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The Labile Nature of Ice Nucleation by Arizona Test Dust

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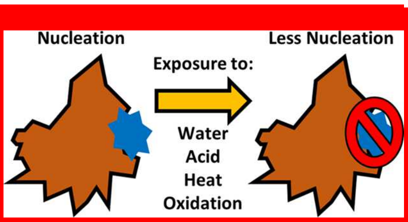
Dust aerosol is an important atmospheric component due to its ice nucleating ability, which affects cloud phase and precipitation. Arizona Test Dust (ATD) is often used as a proxy for environmental dust aerosol in laboratory studies. In this work, we examine how the ice nucleating behavior of ATD is altered with different solution phase and dry aging processes under several conditions. We find that ATD ice nucleating ability is degraded in significant ways under most conditions, including aging for less than a day in deionized water or NaCl solution. Additional studies are detailed for processing in acidic solutions, salt solutions, peroxide treatment, heat treatment, and protease enzyme treatment. We find evidence of warm-temperature ice nucleating particles that share the analytically characteristic attributes of organic ice nucleators but are heat stable beyond the normal range for organic materials. We hypothesize that this is due to binding and stabilization of organic materials at the mineral surface.



ice nucleation, atmospheric aerosol, aerosol processing, cloud physics, dust, soil

INTRODUCTION

The state in which a cloud exists in the atmosphere, as liquid or frozen droplets, is important to both weather and climate due to its impacts upon precipitation and radiative forcing.[1] Small, nearly pure cloud droplets can supercool to around −38 °C in the atmosphere, depending on droplet size, but the degree of supercooling can be reduced by various materials, known as ice nucleating particles (INPs).[2] One very important atmospheric source of INPs is lofted mineral dust, which is often the dominant source of INPs near terrestrial environments.[3−5] Laboratory experiments often use Arizona Test Dust (ATD) as a proxy for diverse natural dust samples due to its known distribution of particle sizes, consistent composition, and convenient availability. In this work, we examine the sensitivity of ATD ice nucleation (IN) activity to a variety of conditions mimicking experimental or atmospheric processing.

Heterogeneous ice nucleation generally has two main distinct modes of action (although further classifications exist): immersion freezing of condensed water and deposition nucleation.[6,7] At a lower relative humidity and low temperatures, deposition mode ice nucleation is possible. In this mode, water vapor is thought to condense and achieve critical embryonic sizes on the IN active sites directly as ice, although the possibility that deposition nucleation is actually freezing of water accumulating in the pores[8] of solid materials via adsorption and capillary condensation has also been tendered. At a higher relative humidity, immersion mode freezing dominates (over both deposition and over collision of insoluble particles with cloud droplets, referred to as contact freezing) and occurs where water is already present in mixed aerosol particles or when a more dilute water droplet (e.g., cloud droplet) forms first and then activates (freezes) on the INPs. ATD ice nucleation has been studied in both regimes as discussed below.

Ice nucleation on mineral dust particles is conceptualized to occur when a small region of the mineral surface is able to lower the local energy required to form an ice surface. This is sometimes thought of as a pre-ordering of the liquid water into an ice-like configuration.[9−12] Because the critical nucleus size of ice is relatively small, the ice nucleating site can generally be much smaller than the surface of a given INP. This makes identifying the freezing site difficult even for simple, pure compounds. To add to the difficulties, environmental mineral dust is very diverse, existing with many different mineral components.[13] ATD mimics the composition of other mineral dusts to a reasonable extent, although there may be better mimics.[6,13,14] One mineral that comprises about one-third of ATD is feldspar,[13] which has been implicated in ice nucleation, particularly the potassium-containing variety, microcline.[15−20] Other components have been shown to have ice nucleating properties as well, although generally at lower temperatures and/or site densities (number per surface area), including illite,[13,21,22] montmorillonite,[22,23] and kaolinite.[22,24−26]

To convolute things further, many IN active sites on mineral particles appear to be defect sites,[15,26−28] which implies that IN properties cannot be considered as solely dependent on the material in a traditional sense. For example, while K-feldspar has been noted above as a good IN material,[15−18,20] the actual IN properties seem to depend on the microscopic structure,

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such that different crystalline arrangements produce differing IN ability.[19] The defect sites on mineral surfaces by definition are less stable, and therefore more reactive, than stable crystal faces. Due to the involvement of defect sites in ice nucleation, IN properties of a given material may be chemically modified, either through reaction or coating formation, under conditions that the bulk material is normally considered inert. ATD is perhaps unique in its surface character because it is collected from the environmental topsoil and then mechanically processed to yield the desired size distribution. In experiments on hematite particles, milling increased ice nucleation efficiency (scaled to surface area) by an order of magnitude.[29] This indicates that the defect sites introduced by mechanical processes are significant and also that they are subsequently lost to weathering in a natural environment.

Processing or solute conditional dependence of particle IN activity in both the deposition and immersion freezing modes has been previously reported. In the immersion freezing regime, solution phase exposure has been investigated in several studies. Studies by Reischel and Vali[30] observed some large changes in IN ability for some combinations of salts and IN materials. Ammonium and iodide salts often had the largest effect, often causing nucleation at warmer temperatures, and inorganic IN were most affected. A work by Gobinathan and Ramasamy[31] on PbI_2 IN indicates strong concentration dependence of salts on IN, with some concentrations increasing freezing temperatures and some decreasing. A more recent work by Zobrist et al.[32] points out that much of the variation that is observed between different solutes is due to changes in water activity, although changes in water activity alone do not account for the entirety of the effects that are observed, indicating ion-specific effects. Experiments by Whale et al.[20] observed relatively consistent effects on a variety of IN, where alkali halide salts generally decrease freezing temperatures, and ammonium salts facilitate freezing at warmer temperatures. Direct comparison between these data sets is difficult due to the variety in salts and IN materials used. In the few cases where direct comparison is possible, there is often poor agreement, although this may be due to differences in the sources or processing of samples that are nominally the same. There is some additional difficulty that arises when comparing ice nucleating results produced by different instrumentation.[33]

Two investigations by Sullivan et al. examined the effects of exposure of ATD to sulfuric acid and nitric acid vapor.[34,35] In these studies, aerosolized ATD particles were exposed to gas-phase acid at variable relative humidity (RH) and temperature. Sulfuric acid studies also included subsequent exposure to a 250 °C thermodenuder and/or ammonia gas is some experiments. Sulfuric acid experiments showed dramatic decreases in immersion mode IN activity except in the lowest processing temperature (45 °C) experiments with no other treatments. Increasing temperature, treatment with ammonia, and thermodenuder exposure all resulted in more dramatic decreases in IN activity.[34] There is evidence that sulfuric acid exposure dissolved the ATD surface, which could explain the irreversible nature of the decrease in IN activity.[36] By contrast, nitric acid exposure at room temperature under similar conditions did not result in significant decreases in immersion mode IN activity.[35]

Experiments examining the effect of chemical treatments on mineral dust IN activity have been reported for the deposition regime under conditions below water saturation.[23,24,37,38] Coating ATD particles with sulfuric acid did not appear to alter the deposition freezing behavior in a study by Knopf and Koop,[37] contradicting the behavior reported in the same humidity regime in the studies by Sullivan et al.[34] and experiments by Cziczo et al.,[38] which observed decreased IN activity for sulfuric acid and ammonium sulfate coatings. It is likely that there are several convoluted effects in these aerosol studies. First, the studies by Knopf and Koop[37] used only the first freezing events above −38 °C, which may bias the data toward lower RH values compared to other studies in that temperature range. Additionally, under water subsaturated conditions, changes to IN activity could be due to changing hygroscopicity of the particles: more hygroscopic particles will condense water below 100% RH, shifting the nucleation mode from deposition to immersion. This is the likely explanation for cases in which a chemical treatment results in IN activity increases for water subsaturated measurements but not for supersaturated ones. Montmorillonite, a modest ice nucleator and component of ATD, showed increased IN ability in the subsaturated region after ammonia gas exposure;[23] however, ammonium sulfate coatings on ATD appear to decrease IN activity.[24] Again, the discrepancy is likely due to changes in particle hygroscopicity leading to an altered nucleation pathway. Experiments with solutions of ammonium salts are consistent with the decrease in IN activity for coated ATD.[20]

There is still much that is not known about the dependencies of ATD IN activity, especially to changes induced by longer-term processing and under less extreme conditions. Through an examination of the sensitivity of ATD to solution phase processing, we hope to gain insight into potential experimental or atmospheric impacts on ice nucleation. Additionally, some information may also be gained about the nature of the IN sites and materials within ATD. Peroxide treatments and heat treatments were used to investigate organic components and IN stability under oxidative and thermally degradative conditions. Next, enzymatic digestions were performed to assay for proteinaceous INPs. Studies were performed in salt solutions and acid solutions of varying composition and strength to test general IN stability under mild and environmentally relevant conditions.

■ MATERIALS AND METHODS

Materials. Materials were purchased and used without purification, unless otherwise noted, although solution phase components were generally filtered through a 100 nm Teflon filter (Puradisc 25 TF, Whatman) prior to ice nucleation assays. ATD (A2 fine test dust; ∼22% <2.75 μm, ∼58% <11 μm, and ∼90% <44 μm) was purchased from Powder Technology Inc. Sulfuric acid (H_2SO_4; 95−98%, ACS reagent grade), hydrogen peroxide (H_2O_2; 30%, stabilized), and sodium chloride (NaCl; ≥97%, ACS grade) were purchased from Sigma-Aldrich. Nitric acid (HNO_3; 65−70%, ACS grade) and potassium chloride (KCl; ≥99%, ACS grade) were purchased from Mallinckrodt. Hydrochloric acid (HCl; 1 M) was purchased from Honeywell Fluka. Aquarium salts (Neomarine Salt Blend) were purchased from Brightwell Aquatics, mixed to 1.025 g mL^{-1}, and filtered with a combined charcoal/HEPA filter (Whatman Carbon Cap 150) to remove organic and particulate matters followed by a 0.22 μm polyethersulfone filter.

Ice Nucleation Assays. The concentrations of INPs per gram of untreated and treated ATD samples were measured using the Colorado State University ice spectrometer (IS).[21,39]

In preparation for IN assays, all ATD suspensions were shaken on a tube rotator (5−20 min) prior to dilution. Visible resuspension occurred in less than 1 min, and variation in shaking time was due to the time taken for other preparations. All further preparation of samples was performed in a laminar flow hood. A series of three 20-fold dilutions of 5% (w/v) ATD mixtures were produced using 0.1 μm filtered DI water, yielding dilutions of 2.5×10^{-3}, 1.25×10^{-4}, and 6.25×10^{-6} g ATD per liter. Fifty microliter aliquots of each mixture were pipetted into 32 wells of a 96-well polypropylene PCR tray (μCycler). In addition to samples, 32×50 μL aliquots of 0.1 μm filtered DI water were tested with each IS experiment to determine background IN activity. The pH of unmodified ATD solutions and DI water were the same, at about 5.5, and are not expected to change with dilution.

The IS uses an immersion freezing method described previously.[21,39] Sample-containing PCR trays were placed inside of temperature-controlled blocks within the instrument. To keep condensation from forming within the instrument, a 650 cc min^{-1} flow of chilled dry nitrogen gas was passed over the samples. The IS ramped down from room temperature at a cooling rate of 0.3 °C min^{-1} to an end temperature of −32 °C. Changes have been made to the CSU IS instruments to implement freezing detection and automation. Three essentially identical instruments have been tested on a variety of samples and performed identically to each other in both precision and accuracy. An enclosure was built around each IS, and a camera was installed (Point Grey BFLY-PGE-20E4M-CS or FBLY-PGE-0552M-CS) to take photos of the sample trays at 1 s intervals as the wells cooled (Neslab Endocal ULT-80 or Fisher Isotemp 6200 chillers). The temperature was recorded and averaged from two to four thermocouples (type T thermocouple wire and NI FP-TC-120 or Omega OM-USB-TC) calibrated against a NIST traceable thermocouple in each IS and attached directly to the chiller blocks using thermal paste. The absolute temperature uncertainty in this instrument is estimated at ±0.5 °C, although the relative temperature uncertainty between data points is much smaller on the order of ±0.01 °C. The freezing of sample wells was detected based on changes in pixel intensity of the photos from initial levels. Images were saved and used for visual verification, especially for wells with higher dust loadings. Control of the instrument was accomplished through a custom code written in LabVIEW. Data was saved in 1 s intervals, including a timestamp, all thermocouple measurements, and number of frozen wells in each user-defined sample.

Raw data was converted to IN number concentrations using the equation put forward by Vali:[40]

$$C_{\mathrm{INP}} = \frac{-\ln(f)}{V}$$

Here, f is the fraction of droplets not frozen, and V is the well volume. Confidence intervals are found using formula 2 recommended by Agresti and Coull.[41] Control samples were performed concurrently with all experiments and consisted of only the solution component of the mixture in the absence of ATD. For example, if the test sample was dilutions of ATD in 0.5 M NaCl solution, the control samples were 0.5 M NaCl solution used for dilution. In these experiments, there are very few cases where there was overlap with control samples. That is, cases in which any control samples were frozen at temperatures where experimental samples were not fully frozen. When those cases did arise, it was only below −23 °C, and the number of INPs in the control was subtracted from both total and frozen wells in the experimental sample. While this treatment is approximate, the errors introduced should be much smaller than the errors that arise from the stochastic nature of the measurements. In some cases when test samples become sufficiently dilute, additional data processing is required to build valid freezing spectra.[42] In these cases, spectra appear disjointed between subsequent dilutions. Because of the relatively high concentrations we measured in this work, and the absence of this feature, we conclude that the simple treatment used was sufficient.

Acid Treatments. All acid treatments were prepared by diluting reagents with 0.1 μm filtered DI water to the desired concentrations, each of which was then mixed with ATD to yield a 5% (w/v) ATD suspension. IN assays of ATD in 420, 168, and 84 mM H_2SO_4 solutions were performed after they had rested for approximately 1, 18, 42, and 66 h. Samples settled over time but were resuspended prior to dilution and assaying. In the same manner, mixtures of 5% (w/v) ATD were prepared in solutions of 314, 126, and 62.8 mM HNO_3 and assayed after approximately 18, 42, and 66 h. HCl dilutions were prepared in concentrations of 40 and 20 mM HCl, and ATD mixtures assayed after approximately 18 and 66 h of treatment. IS samples were run as dilutions of the acidic samples in DI water. This reduced the final concentration of acid to very low levels and altered the final pH compared to the processing pH. If the changes to ATD from acid exposure were reversible or state-dependent, this would result in inconsistencies between the different dilutions. This is not observed, indicating that the changes in acid were irreversible.

Oxidation. Oxidation experiments were attempted to selectively destroy organic materials, ideally leaving only mineral INPs. Hydrogen peroxide digestion of the carbonaceous matter in ATD was performed using the method described by Hill et al.[43] A mixture of 50 mL of 15% H_2O_2 and 10 g of ATD was brought to a boil and slowly stirred. An additional 50 mL of 15% H_2O_2 was added in 25 mL increments after 20 and 40 min of boiling. Once all organic materials and residual H_2O_2 had decomposed (approximately 1 h), the mixture was removed from heat and stirring and allowed to rest. IN assay was performed approximately 18 h after the removal from heat.

Ionic Solutions. Three separate 2.5% (w/v) solutions of either NaCl, KCl, or aquarium salts were prepared by dissolving each solid in a liter of DI water and passing the solution through a 0.22 μm PES filter. The aquarium salt solution was additionally filtered through a Whatman Carbon Cap 75 filter (activated carbon + HEPA) to remove residual organic matter and particles. Each solution was combined with ATD to yield a 5% (w/v) dust mixture. Serial dilutions of ionic solution-treated suspensions of ATD for IN assay were prepared with the respective 0.1 μm filtered solution, in place of 0.1 μm filtered DI water, to maintain constant ionic strength so that colligative and specific effects of ions on freezing remained consistent between dilutions. IN assay was performed on the NaCl-treated ATD mixture after approximately 66 h. Both suspensions of ATD in KCl and sea salt were assayed after 24 h of treatment.

Muffle Furnace. Muffle furnace experiments were performed with the goal of completely removing organic matter from the ATD. Ten grams of ATD was transferred to a glass beaker and placed in a 500 °C muffle furnace for 2 h or a 600 °C furnace for 4 h.[44] The heat-treated dust was left in the furnace to cool overnight, after which 0.1 μm filtered DI water or KCl solution was added to create a 5% (w/v) ATD mixture. Samples were tested both immediately following the addition of water and after the mixture was allowed to rest for approximately 24 h.

TOC Measurements. Heat-treated samples were tested for total oxidizable carbon (TOC) after combustion in the muffle furnace to check the efficiency of the heat treatments. Two grams of ATD was heated to 500 °C for 2 h or 600 °C for 4 h and then suspended in 15 mL of 0.3 M HCl and stirred occasionally for 2 h to remove residual carbonates. Two hundred fifty milliliters of the acid suspension was then diluted with 12 mL of DI water for the test sample. These samples were run in a Shimadzu TOC-Vcsh. The sample was injected into a combustion tube and heated to 680 °C, whereupon the total carbon is oxidized or decomposed to create CO_2. Combustion products were cooled and dehumidified, and CO_2 was quantified using an infrared detector. DI water blanks read 0.20 to 0.32 mg L^{-1} TOC compared to raw sample readings of 4.52 mg L^{-1} TOC untreated, 1.14 mg L^{-1} TOC at 500 °C, and 0.88 mg L^{-1} TOC at 600 °C. Soil TOC percentages were calculated after subtracting the background, with background variation being the dominant source of error.

Papain Digestion. To selectively destroy proteinaceous INPs, particularly those produced by the fungus *Mortierella alpina*, which is likely common in the soils of the region,[39,45] papain digestions were carried out in a slightly modified procedure to that used by Hill et al.[39] Two grams of ATD was mixed with 40 mL of 5 mg mL^{-1} papain (AppliChem) in 0.22 μm filtered 10 mM sodium phosphate buffer (pH 6.3). The same test was performed in DI water rather than in buffer. Two grams of ATD was also prepared in buffer with no papain as a control. Both samples were left in a shaker for 16 h before being diluted in phosphate buffer and assayed. Additional control samples of either buffer solution or DI water were run for these experiments. Although control experiments of papain in the absence of ATD were not performed here, the IN behavior of papain has not been observed previously and would have to be exceptionally efficient to compete with ATD given its excess concentration. Although papain is most active at 60−70 °C, it maintains 20−40% of peak activity at room temperature and ∼65% of peak activity at pH 5.5 when unbuffered, which is sufficient here due to the long digestion time.[46]

RESULTS AND DISCUSSION

Aqueous Aging. Freezing of concentrated ATD samples is recorded as warm as about −2.5 °C, significantly warmer than many natural soil samples.[39,47,48] The first tests of ATD aging were performed simply in deionized water over several days. In the immersion freezing technique used, it is impossible to avoid aging in water entirely. It takes some time to prepare samples and cool them down, so for unaged samples, there is ∼1−2 h between the ATD first mixing with water and the ATD solution freezing, depending on the freezing temperature. In Figure 1, dramatic changes in IN ability of ATD samples can be observed in the first several days of aging, with little to no change in the subsequent week. The decrease in IN is not uniform across the spectrum, and instead there is a large decrease at warm temperatures, above about −5 °C, a region of relatively little change at intermediate temperatures, between about −5 and −12 °C, another decrease between about −12 and −23 °C.

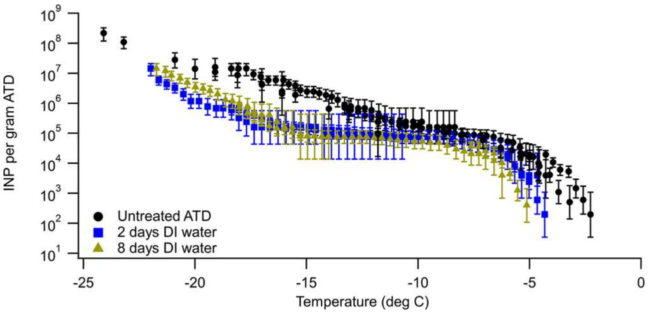

Figure 1. IN spectra of ATD untreated versus aged in DI water. Error bars are 95% confidence intervals.

We also investigated the effects of stirring on ATD aging (as the dust tends to settle out and form a more compact mass, with a clear supernatant) over the course of several minutes to hours depending on the solution composition. Samples were always resuspended before measurements were taken, and there was no difference between samples that were constantly stirred and those that were allowed to age while settled (Figure S1, Supporting Information). The first day of aging for both cases matches the day 2 data shown in Figure 1.

Oxidation. Because aging mechanisms and behaviors differ for mineral and organic INPs, several tests were carried out attempting to characterize the nature of the INPs in ATD. Oxidation, both by heat and peroxide, was used to investigate organic components. Oxidation methods have often been used to test for the presence of organic INPs in soil samples.[39,47,49−51]

Peroxide-treated ATD shows a drastic decrease in INP counts, especially in the warmer temperature region of the spectrum. Addition of KCl solution resulted in no change from the DI water spectrum, which will be discussed later in the manuscript. Any organic material should be readily oxidized by the peroxide treatment, losing its IN properties. Previous experiments on K-feldspar showed no change in IN activity upon similar peroxide treatment,[48] so it is unlikely that the feldspar component of ATD would have been degraded. These peroxide experiments would normally be very strong evidence that ATD contains a significant fraction of organic INPs. However, the 500 °C heat treatment in Figure 2B calls this into question.

After heating to 500 °C for 2 h, the warm-temperature IN is largely preserved. Despite the fact that there is no aging in

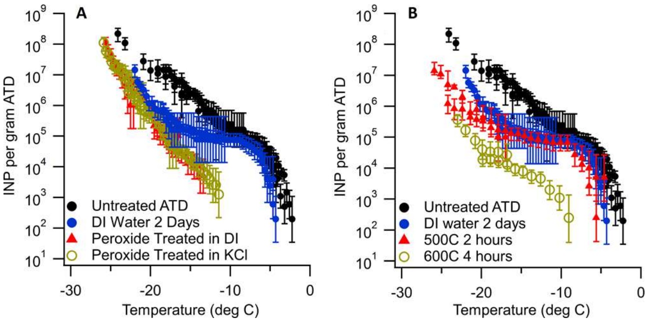

Figure 2. (A) IN spectra of ATD before and after peroxide treatment. The treated sample was tested both in DI water and in 0.5 M KCl solution. (B) IN spectra of ATD before and after heat treatment. A higher temperature and longer muffle furnace heating produced larger changes in the IN spectrum. Error bars are 95% confidence intervals.

water for these heat-treated samples, the 500 °C sample aligns very well with the ATD sample that has been aged in DI water. This is perplexing due to the extremely different nature of these treatments. Upon further exposure to water, there is additional degradation of the INPs in the 500 °C sample, approaching the 600 °C or peroxide-treated samples (Figure S8, Supporting information). In the peroxide treatment, the oxidation and any water-based weathering are inseparable, so the fact that the heating followed by water exposure produces similar results implies some consistency between the effects.

The conclusion from the peroxide and heat treatments is that the warm-temperature INPs are organic but also stable at 500 °C for 2 h. In previous studies of soil dusts, samples heated in a muffle furnace to 300 °C for 2 h showed identical degradation of IN activity to peroxide treatments.[47] In experiments where kaolin particles were coated in IN entities from decayed leaf litter, heat treatment at 500 °C effectively removed all IN effects of the coating.[52] However, there are several types of organic material that have been observed to be relatively stable at these temperatures. In soils, heat-stable organics are usually attributed to either black carbon or humin substances: insoluble, complex, and high-molecular-weight materials.[53,54] A work by Johnson et al.[55] found organics coating manganese oxide particles that were stable at 500 °C but combusted at 600 °C or warmer. They attributed the thermal stability to binding to the manganese surface through carboxylates and found that the coating materials contained higher aliphatic to aromatic/alkene ratios than the bulk, thermally labile material. It is possible that this same type of organic coating plays a role with ATD, perhaps on a Fe surface, since Fe and Mn are both associated with preservation of organic contents in sediments,[56,57] and ATD contains about 3% surface Fe.[14] Binding to aluminum oxide mineral surfaces, which are more abundant in ATD, at ∼24%,[14] is also possible. Studies of bound monolayers to inorganic surfaces have found high thermal stability as well, with 50% carbon loss points exceeding 600 °C for phosphonic acid and alkynes chemically bonded to porous aluminum oxide.[58]

As a rough test of organic removal at these temperatures, TOC analysis was done for both the 500 °C and the 600 °C treatments. The organic content after treatment was found to be 0.032 ± 0.002 and 0.023 ± 0.002%, respectively, compared with 0.154 ± 0.002% with no treatment. This corresponds with 79% TOC removal at 500 °C and 85% removal at 600 °C. While the amount of material removed between the two treatments was low, an organic surface coating would not need to be thick or have extensive coverage to act as a good ice nucleator. Experiments with kaolinite and kaolin show that adsorbed organic ice nucleators retain IN ability while attached to the particle.[52,59] Clay mineral surface areas decrease with adsorption of organic matter relatively quickly, indicating pore/crack filling. Illite and kaolinite show a ∼35% decrease in surface area with 0.1% organic content by mass.[60] So, even at a very low organic content by mass, the surface features can be dominated by the organic components.

If the warm-temperature ice nucleation observed for ATD is instead due to inorganic materials, their identity is not certain. Interestingly, even feldspars and clay mineral IN are not necessarily expected to be preserved well during the heat treatments: feldspars can undergo phase transitions as low as 400 ± 50 °C[61,62] and clay minerals undergo water loss as low as 350 °C.[50] Mineral materials could still satisfy the observed heat resilience while undergoing structural or chemical changes in the presence of water. While feldspar minerals can nucleate ice at warm temperatures, if the warm-temperature IN occurs at feldspar sites, their degradation in peroxide would be inconsistent with a previous study.[48] It should be noted that we expect the main difference between the two heat treatments to be due to the temperature rather than the time. Times for carbon evolution are often assumed to be quite short, with TOC analysis usually only applying heat for several seconds. It is conceivable that the effect of time is important for changes to minerals however.

Further Treatments. In order to examine the organic component of ATD more closely, an enzymatic digestion was used to selectively break down proteins. This treatment has been previously used to destroy ice nucleating proteins produced by the soil-borne fungus *Mortierella alpina*, which is found in the region.[39,45] The enzyme used was papain, a cysteine protease, which is generally used in buffered conditions to maintain activity. The experiment was thus performed both in buffered solution with and without the enzyme. Interestingly, the largest effects observed were between the buffered solution (10 mM sodium phosphate, pH 6.3) and aging in DI water (Figure 3), despite a relatively low buffer concentration and neutral pH. The effect of the papain itself was insignificant and was further confirmed by a test in the absence of buffer (Figure S10). This is strong evidence that the IN material in ATD is not proteinaceous.

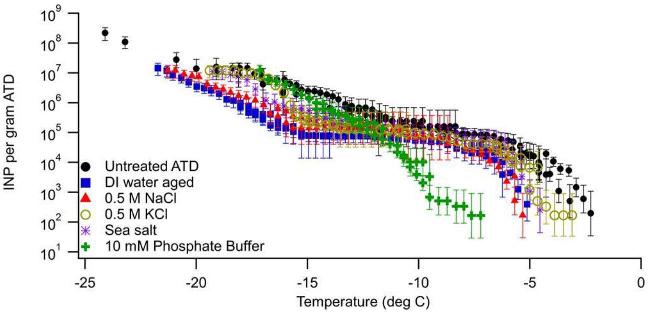

Figure 3. IN spectra of ATD aged in DI water, NaCl, KCl, sea salt (1.025 g mL^{-1}), and phosphate buffer solutions. Concentrated salt solutions were adjusted for colligative freezing point depression. Sea salt freezing point depression was estimated at 1.59 °C by assuming the depression of pure NaCl. Salt solutions were aged 66 h each. The DI water sample was aged 8 days but did not show significant changes in the spectrum after 2 days. Phosphate buffer was aged 16 h. Error bars are 95% confidence intervals.

Because notable changes in ATD INPs are observed in both DI water and dilute phosphate buffer, several other salts were investigated (Figure 3). Relatively concentrated (0.5 M) sodium chloride, when corrected for colligative freezing depression, yielded the same spectrum as aging in DI water alone. Aging in KCl and artificial seawater (which contains a small amount of potassium) produced similar results, with some preservation of INPs in the colder regions of the spectrum, below about −15 °C. Interestingly, this dependence is lost upon peroxide or heat treatment (Figure 2), suggesting whatever IN active components that were preserved by KCl were destroyed upon oxidation. A previous work has observed some depression of K-feldspar IN activity in potassium-free solutions.[51] This may be due to modification of the mineral surface through ion exchange. Potassium-containing feldspar is generally a better ice nucleator than other varieties,[16−19] and defect sites are likely somewhat susceptible to ion exchange. However, a work by Whale et al. has shown that feldspar IN activity was decreased in 15 mM solution of both NaCl and KCl.[20] Our own work (Figure S9, Supporting Information) showed an increase in K-feldspar IN activity with KCl addition, although the effect becomes weaker at concentrations above 10 mM.

The effects of the salts chosen are all relatively small when compared with the effect of the dilute phosphate buffer on the IN spectrum. While the concentrations of salt used in these experiments were quite high, significant differences would be expected in the spectra between DI water and salt if aggregation was involved in IN ability as suggested above. This is particularly true for the peroxide digestions performed in KCl: if the IN spectral changes were due to aggregation changes with organic removal, the KCl and DI water spectra should differ significantly.

To investigate both the effect of different ions and environmentally relevant acidic conditions, aging in different acidic solutions was performed. When aged in dilute sulfuric acid (Figure 4), the IN spectrum shifts in several ways that are different from the shifts in DI water. The warmer end on the spectrum is degraded more, with drastic reductions to INP numbers warmer than about −12 °C. The degradation shows both concentration and time dependence. For 84 mM sulfuric acid, differences were observed between samples tested after 18 and 44 h but not after 66 h (Figure S2, Supporting Information). At higher acid concentrations, the degradation occurred faster, with no significant differences between the time points (Figures S3 and S4, Supporting Information). This degradation also appears to occur via irreversible changes to the material rather than by a pH-dependent deactivation as subsequent dilutions in DI water did not change IN activity. This is true for all acid samples tested. Additional degradation in an acidic, reactive environment is perhaps not surprising, although it contrasts the behavior of pasture soils.[39] What is surprising, however, is the preservation of INP numbers at colder temperatures in dilute acid. The INP numbers are in fact higher in the more dilute acidic samples below about −12 °C than in the sample equivalently aged in DI water.

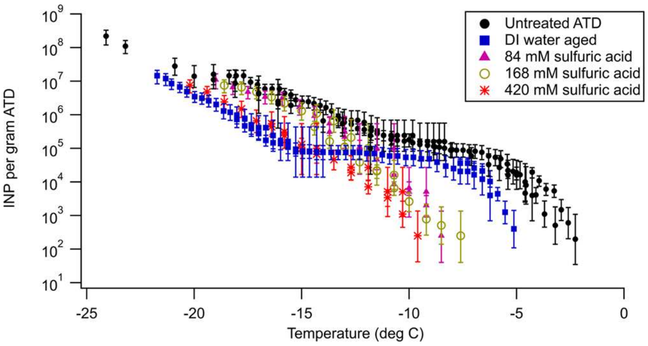

Figure 4. IN spectra of ATD aged in sulfuric acid and DI water compared with unaged ATD. Sulfuric acid samples were aged 66 h each. The DI water sample was aged 8 days but did not show significant changes in spectrum after 2 days. The most concentrated acid samples were run at 1:20 dilution in DI water from processing conditions (i.e., ∼20 mM max), where colligative freezing depression is negligible. Error bars are 95% confidence intervals.

A plausible explanation for the preservation of colder IN activity under acidic conditions is the degradation of the ATD into components that are still IN active. Feldspar minerals are known to weather into clay minerals under aqueous and acidic conditions,[63,64] some of which are still IN active, such as kaolinite. The IN activity that remains is approximately the same in the lowest temperature region which is still somewhat unexpected with this explanation however. Another possibility is that the clay minerals form either with or without the presence of acid, but the acid solubilizes the transformed material and leaves, for example, a feldspar surface exposed to the water.

The change in ATD IN properties appears to be relatively consistent across a range of acids. Data for HCl and HNO_3 are shown in the Supporting Information (Figures S5−S7). The same trend is observed where ATD IN is inhibited at warm temperatures but preserved relative to aging in DI water at lower temperatures for dilute acids. The consistency across acids points to the effect coming from hydrogen ions rather than the anions at least for these acids. This may be due to dissolution or reaction of the ATD surface material at IN active sites. The degradation rate appears to be variable based on the acid used and the concentration, but in all cases, the bulk of the degradation occurs in the first day (Figures S2−S7, Supporting Information).

Other experiments examining acid processing of ATD have had mixed results, with some showing decreases in ATD IN activity, and others showing little change.[34,35,37] This is not inconsistent with our results, especially because previous studies were performed at temperatures colder than −13 °C. Our studies show that in the colder region of the spectrum, IN activity is preserved for dilute acid solutions and can be decreased at higher concentrations. There may be some important differences in the mechanism of degradation, however, when acid vapor is directly deposited on the particles compared to the solution processing investigated here. Because the degradation mechanism and even the materials responsible for IN activity are unclear in both cases, it is very difficult to compare them.

Interestingly, the degradation in phosphate buffer is similar to that observed in dilute acid. The 168 mM sulfuric acid solution produces a similar trace as the 10 mM sodium phosphate solution, although the sulfuric acid solution will have a pH of about 0.8 compared to 6.3 of the buffer. The two solutions appear to be very different conditions but have nearly identical effects on the IN properties of the ATD. The buffer should even be less acidic than the DI water, which is acidified by carbonic acid formation from atmospheric CO_2 to around pH 5−5.5. Assuming that the organic material bound to a Fe surface is responsible for the warm-temperature IN, the mechanism for degradation for both acid and phosphate could be tied to its removal. The acid could remove the organic material bound through carboxyl groups by protonation of the carboxylates or degradation/solubilization of the surface, while the phosphate ions may outcompete iron binding sites.

■ CONCLUSIONS

We have shown that ATD is susceptible to loss of IN activity under a variety of common conditions, with effects summarized relative to ATD aging in water in Table 1. In no cases were INP abundances greater for treated samples than for the untreated, unaged sample. Treatments that resulted in higher INP abundances than a DI water treatment are shown

Table 1. Summary of Treatment Effects on INP Abundance Relative to the Sample Aged in Water, with Treatments Yielding Higher and Lower INP Abundances Shown in Blue and Red, Respectively

Treatment	-10 to 0 °C	-15 to -10 °C	Below -15 °C
Unaged Sample	0 to >1000 fold preservation	0 to 100 fold preservation	0 to 10 fold preservation
Peroxide	>1000 fold reduction	10 to 100 fold reduction	10 to 0 fold reduction
500 °C 2h	No change	No change	Minor reduction
600 °C 4h	>1000 fold reduction	10 to 100 fold reduction	10 fold reduction
Papain	No change	No Change	No Change
10 mM phosphate buffer	1000 fold reduction	100 fold reduction to minor preservation	>10 fold preservation
0.5 M NaCl	No change	No change	No Change
0.5 M KCl	Minor preservation	No change	0 to 10 fold preservation
0.5 M Sea Salt	No change	No change	0 to 5 fold preservation
HCl, H_2SO_4, HNO_3 low conc./time	100 fold reduction	10 fold reduction to 10 fold preservation	0 to 10 fold preservation
HCl, H_2SO_4, HNO_3 high conc./time	1000 fold reduction	100 to 0 fold reduction	No change

in blue (preservation effects), and treatments that produced lower abundances are shown in red.

All of this data taken together illustrates the lability of ATD IN properties, but a clear mechanistic understanding of the aging processes remains elusive. The warmest temperature INPs appear to be the hardest to preserve, while the coldest temperature INPs are well preserved in many treatments. One of the main sources of difficulty is the compositional diversity of ATD. While one of the main entities responsible for the IN behavior is thought to be K-feldspar,[14,16,18] the response of ATD is significantly different than responses observed for a variety of feldspar samples.[19,20] Another complicating factor is the role of defect sites in ice nucleation. Defect sites are generally more easily chemically modified than bulk materials, and their abundance will vary based on both microscopic morphology and mechanical processing. It seems likely that part of the IN activity of ATD comes from the grinding of larger particles to create a fine dust with many freshly cleaved surfaces. We hypothesize that the sensitivity of ATD IN arises partly due to this mechanical processing with little natural weathering occurring afterward. This is expected to dominantly effect the mineral ice nucleating sites, which we expect to act at lower temperatures. Further studies are required to test this hypothesis. Regardless, this work illustrates the necessity of control experiments for aging of IN samples even for relatively short periods of time under very mild conditions. This study also highlights the importance of generation and weathering effects on mineral INP samples. This may be somewhat overlooked in the literature, especially in cases where mineral particles are generated through grinding, which may be especially problematic for pure mineral samples or samples collected from locations protected from water exposure.

Perhaps, the most surprising finding of this work is the existence of warm-temperature INPs that have the characteristic "hump" shape often associated with organic materials, which are lost with peroxide exposure but are thermally stable to 500 °C. We hypothesize that these are heat-stable mineral-bound organic coatings. While heat-stable organic ice nucleating materials have not been observed previously, there is support in the literature both for heat stability[55,58] and ice nucleating abilities[52,59] of mineral-bound organic coatings.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsearthspacechem.9b00304.

Figures demonstrating changes to ATD IN spectra due to stirring in water, aging in different acidic conditions, aging in water after heat treatment, papain treatment and changes to K-feldspar IN spectrum with salt composition and concentration (PDF)

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Notes

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