Manuscript Number: GCA-D-16-00300R1

Title: Dehydroxylation and Diagenetic Variations in Diatom Oxygen Isotope Values

Article Type: Article

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Abstract: Numerous studies have documented changes in the dissolution and reactivity of biogenic silica as it is transferred from the water column to sediment archives; here we present the first experimental data that demonstrate a physical mechanism by which the oxygen isotope ($\delta^{18}\text{O}_{\text{sil}}$) values of biogenic silica (diatoms) are altered during early diagenesis. The $\delta^{18}\text{O}_{\text{sil}}$ value of diatom silica cultured at 19.3°C was $+31.9\% \pm 0.2\%$ ($n = 6$); the same silica experimentally aged in an artificial seawater media at near silica saturation at 85°C had an average $\delta^{18}\text{O}_{\text{sil}}$ value of $+27.1\% \pm 0.6\%$ ($n=20$). The most significant change in the $\delta^{18}\text{O}_{\text{sil}}$ value was coincident with an initial reduction in the total silanol abundance, indicating that the timing of dehydroxylation reactions in natural sedimentary environments is associated with diagenetic changes in the recorded $\delta^{18}\text{O}_{\text{sil}}$ values. The rate of change in the experimental aging environment at 85°C was rapid, with significant changes in both silanol abundance and $\delta^{18}\text{O}_{\text{sil}}$ values. Additionally, the silica-water fractionation relationship recorded by the experimentally-aged samples approaches the equilibrium quartz-water fractionation factor. The linear rate law was used to estimate the timing of these changes in low temperature environments; the initial and most significant change in silica reactivity and $\delta^{18}\text{O}_{\text{sil}}$ values is likely to occur on the order of 10's of years at 4°C. Published silica-water fractionation factors for sedimentary diatoms most likely represent a combination of growth and diagenetic environments, and the $\delta^{18}\text{O}$ value of diagenetic water needs to be addressed when using $\delta^{18}\text{O}_{\text{sil}}$ values to reconstruct paleoceanographic and paleoenvironmental conditions.

Northern Illinois
University

October 3, 2016

Dear Dr. Blake and editorial staff of *Geochimica et Cosmochimica Acta*,

We would like to submit our revised manuscript (GCA-D-16-00300) entitled “**Dehydroxylation and Diagenetic Variations in Diatom Oxygen Isotope Values**” for final consideration.

A detailed list of the changes made/response to reviewers’ comments is included below. Major changes include 1) discussion of the variation in the $\delta^{18}\text{O}$ value of the experimental water and an additional table (Table 3) to clarify how the silica-water fractionation factors relate to the experimental water, 2) clarification of the silanol calculations, and 3) development of our interpretations to reflect the reviewers’ comments. We have included 6 updated figures in .pdf format.

We look forward to your decision regarding the outcome of this manuscript.

Sincerely,

A handwritten signature in black ink, reading "Justin Dodd".

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Response to Comments: Manuscript Number: GCA-D-16-00300

Title: Dehydroxylation and Diagenetic Variations in Diatom Oxygen Isotope Values

Reviewer #1: Liz Webb

This paper examines changes in diatom structure and isotope chemistry in experimental "burial" conditions. The high temperature of the reaction allows them to simulate reactions that would occur between diatoms and "pore waters" throughout 10's to thousands of years of burial. This study follows several that compare the structural and chemical differences of fresh versus fossil diatoms. The experimental evidence presented here explains the discrepancies reported in previous studies that compare the oxygen-isotope fractionation between opal and water for living versus ancient diatoms. This work is very relevant to anyone interpreting paleoclimate from diatom isotope records. The paper is well written and the experiment is well executed. I highly recommend publication after only minor revisions (described below). Most importantly:

1. I feel there should be a more detailed explanation about the perseverance (and proportion) of tetrahedrally coordinated oxygen after exchange/burial

See specific comments below and lines 121 – 123; 328 – 330; 382 - 387

2. Clarification on the calculation to determine the number of internal vs. external silanol groups.

See specific comments below and lines 155 – 158; 223 – 239; Table 1; Figure 4 and caption

3. Take the variation in water oxygen-isotope values into account when calculating fractionation factors - or at least discuss the error this introduces.

See specific comments below and lines 207 – 211; 271 – 277; Table 2 & 3; Fig. 6

Liz Webb

Detailed comments:

Line 25 (and abstract) - the use of 10^1 seems like a complicated way to say "10"

This change has been made (Line 25).

Line 40 - too many parentheses in this list of references

This change has been made (Lines 40 – 42).

Line 98 - diatoms were crushed before dissolution experiment - how fine? Were frustules broken or just disaggregated? How does this compare to diatom conditions when accumulating naturally in sediment?

This issue has been addressed in the text and clarified (Lines 103– 105) with an additional reference (Scherer et al., 2004).

Section 2.2 - you do not say what the ageing periods were (or give the range).

The aging periods have been discussed (Lines 112 – 114; 116).

Section 2.3 - it is not clear what material was used for the titrations - was this done in the middle of an ageing period or at the end of a certain period of aging? If it was performed on the ageing solutions during an ageing period, were the titrations performed on an aliquot?

The titration process has been clarified (Lines 127 – 129).

Line 179 and 180 - you say that the surface charge density is declining but the numbers are getting less negative and therefore are increasing, no? Perhaps this can be re-worded to explain

the reduction of the negative charge?

Surface charge density values are clarified in the revised manuscript (lines 139 – 144; 186 – 188). Surface charge density is reported this way to maintain consistency between our results and others in the literature (i.e. Loucaides et al., 2010).

Line 179 - here you mention the change in surface charge density at 0.5 days, but this time interval is not in Table 1. In Table 2 you show results for both 0.5 day and 1 day so it is a bit confusing which data correlate in these two tables. Likewise in Line 189 (and table 1) does ageing of zero days mean cultured diatoms or 0.5 days?

This issue has been clarified in the text (Lines 188 - 192) and in Table 1.

Table 2 - it is not clear from the methods or results why there are two rows for 0.5 days and two rows for 1 day, when each of these rows also has a $n = 2$. Can you comment on why the two 0.5 day experiments have both the highest and lowest $\delta^{18}\text{O}$ values?

The data for the 0.5 and 1 day experiments have been clarified in Tables 1-3 and the text (revised manuscript Lines 101 – 103; 113 – 114; 217 – 219; Table 1 & 3). Discussion of these $\delta^{18}\text{O}$ values has been added as well (Lines 283 - 287).

Line 204 and the caption for Figure 4 - on Line 204 you say that the ratio of internal to external silanol groups is theoretically constrained to 10:1, but then you go on to say that you can calculate this ratio for your samples. At least I think that is what you are saying (e.g. (external silanol density determined by titration)/((density of silanol determined by FTIR)-(density determined by titration)). I realize these two measurements are not in the same types of units. Can they be converted? Then in the caption for Figure 4 you imply that you have used the theoretical 10:1 ratio to do a calculation. Can you please include this calculation? It would make it easier to follow your logic here. I honestly can't tell if you used your measured data or a theoretical calculation to get the internal:external ratio.

The internal vs. external silanol

Figure 4 you need to explain the units of your silanol density. Is this the % of bonds in the entire structure? The % of silanol groups compared to the cultured sample?

We have clarified the silanol abundance calculations in the text (Lines 223 – 239), Table 1, and in Figure 4 (see figure caption).

Line 215 - The two studies you cite here are for dissolution in fresh water (e.g. Smith et al, 2015; Moschen et al., 2006) dissolution rates can be quite variable between fresh and salt water. If you are going to use these as examples you should comment on these potential differences.

The effects of salinity and other dissolved species have been discussed in lines 308 – 324.

Although these processes are outside the scope of this study, it is noted that there are significant gaps in our understanding of how these other variables relate to the timing and magnitude of diagenetic alteration of bSiO_2 (Lines 320 – 324).

Line 247 - you use a value of 0 ‰ to calculate the fractionation factor between silica and water at 85 °C. But in the results (Line 198) you say that the water value was 0 ± 1.2 ‰ ($n = 7$). That is a spread of 2.4 ‰. Presumably you measured the $\delta^{18}\text{O}$ values of the water that had reacted for different lengths of time? I'm curious is the water $\delta^{18}\text{O}$ values changed progressively with time. Even if they didn't, I'm confused why you didn't use the $\delta^{18}\text{O}$ values of water measured for each experiment rather than the average water $\delta^{18}\text{O}$ when calculating the fractionation factors. This might reduce some of the spread in your alpha values.

Clarification of measured $\delta^{18}\text{O}_w$ values for cultured and experimental aging waters have been included in Table 2. As the reviewer pointed out, it was misleading to present the experimental water as an average of 0.0‰, and this error has been addressed. An

additional table (Table 3) has been added to clarify how the $1000\ln\alpha$ calculations are affected by variability in the $\delta^{18}\text{O}_w$ values in the experimental water and distinguish between results (Table 2) and interpretations (Table 3) $\delta^{18}\text{O}_w$ values are reported more clearly in the results section (3.2; Lines 207 – 211) and discussed in Line 271 – 277.

Line 249 - do you mean to refer to Fig 6 here?

Corrected. Line 277

Line 255 - some of this variation might also be a result of the 2.4 ‰ variation in water $\delta^{18}\text{O}$ values

Variation in the $\delta^{18}\text{O}_w$ values throughout the experiment and the effects on the $\delta^{18}\text{O}_{\text{sil}}$ values have been discussed (Lines 271 – 276) and added to Table 3. Figure 6 has been redrafted to reflect the changing $\delta^{18}\text{O}_w$ values in the $1000\ln\alpha$ calculations.

Line 281 - this comment seems out of place. This one sentence does not explain how Si-isotopes behave or how they are similar to what you observe for the oxygen isotopes. Do you mean that a similar behaviour of precipitation has been observed? In these studies a decrease in $\delta^{30}\text{Si}$ values was observed (but it was not related to temperature dependant fractionation like the O-isotopes). However, by the logic of Figure 6 (which is quite sensible), low temperature exchange between the diatoms and water should increase the $\delta^{18}\text{O}$ of the newly formed silica so that the larger fractionation between silica and water would plot more closely to the quartz-water fractionation line. As you now know Prentice and Webb also report variations in $\delta^{18}\text{O}$ values of phytoliths under near saturation conditions - perhaps you could use this as an example of low temperature behaviour of isotopes instead of a lengthy introduction of the behaviour of Si-isotopes.

These changes have been made (Lines 317 – 329).

Line 281 - The Prentice thesis does not use flow through reactors, and her results can now be cited from the PPP paper.

These changes have been made (Lines 317 – 329) and Prentice and Webb (2016) added to the reference list.

Line 309-310 - you propose that dehydroxylation and coatings of newly precipitated silica are the main controls on the aged diatom $\delta^{18}\text{O}$ values. On Line 264 - you raise an interesting point that the tetrahedrally bound oxygen is not expected to exchange. It is not clear whether you think that there is a "significant" portion of original tetrahedrally bound oxygen still in your samples. In Figure 6 the $1000\ln\alpha$ of the aged samples fall between the cultured samples and the $1000\ln\alpha$ expected for quartz-water and this certainly supports a mixture of new silica and primary tetrahedral oxygen. But it doesn't prove that the $1000\ln\alpha$ for quartz-water is the lower end member of this mixing model either. The close proximity of the $1000\ln\alpha$ value for the aged samples to the qtz-water line seems to suggest that most of the oxygen has been exchanged. How does this compare to the proportion of tetrahedrally bound O versus surface silanols you originally calculated? Do exchanged and newly precipitated silica oxygen now outnumber the original oxygen in tetrahedral?

These comments have been addressed (Lines 329 – 331; 383 – 388)

Line 527 – grammar

Line 530 - grammar (amount of density?)

Line 536 - grammar (remove "that")

These changes have been made (Figure 4 caption; Lines 617 - 622).

Line 540 - grammar (change has to as)

These changes have been made (Figure 6 caption; Line 630).

Line 543 - this might be a good place to explain why your aged 1000lna values are approaching the Sharp and Kirschner line rather than plotting on it. Explain the black arrow.

The arrow has been removed from Figure 6; however, we believe that the discussion of 1000lna should be in the discussion section, not the figure caption.

Reviewer #2:

In this article the authors conducted a suite of experiments that exposed monospecific cultured diatoms to artificial seawater at 85C for up to 37 days in order to accelerate an artificial "aging" process. This experimental set up was designed to allow the freshly grown diatom frustules to go through the natural dehydroxylation process that occurs in the sedimentary column, thereby replicating natural biogenic silica diagenesis on laboratory time scales. The authors used these artificially aged diatom frustules in order to quantify some of the effects of natural diagenesis on $\delta^{18}\text{O}$ measurements from diatom silica.

The authors recognize there is a long standing problem regarding the use of biogenic silica $\delta^{18}\text{O}$ as preserving an environmental signal in that bSiO₂ $\delta^{18}\text{O}$ values significantly change between recently living and sedimentary diatoms. This long standing problem has not been sufficiently investigated by the biogenic silica community, and the authors provide a new approach to exploring this fundamental change. The explanation provided by the authors is narrow in scope, as the data set provided is interpreted for clarity in understanding how the dehydroxylation process directly impacts measured $\delta^{18}\text{O}$ values from the bSiO₂.

What is unexplained by the authors is why their artificially aged diatoms yield $\delta^{18}\text{O}$ values that are depleted relative to their initial, fresh, $\delta^{18}\text{O}$ values. This is interesting as when similar observations of this diagenetic change have been previously reported, the change results in an enrichment of $\delta^{18}\text{O}$ from the biogenic silica. An explanation of this depletion after dehydroxylation is a necessary addition to the paper and would better place these data in context of existing data from natural settings.

These results are predicted by equilibrium isotope theory, please see below and lines 363 – 375.

I do have some clarification questions regarding the methods from the cultured materials: Were the cultured diatoms sieved to isolate a single size fraction, or was the bulk culture material used?

See below and Lines 86 – 105.

I am curious because if there was a large size range of specimens once stationary growth occurred, it is conceivable that different size fractions might have responded differently to grinding and homogenization. This species used (*Stephanopyxis turris*) has a natural size range of 10-115 μm , and the smaller frustules in this size range may have more easily dissolved during the artificial aging process. Clarification of this and possible comment on preferential dissolution of smaller frustules post grinding may clarify that the authors either acknowledge this limitation in their experiments, or are attempting to replicate natural assemblage dissolution.

See below and Lines 86 – 105.

Further clarification on the culturing procedure would also be useful, if the authors are able to

provide this information. For example, were any nutrients/culturing media (such as the commonly used f/2 algal culture mixture) added to the seawater? Were the diatoms cultured in multiple batches, or one large batch? Were the cultured diatoms harvested via centrifuging or filtration, and how much time did this require? This last question is important because if harvesting took longer than 24 hours, it is possible some in-homogeneity of the $bSiO_2$ $\delta^{18}O$ occurred due to diatom growth during harvest and changing water temperatures. **See below and Lines 86 – 105.**

However, these questions are clarifications/points of discussion that do not detract from the significance of the paper, nor the overall contribution that the authors provide. This paper is a good contribution to an understudied area of research.

Other Reviewer Comments from EES File:

Comment — Statement is unsupported by data or the literature. There is no reason why the physical processes couldn't be a loss of some oxygen source instead of an addition. If the process is an addition, it is an addition of $16O$ in order to result in the depletion discussed by the authors. Contrarily, if there is a loss, it could be a loss of $18O$, which this statement totally discounts without discussion.

Line 54 “loss/addition of oxygen atoms” added as per reviewer’s suggestion

Comment — Was just the pure seawater used, or was any algal growth media (such as the very common f/2, or extra sodium silicate nodules to supplement the silica in the water) added to the seawater? I am suspicious of only using the raw seawater for a full culturing cycle without providing additional nutrients, as the diatoms would readily extinguish the nutrient supply of most natural seawater, even in upwelling locations where nutrient density is greatest.

Line 86 – 90: Additional information on the diatom culturing process was added.

Comment — Stationary growth vs log growth harvesting: Collecting algae in stationary growth is the equivalent to harvesting diatoms after the peak algal bloom has already occurred; this may not be representative of the sedimentary fossil assemblage, which would be dominated by algal individuals which grew during log phase growth. Further, isotopic differences have been observed in diatom and other algal species when collected in log vs stationary growth phases (ex: see Sachs and Kawka 2015; Knoechel and Quinn, 1989). These previous studies suggest that stationary growth has significantly different nutrient uptake dynamics in algae between log and stationary growth. Collecting these cultured specimens in stationary growth may therefore include some isotopic offsets not representative of open ocean bloom diatoms that are deposited in the sediment.

The reviewer’s comments are absolutely correct; our experimental goals were not to examine the isotopic variation during diatom growth. It is possible that some of the variability observed in the cultured $\delta^{18}O_{sil}$ is the result of harvesting during the stationary growth phase, but this approach allowed us to have the most $bSiO_2$ possible for our aging experiments. The cultured $bSiO_2$ was homogenized by crushing and mixing prior to isotope analyses and experimental steps (Lines 103 – 105). Variability in the initial growth phase/ $\delta^{18}O_{sil}$ values are discussed as well (Lines 212 – 214; 283 – 287).

Comment — How? Centrifuging? Filtration? How long did the harvesting take? How large were the culturing vessels? From experience, if the samples were harvested over a period of days, this could affect the final measured $d18O$ values from the samples, and could have allowed some algal growth to occur during the harvesting process. Clarifying if the harvest took 24 hours or less is vital to understanding the expected homogeneity of the biosilica $d18O$.

Also, was it just 1 culture batch or several smaller cultures homogenized into one accumulated mass?

We have addressed these comments in Lines 92 – 105 by clarifying the harvesting process and the culturing vessels. As with the previous comment, isotopic heterogeneity was addressed by crushing/homogenizing the frustules (Lines 103 – 105). Two batches of cultured $b\text{SiO}_2$ were used (which were not homogenized/mixed) and the variability in the $\delta^{18}\text{O}_{\text{sil}}$ values have been added to the results and discussion sections (revised manuscript Section 3.2; Lines 212 - 219; Table 2). Variability in the initial growth phase/ $\delta^{18}\text{O}_{\text{sil}}$ values are discussed as well in lines 283 – 287.

Comment — Was this from one culture container, or a multi-batch culture? I am unclear on how much cleaned and purified, dry diatom mass was harvested from the culture.

See above and Lines 86 - 101.

Line 204 – “3.3” changed to “3.2”

Comment — This change is in the opposite direction from all other published data that have observed the sediment dehydroxylation phenomenon. Why is this change a depletion? This opposite direction is an important point that merits comment by the authors, but is not discussed, and as a result, this data set is in contradiction to existing data from natural environments.

In most published studies (natural environments) the dehydroxylation process is taking place at low temperatures ($<20^\circ\text{C}$). Our experiments are taking place at high temperatures (85°C) so that reactions of geologic timescales can be observed in a laboratory setting. Additional discussion has been added to clarify these points (Lines 344 – 356).

Line 270 – “4.1” changed to “4.2”

Comment — Possibly; however the authors do not reference any literature that suggests pore waters of oceanic sediments approach or are at silica saturation, obfuscating the real world validity of this position. This statement is therefore a supposition by the authors in order to progress their interpretations.

References pertaining to pore waters and silica saturation have been added (Lines 335 – 337).

Comment — While the authors provide substantial support for the idea of dehydroxylation as an influencing factor, they do not sufficiently demonstrate that the precipitation of additional SiO_2 occurs in any substantial volume. It is purely theoretical based on equilibrium reactions, and these data do not demonstrate such precipitation occurs.

See above (reviewer #1 comments). Although it is impossible to quantify the amount of silica precipitated/condensed, we have taken care to discuss this as clearly as possible (Lines 329 – 331; 383 – 388).

Reviewer #3: (Mark Brandriss, Smith College)

These are interesting and novel experiments, and the results should be of interest to anyone who studies the climatic and environmental records preserved by oxygen isotopes in diatomaceous sediments. The experiments are well designed and the authors make a convincing argument that they've identified specific structural changes associated with rapid alteration of $\delta^{18}\text{O}$ values of diatomaceous silica during the early stages of sediment maturation. This study thus

provides valuable new experimental constraints on the isotopic and structural changes that occur during maturation and diagenesis of diatomaceous opal in natural environments, and I therefore believe that it's worthy of publication in *Geochimica* (which has a long history of publishing research pertinent to this subject). There are, however, some aspects of the discussion and interpretations that I think should be addressed and/or clarified, and I therefore recommend that the authors make some revisions to the manuscript.

First, I find it difficult to pin down the authors' explanation for the actual MECHANISM of isotopic alteration. In the abstract, the authors claim to "present the first experimental data that demonstrate a physical mechanism by which the oxygen isotope ($\delta^{18}\text{O}$ values) of biogenic silica (diatoms) are altered during early diagenesis"; the remainder of the abstract seems to imply that the loss of silanol is somehow the mechanism. In their discussion, however, they make the case (persuasively) that dissolution and reprecipitation reactions are involved, culminating in the statement: "We propose that dehydroxylation of bSiO_2 and precipitation of additional SiO_2 coatings during sedimentation is the primary mechanism for diagenetic alteration of bSiO_2 $\delta^{18}\text{O}_{\text{sil}}$ values during sedimentation." It's easy to see how diagenetic precipitation of new SiO_2 would be a plausible mechanism for altering the isotopic compositions of diatoms in such a way that they would approach isotopic equilibrium with ambient water during aging. In contrast, it's not clear how dehydroxylation would produce this specific result. Although the loss of isotopically distinct silanol oxygens could certainly change the isotopic composition of the remaining sample, it's not clear why this mechanism should produce the specific values that are observed experimentally (i.e. why loss of silanol should cause the remainder of the sample to re-equilibrate with the water at the temperature of the aging experiment). To summarize, although the authors have convincingly demonstrated that the decrease in silanol abundance is SYNCHRONOUS with a large change in isotopic composition, it doesn't seem to me that they've established it as a causal MECHANISM for the change in isotopic composition. Is it envisioned that the decrease in silanol abundance is directly related to replacement of primary (silanol-rich) silica by secondary (silanol-poor) silica, via dissolution and reprecipitation? If so, it would be good to explain this more clearly and directly.

These points have been clarified throughout the discussion (lines 287– 296; 312 - 324; 329 – 348; 383 – 388)

Following on these points, I can't fully understand the authors' explanations for why the bulk silica $\delta^{18}\text{O}$ should reach quartz-water equilibrium with the ambient fluid during experimental aging. Wouldn't this imply equilibration of ALL oxygen in the silica, including the siloxane oxygen?

This has been clarified (Lines 383 – 388).

If so, then it seems like this would imply some combination of: 1) siloxane oxygen exchanging with the water at 85°C, as seemingly suggested by the phrase "isotopic exchange" in lines 277-282, and/or: 2) new silica being deposited in isotopic equilibrium during dissolution-reprecipitation reactions in the silica-saturated solution, as discussed in lines 303-311. The discussion appears to emphasize the latter, citing "precipitation of additional SiO_2 coatings during sedimentation" as a primary cause for diagenetic changes in $\delta^{18}\text{O}$ — but presumably the precipitation of COATINGS would leave the original siloxane intact. If the original siloxane remains unaffected by diagenetic exchange (e.g. lines 316-320), then it's not clear why the BULK oxygen of the sample should approach isotopic equilibrium with the water during the aging process. On the other hand, could the isotopic similarity between the experimentally aged samples and the equilibrium quartz-water value be coincidental?

For example, what if the original siloxane oxygens incorporated during growth at 19.3°C were

preserved during aging, and ended up mixed with low- $\delta^{18}\text{O}$ oxygen (i.e. the high-temperature end of the Dodd & Sharp 2010 curve) that was incorporated or added during the aging experiment, e.g. via post-mortem isotope exchange, or precipitation of silica? Could this mixture produce a value that is coincidentally close to quartz-water equilibrium? If so, then the implications would be quite different. In any case, I feel that these points require further examination (perhaps I'm simply missing something, in which case further explanation would suffice; or if my comments are actually insightful, then perhaps some of the interpretations should be reconsidered).

The reviewer makes some very good points that we have addressed with additional discussion and reconsidered our interpretations accordingly (Lines 329 – 331; 345 – 362; 376 – 405).

As a follow-up to the previous points: if the samples ARE actually reaching silica-water isotopic equilibrium during the experimental aging process, then wouldn't that imply that nothing remains of the original isotopic signal acquired during growth?

We have clarified these points (Lines 383 – 388).

Or, in the natural analogue, that the final isotopic composition is determined entirely by diagenetic conditions (e.g. Dodd et al., 2012)? If this is among the authors' conclusions, they should state it more directly and emphatically.

We have clarified these points (Lines 383 – 388; 403 – 405).

I also recommend a more detailed examination and discussion of the observed trends in silanol loss over time. For example:

1) Following the very rapid and dramatic loss of silanol during the first 0.5 days of experimental aging, the data in Figure 3 show a continuing (though far more gradual) decrease in silanol as time passes during the longer experiments. If the loss of silanol is related to the mechanism responsible for the changes in O isotope compositions, then wouldn't it be expected that oxygen isotope values should undergo a similarly gradual change after the initial rapid drop? The isotope data in Figure 3 are somewhat erratic, but such a change DOES appear to be crudely discernible: the average $\delta^{18}\text{O}$ values at 20 and 37 days are distinctly lower than at all times <10 days (with the exception of the single anomalous value at 4 days). The authors seem understandably reluctant to attach real significance to these isotopic variations, and instead seem to attribute them to variations in the experimental conditions (i.e. manuscript lines 252-255); as a result, they simply draw a straight line on Figure 6 representing the average isotopic composition of all aged samples. But if the removal of silanol really is a mechanism for changing the oxygen isotope composition, then the silanol data in Figure 4 imply that $\delta^{18}\text{O}$ SHOULD continue to drop gradually as silanol abundance decreases slowly, in which case the hints of a discernible isotopic trend in Figure 3 should be addressed. For example, if the isotopic shift produced by the rapid loss of silanol during the first half-day were extrapolated (based on the total silanol curve in Figure 4), what would be the projected trend on the isotopic plot in Figure 3? This would be useful for evaluating the effects of diagenesis at geologic time scales, i.e. periods of more than 5 years. Perhaps Figure 3 could include such an inferred curve (i.e. theoretical prediction based on measured silanol abundances) in addition to the existing straight line (i.e. conservative fit to noisy data).

Variation in the experimental $\delta^{18}\text{O}_{\text{sil}}$ values have been discussed in much greater detail (lines 271 – 296). As discussed above (Reviewer #1), the measured $\delta^{18}\text{O}_{\text{w}}$ values for cultured and experimental aging waters have been included in Table 2. An additional table (Table 3) has been added to clarify how the 1000ln α calculations are affected by variability in the

$\delta^{18}\text{O}_w$ values in the experimental water and distinguish between results (Table 2) and interpretations (Table 3) $\delta^{18}\text{O}_w$ values are reported more clearly in the results section (3.2; Lines 207 - 211) and discussed in Lines 271 – 296. However, we feel that adding a theoretical curve to Figure 3 could be confusing. As the reviewer pointed out, these data reflect the conservative estimate, and the figure is intended to present the change in the measured $\delta^{18}\text{O}_{\text{sil}}$ values without conflating those values with an interpretation. However, the reviewer does bring up a very good point about using the measured silanol abundance to “predict” the $\delta^{18}\text{O}_{\text{sil}}$ values. These calculations, though perhaps useful, are beyond the scope of our data – the theoretical relationship will be highly dependent on the difference between the growth and diagenetic water. We believe making these calculations with the data we have would be largely speculative, and perhaps not universally applicable.

2) Following on the previous point, the essentially instantaneous loss of 2/3 of the silanol oxygen during aging (i.e. <0.5 days at 85°C, <5 years at 4°C) implies that at geologically interesting timescales (say, >103 years) the growth signal should be completely swamped by the diagenetic signal. A quick visual extrapolation of the silanol trend in Figure 3 suggests that after 100 days of aging (or ~1000 years at 4°C), less than 10% of the original silanol will remain. This brings me back to one of the points I raised earlier: would this imply that, over the timescales of interest, the original isotopic signature acquired during growth is overwhelmed by diagenetic effects, to that point the original signature is (for all practical purposes) entirely lost? If so, the authors should explain this more clearly — including in the abstract, which concludes with the somewhat vague statement that “the $\delta^{18}\text{O}$ of diagenetic water needs to be addressed” when using interpreting isotopic data from natural sediments (and the final sentence of the Conclusion is similarly vague and non-committal).

We have discussed these points (Lines 383 – 388) and committed to our interpretation (Lines 403 – 405; 409 - 410).

Some detailed comments keyed to the text and figures:

* Lines 40-41: delete extra parentheses.

This change has been made (Line 40 – 42).

* The text repeatedly refers to Smith et al. (2016), whereas it's Smith et al. (2016) in the list of references and at the publisher's website.

Changed to Smith et al. (2016) throughout the text.

* Line 74 should probably read “...for up to 37 days...”

This change has been made (Line 74).

* Line 135: delete “have”

This change has been made (Line 143).

* Line 158: delete “during”?

This change has been made (Line 166).

* Line 167: delete extra semicolon.

This change has been made (Line 175).

* Line 186: the bending peak at 147 cm^{-1} is not visible in the figure (i.e. it's beyond the spectral range)

This change has been made. Revised manuscript Lines 195 – 199.

* Lines 197-198: I suggest that the isotopic compositions of the growth water and aging water be reported in the "Methods" section rather than the "Results" section.

The authors have opted to leave these results in section 3.2. We have clarified variations in the culture and experimental water $\delta^{18}\text{O}$ values are an important component of the results (see reviewer 1 & 2 comments). Revised manuscript Lines 205 – 2011.

* Line 241: should the reference to Figure 3 be moved to the end of the previous sentence?
This change has been made. Revised manuscript Line 255.

* Line 249: should this be Fig. 6 instead of Fig. 5?
This change has been made. Revised manuscript Line 265.

* Lines 262-265: the reasoning behind this statement seems unclear to me.
This statement has been moved to later in the discussion (Lines 391 – 395) and the reasoning discussed at length (lines 370 – 405).

* Line 281: "have been" is repeated.
This statement has been replaced (see reviewer #1 comments above) lines 317 – 320.

* Line 282: should read "...flow-through experiments at..."?
This statement has been replaced (see reviewer #1 comments above) lines 317 – 320.

* Line 313: should be "progresses" instead of "progress"?
This section has been revised and the wording changed

* Figure 2: numbers on x-axis are offset from tick marks
The figure has been fixed.

* Figure 4: The units on the Y-axis need clarification; does it represent "Silanol Density (% of initial)"?
Figure 4 and caption (Lines 617 - 622) have been clarified and modified. Silanol density is now represented as silanol abundance.

* Figure 4: lines 530-532 seem to have grammar and/or punctuation errors.

* Figure 5: 2nd sentence (lines 536-538) needs editing.
These changes have been made Lines 617 – 624.

* Figure 6: The fractionation factor for Brandriss et al. (1998) is cited in the caption but not shown on the figure.

* Figure 6: In the caption, lines 544-546 are confusing.

* Figure 6: On the graph, it seems misleading to show (hypothetical) data points for the aged samples at 19.3°C, since the aging was done at 85°C. If the authors want to emphasize that temperature change by itself cannot account for the isotopic effects during aging, then it would seem more logical to simply note that the data after aging do not plot on the high-temperature end of the Dodd and Sharp (2010) curve.

Figure 6 and caption (lines 630 – 637) reflect these changes.

Dehydroxylation and Diagenetic Variations in Diatom Oxygen Isotope Values

Justin P. Dodd^{1*}, Wilson Wiedenheft¹, and Joshua M. Schwartz¹

Abstract

Numerous studies have documented changes in the dissolution and reactivity of biogenic silica as it is transferred from the water column to sediment archives; here we present the first experimental data that demonstrate a physical mechanism by which the oxygen isotope ($\delta^{18}\text{O}_{\text{sil}}$) values of biogenic silica (diatoms) are altered during early diagenesis. The $\delta^{18}\text{O}_{\text{sil}}$ value of diatom silica cultured at 19.3°C was $+31.9\text{‰} \pm 0.2\text{‰}$ ($n = 6$); the same silica experimentally aged in an artificial seawater media at near silica saturation at 85°C had an average $\delta^{18}\text{O}_{\text{sil}}$ value of $+27.1\text{‰} \pm 0.6\text{‰}$ ($n=20$). The most significant change in the $\delta^{18}\text{O}_{\text{sil}}$ value was coincident with an initial reduction in the total silanol abundance, indicating that the timing of dehydroxylation reactions in natural sedimentary environments is associated with diagenetic changes in the recorded $\delta^{18}\text{O}_{\text{sil}}$ values. The rate of change in the experimental aging environment at 85°C was rapid, with significant changes in both silanol abundance and $\delta^{18}\text{O}_{\text{sil}}$ values. Additionally, the silica-water fractionation relationship recorded by the

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experimentally-aged samples approaches the equilibrium quartz-water fractionation factor. The linear rate law was used to estimate the timing of these changes in low temperature environments; the initial and most significant change in silica reactivity and $\delta^{18}\text{O}_{\text{sil}}$ values is likely to occur on the order of 10's of years at 4°C. Published silica-water fractionation factors for sedimentary diatoms most likely represent a combination of growth and diagenetic environments, and the $\delta^{18}\text{O}$ value of diagenetic water needs to be addressed when using $\delta^{18}\text{O}_{\text{sil}}$ values to reconstruct paleoceanographic and paleoenvironmental conditions.

1. INTRODUCTION

The preservation of biogenic silica ($b\text{SiO}_2$) in marine environments on geologic timescales is a topic of considerable interest and importance to the oceanographic and paleoclimate communities; as such, considerable effort has been devoted to the understanding of diagenetic changes observed in $b\text{SiO}_2$ from sediment archives and through laboratory experiments (e.g. Krauskopf 1956; Lewin, 1961; Lawson et al., 1978; Hurd, 1973; Hurd et al., 1981; Hurd et al., 1983; Barker et al., 1994; Tréguer et al., 1995; Koning et al., 1997; Rabouille et al., 1997; Icenhower and Dove, 2000; Dixit et al., 2001a,b; Dixit and Van Cappellen, 2002; Gallinari et al., 2002; Rickert et al., 2002; Van Cappellen et al., 2002; Demaster, 2005; Dove et al., 2008; Loucaides et al., 2008; Passow et al., 2011; Loucaides et al., 2012). Oxygen isotope ($\delta^{18}\text{O}_{\text{sil}}$) values of $b\text{SiO}_2$ have been proposed as a proxy for paleoclimate and paleoceanographic conditions (e.g. Mopper and Garlick, 1971; Labeyrie, 1974; Labeyrie and Juillet, 1982; Wang and Yeh, 1985; Juillet-Leclerc and Labeyrie, 1987; Matheney and Knauth, 1989; Shemesh et al., 1992; Swann and Leng, 2009); however, the utility of $\delta^{18}\text{O}_{\text{sil}}$ values from diatom frustules in reconstructing temperature and water oxygen

isotope ($\delta^{18}\text{O}_w$) variations in both marine and freshwater environments has been limited by potential changes in $\delta^{18}\text{O}_{\text{sil}}$ values that may be the result of diagenetic processes (e.g. Labeyrie and Juillet, 1982; Schmidt et al., 2001; Moschen et al., 2006; Dodd et al., 2012; Smith et al., 2016) and discrepancies in the apparent silica-water fraction factor between fresh/recent $b\text{SiO}_2$ (e.g. Brandriss et al., 1998; Dodd and Sharp, 2010) and sedimentary records (e.g. Juillet-Leclerc and Labeyrie, 1987; Matheney and Knauth, 1989; Shemesh et al., 1992). $b\text{SiO}_2$ is known to undergo a number of physical and chemical changes during sedimentation (e.g. dissolution, dehydroxylation, structural condensation), yet the effects of these processes on $\delta^{18}\text{O}_{\text{sil}}$ values have not been well constrained (e.g. Schmidt et al., 1997). Alteration of the $\delta^{18}\text{O}_{\text{sil}}$ values by post-mortem process must be accompanied by a physical exchange or loss/addition of oxygen atoms to the silica structure, and no mechanism has been proposed that adequately addresses the physical process by which $\delta^{18}\text{O}_{\text{sil}}$ values can be overprinted through diagenetic processes at low temperatures and over short time frames ($<10^2$ years; e.g. Smith et al., 2016). $b\text{SiO}_2$ is composed of two types of oxygen bonds: 1) siloxane bonds ($>\text{Si}-\text{O}-\text{Si}<$) that, once formed, are not likely to undergo isotopic exchange until a phase change occurs (e.g. Murata et al., 1977), and 2) less stable silanol groups ($\text{Si}-\text{OH}$) that may experience isotopic exchange during the above mentioned diagenetic processes (e.g. Juillet-Leclerc and Labeyrie, 1987).

Dissolution and dehydroxylation of the diatom silica has been documented in sedimentary diatoms, resulting in a decline of silica surface reactivity driven by reduction of silanol groups through conversion to siloxane bonds and increased structural ordering (Van Cappellen and Qui 1997a,b; Zhuravlev 2000; Schmidt et al., 2001; Carroll et al., 2002;

Rickert et al., 2002; Dixit and Van Cappellen, 2002; Loucaides et al., 2010). Although oxygen isotopic exchange during dissolution is one potential mechanism for modifying $\delta^{18}\text{O}_{\text{sil}}$ values of fresh diatom silica during transfer from the water column to the sediments, the measured effects of dissolution on $\delta^{18}\text{O}_{\text{sil}}$ values of sedimentary diatoms appears to be relatively small (Moschen et al., 2006; Smith et al., 2016). Precipitation of secondary silica and formation of additional siloxane bonds may also obscure the primary $\delta^{18}\text{O}_{\text{sil}}$ values (e.g. Rickert et al., 2002; Loucaides et al., 2010).

Here we present experimental data from cultured marine diatom silica (*Stephanopyxis turris*) that were subsequently aged in a controlled laboratory environment for up to 37 days at 85°C. Rapid decreases in the surface charge and silanol abundance of the cultured diatom silica are coincident with significant exchange of silica bound oxygen and altered $\delta^{18}\text{O}_{\text{sil}}$ values in the aged $b\text{SiO}_2$ that are in close agreement with the silica-water fractionation relationship reported by Juillet-Leclerc and Labeyrie (1987) and theoretical quartz-water equilibrium fractionation (Sharp and Kirschner, 1994). Dehydroxylation/condensation of silanol bonds, therefore, presents a mechanism for alteration of $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values during diagenesis and over geologic timescales. These observed variations in $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values may also explain the disparate silica-water fractionation factors published for $b\text{SiO}_2$ from culture experiments and sedimentary archives.

2. METHODS

2.1 Culturing Biogenic Silica

Cultures of *S. turris* (Fig. 1; L1 media; National Center for Marine Algae and Microbiota labs) were grown under controlled laboratory conditions where temperature, water

($\delta^{18}\text{O}_w$) values, and pH were periodically monitored. Batches of ~12L of natural seawater (NSW) and f/2 culture medium (NSW/f2) from NCMA labs (Boothbay, ME) were mixed in 20L Pyrex® carboys and used as a growth media. During the exponential growth cycle, incubation temperatures were maintained at $19.3 \pm 0.5^\circ\text{C}$ with a pH of 8.0 (± 0.5). pH was maintained by adjusting the $p\text{CO}_2$ of a filtered airstream that also provided agitation. Diatom cultures achieved the stationary growth phase within 2 to 3 weeks, at which point the culture was harvested. Harvesting was accomplished by filtering the living diatom frustules and immediately removing organic material with 30% H_2O_2 following a modified procedure of Morley et al. (2004). Cultured diatom frustules were rinsed in deionized (DI) water and centrifuged to remove excess salts. Organic material was removed with 30% H_2O_2 at room temperature (~12hr) followed by 15M HNO_3 for 4 hours at 65°C . Samples were rinsed (3x) with DI water after each chemical treatment. The cleaned diatoms were freeze-dried and stored in a desiccation cabinet. Approximately 150mg of bulk $b\text{SiO}_2$ from one *S. turris* culture was used in the aging experiments. Repeat aging experiments at 0.5 and 1 day were conducted on $b\text{SiO}_2$ from a second culture to examine $\delta^{18}\text{O}_{\text{sil}}$ variability during the early phase of the aging experiment. Frustules were crushed with a mortar and pestle (to $\sim 10\mu\text{m}$ $\varnothing$) to reflect fragmented frustules commonly found in sedimentary deposits (e.g. Scherer et al., 2004) and to homogenize $\delta^{18}\text{O}_{\text{sil}}$ variability in the $b\text{SiO}_2$ produced during the culture phase.

2.2 Aging Experiments

S. turris frustules were transferred to 50ml polypropylene reaction vessels and aged at 85°C to accelerate diagenetic changes that normally occur over geologic time scales. The aging media was prepared using deionized water (DI), boiled for 5 minutes to reduce

dissolved CO₂, and then mixed to create a 0.7 M NaCl solution. Na₂SiO₃ was added to form a 1750 µM silicate solution to limit silica dissolution (Loucaides et al., 2010). *S. turris* bSiO₂ (15 to 20 mg) was batch aged in 30 mL of aging media at a pH of 8.0 for each aging period between 0.5 and 37 days, with repeat experiments at 0.5 and 1 day (9 individual reaction vessels). To reduce isotopic exchange between the aging media and water vapor, the headspace was purged with argon prior to sealing the reaction vessel. Oven temperatures were monitored throughout the aging experiment (0.5 to 37 days) with a maximum range in air temperature of ± 0.6°C and water temperature of ± 0.3°C. After completion of each aging period, the diatom silica was separated from the aging media by filtering the suspension through a 5 µm sieve cloth and rinsed with hot DI at 85°C to prevent polymerization and precipitation of silica from the saturated aging media (Kastner et al., 1977). After rinsing, the recovered diatoms were air dried at 60°C for about 2 hours. During the aging experiments, bSiO₂ was lost to dissolution and/or during transfer procedures; it was not possible to calculate the amount of dissolution of bSiO₂ during the experimental aging process.

2.3 Surface Charge Density

The evolution of the bSiO₂ surface chemistry during the aging experiments was measured through potentiometric (acid-base) titrations at a pH endpoint of 7.5 following the procedure outlined by Loucaides et al. (2010). At the end of each aging period, aliquots of bSiO₂ were collected and titrated at 25 ± 0.5°C in a 10mL Polypropylene reactor vessel equipped with a tight fitting Teflon cover and a Teflon-coated magnetic stir bar. Measurements were performed with a Corning 455 pH/ion analyzer calibrated using commercial buffer solutions. The diatom suspension was continuously stirred and purged with

high-purity argon gas injected at the approximate rate of 100 SCCM. Acid and base titrants were added via two Metrohm Dosimat burets containing 0.02 M HCl and NaOH. The NaOH molarity was determined using potassium hydrogen phthalate (KHP) titration. Each aging sample was titrated in a 0.7 M NaCl electrolyte with a silica suspension density in the range of 0.5 to 1.5 g L⁻¹. The specific density was selected based on the anticipated surface charge density, with a resulting impact on precision. Dissolution of diatoms during titration was minimized by the addition of Na₂SiO₃ • 5 H₂O to the 0.7 M NaCl electrolyte to form a 600 µM dissolved silicate solution. Diatom surface area measurements are required when comparing different species with different surface area properties; however, this study utilized a single cultured diatom silica source (*S. turris*) and the average diatom surface area measurements were not necessary. Surface charge density, therefore, is reported as excess H⁺ in µmoles/g instead of µmoles/m² where high surface charge densities are represented by lower (negative) numbers.

2.4 Internal Silanol Abundance

Silanol groups on the surface of the *b*SiO₂ are in contact with external fluids and can be measured through the acid-base titrations described above; progressive changes of the relative number of total silanol bonds in cultured and aged *S. turris* silica were measured using a Mattson ATI Genesis Series FTIR equipped with a Pike MIRacle attenuated total reflection (ATR) device. The ATR allows samples in the solid state to be examined directly using evanescent waves through a diamond crystal plate. The FTIR scan was set to 1400 to 600 cm⁻¹ because the diamond crystal limits sensitivity to spectrum below 600 cm⁻¹. A dry diatom silica sample of about 1 mg was placed on the ATR crystal plate and clamped for

measurement under atmospheric conditions. Integration of the area under the bond absorption peaks was performed using the WinFIRST software supplied with the spectroscope. The internal silanols trapped within the porous structure of *bSiO₂* during formation are then calculated as the difference in the total silanol abundance measured through FTIR and surface silanols measured through acid-base titrations.

2.5 Oxygen Isotope Analyses

Diatom $\delta^{18}\text{O}_{\text{sil}}$ analyses were conducted with the step-wise fluorination (SWF) – CO_2 laser heating described by Dodd and Sharp (2010) in the stable isotope lab at Northern Illinois University. *bSiO₂* (1 – 4 mg/sample) and quartz standards were loaded in a nickel holder and dehydrated *in vacuo* in the laser chamber (>24h) followed by step-wise increases of ~10 kPa of BrF_5 gas to remove hydroxyls prior to $\delta^{18}\text{O}_{\text{sil}}$ analyses (e.g. Hamison and Knauth, 1976; Dodd and Sharp, 2010). Initial studies suggested that BrF_5 would result in isotopic fractionation of the silica; however, an inter-laboratory comparison between F_2 and BrF_5 prefluorination techniques showed no significant differences in $\delta^{18}\text{O}_{\text{sil}}$ values produced by the two techniques (Chapligin et al., 2011). Prefluorination was considered complete when the $\delta^{18}\text{O}_{\text{sil}}$ values of internal *bSiO₂* standards SR2–1B ($\delta^{18}\text{O} = 32.2 \pm 0.2\text{‰}$, $n = 35$) and VT-8C ($\delta^{18}\text{O} = +28.6 \pm 0.2\text{‰}$, $n = 24$) reached a plateau with a reproducibility of $\pm 0.3\text{‰}$ between subsequent pre-fluorination steps (Dodd and Sharp, 2010). The inter-laboratory standard PS1772-8 ($\delta^{18}\text{O} = 42.8 \pm 0.2\text{‰}$) provided an additional check for reproducibility of $\delta^{18}\text{O}_{\text{sil}}$ values (Chapligin et al., 2011). *bSiO₂* samples were heated with a PhotonMachines Fusions 10.6 CO_2 laser in the presence of ~10 kPa of BrF_5 to generate O_2 and SiF_4 gas (e.g., Clayton and Mayeda, 1963; Haimson and Knauth, 1983; Sharp, 1990). Oxygen was transferred

176 cryogenically by adsorption on a zeolite trap to a Thermo MAT253 isotope ratio mass
177 spectrometer for analyses. $\delta^{18}\text{O}_{\text{sil}}$ values are reported in VSMOW and calibrated to
178 international quartz standard NBS-28 ($\delta^{18}\text{O} = +9.6 \pm 0.3\text{‰}$; $n=15$).

179 The oxygen isotope values of culture and experimental waters (media) were measured
180 on a GasBench coupled to a Thermo MAT253 at Northern Illinois University through CO_2
181 exchange and on a Picarro L2130-i Water Isotope Analyzer at Northwestern University. All
182 values are reported relative to VSMOW and calibrated using internal and IAEA water isotope
183 standards (VSMOW2, SLAP2, and GISP).

184 3. RESULTS

185 3.1 Silanol Abundance

186 Since surface charge density is measured as the protonation of the negatively charged
187 surface of the $b\text{SiO}_2$ ($>\text{SiO}^-$), the surface charge density is reported as a negative number (i.e.
188 as negative values approach zero, the surface charge density decreases). The initial (aging
189 days = 0) surface charge density of $-380 \pm 7 \mu\text{moles/g}$ in the cultured *S. turris* $b\text{SiO}_2$ declined
190 to $-244 \pm 17 \mu\text{moles/g}$ in 0.5 days at 85°C (Table 1). From 0.5 to 20 days at 85°C the surface
191 charge density continued to decline to a final charge density of $-16 \pm 13 \mu\text{moles/g}$. Surface
192 charge density at 37 days was below detection limits.

193 The relative abundance of total (internal and external) Si–OH bonds in the $b\text{SiO}_2$ were
194 measured using the ratio of the integrated area under FTIR peaks at 950 cm^{-1} and 800 cm^{-1}
195 (A_{950}/A_{800} ; e.g. Schmidt et al., 2001; Loucaides et al., 2010). The absorption spectra for the
196 $b\text{SiO}_2$ samples analyzed in this study (1200 cm^{-1} to 650 cm^{-1} ; Fig. 2) include the primary
197 vibrational bands associated with stretching of the Si–O–Si bonds (1100 cm^{-1}), stretching of

the Si–O bonds in the Si–OH groups (950 cm^{-1}), and vibration of the inter-tetrahedral Si–O–Si bonds (800 cm^{-1}). The highest A_{945}/A_{800} ratio (1.29) was observed in the cultured *bSiO₂* (aging = 0 days) and decreased throughout the aging experiment to 0.17 at aging day 37 (Table 1; Fig. 2). Conversely, the A_{800}/A_{1100} ratio remained constant throughout the aging experiments indicating that there was no observable change in the degree of organization in the *SiO₂* framework.

3.2 Oxygen isotope values

The $\delta^{18}\text{O}_w$ values for the NSW ($-0.8\text{‰} \pm 0.2\text{‰}$; $n=6$) and NSW/f2 culture medium ($-0.8\text{‰} \pm 0.4\text{‰}$; $n=4$) were identical prior to diatom culture growth. After growth, the NSW/f2 culture medium had $\delta^{18}\text{O}_w$ values of $-1.0\text{‰} \pm 0.1\text{‰}$; $n=3$). The average of all samples ($\delta^{18}\text{O}_w = -0.9\text{‰} \pm 0.2\text{‰}$; $n=12$; Table 2) captures all of the potential variation in the $\delta^{18}\text{O}_w$ values during the culture phase of the study. Experimental aging waters had an initial $\delta^{18}\text{O}_w$ value of $-1.9\text{‰} \pm 0.2\text{‰}$ ($n=2$); however, the $\delta^{18}\text{O}_w$ values changed throughout the 37-day aging experiment by $+2.1\text{‰}$ (-2.0 to $+0.2\text{‰}$; Table 2).

The $\delta^{18}\text{O}_{\text{sil}}$ value of *S. turis bSiO₂* cultured at 19.3°C was $+31.9\text{‰} \pm 0.2\text{‰}$ ($n = 6$) for samples all used in this study. Two cultures of *S. turis* were used, but the $\delta^{18}\text{O}_{\text{sil}}$ values were indistinguishable between the two cultures. *S. turis bSiO₂* subjected to aging experiments at 85°C had an average $\delta^{18}\text{O}_{\text{sil}}$ value of $+27.1\text{‰} \pm 0.6\text{‰}$ ($n=20$); a change of -4.8‰ in the average $\delta^{18}\text{O}_{\text{sil}}$ values occurred during the 37 day aging experiment at 85°C (Table 2; Fig. 3). Experiments at 0.5 and 1 day were repeated on *bSiO₂* from an independent batch of cultures

samples to confirm the observed change in $\delta^{18}\text{O}_{\text{sil}}$ values between the cultured *bSiO₂* and *bSiO₂* during the early stages of diagenesis (noted by *a* and *b* in Tables 1 – 3).

4. DISCUSSION

4.1. *bSiO₂* Dehydroxylation

The decrease in internal and external silanol abundance throughout the aging experiment is consistent with dehydroxylation in previous studies (e.g. Farmer, 1974; Gendron-Badou et al., 2003; Dixit and Van Cappellen 2002; Rickert et al., 2002; Moschen et al., 2006; Loucaides et al., 2010). Internal silanol abundance cannot be measured directly, and must be calculated from the total (FTIR) and external (titration) measurements. Silanol abundance is presented as a percent of the total silanols in the cultured *bSiO₂*, assuming an initial ratio of 10:1 (internal:external) for silanols in the cultured *bSiO₂* (Table 1; Fig. 4). The initial ratio is theoretically constrained from published data (Loucaides et al., 2010); however, it is important to note that the internal silanols represent the largest component of the silanol groups throughout the ageing experiment. As the *bSiO₂* undergoes dehydroxylation, higher-energy silanols are converted to lower-energy siloxane (i.e. condensation), which reduces the surface charge, *bSiO₂* reactivity, and total silanol abundance (e.g. Zhuravlev, 2000). The rapid initial decline in surface charge density is likely the result of a reduction in the abundance of vicinal silanols with lower activation energy. Other surface (isolated and germinal) silanols and internal silanols have higher activation energies, which causes dehydroxylation to occur

at a slower rate. If the experimental relationship continues as observed, the internal silanol abundance of the aged $b\text{SiO}_2$ would approach zero after ~150 aging days (Fig. 4).

Previous experimental data for diagenesis of $b\text{SiO}_2$ at low temperatures (e.g. $<20^\circ\text{C}$) at near neutral pH indicate limited potential for $\delta^{18}\text{O}$ variations (e.g. Smith et al, 2016; Moschen et al., 2006); therefore, in this study experimental temperatures of 85°C were chosen to examine changes in the silica reactivity and $\delta^{18}\text{O}$ values on geologic timescales. Although these temperatures are not likely in shallow sedimentary environments, the temperature dependence of dissolution/precipitation in $b\text{SiO}_2$ reactions is nearly linear at temperatures $<100^\circ\text{C}$ (e.g. Gunnarsson and Arnórsson, 2000, Loucaides et al., 2010). Using silanol properties reported for amorphous silica and the linear rate law (e.g. Rickert et al., 2002; Loucaides et al., 2012), dehydroxylation reaction rates observed in this study can be calculated at lower temperatures (i.e. 4°C) more commonly observed in shallow marine sediments. The Arrhenius law is often used as an expression of the temperature dependence of dissolution/precipitation in $b\text{SiO}_2$ reactions and is discussed at length in previous studies (e.g. Rickert et al., 2002). In short, the Arrhenius law is governed by the general equation:

$$k_d = Ae^{-E_d/RT} \quad (1)$$

where k_d is the rate coefficient that incorporates the temperature dependence of the dehydroxylation reaction, A is the frequency factor, E_d is the activation energy, $R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ and T = temperature ($^\circ\text{K}$). The activation energy for dehydroxylation of amorphous silica varies, ranging between 69 and 209 kJ/mole, depending on the type of silanols present (Fig. 5; Zhuravlev, 2000). The surface of amorphous silica consists of 61% vicinal silanols

with an average activation energy of 87 kJ/mole (69 to 105 kJ/mole), and 39% isolated/geminal silanols with a higher activation energy of 157 kJ/mole (105 to 209 kJ/mole). Using the average activation energies for the different silanols, the reaction rates can be recalculated using the following equation, where $T_1 = 277$ K (4°C) and $T_2 = 358$ K (85°C):

$$\ln (k_2/k_1) = E_d/R [1/T_1 - 1/T_2] \quad (2)$$

For vicinal silanols, where $E_d = 87$ kJ mole⁻¹ and $k_2/k_1 = 5,153$, one aging day at 85°C is equivalent to 5,153 days (~10¹ years) at 4°C (Fig. 3). The geminal and isolated silanols (ave. $E_d = 157$ kJ/mole) yield $k_2/k_1 = 4.9 \times 10^6$ which is ~10⁴ – 10⁶ years. Our results mirror other studies that have noted measurable Si-OH (silanol) peaks in FTIR spectra of sedimentary *bSiO₂* that is on the order of 10³ years old (e.g. Schmidt et al., 2001; Moschen et al., 2006; Dodd et al., 2012).

4.2. Diagenetic variation in *bSiO₂* δ¹⁸O values

The cultured *S. turris bSiO₂* from this study ($T = 292.5$ K; $\delta^{18}\text{O}_w = -0.9\text{‰}$) has a measured silica-water fractionation factor ($\alpha_{\text{silica-water}} = 1.0329$) that is identical to that reported for other diatom silica from cultured and modern natural environments (e.g. Dodd and Sharp, 2010; Brandriss et al., 1998). Changes in the $\delta^{18}\text{O}_w$ values throughout the 37-day aging experiment (from -2.0 to +0.2‰; Table 2) are likely due to evaporation, and the $\delta^{18}\text{O}_w$ value measured at the end of each aging period has been used to calculate the silica-water fractionation factor. The average silica-water fractionation factors for the experimentally-aged silica ($T=358.2$ K; ave. $\delta^{18}\text{O}_w = -1.2\text{‰}$; ave. $\alpha_{\text{silica-water}} = 1.0284$) approach the quartz-water equilibrium conditions (Kita et al., 1985; Sharp and Kirschner, 1994; Table 3; Fig. 6). The largest

280 observed decrease in $\delta^{18}\text{O}_{\text{sil}}$ values (-3.8‰ in 0.5 days) during the aging experiment
 281 coincides with the initial rapid decline in silanol density driven by the dehydroxylation of the
 282 cultured $b\text{SiO}_2$. The total range of $\delta^{18}\text{O}_{\text{sil}}$ values between the cultured diatom silica and the
 283 lowest experimental $\delta^{18}\text{O}_{\text{sil}}$ value is 5.6‰ . Although all cultured $\delta^{18}\text{O}_{\text{sil}}$ values were within
 284 the standard analytical error ($\pm 0.2\text{‰}$), repeat aging experiments at 0.5 and 1 day recorded
 285 $\delta^{18}\text{O}_{\text{sil}}$ values that were different by as much as 1‰ (Table 2); lower $\delta^{18}\text{O}_{\text{sil}}$ values were
 286 observed in samples from the second culture batch (0.5*b* and 1*b*) and could reflect different
 287 frustule growth (e.g. silica density) between the two cultures. Although the measured $\delta^{18}\text{O}_{\text{sil}}$
 288 values (and calculated $\alpha_{\text{silica-water}}$; Table 3) generally continue to decrease throughout the aging
 289 experiments, there is notable variability throughout the aging experiments (e.g. day 4 and 20).
 290 Additional work is needed to “calibrate” the effects of decreasing silanol abundance on the
 291 measured $\delta^{18}\text{O}_{\text{sil}}$ values; however, our data suggest that changes in the $\delta^{18}\text{O}_{\text{sil}}$ values will
 292 asymptotically approach quartz-water equilibrium until no measureable silanol remains. For
 293 sedimentary $b\text{SiO}_2$ with measureable silanols (e.g. Si-OH peaks via FTIR) it is likely that the
 294 measured $\delta^{18}\text{O}_{\text{sil}}$ values represent a combination of the growth and diagenetic conditions,
 295 which may explain some of the variability in published silica-water fractionation curves (e.g.
 296 Matheney and Knauth, 1989; Schmidt et al., 2001; Dodd and Sharp, 2010).

297 Although not a primary goal of this research, the $\delta^{18}\text{O}_{\text{sil}}$ values recorded by the
 298 cultured *S. turris* $b\text{SiO}_2$ indicate that the same silica-water fractionation relationship observed
 299 in modern/cultured freshwater diatoms (e.g. Dodd and Sharp, 2010) is also valid for marine
 300 diatom species growing in higher salinity water. Therefore, the apparent difference in the

silica-water fractionation relationship reported by Juillet-Leclerc and Labeyrie (1987) and modern/cultured diatoms (e.g Dodd and Sharp, 2010; Brandriss et al., 1998) is not an artifact of freshwater, marine, or cultured environments.

Oxygen isotope exchangeability in diatom $b\text{SiO}_2$ was initially reported by Labeyrie (1979) and discussed thermodynamically by Labeyrie and Juillet (1982). These studies and subsequent examinations of oxygen isotope exchangeability in $b\text{SiO}_2$ focused on environments that were significantly undersaturated with respect to dissolved silica and the exchange reactions were driven by dissolution kinetics. Although many of these studies have focused on freshwater environments (e.g. Moschen et al., 2006; Dodd and Sharp, 2010; Smith et al., 2016). Dissolution of $b\text{SiO}_2$ appears to be damped by the presence of dissolved salts and other cations (e.g. Al), and there is considerable variability observed in $b\text{SiO}_2$ dissolution rates in natural systems (e.g. Rickert et al., 2002). If dissolution is the only process acting on $b\text{SiO}_2$, dissolution should result in the formation new external silanols until the $b\text{SiO}_2$ is completely dissolved or suspended in colloidal form (e.g. Williams et al., 1985; Williams and Crerar, 1985); however, changes in the abundance of external silanols are obviated by the removal of the hydroxyl oxygen during the prefluorination process (Dodd and Sharp, 2010). Dissolution experiments of diatoms other $b\text{SiO}_2$ material (i.e. phytoliths) resulted in a significant change (up to + 4 to +5‰) in the $\delta^{18}\text{O}_{\text{sil}}$ values, which has been attributed to a selective removal of ^{16}O rich hydroxols; however, it is not clear how this kinetic effect is preserved in the siloxane (tetrahedral) oxygen (Prentice and Webb, 2016). Continued experimental work needs to be undertaken to address how changes in salinity, cation concentrations, and other variables relate to the timing and magnitude of diagenetic alteration

of $b\text{SiO}_2$ in natural settings; however, these processes are outside the scope of the current manuscript.

The oxygen isotope exchange reported in this study was conducted near silica saturation, where molecular-scale dissolution and precipitation of $b\text{SiO}_2$ occur simultaneously (e.g. Williams et al., 1985). Other studies have shown that once silica saturation has been reached, new silica is precipitated with $\delta^{18}\text{O}_{\text{sil}}$ values that appear to follow equilibrium silica-water fractionation (Prentice and Webb, 2016). Although the proportion of original versus secondary siloxane cannot be determined for this study, both dehydroxylation and reprecipitation results in the formation of new siloxane bonds (Table 1; Fig. 2 & 4). In contrast to experimental environments where dissolution kinetics are the dominant control, the experimental conditions in this study more closely mimic sea floor/sedimentary conditions, where a decrease of several orders of magnitude in the reactivity of the $b\text{SiO}_2$ is observed (e.g. Dixit et al 2001a&b; Rickert et al., 2002; DeMaster, 2005). Decreases in $b\text{SiO}_2$ reactivity during sedimentation (e.g. Zhuravlev, 2000; Gunnarsson and Arnórsson, 2000; Rickerts, et al., 2002; Loucaides et al., 2010) are in agreement with observed decreases in silanol density and coincident changes in $\delta^{18}\text{O}_{\text{sil}}$ values in this study, which provide the first empirical evidence that dehydroxylation is accompanied by oxygen isotope exchange. We propose that dehydroxylation of $b\text{SiO}_2$ and precipitation of additional SiO_2 during sedimentation is the primary mechanism for diagenetic alteration of $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values during sedimentation. Of particular interest is the role of internal silanols that may be trapped inside the $b\text{SiO}_2$ structure (Fig. 5). Our assumption that the initial ratio internal to external silanols was ~10:1, seems to be supported by the final (37 day) measurement where external silanols were below

detection and internal silanols were at ~13% of the initial silanol abundance. The dehydroxylation/condensation, by which internal silanols are converted to siloxane bonds, combined with reprecipitation of dissolved silica likely results the changing apparent silica-water fraction seen throughout 37-day aging experiment.

Variability in the apparent silica-water fraction factor within the Juillet-Leclerc and Labeyrie (1987) dataset is also potentially explained by this process. Shemesh et al. (1992) proposed a different silica-water fractionation factor (Fig 6) based on a subset of the Juillet-Leclerc and Labeyrie (1987) dataset from high-latitude marine cores; however, these variations may be the result of diagenetic timing rather than a fundamentally different silica-water fractionation relationship for high-latitude $b\text{SiO}_2$ (e.g. Clayton and Shemesh, 1992). Rickert et al. (2002) demonstrate variable kinetic controls of $b\text{SiO}_2$ reactivity in Southern Ocean and Norwegian Sea sediments, and the strong correlation between the initial changes in silanol abundance and $\delta^{18}\text{O}_{\text{sil}}$ values in our experimental data suggest a strong link between $b\text{SiO}_2$ reactivity and diagenetic control on $\delta^{18}\text{O}_{\text{sil}}$ values. Equilibration of $\delta^{18}\text{O}_{\text{sil}}$ with benthic conditions may resolve some of the discrepancies observed in marine sediment $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ and surface water $\delta^{18}\text{O}$ values at high latitudes (e.g. Shemesh et al., 1992; Crespin et al., 2014) and resolve unanswered questions about the relationship between the silica-water and carbonate-water oxygen isotope fractionation (e.g. Clayton and Shemesh, 1992).

The experimental reactions in this study were conducted at higher temperatures than are expected in most marine sediment records so that changes in $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values over geologic-time scales ($>10^3$ yrs) can be explored. In our study, the aged $b\text{SiO}_2$ has lower $\delta^{18}\text{O}_{\text{sil}}$ values, because equilibrium fractionation of the oxygen isotope values results in less offset

(smaller $\Delta^{18}\text{O}$; Table 3) between the water and the silica as temperatures increase. The decreased silica-water fractionation factor ($1000 \ln \alpha$) in the experimental $b\text{SiO}_2$ relative to the cultured diatom $b\text{SiO}_2$ is predicted by equilibrium isotope fractionation (e.g. Sharp and Kirschner, 1994). In sedimentary environments where diagenetic and growth temperatures are similar, changes in $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values in the sediments often result in an increase in the $1000 \ln \alpha$; our results predict that the fractionation factor from fresh $b\text{SiO}_2$ (e.g. Dodd and Sharp, 2010; Brandriss et al., 1998) should increase to that of sedimentary opal (e.g. e.g. Juillet-Leclerc and Labeyrie, 1987) or quartz-water equilibrium (e.g. Sharp and Kirschner, 1994) at low temperatures (Fig. 6).

It is important to note that the change in the silica-water fractionation factor reflects more than reequilibration of $\delta^{18}\text{O}_{\text{sil}}$ values at 85°C . Our experimental data show that there is a fundamentally different silica-water fractionation relationship between fresh/cultured $b\text{SiO}_2$ and $b\text{SiO}_2$ from sedimentary archives (Table 3; Fig. 6). Although the apparent silica-water fractionation in the aged $b\text{SiO}_2$ continues to change throughout the aging experiment (Table 3; Fig. 6), the lowest measured $\delta^{18}\text{O}_{\text{sil}}$ values (aging days 4, 20 and 37) reflect equilibrium quartz-water fractionation (Sharp and Kirscher, 1994), not the silica-water fractionation relationship seen in the cultured samples (of Dodd and Sharp, 2010). Intermediate silica-water fractionation factors reflect a combination of the growth $\delta^{18}\text{O}_{\text{sil}}$ values and aged $\delta^{18}\text{O}_{\text{sil}}$ values; however, the equilibrium quartz-water fractionation can only be achieved if the original $\delta^{18}\text{O}_{\text{sil}}$ values are completely overprinted during diagenesis through dehydroxylation/condensation and precipitation of additional silica (i.e. secondary siloxane >

primary siloxane). Although the most significant change in the $\delta^{18}\text{O}_{\text{sil}}$ values occurs rapidly (0.5 days), small changes in the $\delta^{18}\text{O}_{\text{sil}}$ values and the evaporative enrichment increase in $\delta^{18}\text{O}_{\text{w}}$ values, results in silica-water fractionation factors ($1000 \ln \alpha$) that continue to change until dehydroxylation is complete. One implication of these results is that silica-water fractionation relationship reported for core top/shallow sedimentary $b\text{SiO}_2$ (e.g. Juillet-Leclerc and Labeyrie, 1987; Shemesh et al., 1992; Schmidt et al., 2001) may represent an incomplete dehydroxylation and oxygen isotope exchange, where original siloxane (tetrahedral) oxygen atoms in the $b\text{SiO}_2$ retain the growth diatom $\delta^{18}\text{O}_{\text{sil}}$ value. Once dehydroxylation has occurred, it could then be deduced that the $\delta^{18}\text{O}_{\text{sil}}$ values are relatively stable until the phase change from opal-A to opal-CT occurs, which is in agreement with silica-water fractionation relationships from $b\text{SiO}_2$ in the Monterey Shale (Murata et al., 1977) and other natural environments (e.g. Juillet-Leclerc and Labeyrie, 1987; Labeyrie and Juillet, 1982). Further work is needed to constrain the degree and timing of diagenetic alteration to $b\text{SiO}_2$ $\delta^{18}\text{O}_{\text{sil}}$ values in natural marine environments, where organic and detrital mineral content of the sediment is directly related to changes in the reactivity and preservation of diatom silica and may affect $\delta^{18}\text{O}_{\text{sil}}$ values (e.g. Rickert et al., 2002); however, these results indicate that sedimentary $\delta^{18}\text{O}_{\text{sil}}$ values likely reflect diagenetic water ($\delta^{18}\text{O}$ and temperature) rather than surface/growth water.

5. CONCLUSIONS

Cultured $b\text{SiO}_2$ from marine diatom frustules (*S. turris*) were subjected to experimental aging in a controlled laboratory setting at 85°C and recorded diagenetic changes

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in $\delta^{18}\text{O}_{\text{sil}}$ values of 5.6‰. The $\delta^{18}\text{O}_{\text{sil}}$ values of the aged $b\text{SiO}_2$ reflect quartz-water equilibrium fractionation values and represent a complete overprinting of the growth $\delta^{18}\text{O}_{\text{sil}}$ values. Dehydroxylation/condensation and precipitation of abiogenic silica during sedimentation of $b\text{SiO}_2$ likely overprint primary $\delta^{18}\text{O}_{\text{sil}}$ values. The rate of change in the experimental aging environment at 85°C was rapid, with significant changes in both silanol abundance and $\delta^{18}\text{O}_{\text{sil}}$ values in 0.5 days to 37 days. As has been done with other $b\text{SiO}_2$ reactivity studies, the linear rate law was used to estimate the timing of these changes in low temperature environments. The initial and most significant change in $b\text{SiO}_2$ reactivity and $\delta^{18}\text{O}_{\text{sil}}$ values is likely to occur on the order of 10's of years at 4°C; however, our data also suggest that the dehydroxylation process has the potential to continue to alter $\delta^{18}\text{O}_{\text{sil}}$ values for $\sim 10^4$ to 10^6 years. The experimental aging data demonstrate that diagenetic water needs to be addressed when using $\delta^{18}\text{O}_{\text{sil}}$ values as a paleoceanographic and paleoenvironmental proxy.

ACKNOWLEDGEMENTS

The current manuscript benefitted from the thoughtful feedback of Dr. Elizabeth Webb, Dr. Mark Brandriss, associate editor Dr. Ruth Blake, and one anonymous reviewer. Funding for this research was provided by Northern Illinois University, American Chemical Society Petroleum Research Fund Grant# 53798-DNI2 (Dodd), National Science Foundation-Antarctic Earth Sciences Grant# 1443420 (Dodd), and a Geological Society of America Graduate Student Research Grant (Wiedenheft).

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Table 1. FTIR spectroscopy and surface charge density measurements for *S. turris* experiments at 85 °C are shown. Both silanol abundance (A_{950}/A_{800}) and surface charge density decrease throughout the experiment from cultured $b\text{SiO}_2$ (day = 0) to day 37.

Experimental Time (days)	FTIR		Surface Charge ($\mu\text{moles/g}$)	Silanol Abundance (%) [†]		
	A_{800}/A_{1100}	A_{950}/A_{800}		Total	External	Internal
0	0.044	1.29	-379.9 ± 7.1	100.0	9.1	90.9
0.5 _a	0.043	0.63	-244.4 ± 17	48.8	5.8	43.0
2	0.05	0.51	--	39.5	--	39.5
4	--	--	-146.9 ± 14.1	--	3.5	--
7	0.046	0.37	-68.3 ± 8.3	28.7	1.6	27.1
14	0.045	0.35	-25 ± 15.6	27.1	0.6	26.5
20	0.045	0.32	-15.9 ± 13	24.8	0.4	24.4
37	0.045	0.17	BD*	13.2	0	13.2

* surface charge was below detection w/ acid-base titration; [†] calculated as percent of total initial silanols

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Table 2. Oxygen isotope values *b*SiO₂, culture water, and aging (experimental) water.

Aging days	Diatom $\delta^{18}\text{O}^*$	σ	n	Cult. Water $\delta^{18}\text{O}^*$	Aging Water $\delta^{18}\text{O}^*$	σ	n
0	31.9	0.2	6	-0.9	--	0.2	12
0.5 _a	28.0	0.0	2	--	-2.0	--	1
0.5 _b	27.0	0.3	2	--	-1.8	--	1
1 _a	27.9	0.0	2	--	-1.6	--	1
1 _b	27.1	0.1	2	--	-1.5	--	1
2	27.4	0.5	2	--	-1.5	0.0	2
4	26.2	0.0	2	--	-1.5	--	1
7	27.5	0.3	3	--	-1.8	0.0	2
20	26.3	0.3	2	--	-1.0	--	1
37	26.9	0.3	3	--	-1.0	--	1
Ave. Aged Samples	27.1	0.6	20	--	-1.2	0.8	9

*values reported in ‰ relative to VSMOW; a & b repeat experiments at 0.5 and 1 day

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Table 3. Oxygen isotope fractionation factors for cultured and experimentally aged *b*SiO₂.

Aging days	Diatom $\delta^{18}\text{O}^*$	Water $\delta^{18}\text{O}^*$	T (°C)	$\alpha^{(1)}$	1000 ln α	$\Delta^{18}\text{O}^{(2)}$
0	31.9	-0.9	19.3	1.0330	32.3	32.8
0.5 _a	28.0	-2.0	85.0	1.0300	29.5	29.9
0.5 _b	27.0	-1.8	85.0	1.0288	28.4	28.8
1 _a	27.9	-1.6	85.0	1.0295	29.1	29.5
1 _b	27.1	-1.5	85.0	1.0286	28.2	28.6
2	27.4	-1.5	85.0	1.0289	28.5	28.9
4	26.2	-1.8	85.0	1.0280	27.6	27.9
7	27.5	-1.0	85.0	1.0286	28.2	28.5
20	26.3	0.3	85.0	1.0260	26.0	26.0
37	26.9	0.2	85.0	1.0267	26.4	26.7
Ave. Aged Samples	27.1	-1.2	85.0	1.0284	28.0	28.3

*values reported in ‰ relative to VSMOW; a & b repeat experiments at 0.5 and 1 day; ⁽¹⁾ $\alpha = (\delta^{18}\text{O}_{\text{sil}} + 1000)/(\delta^{18}\text{O}_{\text{w}} + 1000)$; ⁽²⁾ $\Delta^{18}\text{O} = \delta^{18}\text{O}_{\text{sil}} - \delta^{18}\text{O}_{\text{w}}$

FIGURE CAPTIONS

Figure 1. Scanning electron microscope images of cultured *S. turris* *bSiO₂* frustules grown in this study.

Figure 2. FTIR spectra of experimentally aged *S. turris* show decreases in the amount of silanol (Si-OH) bonds over the course of the 37-day experiment at 85°C. Silanol abundance is calculated as the ratio of the integrated area under the 950 and 800 cm⁻¹ peaks (gray bars).

Figure 3. The $\delta^{18}\text{O}_{\text{sil}}$ values of *bSiO₂* grown at 19.3°C ($+31.9\text{‰} \pm 0.2\text{‰}$; $n = 6$) rapidly decreased by 3.8‰ within 0.5 days of experimental aging. Aging experiments at 85°C had an average $\delta^{18}\text{O}_{\text{sil}}$ value of $+27.1 \pm 0.6\text{‰}$ ($n=20$). Error bars represent 1σ .

Figure 4. The ratio of internal to external silanols was constrained to 10:1 in the cultured *bSiO₂* (red circles) and used to calculate the silanol abundance (%) at each experimental aging period. The total silanol abundance (solid line) decreases from the initial value (red circle) rapidly (0.5 days) and then following the exponential decay curve over the experimental aging period. Internal silanols (dashed line) are inferred from the FTIR data (total silanols; solid circles) and titration data (external silanols; open circles).

Figure 5. Schematic of a *bSiO₂* nanosphere and internal/external silanols. The average activation energies (E_d) apply to the dehydroxylation reaction that converts silanols to lower energy siloxane. Internal silanols form in sealed pores and crevices within the silica structure,

which may be exposed to diagenetic water through dissolution (adapted from Zhuravlev, 2000).

Figure 6. The silica-water fractionation factor (shown here as $1000\ln\alpha$) for the cultured *S. turris* $b\text{SiO}_2$ is nearly identical to natural and cultured material from previous studies (e.g. Dodd and Sharp, 2010). Observed changes in the silica-water fractionation for the aged samples approach the quartz-water equilibrium fractionation factor (Sharp and Kirschner, 1994). For the experimentally aged $b\text{SiO}_2$, the $1000\ln\alpha$ does not match Dodd and Sharp (2010), but approaches quartz-water equilibrium (solid circles). The silica-water fractionation relationships of Juillet-Leclerc and Labeyrie (1987), Matheny and Knauth (1989), and Shemesh et al. (1992) are also shown for comparison.

Dodd et al. Figure 1

Figure

Dodd et al. Figure 2

Figure

Dodd et al. Figure 3

Figure

Dodd et al. Figure 4

Dodd et al. Figure 5

Figure

Dodd et al. Figure 6