



ISOLATION AND FRUSTUL CHARACTERIZATION OF DIATOMS ISOLASI DAN KARAKTERISASI FRUSTUL DIATOMS *Navicula* sp. TAD STRAINS

Ivonne Telussa*¹, Rahayu¹

¹Departmen Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas
Pattimura, Ambon, Indonesia

*¹e-mail : ivonnetelussa@gmail.com
²e-mail : rahayumahmud09@gmail.com

Abstract

*frustul diatom Navicula sp TAD was isolated by acid washing and burning. Cells of Navicula sp TAD were cultivated for 7 days at room temperature under a light intensity of 5000 lux to obtain high cell density and biomass. The highest cell density was obtained at $72.167 \times 105 \pm 0.946$ cells mL^{-1} . Dry biomass was obtained at 11.459 g with biomass productivity of 0.1364 $gL^{-1} h^{-1}$. Frustul *Navicula* sp TAD results from 20% HCl washing and burning at 600 oC has a whiter frustul color. The frustul characterization showed that the structure with nanopores on the frustul surface was clearly visible. Frustul from *Navicula* sp TAD is composed of silicon and oxygen*

Keywords: Diatom, Frustule, Isolation, Characterization, *Navicula* sp. TAD

Abstrak

Frustul diatom *Navicula* sp. galur TAD diisolasi melalui proses pencucian dengan asam dan pembakaran. Sel *Navicula* sp. galur TAD dikultivasi sel selama 7 hari pada temperature ruang di bawah intensitas cahaya 5000 lux untuk mendapatkan kerapatan sel dan biomassa yang tinggi. kerapatan sel tertinggi diperoleh sebesar $72,167 \times 105 \pm 0,946$ sel mL^{-1} . biomassa kering diperoleh sebesar 11,459 g dengan produktivitas biomassa sebesar $0,1364$ $gL^{-1} h^{-1}$. Frustul *Navicula* sp. galur TAD hasil pencucian HCl 20% dan pembakaran pada 600 oC memiliki warna frustul yang lebih putih. Karakterisasi frustul menunjukkan struktur dengan nanopori pada permukaan frustul terlihat jelas. Frustul dari *Navicula* sp galur TAD tersusun atas silikon dan oksigen

Kata Kunci: Diatom, Frustul, Isolasi, Karakterisasi, *Navicula* sp. galur TAD

1. INTRODUCTION

Diatoms are a large group of unicellular microalgae that can absorb silicate and turn it into a silicate framework that makes up the cell wall with significant ornaments called frustules. Nano-sized porous frustules of diatoms play an important role in mechanical protection and exchange of nutrients in diatom cells (Dolatabadi & de la Guardia, 2011). In natural habitats, diatoms contribute greatly to the silicon cycle, as well as a large part of carbon fixation in the oceans (Mohammed, 2015). Diatoms capture atmospheric CO₂ and dissolved carbonates as

a carbon source for growth, which directly reduces emissions of the problematic greenhouse gas (CO₂). Thus, diatoms become a sustainable source of land for the synthesis of porous materials with nanostructures. Diatom biosilica is a bionanomaterial with nanostructure morphology that varies from each diatom species. (Meyers, Chen, Lin, & Seki, 2008). Diatom biosilica has superior properties in chemical and thermal stability, porosity, non-toxicity and compatibility compared to other materials. This allows diatom biosilica to be proposed in various fields of

application such as biotechnology and biomedicine as natural-made nanodevices (Gordon, Losic, Tiffany, Nagy, & Sterrenburg, 2009). The properties of diatoms which have a high surface area, light weight and strong mechanical strength caused by nanopores vary from each species (Lu, Sun, & Wang, 2015). These advantages make diatoms an ideal biosilica source (Meyers, Chen, Lin, & Seki, 2008); (Dolatabadi & de la Guardia, 2011)

Diatomite or *diatomaceous earth* (DE), which is a fossil diatom, is usually used for the preparation of porous biosilica nowadays (Sheng, et al., 2009). This porous silica material has excellent properties and has been widely used as a drug delivery carrier, biosensor, biocatalyst and adsorbent (Dolatabadi & de la Guardia, 2011). Diatom frustules have a unique pore arrangement that varies greatly with the diatom species, and their pore sizes range from 50 nm to several microns. Diatom frustules are composed of amorphous hydrated silica ($\text{SiO}_2 \cdot 2\text{H}_2\text{O}$) with organic macromolecules (Wu, Coca, Suen, Chang, & Lin, 2012). These superior physicochemical properties make it easier to extract Frustul from diatomaceous biomass (De Stefano, De Stefano, & Congestri, 2009).

2. RESEARCH METHODS

2.1 Kultivasi *Navicula sp. galur TAD*

The equipment used was sterilized using an autoclave at 121°C to avoid contamination. cells *Navicula sp. TAD* strains, grown in a photobioreactor using modified medium (Telussa, Rusnadi, & Nurachman, 2019). Cultivation was carried out at room temperature under a light intensity of 5000 lux. with an initial cell density of 5×10^5 cells mL^{-1} with a photoperiod of 12:12 hours (dark: light), salinity 28 ppt, pH 8.2–8.5 and aerated with free air bubbles. Cell growth in culture was observed for cell shape using a *Neubauer Haemocytometer* under a light microscope.

2.2 Harvesting biomass *Navicula sp. galur TAD*

sp Navicula . TAD lines, which have been cultivated are harvested using sedimentation and filtration techniques. wet cell biomass *Navicula sp. TAD* lines, weighed to obtain the weight of wet biomass..

2.3 Isolation and characterization frustulm *Navicula sp. galur TAD*

2.3.1 Chemical Treatment

The initial organic mass of diatoms was removed by washing with an acid solution. HCl acid solution was chosen as the general washing solvent. The effect of acid concentration to remove biomass impurities was carried out by adding 2.50 g of biomass to a 250 mL Erlenmeyer. The concentration of the various acid solutions is 20%. The mixture was shaken at 180 r/min at room temperature (about 25°C) for 30 minutes. The reaction product was centrifuged at 4000 rpm for 10 minutes. Then the precipitated sediment was washed with deionized water and centrifuged for 3 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 retained moisture.

2.3.2 Thermal Treatment

After the acid wash treatment, the dry washed frustules samples are treated in the furnace for 3 hours. Optimized combustion temperature. The combustion temperature is set at 600°C .

2.3.3 Characterization of frustul

Characterization of diatom frustules was analyzed using SEM-EDS (Hitachi Flexsem 100) and FTIR (Shimadzu, Type: IRPrestige 21)

4. RESULTS AND DISCUSSION

4.1 Kultivasi *Navicula sp. galur TAD*

Navicula sp. TAD strain is a microalgae from the class Bacillariophyceae (diatoms), isolated from deep Ambon Bay waters (Telussa, Hattu, & Sahalessy, 2021), has a cell wall composed of silica (SiO_2) called frustules. The frustules of each diatom have a different morphology. *Navicula sp.* the *TAD* strain has frustules that are oval in shape with nanopores scattered on the

surface (Figure 1). The light and electron microscopy (SEM) image shows that the frustules are asymmetrical with the spread of nanopores on the upper surface being wider and the lower surface being smaller so that they are shaped like the letter D.

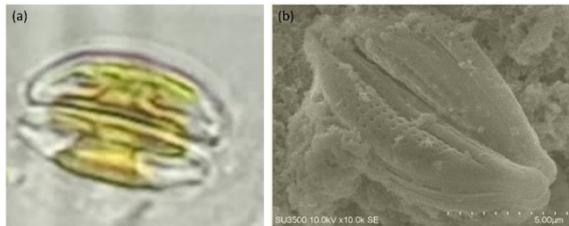


Figure 1. *Navicula* sp. cells. TAD strain. (a) Observation under a light microscope; (b) SEM observation

cell cultivation *Navicula* sp. TAD strains to obtain biomass are carried out through a cell cultivation process. Cell cultivation was carried out in a room with a light intensity of 5000 lux, photoperiod of 12:12 hours (dark:light), salinity 28 ppt, pH 8.2–8.5 and aerated with free air bubbles. Cell cultivation *Navicula* sp. TAD strains increased with time with darker culture discoloration (Figure 2). The change in culture color on the 7th day confirmed the change in cell density on the growth of *Navicula* sp. the TAD line, where the darker culture color indicates a higher number of cells and higher biomass productivity.

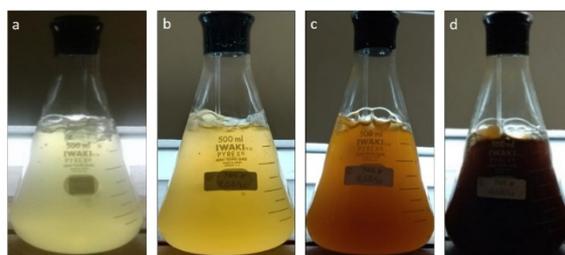


Figure 2. cell cultivation *Navicula* sp. TAD strain, Culture on day 0 (a), day 2 (b), day 4 (c), and day 7 (d)

cells *Navicula* sp. TAD lines were grown in modified medium (Telussa *et al.*, 2019) to obtain high cell growth and biomass. Modified medium is a simple medium containing a mixture of nitrate, silicate, iron and phosphate. Using a starting cell of 5×10^5 cells mL^{-1} at a light intensity of 5000 lux,

Navicula sp. TAD strains were grown in modified medium. Cells were grown for 7 days and showed different culture color changes and cell densities during growth (Figure 3). The change in color of the culture on day 7 confirmed the change in cell density on the growth of *Navicula* sp. TAD strain, where the darker culture color indicates a higher number of cells and higher biomass productivity. The highest cell density was obtained on day 7 of $72.167 \times 10^5 \pm 0.946$ cells mL^{-1} .

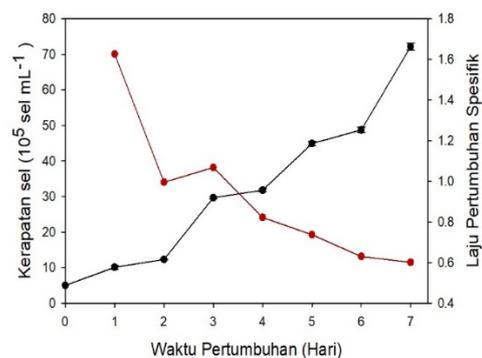


Figure 3. Growth curve of *Navicula* sp. TAD line (black) and Specific growth rate (red)

Specific growth rate of *Navicula* sp. TAD strains can be seen in Figure 3 (red), where the specific growth rate of *Navicula* sp. TAD strains decreased every day. This indicates that *Navicula* sp. TAD strains more optimally adapt to the conditions of the growth medium so that the availability of nutrients in the medium is more quickly absorbed for the process of cell division. The environmental conditions of the culture media with reduced nutrient content caused *Navicula* sp. The more TAD strains, the slower the cell divides.



Figure 4. *Navicula* sp. cell cultivation. TAD strains *scale-up*

Biomass production in large quantities is carried out by increasing the growth medium volume (*scale-up*) (Figure 4). This method is used with a gradual increase in the volume of cultivation medium to obtain large amounts of biomass with good cells.

4.2 Harvesting Biomass

The process of harvesting *Navicula* sp. TAD lines were carried out on the 7th day, where the cells reached their maximum point with very good cell shape in the exponential phase. The culture harvesting process was carried out using sedimentation and filtration techniques (Figure 5). The sedimentation technique was carried out for 30 minutes. The time needed in the sedimentation process is not too long because *Navicula* sp. TAD strains have a large cell shape and size, so it doesn't take a lot of time in the sedimentation process (Santoso, 2017). This technique is used in the process of harvesting *Navicula* sp. TAD strains because the cell size is large enough so that the harvesting process can be done more easily. The yield from 800 ml of culture obtained 0.933 ± 0.062 g of dry biomass with a biomass productivity of 0.166 ± 0.011 gL⁻¹h⁻¹. While harvesting from 10 L of culture obtained the weight of wet biomass obtained was 65.217 g with a biomass productivity obtained of 0.932 gL⁻¹ h⁻¹ and dry biomass obtained of 11.459 g with a biomass productivity obtained of 0.1364 gL⁻¹ h⁻¹.



Figure 5. *Navicula* sp. biomass harvesting process. TAD strain. (a) Culture on the 7th

day, (b) Screening process, (c) Alkaline biomass, (d) Dry biomass.

4.3 Isolation of the frustul diatom *Navicula* sp. strain TAD

Isolation of frustules from biomass through acid washing and burning processes can be seen in Figure 6a-d. Prior to treatment, the biomass sample of *Navicula* sp. TAD line is green. Changes in the color gradient of the sample after washing and burning treatment showed a whiter frustule color. The results showed that acid leaching pre-treatment was required to remove the impurities from the biomass. The removal of impurities by washing with 20% HCl solution was $78.00 \pm 0.514\%$ and the accumulation rate of removal of impurities after the combustion treatment was 93.072%.

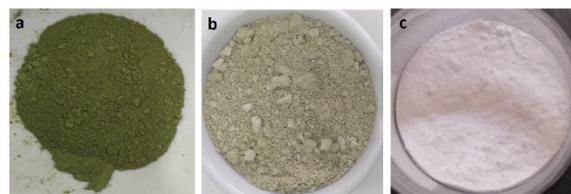


Figure 6. Sample (a) *Navicula* sp. strain TAD, (b) frustules washed with 20% HCl, (c) frustules washed with 20% HCl, and firing at 600°C.

4.4 Characterization of Frustul *Navicula* sp. TAD strain

Morphology of the biomass, washed with 20% HCl, and fired at 600 °C, observed under the electron microscope (SEM) (Fig. 7a-b). In Figure 4a which shows the cell surface of *Navicula* sp. the untreated TAD line had a non-porous surface. Meanwhile, after washing treatment with 20% HCl, structure and further burning at 600 °C, micro-pores can be seen clearly on the surface (Figure 7b). This confirms the decomposition of surface impurities. The results showed that the combustion treatment at 600 °C can remove impurities effectively and keep the frame structure intact. Thus, the acid wash treatment method followed by a 600 °C firing treatment is good for obtaining more clearly exposed frustules.

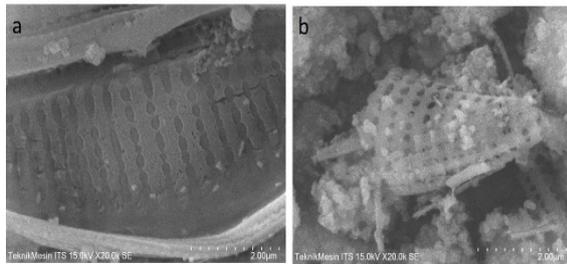


Figure 7. SEM image (a) untreated diatom biomass, (b) frustules washed with 20% HCL fired at 600 °C

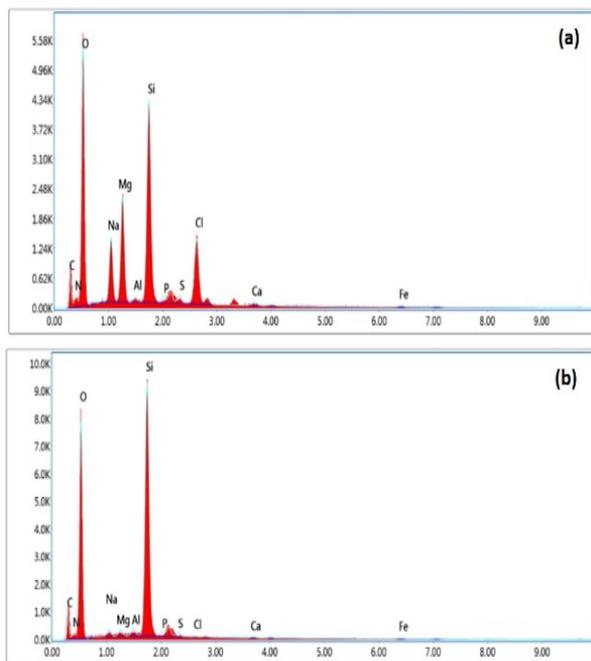


Figure 8. EDX spectrum (a) Biomass (b) Frustules Diatom *Navicula* sp. strain TAD

Energy dispersion X-ray spectroscopy (EDS) was used to analyze the chemical composition of gross frustules (Fig. 8). The atomic content of N elements decreased after treatment, while the atomic content of Si and O increased, indicating that impurities were successfully removed. But the atomic content of element C slightly increases. The results showed that the combustion temperature at 600°C resulted in an increase in Si atomic content from 15.72% to 28.82%,

so that the surface of frustul *Navicula* sp. can be seen clearly.

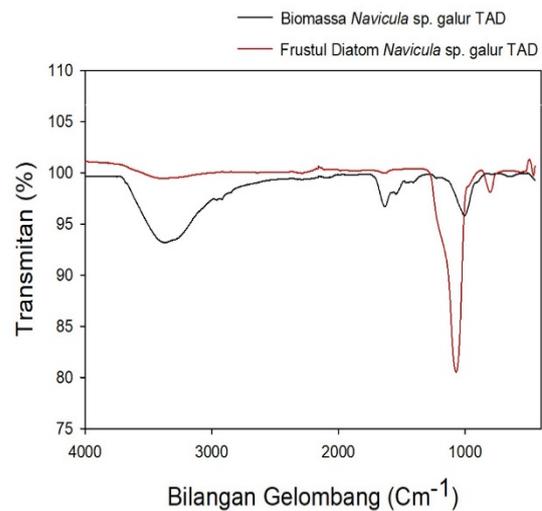


Figure 9. FTIR Spectrum of Biomassa and Frustul Diatom *Navicula* sp. TAD strain

Functional group analysis using a *Fourier Transform Infra-Red* (FTIR) spectrophotometer showed characteristic peaks of biomass and frustul *Navicula* sp. strain TAD (Figure 9). Chemical groups in *Navicula* sp. biomass. TAD strains include *stretching* Si-O-Si OH Si-O-Si bending at 1040–1095/cm, and *bending* on hydroxyl groups at 3435/cm, -COOH at 1734–1887/cm, -COO - at 1400–1651/cm and -OH of -COOH at 2523/cm, -SO- at 1231/cm, -R₃C-OH at 1072/cm, and -CH₂ -, -CH₃ -, -CH₄ - at 2850–2949/cm (De Stefano, De Stefano, & Congestri, 2009).

Navicula sp. the TAD strain that has been washed with HCL solution and fired, the -COOH, -COO-, -OH groups from -COOH, -SO-, or -R₃C-OH are removed. The frustul functional groups include *stretching* of Si-O-Si at 470/cm, Si-O-Si at 797–800/cm, *bending* Si-O on Si-OH groups at 950/cm, Si-O-Si at 1040– 1095/cm, and the OH hydroxyl group at 3435/cm, containing adsorbed water and stretching Si-OH. The peaks of 1630–1640/cm and 3740–3780/cm represent the Si-OH bonds. Compared to biomass, most of the organic chemical groups disappear, indicating the successful removal of impurities from crude dirty

diatoms through acid leaching and combustion.

5. CONCLUSION

Cultivation of *Navicula* sp. the TAD strain had the highest cell density obtained on day 7 of $72.167 \times 10^5 \pm 0.946$ cells mL⁻¹. *Navicula* sp. 65.217 g of wet biomass was obtained with a biomass yield of 0.932 gL⁻¹ h⁻¹ and 11.459 g of dry biomass obtained with a biomass productivity of 0.1364 gL⁻¹ h⁻¹. Frustul *Navicula* sp. the TAD line washed with 20% HCl and fired at 600°C had whiter frustules. Characterization of the diatom frustul *Navicula* sp. the TAD strain exhibits a clearly visible nanoporous structure of the surface and is composed of silicon and oxygen. This indicates that through washing and burning can produce impurities free frustules so that the surface of *Navicula* sp. frustules. TAD strains can be seen clearly.

ACKNOWLEDGMENTS

Thanks to the Head of the Biochemistry Laboratory, Chemistry Department, Faculty of Mathematics and Natural Sciences and the Research Team who have assisted in the process of completing this research.

DAFTAR PUSTAKA

- De Stefano, M., De Stefano, L., & Congestri, R. (2009). Functional morphology of micro- and nanostructures in two distinct diatom frustules. *Superlattices and Microstructures*, 46 (12): 64-68.
- Dolatabadi, J. N., & de la Guardia, M. (2011). Applications of diatoms and silica nanotechnology in biosensing, drug and gene delivery, and formation of complex metal nanostructures. *TrAC Trends in Analytical Chemistry*, 30 (9): 1 538-1 548., 30 (9): 1 538-1 548.
- Gordon, R., Losic, D., Tiffany, M. A., Nagy, S. S., & Sterrenburg, F. S. (2009). The Glass Menagerie: diatoms for novel applications in nanotechnology. *Trends Biotechnol*, 27 (2): 116-127.
- Lu, J., Sun, C., & Wang, Q. J. (2015). Mechanical simulation of a diatom

- frustule structure. *Journal of Bionic Engineering*, 12 (1), 12 (1): 98-108.
- Meyers, M. A., Chen, P. Y., Lin, A. M., & Seki, Y. (2008). Biological materials: structure and mechanical properties. *Progress in Materials Science*, 53 (1): 1-206.
- Mohammed, S. J. (2015). Micro- and nanotechnologies in plankton research. *Progress in Oceanography*, 134 : 451-473.
- Santoso, A. D. (2017). Pemanenan Mikroalga Dengan Metode Sedimentasi. *Jurnal Rekayasa Lingkungan*, 10:1.
- Sheng, G. D., Wang, S. W., Hu, J., Lu, Y., Li, J. X., Dong, Y. H., & Wang, X. K. (2009). Adsorption of Pb(II) on diatomite as affected via aqueous solution chemistry and temperature. *Colloids and Surfaces A : Physicochemical and Engineering Aspects*, 339 (1-3): 159-166.
- Telussa, I., Hattu, N., & Sahalessy, A. (2021). Morphological Observation, Identification and Isolation of Tropical Marine Microalgae from Ambon Bay, Maluku. *Indonesian Journal Of Chemical Research*, 9(3) 137-243.
- Telussa, I., Rusnadi, & Nurachman, Z. (2019). Dynamics Of β -Carotene And Fucoxanthin Of Tropical Marine *Navicula* Sp. As A Response To Light Stress Conditions. *Algal Research*, 41, 101530.
- Wu, M. C., Coca, J. P., Suen, S. Y., Chang, G. L., & Lin, C. F. (2012). Chemical modification of *Nitzschia panduriformis*'s frustules for protein and viral nanoparticle adsorption. *Process Biochemistry*, 47 (12): 2 204-2 210.