



Supplement of

Lignin's ability to nucleate ice via immersion freezing and its stability towards physicochemical treatments and atmospheric processing

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Figure S1: IC calibration curves. Linear fits have R² > 0.99 for all four analysed small low-molecular weight organic acids.

Section S2. Freezing of background water filtered with PTFE and cellulose acetate filters



Figure S2: Freezing temperatures determined by FINC with background water after filtration with PTFE membrane (BGB®, USA) and cellulose acetate filters (VWRTM, USA). The use of these filters for lignin size filtration was discontinued as their background IN activity was the same as lignin's IN activity at 20 mg C L⁻¹. The high background of these filters is likely due to abraded IN active filter material or insufficient rinsing before use.



Figure S3: Spectral irradiance as a function of wavelength of the photochemical setup using eight 300 nm UVB light bulbs. The spectrum is measured by a portable UV/Vis spectrophotometer (Ocean Optics) and calibrated with actinometry experiments (see Sections S3.1 and S3.2).



35 Figure S4: Logarithmic plot of concentration decay with time of actinometer *para*-nitroanisole (PNA) quantified by HPLC. The linear regression fit was calculated for triplicate experiments and each regression has a $R^2 > 0.99$. The slope of each fit provides one pseudo first-order degradation constant $k_{deg,PNA}$. The average value of all three experiments with one standard deviation was $k_{deg,PNA} = -0.568 \pm 0.004 h^{-1}$.

S3.1 Calculation of absolute spectral irradiance

The light intensity of the photochemical setup using eight 300 nm light bulbs was used to calculate the absolute spectral irradiance $I_{\lambda,m}$. I_{λ} is scaled (factor s) from the known spectral irradiance $I_{\lambda,m}$, which was previously measured in the photoreactor with a calibrated Jaz spectrophotometer (Ocean Optics) using two 300 nm UVB bulbs and was corrected for the absorption of the borosilicate test tubes between 280-300 nm (Borduas-Dedekind et al., 2019). The scaling factor s is calculated with Eq. S1.

Eq. S1:
$$\mathbf{s} = \frac{k_{deg,PNA} * [PNA]_0 * l}{\phi_{deg,PNA} * \sum_{\lambda} (I_{\lambda,m} * f_{\lambda,PNA}) \Delta \lambda}$$

- 50 In Eq.S1, [PNA]₀= (23.5 ± 0.18) μ M is the average PNA concentration at timepoint 0 h from 3 experiments and l = 1.14 cm is the light pathlength of the borosilicate test tube. $\Phi_{deg,PNA}$ = 3.63 x 10⁻⁴ is the direct photolysis quantum yield which was calculated following Laszakovits et al., 2016. $f_{\lambda,PNA}$ is the fraction of light absorbed by the starting PNA solution and was calculated with Eq.S2, where $\varepsilon_{\lambda,PNA}$ is the molar extinction coefficient of PNA. $I_{\lambda,m}$ and $f_{\lambda,PNA}$ are summed up from 280 nm to 400 nm in steps of $\Delta\lambda$ = 1 nm.
- 55 Eq. S2: $f_{\lambda,PNA} = 1 10^{-\epsilon_{\lambda,PNA} + [PNA]_0 + l}$

With a scaling factor of s = 375.89, this calculation resulted in an absolute spectral irradiance I_{λ} = (109.94 ± 0.85) W m⁻².

S3.2 Calculation of equivalent sunlight conditions

60 To compare the experimental conditions to environmental sunlight conditions, a conversion factor c_{sun} from the irradiation time in the photoreactor into the equivalent irradiation time in natural sunlight is introduced. c_{sun} is the ratio of the rate of light absorption in lignin solutions irradiated by I_{λ} ($R_{abs}^{photoreactor}$, Eq.S3) over the equivalent rate by irradiation with $I_{\lambda,sun}$ (R_{abs}^{sun}), the simulated solar irradiance (Apell and McNeill, 2019).

65 Eq. S3:
$$R_{abs}^{photoreactor} = \frac{1}{l} \sum_{\lambda} I_{\lambda} (1 - 10^{-A_{\lambda, \text{lignin}}})$$

In Eq. S3, $A_{\lambda, \text{ lignin}}$ is the absorbance of a 20 mg C L⁻¹ lignin solution at 0 h of UVB irradiation. For R_{abs}^{sun} , I_{λ} in Eq. S3 is replaced by $I_{\lambda,sun}$, which was simulated as in Borduas-Dedekind et al., 2019, giving an integrated irradiance of 59.2 J s⁻¹ m⁻² between 280 nm and 400 nm. Using the resulting conversion rate of $c_{sun} = 3.14$, 25 h in the photoreactor equipped with eight UVB light bulbs is equivalent to an irradiation time of 78.6 h of surface to a short day.

time of 78.6 h of sunlight, or 6.5 days in the environment. This conversion assumes 12 h of sunlight on a clear day.

Section S4. Details FINC - Ice Nucleation Setup



Figure S5: Ice Nucleation Setup (a) FINC instrument; (b) Picture of piko PCR travs taken by the FINC camera during an ongoing sample run showing the change in contrast between liquid (light) and frozen state (dark) of each well.

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S4.1 FINC data processing

FINC's raw data are the pictures taken during cooling and the corresponding recorded Lauda bath temperature. These images are processed with automated picture analysis in MATLAB®, first developed by David et al. (2019). First, each well location is detected and then a freezing event is assigned to each well at the time the greatest change in light intensity occurred between 80 two pictures (details in Miller et al., 2020). A vector containing the freezing temperatures of all 288 wells is the main output. These freezing temperatures are then calibrated with a temperature calibration (Eq. S4) to account for the difference between the Lauda recorded bath temperature (T_{bath}) and the temperature within each well (T_{well}). The temperature calibration was conducted with a 12-channel thermo-couple logger (Lutron Electronic, BTM-4208SD) before starting the freezing experiments for this study and validated again after the freezing experiments were completed (Miller et al., 2020). The reported freezing temperatures in the manuscript are all calibrated with Eq. S4 and shown as Twell.

Eq. S4: $T_{well} = 0.75 + 0.95 * T_{bath}$

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S4.2 FINC limit of detection determination

The background of FINC was determined by averaging 10 consolidated freezing curves of Sigma Aldrich molecular biology water (background water), used as a pure background water standard (Figure S6). One standard deviation at each 1/288 step

95 describes the uncertainty in FINC's limit of detection. On average, the lower temperature limit of FINC is at -23.40 ± 0.58 °C when 50% wells are frozen for a well volume of 20 µL (Miller et al., 2020).



Figure S6: FINC background determination results based on averaged background water (bg water average). 10 freezing curves of bg water are shown in blue color as stepwise function, titled after the measurement date. One standard deviation of the average represents the uncertainty of the limit of detection and is shown in grey shading.

S4.3 Reproducibility within FINC of 20 mg C L⁻¹ lignin solutions



Figure S7: FF curves for seven FINC experiments with 20 mg C L⁻¹ untreated lignin solutions from the same bulk solution conducted over a 2-month period. The T_{50} is -20.8 ± 0.2 °C showing the stability of the IN activity in the lignin bulk solution and the reproducibility within FINC experiments.

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Section S5. TOC analysis

To record changes in the carbon content of lignin sample solutions after treatments and atmospheric processing, we attempted to quantify its total organic carbon content, specifically the non-purgeable organic carbon (NPOC). The measurement was done using a total organic carbon (TOC) analyser (Shimadzu, model TOC-L CSH). The instrument's method was set to 50 µL

- 110 injections up to 4 times per sample, the sparge time was 90 sec and the sparge gas flow was 80 mL min⁻¹. The results are reported as concentrations in mg C L⁻¹ with standard deviation and calibrated using a linear calibration curve from recrystallized potassium phthalate that ranged from 5 to 200 mg C L⁻¹ ($R^2 = 0.9997$).
- In preliminary measurement runs with freshly prepared aqueous lignin solutions, we noticed that the instrument's platinum oxidation catalyst for the combustion process did not efficiently nor reproducibly combust lignin. The quantified NPOC values from the TOC analyzer was only ~ 60 % of the expected NPOC value of 20 mg C L⁻¹, based on the weighed solid lignin and on the manufacturer's carbon content analysis. For lignin solutions of 200 mg C L⁻¹, the TOC analysis reported values of 5 mg C L⁻¹ for all samples, indicating an even worse conversion in the combustion process within the TOC instrument. In another attempt, we pre-treated the lignin solutions with 0.5 mL of 35-w% of H₂O₂ as an oxidant to help decompose lignin and consequently help the TOC combustion process. However, this addition did not have an effect on the quantification. Lastly, we added an acid digestion pre-treatment. In this pre-treatment, 1 mL of pure nitric acid and 0.5 mL of 25% sulphuric acid (Sigma Aldrich) were added to 5.5 mL of sample solution prior to the carbon content quantification on the TOC analyser. This method was indeed able to help decompose lignin and the recovery rates were increased substantially. However, the measured
- values still showed variability with a suspected NPOC contribution from the acids themselves. Consequently, open questions remain on the decomposition of lignin in a TOC analyser which is why we chose to report the mass of lignin weighed.

Currently, we hypothesize that this TOC analyzer's setup is not yet apt to reliably analyze lignin due to its robust and recalcitrant polymeric chemical structure. Thus, the acid digestion method optimization attempts still only provide preliminary results. We were not able to thoroughly track changes in the carbon content of the lignin solutions and rely instead on the

130 carbon content as specified from Sigma-Aldrich. However, we show in Figure S8 (and in Figure 4c) that the ice-active mass site density (n_m) of the dilution series is clearly significant. Even a hypothetical maximum variability of 50 % in organic carbon content is not large enough to affect the data interpretation. This result solidifies that the n_m values of the dilution series are statistically significant from one another.



Figure S8 Ice-active mass site density n_m as a function of temperature of the aqueous dilution series of lignin. At the T_{10} , T_{50} , T_{90} values of each experiment, the uncertainty is included representing the freezing temperature x-axis uncertainty of 0.5 °C and the n_m uncertainty based on a 50 % variation in carbon content. For comparison, a fit for biogenic particles in sea-spray aerosols from Wilson et al. (2015), a lignin parametrization based on 20 mg C L⁻¹ solutions from Miller et al. (2020), where n_m =exp(-0.558*T-3.12), and n_m values from dissolved organic matter (Borduas-Dedekind et al., 2019) are included.

Section S6. Effect of sonication on lignin's IN activity: Freezing temperature boxplot



140 Figure S9: Freezing temperature boxplots after sonication of aqueous lignin solutions as physicochemical treatment. *Bg* refers to background water. Sonication did not introduce a distinct change on lignin's IN activity.

Section S7. Effect of H₂O₂ on lignin's IN activity: Freezing temperature boxplots and UV/Vis absorption spectra

In the reaction series of lignin with hydrogen peroxide (H_2O_2) no change in IN activity was observed up to the ratio of 1 g lignin : 100 mL H_2O_2 . At higher concentrations of H_2O_2 , an odd freezing point depression governed the decrease of the freezing

- 145 temperatures down to the T₅₀ value of the H₂O₂ background at 27.9 °C (Figure S11). This H₂O₂ background was prepared in the same manner as a lignin solution sample in the ratio 1 g lignin : 750 mL H₂O₂ but without adding any lignin. Notably, the determined T₅₀ value of this H₂O₂ background falls 4.1 °C below the background water's T₅₀ value. Increasing the H₂O₂ ratio in the background to 1 g lignin : 1000 mL H₂O₂ further enhanced the depression, so much so that the lower temperature limit in FINC was reached (- 36 °C) before complete freezing of all wells was observed. Similar incomplete freezing of the 288
- 150 wells was also observed in the 1 g lignin : 1000 mL H₂O₂ experiment.

The lower background of the H_2O_2 control complicates the interpretation of lignin's IN activity at ratios higher than 1 g lignin : 100 mL H_2O_2 . It is difficult to deconvolute a possible freezing point depression with reaction with H_2O_2 . Indeed, lowering the lignin concentration would lower the IN activity (see Sect. 3.1.1), but tracking this possible decrease in concentration was unsuccessful



155 unsuccessful.

Figure 10: Freezing temperature boxplots for the control reaction series with H₂O₂ with prolonged reaction time to 4 days. The additional 3 days of reaction time did not introduce any change in the freezing temperatures. The log x-axis displays the reaction ratio of 1 g lignin to x mL of H₂O₂ (35-w%) before dilution with background water to 200 mg C L⁻¹. *Bg water* refers to background water. *Bg lignin* refers to a measurement of a 200 mg C L⁻¹ lignin solution without H₂O₂. *Bg H₂O₂* is a background control prepared like a lignin sample in the ratio 1 g lignin : 750 mL H₂O₂ but without actual addition of lignin.





Figure S11: Freezing temperature boxplots for the reaction series with H₂O₂. The log x-axis displays the reaction ratio of 1 g lignin to x mL H₂O₂ (35-wt%) before dilution with background water to 200 mg C L⁻¹. *Bg lignin* refers to a measurement of a 200 mg C L⁻¹ lignin solution without H₂O₂. *Bg* H₂O₂ is a background control prepared like a lignin sample in the ratio 1 g lignin : 750 mL H₂O₂ but without any lignin. *Bg water* refers to background water.

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Figure S12: Absorption spectra for reaction series with H_2O_2 illustrating the increasingly overlaying signal of H_2O_2 over lignin. The reaction ratio is given in the legend as 1 g lignin : 5-1000 mL H_2O_2 . The dashed lines of *bg lignin* and *bg H_2O_2* refer to background measurements of only lignin concentrated 200 mg C L⁻¹ in background water and only H_2O_2 prepared like a lignin sample in the ratio 1 g lignin : 750 mL H_2O_2 but without addition of lignin.

Section S8. Effects of atmospheric processing on lignin's IN activity: Freezing temperature boxplots



Figure S13: Freezing temperature boxplots of 20 mg C L^{-1} lignin solutions after exposure to UVB irradiation as atmospheric processing experiment. Includes two 25h-experiments equivalent to 6.5 days in the environment. After 25 h UVB irradiation, the change in T₅₀ value was less than -1 °C.



Figure S14: Freezing temperature boxplots of 20 mg C L⁻¹ lignin solutions after exposure to ozone in an atmospheric processing experiment. (a) Background control experiments were conducted to ensure stable background conditions: In *Bg water setup*, the bg water was collected after flushing through the glassware in the ozonation setup. *Bg water air* and *lignin air* refer to controls where filtered air was bubbled through the solutions for 30 min, but the ozone generator stayed turned off. (b) Freezing temperature boxplots for ozonation series with 20 mg C L⁻¹ lignin bulk solution.



190 Figure S15: Absorption spectra for 20 mg C L⁻¹ lignin solutions after atmospheric processing by UVB irradiation. With increasing exposure, lignin's absorbance decreases.



Figure S16: Formation of low molecular weight organic acids from lignin upon (a.) UVB irradiation and (b.) exposure to ozone (O₃). Pyruvic acid was below the detection limit in all samples, acetic acid was detected in all samples but the samples from exposure to 100 ppb O₃. Note that in (b.) different time points were taken for different experiments.

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