Influence of Baking and Acid Cleaning on the Morphology and Composition of Diatom Biosilica

Wenkai Jiang and Xiangyun Deng

ABSTRACT

Living diatoms show a promise for applications in various fields in particular for the fabrication of micro/nano devices. This study acquired pure diatom walls (frustules) by several steps including collection, separation, and pure culture of marine diatoms. A two-step acid cleaning and baking method was adopted to purify biosilica structures from the diatoms. Results indicated that the morphology of the diatom frustules began to change at a baking temperature of 700 °C. With rising temperatures, the damage to the diatom frustules was more severe, but the composition was less affected by temperature. Therefore, an optimal parameter of 600 °C, 1 °C min⁻¹, and 2 h was set for our study. However, because of the species-specific differences, the morphology and composition of the different frustules were not identical at the optimal parameter.

INTRODUCTION

Diatoms are unicellular, eukaryotic algae found in waters worldwide [1]. Their cell shells, called frustules, have raised interest in the field of material science because of their novel porous biosilica structures [2-3]. Moreover, in some respects such as regularity and materials properties, frustules are hardly matched by artificial synthetic materials [4]. Therefore, diatoms are praised masters of bio-nanotechnology by some scientists [5]. In recent years, several diatom products have been developed including gas sensors [6], filters [7], drug delivery systems [8], photoelectric devices [9], and others.

Diatom frustules are obtained from two sources: diatomaceous earth and living diatom cells. Compared with diatomite, frustules from living diatom cells show some advantages, including sustainability, high quality, and pure cultivation [10]. Therefore, they have been considered the best materials for use in diatom research on nanotechnology and device application [11].

Wenkai Jiang¹,a and Xiangyun Deng¹,²,b
¹Key Laboratory of Ministry of Education for Advanced Material in Tropical Island Resources, Hainan University, Haikou 570228, China
²College of Physics and Materials Science, Tianjin Normal University, Tianjin 300387, China
¹jiangwenkai555@126.com¹, bxiangyundtj@126.com
Sulfuric acid, hydrogen peroxide, nitric acid, and SDS/EDTA solution are the common oxidants for cleaning biosilica structures from frustules [12]. Moreover, in a previous study from our lab [10], hydrochloric acid was used to clean frustules. In addition, two methods (acid cleaning and baking) were combined to achieve better results.

Baking is another important method to remove organic matter during the purification of diatom frustules. Moreover, the preparations of diatom products often involve high temperature processes. Umemura et al. [13] found that the nanoporous structure of frustules was unchanged after baking for 6 h at 400°C; however baking at 800 °C for only 2 h induced a dramatic change in the nanoporous structure of the frustules. Furthermore, Bioclastic and Shape-preserving Inorganic Conversion (BaSIC) was introduced by Sandhage et al. [14]. Almost all shape-preserving, chemical conversion processes need to be done at high temperature, and mainly include the synthesis of MgO at 900 °C for 4 h [15] or at 650 °C for 2.5 h [6], ZrO₂ at 250-800 °C for 2 h [16], TiO₂ at 350 °C for 2 h [17] and SiC at 1200 °C for 2 h [18]. In addition, Liu et al. [19] and Lee et al. [20] synthesized ZnFe₂O₄/SiO₂ composites at 1000 °C for 3 h and Y₂SiO₅:Eu³⁺ coated frustules at 1000 °C for 4 h respectively. Somewhat unexpectedly, these results showed that the frustules can be converted into a new nanocrystalline material without loss of their bioclastic shape or fine features. Differences in the ultimate breakage temperature may be related to the differences in diatom species, protective gases, and conversion products used in the experiments. Unfortunately, there is very little information available about systematic studies on the diatom breakage temperature and damage process.

EXPERIMENTAL

Diatom source and observation: Diatom sampling was carried out from major coasts in China’s southern island province of Hainan. A portion of the samples were expanded via culture to determine which species were dominant microalgae species from their respective coasts; the remaining samples were observed by SEM via a rapid sample processing method to research the diversity of marine diatoms [21]. This method not only can be used for the observation and classification of the diatoms, but can also be used in the preliminary identification of purified diatoms (shown in Fig. 1a-i).
Diatom purification and culturing: The cells (expanding culturing) were cultured according to our previous report [22]. Following two weeks of incubation, a number of diatom species were extracted by a simple isolation method (shown in Fig. 1j). The experimental procedure is as follow:

Step 1: **Dilution.** The high concentration algae fluid was diluted at the ratio of 1:20 (algae fluid / sea water).

Step 2: **Extraction.** A drop of algae fluid was taken from the diluted samples by a microcapillary, transferred to a slide, and observed by a light microscope (LM). If the diatom cell number was not greater than 2, it was swashed into a test tube.

Step 3: **Cultivation.** The diatoms in the test tube were cultured for an additional 10-14 days.

Step 4: **Testing.** At the end of the culture period, a small amount of algae fluid was extracted to detect whether the diatom species in the test tube were pure. Otherwise, it would be processed by the previous procedures. Purified diatoms were cultured in a large-scale to provide an abundant amount of raw materials for the collection of frustules.

Acid cleaning and baking of diatom cells: In order to remove inorganic salts and unclog the pores, the collected algae fluid was processed according to our previous method [10]. Then, the *Navicula* sp. frustules were baked at 500, 600, 700, 800, 900, 1000 and 1100 °C respectively with a heating rate of 3 °C min⁻¹ for 2 h. Furthermore, four species of diatoms (*Navicula* sp., *Thalassiosira weissflogii*, *Chaetoceros*...
muelleri, and Closterium sp.) were processed at the same parameter (600 °C, 1°C min⁻¹, and 2 h), for comparison.

Characterization methods: The morphology and composition of diatom frustules in the different stages of processing were examined by scanning electron microscopy (SEM), energy dispersive x-ray analysis (EDS) and Fourier transform infrared spectroscopy (FTIR) [10, 21].

RESULTS AND DISCUSSION

The morphology of Navicula sp. frustules in the different stages of treatment process are shown using scanning electron microscope in Fig. 2a-2j. Fig. 2c and 2d show the diatom frustules baked at 500 and 600 °C respectively. Compared with Fig. 2b, no obvious shape and size changes occur in the two figures. Diatom frustules baked at 700 °C show pits in the surface, especially around at the pores suggesting excessive heat lead to partial melting of the biosilica structure. When the diatom frustules were baked at 800 and 900 °C respectively, the pits became deeper and more numerous leading to the collapse of some pores. At 1000 °C, the diatom frustules are collapsed completely with only backbone-like structures. The results indicate that melting and evaporation began from the edges of the frustules, in agreement with a previous report [13], just details and temperature are slightly different. At 1100 °C, the morphology of the frustules was completely changed, with even backbone-like structures have been melted into the floccules. Nevertheless, the cross section of the sample baked at 1100 °C can be observed in Fig. 2j, a large amount of diatom frustules covered by a layer of molten frustules are in almost pristine condition.

Element weight percentages of Navicula sp. frustules in the different processing stages analyzed by EDS are presented in Fig. 2k. The results reveal a marked increase in terms of the element content of Si and O after acid cleaning and baking. With increasing baking temperature, the proportion of Si and O increased even further, and when the temperature reaches more than 900 °C, the atomic ratio of Si/O is close to 1:2 by conversion of the weight percentage.

The FTIR spectra of Navicula sp. frustules in the different stages of treatment process are shown in Fig. 3a and 3b. The intense bands located at 470, 810 and 1090 cm⁻¹ are due to the bending vibration, and the symmetric and asymmetric stretching vibration of Si-O-Si network of the biosilica [23], while the shoulder in the 1100-1200 cm⁻¹ region is related to the out-of-phase Si-O stretching vibrations [25]. Another similarities are the broad band observed around 3435 cm⁻¹ and the weak band located at 1640 cm⁻¹, they are ascribed to the O-H stretching and bending vibration of hydroxyl groups of surface-bound water and water adsorbed in the porous structures [19, 24], which also includes the H-O-Si configuration [13]. After acid cleaning, the bands located at 470, 810 and 1090 cm⁻¹ for Si-O-Si network are more pronounced, but the bands around 3435 and 1640 cm⁻¹ weakened. The peak located at 570 cm⁻¹ may be attributed to inorganic substances. The band located at 950 cm⁻¹ is ascribed to Si-O stretching vibration of Si-OH groups [26], which is noticeable in the “acid-cleaned” curve and possible partially masked by the broad band around 1090 cm⁻¹ in the “original” curve. The most obvious difference between Fig. 3a and 3b is the disappearance of some peaks upon baking, such as the peaks ascribed to C-H bending, N-H bending, C=O stretching, and the symmetric and
asymmetric stretching of C-H located at 1400, 1545, 1745, 2855, 2925 cm$^{-1}$ respectively [27]. With increasing baking temperature, the bands located at 950, 1640 and 3435 cm$^{-1}$ decreased and even disappeared, while the bands located at 470, 810 and 1090 cm$^{-1}$ increased in intensity.

Fig. 2. SEM images of Navicula sp. frustules a untreated, b acid cleaned, and baked at c 500 °C, d 600 °C, e 700 °C, f 800 °C, g 900 °C, h 1000 °C, and i, j 1100 °C for 2h. k EDS analysis of Navicula sp. frustules in the different stages of processing.

Fig. 3c shows the TG-DSC curves of the original diatom cells from Navicula sp.. There are three main weight-loss stages before heating to 600 °C corresponding to three peaks as can be seen from DSC curve. An endothermic DSC peak was observed at 70.5 °C, which was attributed to the evaporation of body water and physically absorbed water. The following two stages from 200 to 600 °C with an exothermic peak at 338.3 and 485.2 °C are due to the thermal degradation of the organic constituents [20]. Finally, there is little weight loss during the temperature rising to 1100 °C, but with an endothermic process owing to the frustules melting, that can be evidenced by the morphology analysis of the diatom frustules baked at 700-1100 °C.

SEM images and EDS analyses of Navicula sp. frustules baked at 600 °C with a heating rate of 1 °C min$^{-1}$ for 2 h are given in Fig. 4a-4c. Compared with original and acid-cleaned diatoms (Fig. 2a and 2b), the baked frustules have no obvious shape and size changes after this treatment (Fig. 4a and 4b). EDS results (Fig. 4c) reveal Si and O are the main elements of the baked frustules, with a total Si and O content of about 94%. Accordingly, C and other elements dropped to 6%, which indicates that organic matter and other oxide components are little.
Figure 3. a-b Infrared spectra of Navicula sp. frustules in different treatments. c TG–DSC curves of the original cells from Navicula sp.. d Infrared spectra of the four species diatom frustules baked at 600 °C with a heating rate of 1 °C min⁻¹ for 2 h.

The SEM images in Fig. 4d-4h and the data in Table 1 indicate that different morphology and composition changes happened to different species of diatom frustules after the same processing. The reasons of these results mainly include the differences of structures and element contents between four species of frustules. For Thalassiosira weissflogii, organic matter was difficult to be removed, due to the diameter of pores were too small. In contrast, Navicula sp. and Closterium sp. possess many pores with average lengths (or diameters) of more than 100 nm. Moreover, for the two diatoms, the separation of valves and girdle bands is another reason for success of acid cleaning. However, the baked Closterium sp. frustules were seriously damaged, perhaps due to the fragility and thinness of the frustules. For the same reason, the frustules of Chaetoceros muelleri were also difficult to keep the original morphology by acid cleaning (Fig. 4f).

The FTIR spectra of the four species of diatom frustules baked at 600 °C with a heating rate of 1 °C min⁻¹ for 2 h are presented in Fig. 3d. The O-H stretching and bending vibration, located at 3435 cm⁻¹ and 1640 cm⁻¹, are visible in every curve. However, the bending vibration, and the symmetric and asymmetric stretching vibration of Si-O-Si network of the biosilica, located at 470, 810 and 1090 cm⁻¹, are diverse from each other. The three peaks in the curve of Navicula sp. are strongest, but in the curve of Thalassiosira weissflogii are weakest. Unlike other curves, one weaker band at 617 cm⁻¹ appears in the curve of Thalassiosira weissflogii. The band can be ascribed to the diagnostic band for cristobalite due to SiO-H vibration [19], which indicates high temperature could lead to a crystal structure transformation.
Figure 4. a, b SEM images and c EDS analysis of *Navicula* sp. frustules baked at 600 °C with a heating rate of 1 °C min⁻¹ for 2 h. SEM images of *Thalassiosira weissflogii* frustules d untreated, and e baked (600 °C, 2 h, 1 °C min⁻¹). SEM images of *Chaetoceros muelleri* frustules f acid-cleaned. SEM images of *Closterium* sp. frustules g untreated, and h baked (600 °C, 2 h, 1 °C min⁻¹).

Table 1. Data of eds analyses from different frustules in different treatments.

| Diatom species in different treatments | Atomic percentage (at%) |
|---------------------------------------|-------------------------|
|                                       | O                       | Si          | C           | Others   |
| *Chaetoceros muelleri*                | Acid-cleaned            | 40.68       | 9.55        | 46.73     | 3.04      |
|                                       | Baked                   | 63.88       | 25.79       | 8.45      | 1.88      |
| *Closterium sp.*                      | Acid-cleaned            | 33.59       | 5.61        | 56.04     | 4.76      |
|                                       | Baked                   | 65.27       | 26.93       | 6.20      | 1.60      |
| *Thalassiosira weissflogii*           | Acid-cleaned            | 37.02       | 4.78        | 45.08     | 13.12     |
|                                       | Baked                   | 60.56       | 21.61       | 13.17     | 4.66      |
| *Navicula sp.*                        | Acid-cleaned            | 36.21       | 14.92       | 46.85     | 2.02      |
|                                       | Baked                   | 64.75       | 29.02       | 5.03      | 1.20      |

CONCLUSIONS

A two-step acid cleaning and baking method is effective for purifying diatom frustules. An optimal parameter of 600 °C, 1°C min⁻¹, and 2 h was set for *Navicula* sp.. However, because of the species-specific differences, the morphology and composition of the different frustules were not identical at the same parameter. More methods for various different kinds of diatoms will be carried out in the near future.

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REFERENCES

[1] E.V. Armbrust. Nature 459 (2009) 185-192.
[2] T.X. Fan, S.K. Chow, D. Zhang. Prog. Mater. Sci. 54 (2009) 542-659.
[3] M. Hildebrand. Chem. Rev. 108 (2008) 4855-4874.
[4] E. Brunner, C. Gröger, K. Lutz, et al. Appl. Microbiol. Biotechnol. 84 (2009) 607-616.
[5] R. Gordon, D. Losic, M.A. Tiffany, et al. Trends. Biotechnol. 27 (2009) 116–127.
[6] Z. Bao, M.R. Weatherspoon, S. Shian, et al. Nature 446 (2007) 172-175.
[7] D. Losic, G. Rosengarten, J.G. Mitchell, et al. J. Nanosci. Nanotechno. 6 (2006) 1-8.
[8] D. Losic, Y. Yu, M.S. Aw, et al. Chem. Commun. 46 (2010) 6323-6325.
[9] C. Jeffryes, R. Solanki, Y. Rangineni, et al. Adv. Mater. 20 (2008) 2633-2637.
[10] W. Jiang, S. Luo, P. Liu, et al. J. Appl. Phycol. 26 (2014) 1511-1518.
[11] Y. Wang, D. Zhang, J. Cai, et al. Appl. Microbiol. Biotechnol. 95 (2012) 1165–1178.
[12] Y. Wang, J. Cai, Y. Jiang, et al. Appl. Microbiol. Biotechnol. 97 (2013) 453-460.
[13] K. Umemura, Y. Noguchi, T. Ichinose, et al. J. Nanosci. Nanotechno. 10 (2010) 5220-5224.
[14] K.H. Sandhage, S.M. Allan, M.B. Dickerson, et al. Int. J. Appl. Ceram. Tec. 2 (2005) 317-326.
[15] K.H. Sandhage, M.B. Dickerson, P.M. Huseman, et al. Adv. Mater. 14 (2002) 429-433.
[16] S. Shian, Y. Cai, M.R. Weatherspoon, et al. J. Am. Ceram. Soc. 89 (2006) 694-698.
[17] R.R. Unocic, F.M. Zalar, P.M. Sarosi, et al. Chem. Commun. 7 (2004) 796-797.
[18] Z. Bao, M.K. Song, S.C. Davis, et al. Energ. Environ. Sci. 4 (2011) 3980-2984.
[19] Z. Liu, T. Fan, H. Zhou, et al. Bioinspir. Biomim. 2 (2007) 30-35.
[20] D.H. Lee, W. Wang, T. Gutu, et al. J. Mater. Chem. 18 (2008) 3633-3635.
[21] W. Jiang, H. Pan, F. Wang, et al. J. Appl. Phycol. 27 (2015) 243-248.
[22] G. Zhang, W. Jiang, L. Wang, et al. Mater. Lett. 110 (2013) 253-255.
[23] Y. Li, W. Chian, X. Wang, et al. Photochem. Photobiol. 87 (2011) 618-625.
[24] T. Qin, T. Gutu, J. Jiao, et al. Nanosci. Nanotechno. 8 (2008) 2392-2398.
[25] S. Lettieri, A. Setaro, L. De Stefano, et al. Adv. Funct. Mater. 18 (2008) 1257-1264.
[26] G.E. Swann and M.J. Leng. Quaternary. Sci. Rev. 28 (2009) 384-398.
[27] D.K. Gale, T. Gutu, J. Jiao, et al. Adv. Funct. Mater. 19 (2009) 926-933.