Research Article
Effect of Silver Nanoparticles on Tropical Freshwater and Marine Microalgae

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The increase in synthesis and application of silver nanoparticles (AgNPs) in the last decade has resulted in contamination of AgNPs in the aquatic environment. The presence of AgNPs in aquatic environments has posed toxic effects to aquatic organisms and ecological damage. In this study, two tropical microalgae species including the freshwater Scenedesmus sp. and the marine diatom Thalassiosira sp. were employed to examine the toxic effects of AgNPs. The toxic effects were determined by analyzing different end points, such as half maximal effective concentration (EC$_{50}$), algae growth inhibition, algae cell size, chlorophyll-$a$ content, and total lipid accumulation. The results suggested that AgNPs presented different toxicity mechanisms for microalgae and showed to be more toxic in freshwater than in marine environment. The EC$_{50}$ values of AgNPs after 72h for the growth inhibition of Scenedesmus sp. and Thalassiosira sp. were 89.92 ± 9.68 and 107.21 ± 7.43 μg/L, respectively. AgNPs at a certain concentration have resulted in change in cell diameter, reduction in chlorophyll-$a$ content, and enhancement of the total lipid production in the tested microalgae. Thus, local species should be involved in the toxic assessment. This research contributes on understanding the toxicity of AgNPs on freshwater and marine environments.

1. Introduction

The rapid development and application of nanomaterials in different industrial sectors have resulted in contamination of nanomaterials in the aquatic environment [1, 2]. In particular, nanomaterials containing silver have been widely used in various industries and applications [3], because of its antibacterial activity. More than 1300 products associated with NP technology are now available [4], in which 438 products (account for 24%) contain AgNPs [5]. In the USA, over 50% of the registered biocidal silver products by the Environmental Protection Agency (EPA) likely contain AgNPs. And, about 230 tons production with AgNPs is produced each year from European countries [6]. Although the concentration from nondetectable to over 0.1 mg/L of AgNPs has been detected from surface waters, the quantitative monitoring program for AgNPs in water environment is not currently available [7]. Therefore, the contamination of these nanomaterials has recently become environmental concerns due to the potential hazards in aquatic ecosystem [8, 9].

In ecological ecosystems, the microalgae are primary producers and play a vital role in the food chains. Thus, the bad effects of pollutants on microalgae species may break the balance of whole ecosystems [10]. The toxic effect and behavior of nanomaterial in the ecosystems on microalgae have been recently reviewed [3, 11]. Previous studies have indicated that AgNPs caused inhibition of growth in freshwater green microalgae [12], decrease photosynthesis activity in the unicellular green algae Chlamydomonas reinhardtii [13], and stimulate the antioxidant activities in the marine flagellate Chattonella marina [14]. AgNPs caused depletion in chlorophyll content and morphological malformations in the freshwater green alga Pithophora oedogonia [15]. Decrease in chlorophyll content, viable algal cells, increased reactive oxygen species (ROS) formation, and lipid peroxidation in the freshwater microalga Chlorella vulgaris and marine microalga Dunaliella tertiolecta were observed after exposure to AgNPs for 24h [16]. Huang et al. [10] reported that AgNPs at a concentration of 0.5 mg/L caused both significantly induced excess intracellular ROS and
decreased cell viability in the marine diatom *Skeletonema costatum*.

Although the adverse effects of AgNPs on different organisms have been reported, previous studies however focus largely on freshwater environment [6]. There is little understanding about toxic effects of AgNPs on marine species, particularly the ones from tropical region. Therefore, the aims of this study was to investigate the toxicity of AgNPs to different species including the freshwater microalgae, *Scenedesmus* sp. (Chlorophyceae), and the marine diatom, *Thalassiosira* sp. (Bacillariophyceae). The toxic effects were determined by analyzing different end points, such as half maximal effective concentration (EC50), algae growth inhibition, algae cell size, chlorophyll-α content, and total lipid production.

2. Materials and Methods

2.1. Silver Nanoparticles. Silver nanoparticles were prepared as previously reported by Becaro et al. [17]. Briefly, stock solution of AgNPs was prepared by dissolving 10 g/L of AgNO3 (Aldrich Chemical Co., MO, USA) in MQ-water and heating at 80°C for 5 min. To this solution, 5 g/L of polyvinyl alcohol (PVA) as a stabilizing agent was added with constant stirring. Ultraviolet-visible (UV-Vis) absorption was monitored from 300 to 600 nm by using a Bio)K_hermo spectrophotometer. Diameter of particle size and morphology were characterized by a transmission electron microscope (TEM). Samples were kept in dark at 4.0 ± 1°C and used within 6 months. The physicochemical properties of AgNP were stable under this condition [18].

2.2. Test Species. In this study, the freshwater microalgae *Scenedesmus* sp. isolated from the Saigon River, Ho Chi Minh City, Vietnam, and the marine diatom *Thalassiosira* sp. isolated from coastal waters of the Can Gio Sea, Ho Chi Minh City, Vietnam, were used. The green algae *Scenedesmus* sp. was maintained in COMBO medium (pH: 7.5 ± 0.3) [19], and the diatom *Thalassiosira* sp. was maintained in F/2 medium (pH: 8.0 ± 0.3) enriched with 0.2 μm filtered seawater. Both cultures were grown on a 12h light:12h dark cycle at a temperature of 28°C for 72 h under a 12h light-dark cycle. The pH of all test solutions was measured at the beginning and end of the test. After 72 h, subsamples of culture medium (about 10 mL) were fixed with Lugol’s solution and cell density was determined by using a Sedgewick Rafter counting chamber. About 200 mL of culture medium was collected at the end of the test, filtered onto glass fiber filters (GF/C, Whatman, England), dried at 45°C for 48 h, and stored at −20°C prior to total lipid extraction and analysis. The cell size of microalgae was measured directly by using an ocular micrometer. The graduation in arbitrary units is calibrated by using a microscope calibration slide.

The AgNPs concentration that resulted in 50% inhibition growth of algae over 72 h (EC50, 72 h) was calculated by using the specific growth rate (SGR). The SGR for each species was evaluated by the difference cell density after 72 h as follows:

\[
\delta_{x-y} = \frac{\ln C_y - \ln C_x}{t_y - t_x},
\]

where \(\delta_{x-y}\) is the SGR from time \(x\) to time \(y\), \(t_y - t_x\) is the duration of exposure periods in \(h\), and \(C_y\) and \(C_x\) are the cell density at times \(x\) and \(y\), respectively.

The inhibition growth rate (IGR) of algae was determined as follows:

\[
IGR = \frac{\delta_c - \delta_T}{\delta_c} \times 100,
\]

where IGR (%) is the inhibition growth rate of each microalgae species, \(\delta_c\) is the average growth rate of the control, and \(\delta_T\) is the average growth rate of the exposures.

2.4. Chlorophyll-α Determination. To determine the chlorophyll-α fraction, a known volume of culture samples (10 mL) was filtered using filter paper (GF/C, Whatman, England), and then the chlorophyll-α was extracted using 80% acetone for overnight in dark (4°C). After centrifugation, the supernatant was used to measure chlorophyll-α at 630–750 nm by using a spectrophotometer (UV-VIS, Harch, 500), and the concentration of chlorophyll-α (% of control) was calculated according to Metzner et al. [21].

2.5. Total Lipid Extraction and Measurement. The dried biomass was ground into powder; the total lipid in the homogenized cells was extracted according to the method of Bligh and Dyer [22] and analyzed using gravimetric quantification methods according to the procedure of Han et al. [23]. Briefly, about 50 mg dry weight (DW) of algae biomass (M1) was homogenized and digested with 3 mL of 1 mol/L HCl in 50 mL centrifuge tube at 80°C for 30 min. Then, the cell debris was collected after centrifugation. Lipid was then extracted with 3 mL methanol: chloroform (2:1 v/v) for 3 h and mixed well. Lipids settle in the upper phase was collected to a new dish that had been preweighed (M2). The dish was then dried completely and rewighted (M3). Lipid concentration (LC) was determined gravimetrically as follows:

\[
LC (%) = \frac{(M3 - M2)}{M1} \times 100.
\]
2.6. Statistical Analysis. The growth inhibition (%) of each species was determined according to the method of OECD [20]. The effective concentration (EC$_{50}$) values after 72 h for each species were evaluated by using the IGR at 50% by nonlinear regression. The data in tested treatments were presented as the mean ± SD. One-way analysis of variance (ANOVA) and post hoc Tukey’s honestly significant difference (HSD) were applied to identify the significant different between exposure and control treatments. All data were log-transformed to normalize the distribution before analysis. Significant different was considered at $P$ values $<$ 0.05.

3. Results

3.1. Characterization of AgNPs. The morphology, absorbance wavelength, and size distribution of AgNPs are shown in Figure 1. The TEM image of AgNPs is shown in Figure 1(a). The UV-Vis absorbance wavelength showed that the samples of AgNPs generated a single peak with maximum absorbance at 410 nm (Figure 1(b)). The particle size of AgNPs stock solution under TEM measurements indicated the dominant of particles with diameter from 6 to 10 nm (Figure 1(c)).

3.2. Growth Inhibition. The inhibition of AgNPs at the concentration from 5 to 200 μg/L on the Scenedesmus sp. and Thalassiosira sp. after a 72 h exposure is shown in Figures 2(a) and 2(b). A 72 h exposure of Scenedesmus sp. and Thalassiosira sp. to sublethal concentrations of AgNPs caused a concentration-dependent inhibition of growth. The 72 h EC$_{50}$ values of AgNPs for the growth inhibition of Scenedesmus sp. and Thalassiosira sp. were 89.92 ± 9.68 and 107.21 ± 7.43 μg/L, respectively. AgNPs from the concentration of 20 μg/L or higher resulted in significant inhibition on the growth of both algae. The freshwater green algal Scenedesmus sp. showed greater sensitivity than the marine diatom Thalassiosira sp. to AgNPs. Exposure to AgNPs at 200 μg/L inhibited completely the growth of Scenedesmus sp., whereas this concentration of AgNPs inhibited almost 100% growth of Thalassiosira sp. (Figure 2). The pH value rises slightly from 7.5 ± 0.3 at the beginning of the experiment to 7.8 ± 0.3 at the end in the test with Scenedesmus sp. (in COMBO medium), while with Thalassiosira sp. (in F/2 medium), the pH value remains at approximately 8.0 ± 0.4 throughout the period of incubation.

3.3. Algal Cell Diameter. Cell diameter of the green algal Scenedesmus sp. was 4.31 ± 0.15 μm. There was no difference in cell diameter of the Scenedesmus sp. between the control and treatments after 72 h exposure to AgNPs (Figure 3(a)). In the case of the marine diatom Thalassiosira sp., the cell diameter was not different from the control after 72 h exposure to AgNPs with concentration of 5, 20, and 50 μg/L, but AgNPs with concentration of 100 and 200 μg/L caused significant increase in cell diameter with respect to the control. The control treatment had an average cell diameter of 8.98 ± 0.17 μm, while the cell diameter increased up to 9.12 ± 0.31% and 8.05 ± 0.39% in the exposures with 100 and 200 μg/L of AgNPs, respectively (Figure 3(b)).

3.4. Chlorophyll-a Content. Results of chlorophyll-a contents in Scenedesmus sp. and Thalassiosira sp. were changed significantly upon exposure to AgNPs. In the freshwater green algal Scenedesmus sp., exposure to AgNPs at the concentration of 5 and 20 μg/L was not different with the control, but exposure to AgNPs at the concentration of 50, 100, and 200 μg/L resulted in significant decrease in chlorophyll-a content (the highest decrease of 21.5% in the treatment with 200 μg/L of AgNPs, with respect to the control) (Figure 4(a)). Chlorophyll-a content varied almost the same trend in the marine diatom Thalassiosira sp. The chlorophyll-a content in the lowest treatment of AgNPs (5 μg/L) was not different from the control but the treatments with AgNPs from 20 to 200 μg/L resulted in significant decrease in chlorophyll-a contents. The lowest level of chlorophyll-a (14.5%) was found in the treatment with 200 μg/L (Figure 4(b)).

3.5. Lipid Accumulation. Silver nanoparticles had great influence on the lipid production of Scenedesmus sp. and Thalassiosira sp. AgNPs at the concentration of 5 and 20 μg/L led to a significant increase in total lipid production in Scenedesmus sp., with the maximum total lipid increase of 8.1% and 7.6%, compared with the control, respectively. In contrast, AgNPs at higher concentration (100 and 200 μg/L) resulted in significant decrease in total lipid production. The highest concentration of AgNPs (200 μg/L) resulted in the lowest total lipid production (Figure 5(a)). In the marine diatom exposed to AgNPs, total lipid production was significantly increased (from 11.6 to 17.4%, compared with the control) in all treatment with AgNPs. The highest increase of total lipid production (17.4%) was found in the treatment with 100 μg/L (Figure 5(b)).

4. Discussion

In aquatic environment, green algae and diatom are not only the primary producers for higher tropic levels but also play an important role for the normal functioning of aquatic ecosystems [10]. Numerous studies have demonstrated that NPs generated adverse effects on freshwater and marine microalgae species [3, 24–26]. NPs are well known for their involvement of ROS in both animal and plant cells, causing oxidative stress and cellular damage [27, 28]. Zhang et al. [29] demonstrated the mechanism of the photosynthetic toxicity against the green algae Scenedesmus obliquus by using silver nanoclusters (AgNCs). The authors found that the photosynthetic toxicity of AgNCs was largely attributed to the “joint-toxicity” effect of particulate form of AgNCs and their released Ag’ which resulted in the disruption of the electron transport chain of light reaction and affected the content of main enzymes of the Calvin cycle of algae cells. Other toxicity mechanisms of NPs in algae cells are the interaction with the algae cell wall or binding to the cell surface that resulted in the formation of large aggregates.
[16, 30]. The formation of aggregates could entrap algal cells and block cell division with consequent inhibition of cell division [31, 32]. Sendra et al. [25] indicated that AgNPs have	he toxicity of NPs on algae may vary from algae species, exposure duration and dose, coating, and size [11, 24, 26, 33].
generated direct and indirect deleterious impacts on algae in different exposure environment. Generally, AgNPs tend to dissolve to Ag⁺ under the presence of oxygen in aqueous environment [34]. Thus, both AgNPs and Ag⁺ are present in the environment and hazardous effects of AgNPs to algae cells were contributed from the effect of AgNPs and their released Ag⁺. In freshwater algae, the toxic effect of Ag⁺ was the main mechanism, while under marine condition with the present of high chloride ions (Cl⁻), large amount of AgNPs are oxidatively dissolved and generate Ag/AgCl-NPs and AgClₓ⁻uestoside compound. The formation of silver/silver chloride nanoparticles decreases the toxic effect of AgNPs to organisms [25, 26, 34, 35]. In addition, the toxic effects of NPs may depend on various factors such as exposure duration, dose of exposure, and environmental conditions [29]. When exposing the marine algae Dunaliella tertiolecta to both ZnO NPs and the bulk ZnO, Manzo et al. [33] reported that the toxic effects of ZnO NPs on the tested algae were stronger than of the bulk ZnO, and the toxicity mechanisms of bulk ZnO may not relate to the ion of zinc. In addition, when using different marine and freshwater species for the toxicity test, Aravantinou et al. [24] found that toxic effects of ZnNPs obviously depended on the microalgae species as well as the exposure duration and dose. Results of the present study agree well with Sendra et al. [25] that AgNPs is more toxic in freshwater than in marine environment. Probably, the high generation of the dissolved ion Ag⁺ in freshwater media resulted in higher toxicity of AgNPs in freshwater microalgae [25, 29].

Previous studies often used physiological alterations as indicators for assessment toxicity of NPs in microalgae [10, 25, 35]. Chlorophyll-a content is commonly used to indicate the photosynthesis efficiency or the cell division of algal species [10, 36]. Exposure of microalgae to NPs has resulted in the reduction of chlorophyll-a content due to the damage in the pigment of the exposed cells or reduction of the pigment-protein complexes [10, 36]. The decrease in chlorophyll-a content in both tested algae upon exposure to AgNPs may be a result of accelerating the activity of the chlorophyllase or stimulating ROS production [10]. Results of the present study are in agreement with the observations of Wei et al. [36] and Huang et al. [10] that AgNPs has induced a physiological response in the freshwater and marine algae through reduction of chlorophyll-a content [10]. Under stress condition, living cells have the ability to...
generate enzymatic and nonenzymatic antioxidants to regulate ROS adverse effects. Thus, intracellular ROS have been commonly used as biomarkers to quantify the toxic effects of pollutants [10, 25, 35]. In addition, morphological characteristics such as cell size, cell teratology, or deformity have been used as bioindicators for evaluating toxicity of hazardous substances [37]. In this study, exposure to a specific concentration of AgNPs resulted in elevation in cell size of the marine diatom *Thalassiosira* sp. as well as depletion in chlorophyll-α content. These results indicated chlorophyll-α content and the morphological response in diatom frustule can be used to monitor hazardous substances in water environment.

Green algae and diatoms are known for storing lipid level up to 50% as a reserve food material under stress condition [28, 38]. Higher lipid contents in green algae and diatoms under heavy metals or nanoparticles stress have been reported [28, 38]. Under stress condition, algal cells tend to adjust the cellular metabolism and store more large molecules such as proteins and lipid [10]. Algae can tolerate toxic effects typically at lower doses of toxicants [28]. In this study, the lipid content in the green algae *Scenedesmus* sp. increased significantly at low dose of AgNPs, but decreased as higher dose of AgNPs could be reflected from the above mechanisms. He et al. [28] demonstrated that a specific concentration of NPs could promote neutral lipids accumulation in the green algae *S. obliquus*, but further increase of NPs has resulted in the inhibition of cell growth and reduction of lipid production. Results of this study were also in agreement with observations of Huang et al. [10] that there is a reduction in the chlorophyll-α content but an elevation in lipid contents in a dose-dependent manner upon exposure the marine diatom *S. costatum* to AgNPs. Further studies are needed to determine the optimal growth rates as well as for lipid production in the tested species, which could be used to make biodiesel in tropical regions.

5. Conclusions

By using two tropical microalgae species including the freshwater *Scenedesmus* sp. and the marine diatom *Thalassiosira* sp., the present study demonstrated the different toxic effects of AgNPs in freshwater and marine environments. The present results indicated the toxic effects of AgNPs may vary in different aquatic environments. Results indicated that AgNPs are more toxic in freshwater than in marine environment. AgNPs at a certain concentration have resulted in change in cell diameter, reduction in chlorophyll-α content, and stimulation of the total lipid production in the tested microalgae. Thus, morphological characteristic of microalgae can be used as an effective tool for monitoring nanoparticles in water.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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References


