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Fabrication of SiO_2 Materials used in Diatom Culture

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ABSTRACT

Diatoms are one of the microalgae species commonly used as food for many aquatic organisms during the hatchery stage. The culture of diatoms usually uses silicon from Na_2SiO_3, but this salt compound easily causes the precipitation of trace ions in the culture medium. This study is to fabricate SiO_2 material particles used to replace Na_2SiO_3 in the period of diatom proliferation and application in aquiculture. The material particles were synthesized based on the Stober method using the surfactant compound CTAB with a spherical particle structure with the particle size of about 700 nm. Testing using SiO_2 material particles showed faster growth of diatoms compared with conventional culture media using Na_2SiO_3.

Keywords: Diatoms; SiO_2; Culture; Sea food; UV-Vis.

1. Introduction

Diatoms (diatoms) are single-celled photosynthetic organisms, belonging to the class Bacillariophycease in the phylum Heterokont, with a distinctive feature from other microalgae that their cell walls contain silicon [1-2]. In aquaculture hatcheries, diatoms are used as the main food for oyster larvae, geoduck, mussels and shrimp. Diatoms are said to be the best indicator zooplankton in shrimp ponds. When combining diatoms with biofloc, shrimp grow faster and have higher survival rates [3-5]. Currently, there are many research projects focusing on isolation, seeding, storage and cultivation of algal biomass and the production process of algae species is also relatively complete. However, limited applied research in production facilities needs to be conducted further to find a better active nutrient supply for diatoms. In addition to optimizing the process of cultivating aquatic animals based on changing food sources and changing farming methods, finding a new solution combined with new technology to speed up the process is important. Diatom farming helps save farming time while supporting increased productivity in aquaculture farms [5].

Traditional algae farming environments often use silicon sources from Na_2SiO_3, however, when Na_2SiO_3 concentration increases, it can easily cause silicate precipitation of trace ions such as Mn^{2+}; Co^{2+}; Fe^{3+}, changes the nutritional composition of the culture medium [6-7]. Meanwhile, SiO_2 particles can be a suitable source of Si for diatom culture because they do not react to precipitate with trace ions, so increasing the concentration does not have much effect on the ingredients in the farming environment. Therefore, SiO_2 nanoparticles have the ability to accelerate the proliferation process of diatoms. This research aims to manufacture SiO_2 material particles and test using them as food in cultivating diatoms on a laboratory scale.

2. Experiments and Methods

2.1. Fabrication process of SiO_2 nanoparticles

Prepare stock solution: 20ml CTAB 0.1M in solvent C_2H_5OH and C_3H_7OH (ratio 1:1). First, prepare solution A: measure 44.3 ml of solvent C_2H_5OH and C_3H_7OH (ratio 1:1) into a triangle flask, add 10 ml TEOS into the flask.

Add 0.7 ml CTAB 0.1 mM. After stirring for 10 minutes, set the magnetic stirrer to level 2. Prepare solution B: Add 9 ml of distilled water to the beaker, then add 6 ml of NH4OH to the beaker. Then drop solution B into solution A, stir magnetically for 4 hours, and place the machine at 60oC for 4 hours [8]. The synthetic sample is cleaned with alcohol mixed with distilled water (1:1), then diffused again in distilled water to completely remove surfactants and unwanted products of the reaction to obtain a biological system. SiO_2 material (Figure 1). The general reaction taking place during the synthesis is presented as equation 1: $Si(OC_2H_5)_4 + 2\ H_2O \rightarrow SiO_2 + 4C_2H_5OH$ (1)

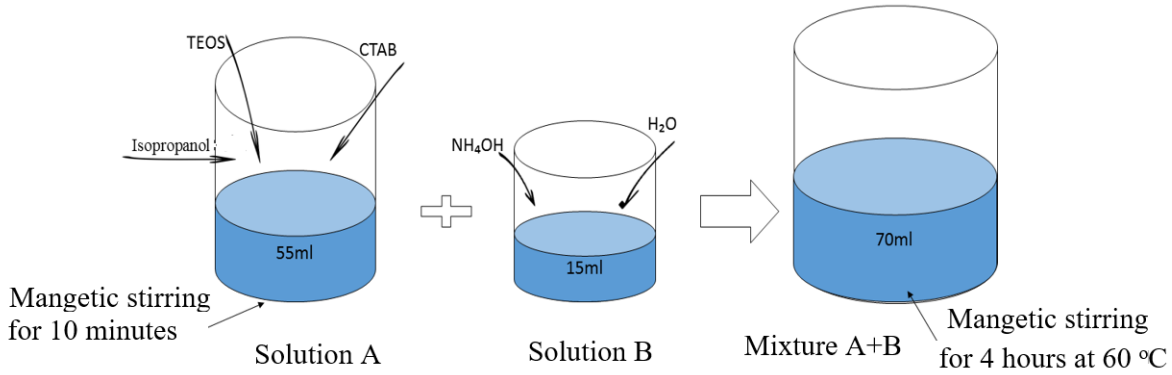

Figure 1. Fabrication diagram of SiO_2 material system

2.2. Algae farming process

The culture medium is mixed from stock solutions and supplemented with silicon sources from Na_2SiO_3 or spherical SiO_2 particles [5]. Samples supplemented with silicon from Na_2SiO_3 (environment symbol F2); Sample addition of SiO_2 particles (SiO_2 nano media, respectively denoted $15SiO_2$, $30SiO_2$ and $120SiO_2$ corresponding to the mass of SiO_2 nanoparticles added into the solution is 15, 30, and 120 mg. The experimental model is Arranged with the culture vessels arranged symmetrically, 15W compact light bulbs are arranged at the 4 corners of the outer rectangle to ensure that the light reaching the experimental vessels is the same. Air is provided. Supplied into the vessels from a branched aeration system to ensure the air pressure is the same in every vessel. Air and light are supplied continuously throughout the culture process to ensure photosynthesis of the algae Room temperature is kept between 28-31^oC (Figure 2).

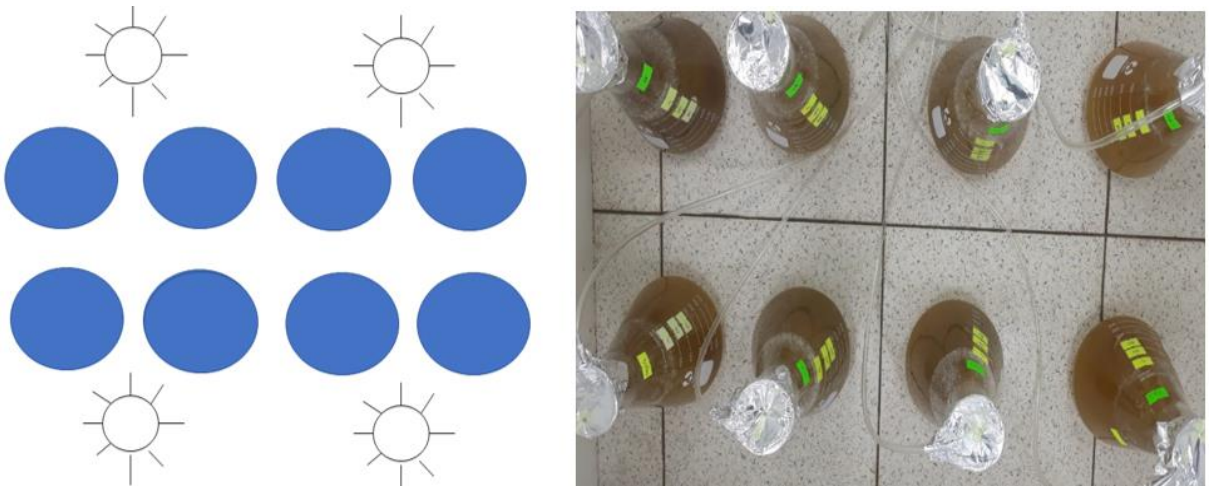

Figure 2. Diagram of experimental setup and pictures of experimental diatom farming

2.3. Evaluation methods

The morphology of SiO_2 grain structure was measured on a scanning electron microscope SEM and transmission electron microscope TEM. Algae absorption spectrum measurements were performed on a UV-Vis meter. Measure culture images on the Axio Scope A1 microscope and counting chamber system.

3. Results and Discussions

3.1. Morphology of SiO_2 particle

Figure 3 presents the structural morphology of SiO_2 material, evaluated by scanning electron microscope (SEM - Scanning Electron Microscope) and transmission electron microscope (TEM - Transmission Electron Microscope). Observing SEM images shows the 3D structure of the composite material system; consists of fairly uniform spherical SiO_2 particles; The particles grow independently and do not stick together; Particles arranged on top of each other create gaps and pores in the composite material system. The 2D structure of the material system observed by TEM images gave results consistent with the analysis by SEM images, confirming that the SiO_2 spheres were formed and developed independently.

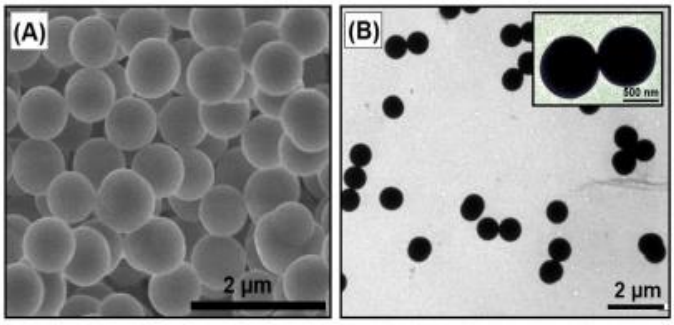

Figure 3. Observation by SEM and TEM of sample SiO_2-CTAB 1mM

The particle size and particle size distribution are shown in Figure 4. The results show that the average particle size of SiO_2 material particles is 700 nm ± 50 nm, with a Poisson distribution. The Poisson size distribution line is consistent with the assessment of the independent growth of SiO_2 particles during the material fabrication process as observed by structural morphology using SEM and TEM images.

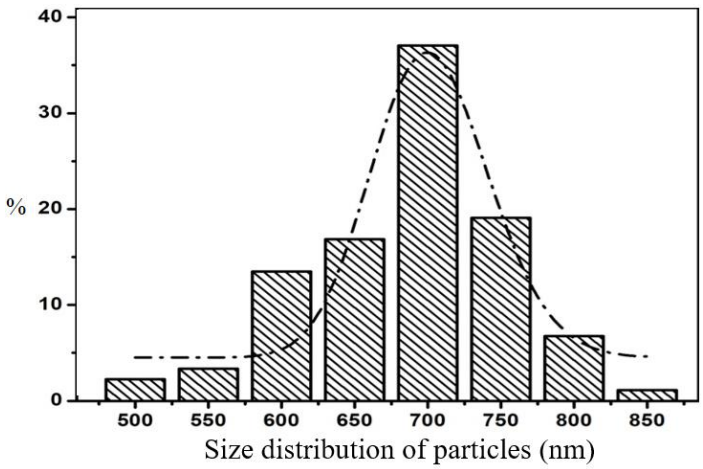

Figure 4. Particle size distribution curve of SiO_2 material – CTAB 1mM

In previous research, SiO_2 material system was synthesized with size controlled through CTAB surfactant content [8]. CTAB surfactant helps regulate particle morphology, acting as a template during particle synthesis. Adjusting the CTAB surfactant content can produce SiO_2 particles with sizes below 200 nm. In this study, SiO_2 particles with a size of about 700 nm were synthesized for use in cultivating diatoms as food for oyster larvae, geoduck, mussels and shrimp in aquaculture. SiO_2 particles with the above size are added to diatom culture environments as food in aquaculture.

3.2. Determine the concentration of diatoms using a microscope and measuring the absorption spectrum

Electron microscope image and absorption spectrum of a standard algae sample for studying the growth of algae in a culture environment containing SiO_2 material system are presented in Figure 5. Microscope image of algal cells taken on the counting chamber shows that the standard sample has an algal cell concentration of $6.52 \pm 1.58 \times 10^6$ (cells/ml) (Figure 3A). The method of using a cell counting chamber in electron microscope imaging technique to determine algae concentration is a popular method. However, determining concentration using a counting chamber often takes a lot of time, along with the counting error is also very large because it usually only counts on a small volume (about 10µl). With the same algae sample above, the absorption spectroscopy method was used to determine algae concentration by measuring the spectrum in the light wavelength range from 550 - 850 nm (Figure 3B). The results of measuring the absorption spectrum of the algae sample show an absorption peak at 685 nm and an absorption intensity of 0.72 (a.u). The wavelength of 685 nm is the wavelength corresponding to the characteristic absorption peak of Chlorophyll a - the photosynthetic nucleus of diatoms [9].

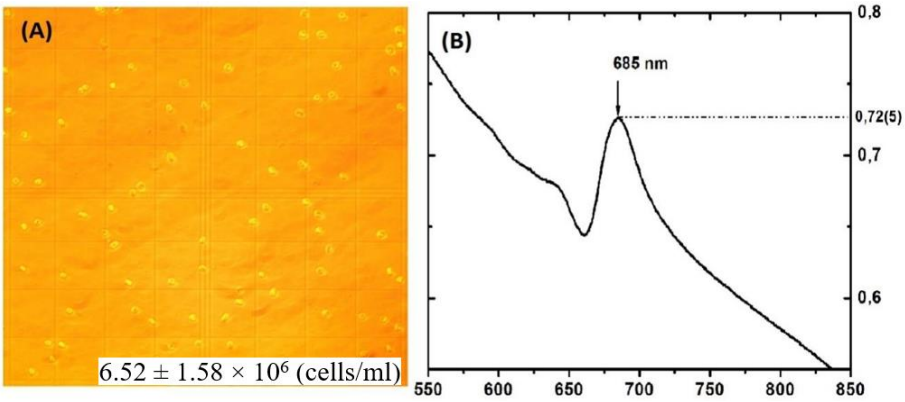

Figure 5. Microscope image and absorption spectrum with absorption intensity measured at peak 685 nm of standard algae sample

From the original standard algae sample, mix a solution containing diatoms with different concentrations and investigate the dependence of the absorption intensity at the peak of 685 nm. In particular, the algal concentration is determined using a cell counting chamber and the absorption intensity is taken from the absorption spectrum. It can be seen that, when the cell concentration is less than 11×10^6 cells/ml (corresponding to an absorption intensity of 1.1), the cell concentration is proportional to the absorption intensity (Figure 6). Because the height of the counting chamber is 100 µm, when the cell concentration is greater than 11×10^6 cells/ml, the number of cells outside the focusing field of the microscope increases, easily causing missed cell counts. Therefore, the measured points are usually located below the linear fit line. By linear fitting method of data points in Figure 4, when the absorption

intensity is less than 1.1 (a.u) - C = a. Abs, where C is the cell concentration, Abs is the absorption intensity at 685 nm, we get coefficient a = 11.13 ± 1.38 (10^6 cells/ml. absorption units). Thus, the method of determining cell concentration using absorption intensity at wavelength 685 nm shows more efficiency than using a counting chamber. Furthermore, the method using absorption intensity gives smaller errors when measuring on a much larger volume than using a cell counting chamber (3ml compared to about 10 µl). From the linear fit results, algae concentration was calculated and plotted against culture time.

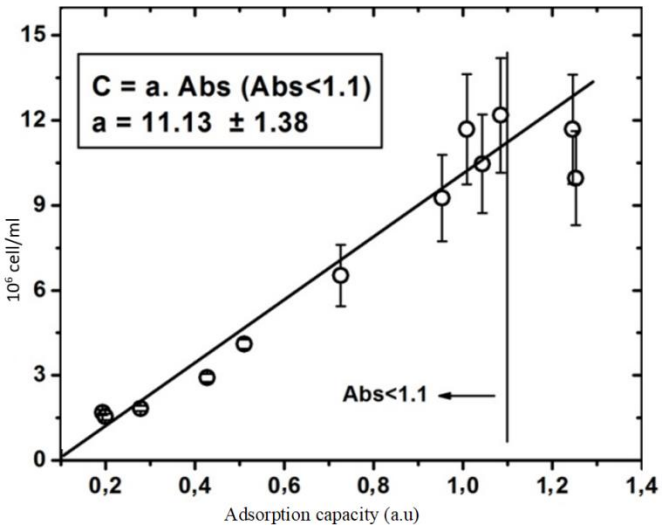

Figure 6. Dependence of algae concentration on absorption intensity determined based on sample absorbance at 685 nm

3.3. Development process of diatoms when cultured with different concentrations of SiO_2 particles

Figure 7 depicts the growth of diatoms carried out in different culture media denoted as F2; $7.5SiO_2$; $30SiO_2$; $60SiO_2$ and $120SiO_2$. It can be seen that, in the presence of SiO_2 nanoparticles in the culture solution, diatoms showed faster growth than when grown in a medium containing Na_2SiO_3 (sample F2) even when the concentration of silicon was present in the medium. The culture medium is smaller than that in the F2 environment. The results show the promise of using synthetic SiO_2 materials in cultivating diatoms as food for larvae in aquaculture [10].

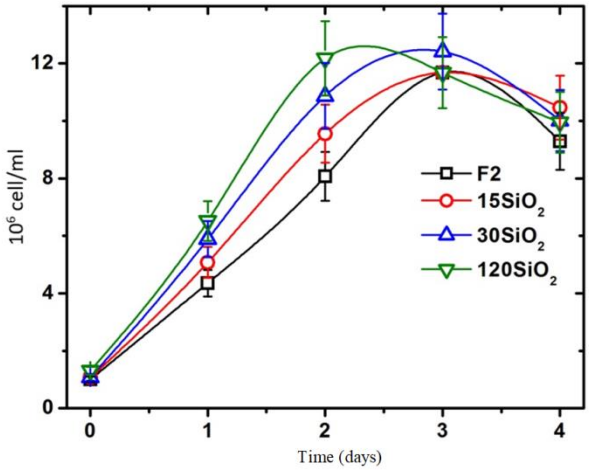

Figure 7. Dependence of algae concentration on culture time and amount of SiO_2 nanoparticles in the culture medium

4. Conclusion

The research has successfully produced SiO_2 nanoparticles that are uniform in both size and structural morphology. The SiO_2 particles are uniformly spherical with an average particle size of 700 nm ± 50 nm. Initially, SiO_2 particles were successfully used to replace Na_2SiO_3 in improving the farming environment to speed up the diatom growth process. The introduction of SiO_2 nanoparticles not only creates a new food source but also introduces technology to increase efficiency and productivity in the aquaculture process.

Declarations

Source of Funding

The study has not received any funds from any organization.

Competing Interests Statement

The author has declared no competing interests.

Consent for Publication

The author declares that he/she consented to the publication of this study.

Ethical Approval

Not applicable.

Authors' Contributions

Author's individual contribution.

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