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Morphology and Physical-Chemical Properties of Baked Nanoporous Frustules

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We investigated the morphology and physical-chemical properties of baked and unbaked nanoporous frustules. Scanning electron microscopy (SEM) observations showed that the nanoporous structures of frustules unchanged at 400 °C even after baking for 6 h. During baking at 800 °C, the frustule structures changed dramatically. On the other hand, Fourier transform infrared spectroscopy (FTIR) of bulk frustule samples indicated that physical-chemical properties of the frustules had clearly changed after baking at not only 800 °C but also 400 °C. These results showed that the reconstruction of the structures had occurred inside the frustules, even though the morphology of the frustules had not apparently changed at 400 °C. In order to characterize the exact shape of the frustules, living diatom cells were grown on a functionalized mica surface, and then baked without any chemical treatment for SEM study. This 'direct baking' technique is effective for comparing minute structures of the frustules, because completed combination of every part of the frustules can be observed.

Keywords: Diatom, Frustule, Nanotechnology, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy.

1. INTRODUCTION

Nanoporous structures of diatom frustules are one of the most unique biological architectures that are fabricated using marine/fresh water under the sun's rays and without drastically treating them under high temperature or pressure.[1–5] A fossilized diatom frustule, diatomite, has been widely used in industrial equipment such as filters and carriers, as a support for chromatography, and in building materials.[6–9]

Recently, by applying nanotechnology, the use of diatom frustules as nanobiomaterials has been intensively proposed by several pioneer researchers.[10–17] To obtain excellent results, purified frustules are used instead of diatomite, because a detailed characterization of the structures and physical-chemical properties of the frustules is necessary. Anderson et al.[10] made pioneering efforts in this field; they functionalized frustule surfaces by annealing the frustules with zeolite nanoparticles at 175 °C. In 2007, Bao et al.[16] proposed a new NO_x sensor using a single frustule; in these experiments, they annealed purified frustules at 650 °C in the presence of magnesium in order to convert SiO_2 into Si. Annealing frustules is one of the important processes to modify their structures and physical-chemical properties for nanotechnological applications.

Although frustules are recognized as new nanobiomaterials having functionalization with metals and other atoms or molecules, nanoscopic physical-chemical properties of diatom frustules have not been well investigated. Recent studies have shown that frustules contain organic components such as polyamine in addition to silica;[3] however, the effect of organic components on nanoporous structures has not been well understood at the nanoscale.

In the past, we proposed new methods for nanoscopic characterization of diatoms.[17–19] For example, we proposed a method for culturing adhesive diatoms on self-assembled monolayers (SAMs) prepared on a mica or glass surface.[18] Cells of *Navicula* sp. were successfully grown on SAMs such as an amino-terminated SAM. We also proposed a new method for frustule purification. After the cultivation of the cells on the SAM surfaces, we directly baked diatom cells at 400 °C and 800 °C for 2 h each.[19] Organic components of diatom cells were successfully eliminated during baking, and clear frustules were obtained on the mica surface. While baking at 800 °C, it was observed that the frustules shrank slightly in size. One of the advantages of our methods is that individual parts of the frustule, such as epitheca and hypotheca did not dissociate during baking.

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Although there are various methods for the purification of frustules by chemical etching, the parts usually dissociate after purification.

Here, we report the detailed characterization of baked diatom frustules. We baked diatom frustules at 400 °C and 800 °C for up to 6 h, in order to verify their structural changes; then, we characterized their structures and physical-chemical properties both by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).

2. MATERIALS AND METHODS

2.1. Culture Medium

The marine diatom *Navicula* sp. was cultured in sea water with the Daigo IMK culture medium (Nihon Pharmaceutical Co., Ltd., Osaka, Japan).[18] One millimolar Na_2SiO_3 (Wako, Japan), which acts as the Si source, was added to the culture medium. The sea water was filtrated using a 0.2 μm Millipore filter and sterilized in an autoclave before cultivation. All the facilities used for the culture were sterilized before use.

2.2. Preparation of Frustules in Bulk

Navicula sp. was cultured in a bottle for almost one month. Then, the culture medium was replaced with pure water by carrying out centrifugation (4000 rpm, 10 min) 3 times. Next, frustules were purified by the previously described method.[20–21]

2.3. Preparation of Frustules on a Mica Surface

A freshly cleaved mica surface was functionalized with 1% 3-aminopropyltriethoxysilane (APS, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) aqueous solution for 3 h and rinsed with water 3 times.[18] The mica substrate was then baked at 95 °C for 1 h and rinsed with ethanol. The functionalized mica surface was immersed overnight in a diatom suspension; subsequently, the surface was rinsed 3 times with the culture medium. Then, the mica substrate was incubated in the culture medium for one month. After the cultivation, the mica substrate was rinsed with pure water in order to remove the culture medium.

2.4. Baking Diatoms

In order to obtain purified frustules in bulk, 1 ml of the frustule suspension in water was dropped into a crucible and maintained overnight at 60 °C in order to evaporate water from it. Next, the frustules in the crucible were baked using an electric furnace (Super 100, Shirota Denki Co., Tokyo, Japan) at either 400 or 800 °C for 2, 4, and 6 h. In the case of diatom cells grown on a functionalized mica surface, the samples were directly baked using the electric furnace.

2.5. Electron Microscopy

In the case of bulk samples, an aliquot of the frustule suspension was placed on a mica surface and dried before carrying out scanning electron microscopy (SEM, S-4100, Hitachi Co., Tokyo, Japan). In the case of baked diatom cells on a functionalized mica surface, no pretreatment was carried out. In the case of unbaked diatom cells grown on a functionalized mica surface, the samples were fixed with 10% glutaraldehyde for 1 h at room temperature and were gradually dehydrated with 10%, 30%, 50%, 70%, and 100% methanol. In all cases, the samples were coated with PtIr before SEM observations.

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

Bulk samples were characterized by the universal ATP FTIR (Spectrum One, Auto IMAGE, Perkin Elmer Co., Fremont, CA). Frustules suspended in pure water were dried in a sample tube at 60 °C prior to the measurements.

3. RESULTS AND DISCUSSION

Figure 1 shows SEM images of unbaked diatom frustules. In the case of bulk samples that were prepared from a frustule suspension, as is commonly observed, rolled, slender-shaped, partially assembled structures were observed (Fig. 1(A)). Because frustules were purified by centrifugation more than seven times in aqueous solution after chemical decomposition of organic parts of diatom cells, frustule parts such as epitheca and hypotheca should be separated during the purification process.

On the other hand, when a living diatom cell was fixed with glutaraldehyde and dehydrated with alcohol, some of

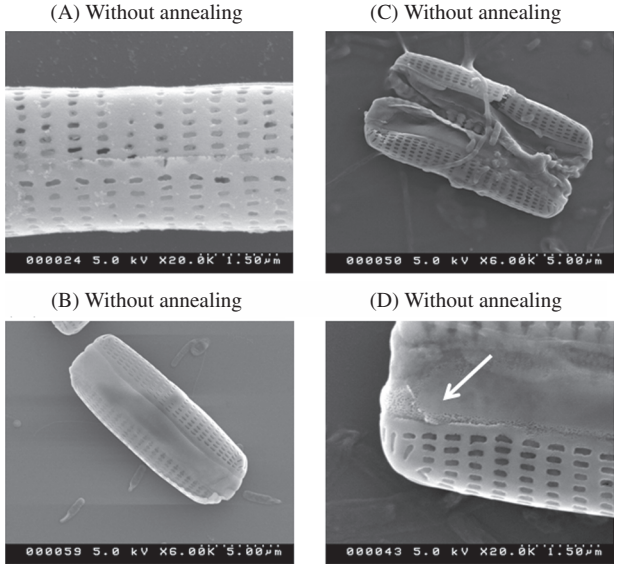

Fig. 1. SEM images of unbaked frustules. (A) Chemically purified frustule after culturing in a bottle. (B), (C) and (D) fixed diatom cell grown on an APS surface.

the frustules were formed completely without the separation of individual frustule parts. However, as reported in our previous paper, many of the cells collapsed during sample preparation (Fig. 1(C)). Even in the uncollapsed cells, small cracks (see the white arrow in Fig. 1(D)) were observed. Hence, more sophisticated techniques such as rapid freezing are necessary for the preparation of such marine diatoms.

With regard to nanostructures on the frustule surfaces, Figures 1(A and D) shows typical arrays of nanopores. A difference in the purification process of the samples did not affect the pore structures, as there was no significant difference between the shape and size of the samples shown in Figures 1(A and D).

Figure 2 shows typical SEM images of baked diatom cells that were grown on a functionalized mica surface. When the diatom cells were baked at 400 °C for 2 h, ordered nanoporous structures were clearly observed (Fig. 2(A)). Most of frustules revealed similar structures without collapse, and completed combination of every part of frustules were observed. From the results, we think that frustules were stably purified on the mica surface by the baking procedure although carbonized organic components might be remained inside of the frustules. Furthermore, there was no significant difference in the morphology of the frustules even when the baking periods were varied (Figs. 2(A–C)). This indicates that the frustule structures were stable at 400 °C from the viewpoint of their morphologies, although the frustules in the living diatom cells included water and other organic molecules such as polyamine. In addition, there were no differences in the nanoporous features of these diatom cells compared to the unbaked diatom cells (Fig. 1). On the other hand, cracks that were observed in the unbaked cells (Fig. 1(D)) were absent on the baked frustule surfaces.

When the diatom cells were baked at 800 °C, the size of nanoholes increased even after baking for 2 h (Fig. 2(D)). While baking for 4 h, some holes combined with each other, and hence, the shapes of the frustules were slightly distorted (Fig. 2(E)). Next, while baking for 8 h, big holes were formed because of the merger of melted nanopores (Fig. 2(F)). These images suggested that the frustules melted and evaporated. Additionally, the SEM images indicated that melting and evaporation began from the edges of the frustules. If we assume that the composition of the frustules is uniform, it is possible for the edges of the frustules to be thinner than the central area.

When the chemically purified frustules were baked as bulk samples, almost similar results were obtained during baking at both 400 °C and 800 °C. The baked frustules clearly shrank during baking at 800 °C while no structural change was observed during baking at 400 °C (Fig. 3(A)). During baking at 800 °C, the frustules melted from the edge, and after baking for 6 h, only backbone-like structures of the frustules remained (Fig. 3(B)). The melting at 800 °C was also observed in the case of baking in a crucible (Fig. 3(C)). The observed structures resembled intermediate structures that appear during frustule formation in living diatom cells.

Figure 4 shows FTIR spectra of unbaked and baked diatom frustules. In order to clearly identify the peaks in the spectra, a sufficient amount of dried bulk frustules was used for the measurement. By comparing the peaks of the unbaked and baked samples, it was found that three peaks that were observed in the unbaked sample disappeared after the baking. One of these peaks was the broad peak due to SiOH, which was visible in the region from 3000 cm^{-1} to 3700 cm^{-1}. When the samples were baked at 400 °C, the height of the peak decreased gradually as the baking period increased. After baking for 6 h at 400 °C, the height of the broad peak became almost half of the original height (denoted by a bold solid line in Fig. 4(A)). The data revealed that SiOH structures of the frustules disappeared to some extent during baking at 400 °C although the morphology of the frustules was preserved, as confirmed by the SEM images (Figs. 2(A–C)). In the case of the baking at 800 °C, the SiOH peak clearly disappeared even in the case of baking for 2 h. It is reasonable results

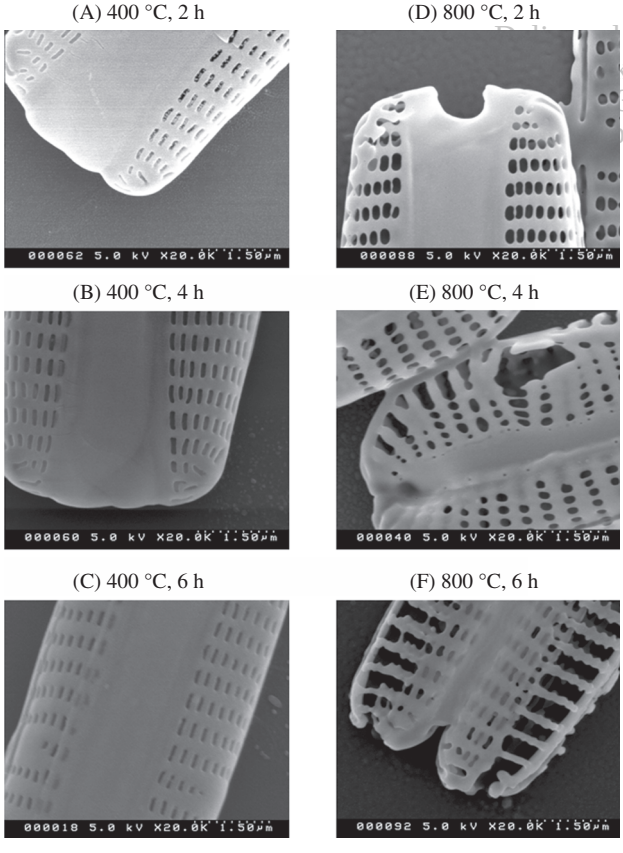

Fig. 2. SEM images of baked frustules. Diatom cells were grown on a functionalized mica surface and then baked without chemical purification. (A) 400 °C, 2 h. (B) 400 °C, 4 h. (C) 400 °C, 6 h. (D) 800 °C, 2 h. (E) 800 °C, 4 h. (F) 800 °C, 6 h.

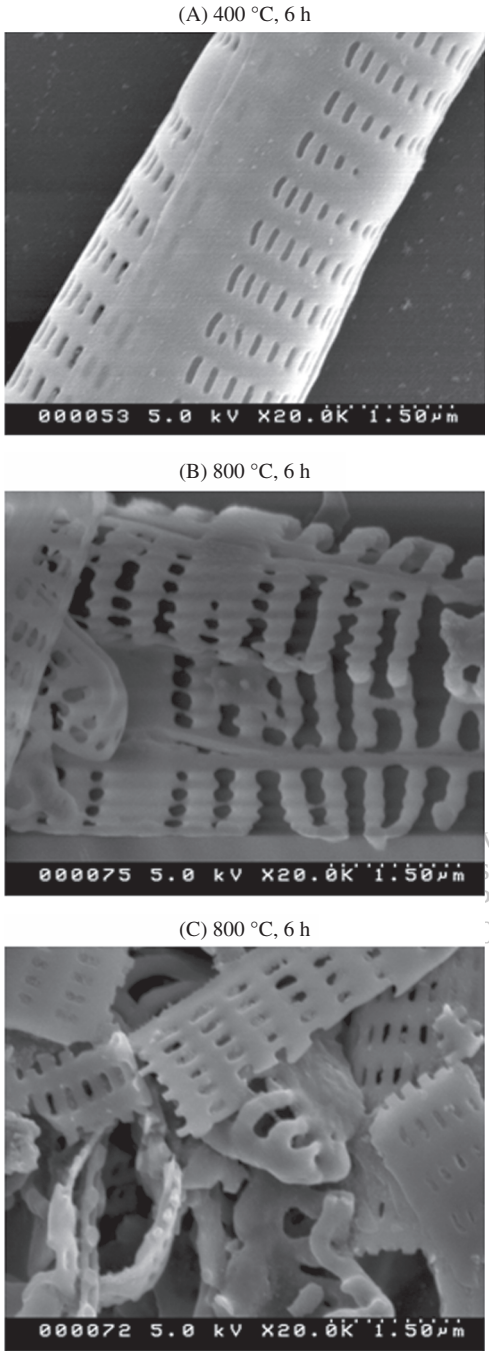

Fig. 3. SEM images of baked frustules. (A) and (B) Chemically purified frustules baked at 400 °C and 800 °C on, respectively, on a functionalized mica surface for 6 h. (C) Chemically purified frustules baked at 800 °C in a crucible for 6 h.

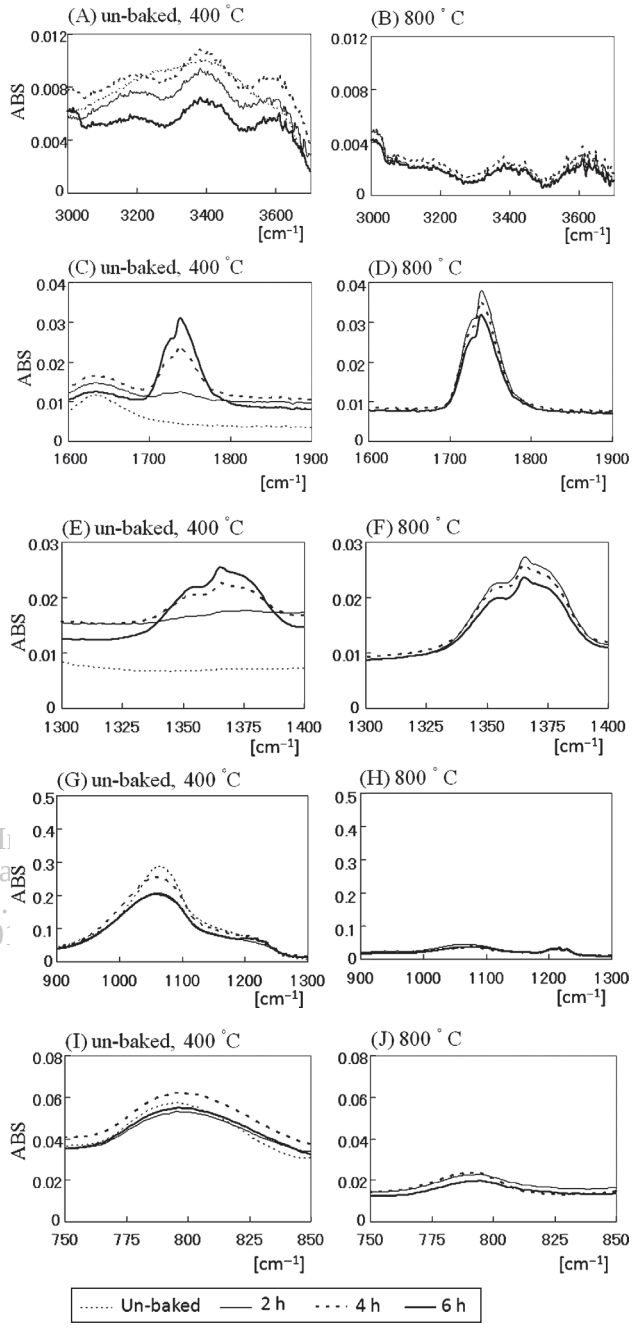

Fig. 4. FTIR spectra of baked and unbaked frustules. (A, C, E, G, and I) unbaked and baked frustules at 400 °C. (B, D, F, H, and J) baked frustules at 800 °C. Dotted lines: Unbaked. Solid lines: Baked for 2 h. Broken lines: Baked for 4 h. Bold solid lines: Baked for 6 h.

because dehydration of SiOH above 200 °C is well known phenomenon in usual silica studies.[22]

A peak at around 1070 cm^{-1} attributed to SiOR also disappeared on baking (Figs. 4(G and H)). In this case, the height of the peak did not decrease significantly at 400 °C even after baking for 6 h (Fig. 4(G)). This observation was different than that in the case of SiOH. During baking at 800 °C, the peak clearly disappeared after 2 h.

Finally, the height of a small peak at 800 cm^{-1} decreased in the case of the baking at 800 °C although it remained unchanged during baking at 400 °C. The peak probably indicated SiOSi bonding, which is typically observed in common glass materials. The quenching of the three peaks of SiOH, SiOR, and SiOSi described above suggested that the SiO bonding was strongly affected by heat, particularly at 800 °C. Because this peak of quartz

or glass is stronger than that of silica gel in general, increase of this peak was expected after the baking. Further experiments are necessary to explain mechanism of this phenomenon.

Conversely, two peaks were formed due to baking. A peak with a shoulder at around 1750 cm^{-1} formed gradually during baking at 400 °C, although it was not observed in the unbaked sample (Fig. 4(C)). The observed peak was small after the sample was baked for 2 h, and then, it became distinct after the sample was baked for 4 h. The formation of this peak probably indicated the formation of a C=O bond. During baking at 800 °C, this peak was almost completely formed within 2 h of baking.

A broad peak was also formed around 1370 cm^{-1} during the baking. There are several possible explanations for the cause of the formation of this peak. One is bending vibration of CH groups, and the other is bending vibration of OH groups such as alcohol or carbonic acid. For final conclusion, additional data from other analytical methods might be necessary. Although structures of frustules/diatomite have been intensively investigated, spectroscopic studies in order to determine physical/chemical properties of the frustules/diatomite were rarely carried out. A few papers reported infrared spectra of unbaked diatomite, but spectroscopic study of baked and unbaked frustules is still in the primitive stage.[23–25]

In our experiments, no significant change of frustule structures was observed in the SEM images during baking at 400 °C. However, the FTIR data clearly revealed that the chemical properties of the frustules were affected by baking even at 400 °C. These two different results suggest that changes occurred inside the frustule structures, without any apparent change in their shapes.

From the viewpoint of nanotechnology, our results suggested potentials of nanobiological applications using frustules. When surface modification of frustules without changing frustule structures is expected, chemical reaction at 400 °C is no problem at least 6 h. On the other hand, when removal of frustules is expected after preparation of frustule replica, baking at 800 °C is available. Frustules should be evaporated after the 800 °C baking. We hope our experimental results will be a basis of developing various nanobio devices using diatom frustules.

4. CONCLUSION

In summary, we conclude that the physical-chemical properties of the frustules changed even at 400 °C, although the shape of the frustules remained unchanged. At 800 °C, both the morphology and the physical-chemical properties of the frustules changed dramatically with baking. Especially, SiO bonding such as SiOH, SiOR, and SiCSi were strongly affected by the baking. For the SEM observation, direct baking of living diatom cells on a functionalized mica surface was effective. These results will prove useful for various applications in which diatom frustules are used, such as gas sensors, electronic devices, and nanomedicines.

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