2.3.4, we added “Experiments of blanks and samples were typically run in a triplicate. The reproducibility of the whole analysis showed that the relative standard deviation of the DTT consumption rate analysis was 3-4%.”

3. Too many results are presented with no clear links, and sometimes with no appropriate discussion, but only in reference to other papers, mostly from their own group. For example, the comparison between the kinetics of photolysis vs OH-oxidation vs reactivity towards ³C* where no discussion is performed on the amount of OH and ³C* precursors, neither the lamp's actinic flux.

Response: We have cited many references to make some conclusion sense. For example, we cited (Gilardoni et al., 2016; He et al., 2019; Jiang et al., 2021; Li et al., 2014; Li et al., 2021; Mabato et al., 2022; Tang et al., 2020; Yang et al., 2021; Yu et al., 2016) published recently, related to reactions of phenolic carbonyl compounds in aqueous-phase; References (Laurentiis et al., 2013; Vione et al., 2019; Wu et al., 2021) were cited to indicate EEM spectra at Em of 400-500nm; The amounts of radicals can be identified and quantified by the peak patterns in EPR spectra, such as quarter line with a height ratio of 1:2:2:1 for DMPO-•OH, 1:1:1:1 for DMPO-O₂⁻ and 1:1:1 for TEMP-¹O₂ (Guo et al., 2021);

Actually, Rayonet photoreactor (model RPR-200) in this work (See photo below) was frequently used for photochemical reaction and described in detail by several groups (George et al., 2015; Hong et al., 2015; Huang et al., 2018; Jiang et al., 2021;

Zhao et al., 2014) to mimic sunlight. The photoreactor and light sources were the same as above mentioned. The normalized distribution of the photon fluxes inside reactor have been reported elsewhere (George et al., 2015, see below Figure). According to their description, the wavelength of photon fluxes was over 280 and 500 nm range. In order to avoid mistaking, we deleted Fig. S1.



Normalized distribution of the photon fluxes inside our RPR-200 illumination system, and, for comparison, the fluxes from our solar simulator and for midday, winter solstice, actinic flux in Davis, CA (George et al., 2015, Atmos. Environ., 100, 230-237)

4. Many small errors can be mentioned. See for example confusion between water solubility and water miscibility, confusion between a radical and triplet state species, ... There are too many English errors and typos;

Response: Sorry for our carelessness. In revised manuscript, we have corrected typographical errors, grammatical inaccuracies, misspelling, and so on, we hope the new version meets the journal's requirement.