

ISOLATION AND CHARACTERIZATION OF BIOSILICA AS BIONANOMATERIAL FROM THE WASTE OF FRUSTULES DIATOM *Navicula* sp. TAD

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ABSTRACT

Biosilica from frustul diatom *Navicula* sp. TAD was isolated under optimal conditions by washing with 20% HCl and baking at 600 °C. The treatments successfully removed $78.00 \pm 0.514\%$ of impurities from the biosilica during washing, and $93.072 \pm 0.347\%$ were further removed after baking. Biosilica Frustule *Navicula* sp. TAD resulting from HCl washing and burning at 600°C has a whiter silica color. Biosilica was identified by Fourier transform infrared spectroscopy (FTIR) at wave numbers 1601, 1062, 962, 799, and 470 cm^{-1} , which were associated with stretching, bending, and vibrational groups of Si-O-Si bonds. Scanning electron microscopy (SEM) observations show that the silica frustule nanoporous surface structure can be seen, and the results of Energy Dispersive X-Ray (EDX), silica from *Navicula* sp. TAD is composed of silicon and oxygen. This shows that silica was obtained from the diatom *Navicula* sp. TAD. X-ray diffraction (XRD) analysis shows that the morphology of SiO_2 in biosilica is a typical amorphous silica

Keywords: Bionanomaterial, Biosilica, Diatom, Frustule, *Navicula* sp. TAD, Waste

RASAYAN J. Chem., Special Issue, 2022

This manuscript is focusing **SDG-12: Responsible Consumption and Production**

INTRODUCTION

Diatoms are a large group of unicellular microalgae that absorb silicates and then convert to silica skeletons that make up significantly ornate cell walls called frustules. Frustule diatoms have nanopores that exchange nutrients or other materials in diatom cells.¹ In natural habitats, diatoms contribute significantly to the silicon cycle and a large part of carbon fixation in the oceans.² Diatoms capture atmospheric CO_2 and dissolved carbonates as a carbon source for growth, directly reducing emissions of the problematic greenhouse gas (CO_2). Thus, diatoms become a sustainable land source for synthesizing porous materials with nanostructures. Diatom biosilica is a bionanomaterial with nanostructure morphology that varies from each diatom species.^{3,4} Diatomaceous biosilica has preferable properties in the stability of chemical and thermal, porosity, non-toxicity, and compatibility when compared to other substances. This allows diatom biosilica to be proposed in various fields of application, such as biotechnology and biomedicine as natural-made nanodevices.⁵⁻⁸ The properties of diatoms which have a high surface area, lightweight, and muscular mechanical strength caused by nanopores, vary from species to species.⁵ These advantages make diatoms an ideal biosilica source.¹⁻³ Natural biological synthesis of porous silica materials with diatomic cations provides an alternative approach.³ Frustule diatoms have a unique pore arrangement that varies significantly with the diatom species, and their pore sizes range from 50 nm to several microns. They are composed of amorphous hydrated silica ($\text{SiO}_2 \cdot 2\text{H}_2\text{O}$) with organic macromolecules.¹¹⁻¹³ These superior physicochemical properties make it uncomplicated to extract pure biosilica from diatomaceous biomass.¹⁴ Apart from being a source of biosilica, diatom biomass also contains various high-energy biological compounds such as carbohydrates and lipids. In the diatom-based industry, it has been widely used as a raw material for making biofuels. This biofuel processing process produces waste in diatom frustules that are not utilized. This is an excellent opportunity to obtain biosilica with superior properties through extraction. Therefore, Isolation and characterization of biosilica as bionanomaterial frustules diatom *Navicula* sp. TAD through an effective

extraction process while still considering the biosilica structure and optimizing the use of chemical reagents is very important.

EXPERIMENTAL

Sample Preparation

The frustule diatom samples used in this study were collected from biodiesel production waste from *Navicula* sp. TAD is one of the species collected at the Biochemistry Laboratory of the Chemistry Department, Universitas Patimura. The collected frustules were resuspended in deionized water and centrifuged three times. Then, frozen pellets are dried in a refrigerated pressurized dryer.

Isolation and Characterization of Biosilica

Remove the initial mass of diatoms by washing the acid solution. The acidic HCl solution was chosen as the general washing solvent. The effect of acid concentration to remove impurities from frustule diatom waste was carried out by adding 2.50 g of diatom fractured waste into a 250 mL Erlenmeyer. The concentration of the solution that was varied was 1%, 2%, 3%, 4%, 5%, 10%, 15%, and 20%. The mixture was shaken at 180 rpm at room temperature (about 25 °C) for 30 min. The reaction products were centrifuged at 4000 rpm for 10 min. Then the precipitated sediment was washed with deionized water and centrifuged three times to remove traces of acid. After the washing process, the samples were dried in an oven at 60°C for 48 hours to remove any retained moisture. After the acid washing treatment, the dry-washed frustule diatom waste samples were treated in a kiln for 3 hours, optimized combustion temperature. The combustion temperature is set to 200°C, 400°C, and 600°C, respectively. The surface chemical groups of biosilica samples were measured by Fourier Transform Infra-Red spectroscopy (FTIR; (Shimadzu, Type: IRPrestige 21). X-ray diffraction (XRD) analysis was performed with an X-ray powder diffractometer. A dispersive Spectrometer analyzed the biosilica sample's elemental composition, and the samples' surface morphology was examined by SEM-EDS (Hitachi Flexsem 100).

RESULTS AND DISCUSSION

Isolation of Biosilica by Chemical and Thermal Treatment

Frustule waste of *Navicula* sp. TAD was washed with hydrochloric acid (HCl) solution to remove acid-soluble organic or inorganic impurities. Washing with a hydrochloric acid solution was carried out with various acid concentrations ranging from 1%, 2%, 3%, 4%, 5%, 10%, 15%, and 20%. Figure-1 shows the mass loss of impurities from the frustule waste increases slowly up to a concentration of 20%, where the total mass of impurities lost reaches 78%. Thus, the optimal HCl concentration is to wash the frustule *Navicula* sp. TAD waste is 20%.

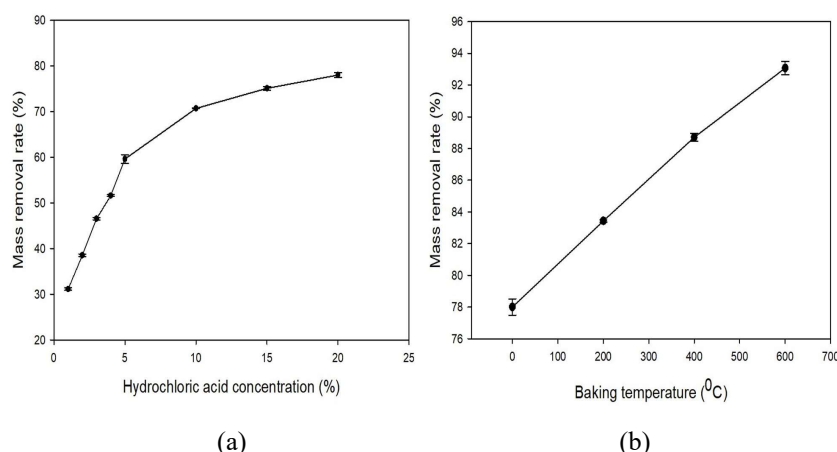


Fig.-1: Washing of Frustule Waste with HCl Solution (a), Baking the Results of Washing the Frustule Waste with 20% HCl Solution (b)

After washing with acid, the color of the residue is bright green, indicating that there are still organic impurities in the frustule waste. Hence, it needs to be washed longer to remove the organic impurities. These results indicate that the chemical method has not effectively removed acid-soluble organic impurities

from the waste of frustule *Navicula* sp. TAD, so it needs further steps for biosilica purification from frustule. Figure-1b shows the removal rate of impurities from the frustule waste washed with 20% HCl after being burned under different temperatures. The removal of impurities that were washed with 20% HCl and burned at 200, 400, and 600 °C reached 83.430, 88.706, and 93.072%, respectively. The results showed that the baking treatment at 600 °C for 3 hours was sufficient to decompose the remaining impurities from the frustule diatoms washed with 20% HCl.



Fig.-2: Biosilica Washed with 20% HCl on the Baking of (a) 200, (b) 400, and (c) 600°C

The color of samples of raw diatom biomass, frustule waste, 20% HCl-washed frustule waste, and biosilica can be seen in Fig.-3. Before treatment, the biomass sample of *Navicula* sp. TAD is green. Changes in the color gradient of the sample after washing and burning treatment can be observed. The results showed that acid-leaching pre-treatment was required to manufacture biosilica from biomass. The removal of impurities by washing with 20% HCl solution was $78.00 \pm 0.514\%$, and the accumulated rate of removal of impurities after baking treatment was 93.072%. This condition is an optimal method of biosilica preparation from the waste of frustule *Navicula* sp. TAD.



Fig.-3: Samples (a) biomass of *Navicula* sp. TAD, (b) Frustule Waste, (c) 20% HCl Washed Frustule Waste, (d) Biosilica of Frustules Waste After Baking

Characterization of Biosilica

SEM images of raw diatoms, waste frustule, waste frustule washed with 20% HCl, and diatom biosilica roasted at 600°C are shown in Fig.-4, respectively. Surface biomass of *Navicula* sp. TAD untreated had a non-porous surface (Fig.-4a).



Fig.-4: SEM Images of (a) Untreated Diatom Biomass, (b) Waste Frustule, (c) Biosilica of Frustule Washed with 20% HCL Baked at 600 °C

Meanwhile, the frustule waste from lipid extraction has a less clear surface because it is covered with impurities, solvent crystals, and the destruction of cell debris due to the extraction process. However, after washing treatment with 20% HCl and further burning at 600°C, the surface structure with nanopores could

be clearly seen, this confirms the impurity on the frustule surface can be removed. The results showed that the combustion treatment at a temperature of 600°C could remove impurities effectively and keep the skeletal structure intact. Thus, the acid leaching method followed by a combustion treatment of 600°C is preferred for preparing marine diatom biosilica. The chemical composition of diatom frustules was analyzed by energy dispersion X-ray spectroscopy (EDS). Figure-5 shows the percentage of Si, O, C, and N atoms that changed during the biosilica preparation process from frustule waste. The atomic content of N elements decreased after treatment, while the content of Si and O atoms increased, indicating that the impurities were removed successfully. But the atomic content of element C is slightly increased. The results showed that the combustion temperature at 600°C increased Si atomic content from 15.72% to 28.82%.

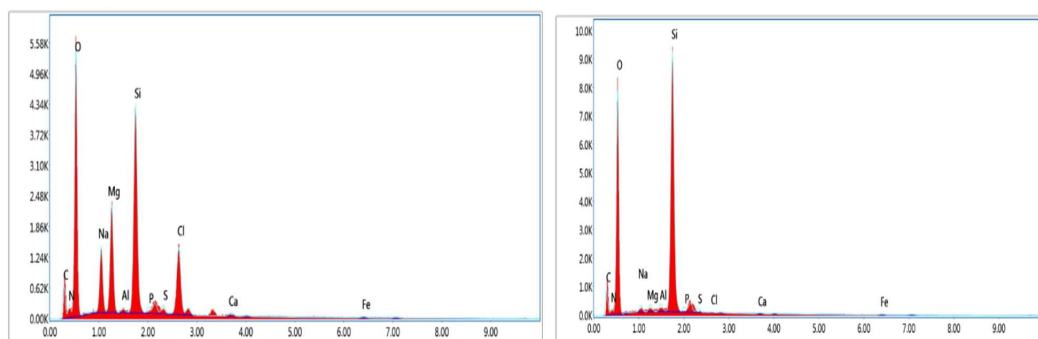


Fig.-5: EDS Spectrum (a) Frustule Waste, (b) Biosilica of Frustule Waste

Functional group analysis with Fourier Transform Infra-Red Spectrophotometer showed peak characteristics of biomass, frustule waste, and biosilica from *Navicula* sp. TAD (Fig.-6a). Chemical groups in the biomass of *Navicula* sp. TAD includes stretching vibrations of Si-O-Si at 470 cm^{-1} , bending Si-O-Si at 1040–1095 cm^{-1} , and bending OH on the hydroxyl group at 3435 cm^{-1} , -COOH at 1734–1887 cm^{-1} , -COO- at 1400–1651 cm^{-1} and -OH from -COOH at 2523 cm^{-1} , -SO- at 1231 cm^{-1} , -R₃C-OH at 1072 cm^{-1} , and -CH₂-, -CH₃-, -CH₄- at 2850–2949 cm^{-1} . After biosilica preparation, the -COOH, -COO-, -OH groups of -COOH, -SO-, or -R₃C-OH are removed. The biosilica functional groups include the stretching vibration of Si-O-Si at 470 cm^{-1} , Si-O-Si at 797–800 cm^{-1} , bending Si-O at the Si-OH group at 950 cm^{-1} , Si-O-Si at 1040–1095 cm^{-1} , and OH hydroxyl group at 3435 cm^{-1} , containing adsorbed water and stretching Si-OH. The peaks of 1630–1640 cm^{-1} and 3740–3780 cm^{-1} represent the Si-OH bond.¹⁵ Compared to biomass, most organic chemical groups disappeared, indicating the successful removal of impurities from crude dirty diatoms through acid washing and combustion. X-ray diffraction pattern of biosilica from waste frustule *Navicula* sp. TAD is shown in Fig.-6b. The typical biosilica diffraction spectrum for amorphous silica (broad reflection centered at $2\theta = 20^\circ\text{--}25^\circ$) with no other apparent peaks.^{16,17}

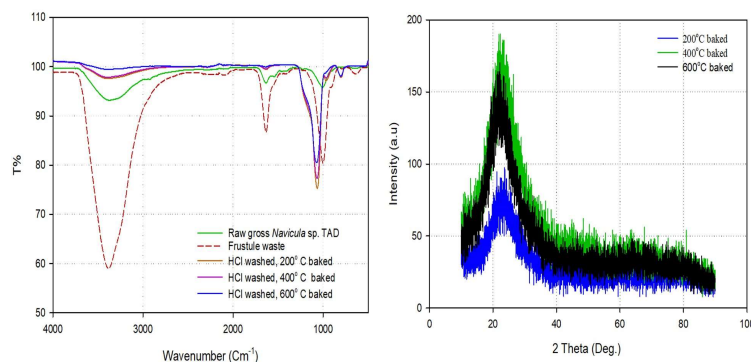


Fig.-6: FTIR Spectrum (a) and X-ray Diffractogram (b)

The results showed that the biosilica from the waste of frustule *Navicula* sp. TAD is mainly composed of SiO₂. The amorphous bands described in Fig.-6b are diffractogram curves of the biosilica waste of frustule

Navicula sp. TAD line with various hydrothermal treatment temperatures of 200°C, 400°C, and 600°C resulted in a curve with a comprehensive profile with peaks at $2\theta = 22.6552^\circ$; 21.7721° ; and 21.4927° . These results indicate that marine diatom biosilica extracted using the exploration method has a high purity. The amorphous nature of the biosilica waste of frustule *Navicula* sp. TAD analysis using XRD showed that frustule, pretreated with hydrochloric acid washing and baked at temperatures 200°C, 400°C, and 600°C, had an amorphous solid structure. There is no change in the structure of amorphous silica in the combustion process up to 600°C.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that Optimum biosilica conditions from frustule diatom waste *Navicula* sp. TAD can be determined by washing HCl with a concentration of 20% and burning at 600°C for 3 hours. Physical characteristics of the biosilica of frustule *Navicula* sp. TAD from HCl washing and burning at 600°C had a whiter silica color. Biosilica was identified by infrared spectroscopy at wave numbers 1601, 1062, 962, 799, and 470 cm^{-1} , which corresponded to the stretching, bending, and rocking vibration groups of Si-O-Si bonds, respectively. Based on its chemical characterization, no peaks of impurities were identified in the silica, indicating that silica was successfully isolated from the diatom *Navicula* sp. TAD. SEM observations showed that the nanoporous structure of the silica frustule surface could be seen clearly, and the EDX results, silica from the culture of *Navicula* sp. TAD was composed of silicon and oxygen, indicating that silica was successfully obtained from the diatom *Navicula* sp. TAD. XRD analysis showed that the SiO_2 morphology of the biosilica is typical of amorphous silica.

ACKNOWLEDGMENTS

Thank you to the dean of the Faculty of Mathematics and Natural Sciences for funding research through PNBPMIPA and the research team who have assisted in the process of completing this research.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the faculty authors can be verified from their ORCID ids, given below:

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[RJC-8109/2022]