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Effect of silver nanoparticles on water quality and phytoplankton communities in fresh waterbody

Ảnh hưởng của vật liệu nano bạc đến chất lượng nước và quần xã thực vật phù du trong thủy vực nước ngọt

Research article

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This study aims to investigate the potential effects of environmental variables and the toxicity of nanosilver colloids synthesized by chemical reduction method on growth and development of phytoplankton community (the *Microcystis* genus dominance) in the eutrophication Tien lake water, Hanoi city, Vietnam. The variables analyzed including: physical (pH and Turbidity), chemical (content of NH_4^+, PO_4^{3-} and silver metal), biological (content of Chlorophyll-a, cell density). The characteristic of nanomaterial was confirmed by using UV-visible spectrophotometer, TEM and HR-TEM methods. The obtained silver nanoparticles (AgNPs) showed that their spherical form and uniform size varied from 10 to 15 nm. The experimental results showed that the samples treated with AgNPs inhibition on growth against *M. aeruginosa* at concentration 1 mg/l after 8 days. The content of silver in aquarium water decreased from 1 mg/l (D0) to 0.8 mg/l (D8). The contents of chlorophyll-a of phytoplankton community, including *Microcystis* genus in samples exposed with AgNPs were declined from 11.27 ± 0.56µg/L (D0) to 1.98 ± 0.37 µg/L (D8) . The environmental variables such as: pH, temperature, dissolved oxygen, turbidity, ammonium, phosphate...in the experiment were below the limit of the Vietnam Standard 08:2015/MONRE for surface water quality.

Mục đích của nghiên cứu này là khảo sát ảnh hưởng của vật liệu nano bạc tổng hợp bằng phương pháp khử hóa học đến sinh trưởng và phát triển của quần xã thực vật nổi (chủ yếu là chi Microcystis) trong nước hồ Tiền phú dưỡng, tại Hà Nội, Việt Nam. Các thông số phân tích bao gồm: thủy lý (pH và độ đục), hóa học (hàm lượng amoni, photphat và hàm lượng bạc kim loại), sinh học (hàm lượng chất diệp lục, mật độ tế bào). Đặc trưng của vật liệu được xác định bằng các phương pháp quang phổ UV-VIS, TEM và HR-TEM. Vật liệu nano bạc có dạng hình cầu, kích thước đồng nhất trong khoảng 10-15nm. Kết quả thử nghiệm sau 8 ngày cho thấy các mẫu có bổ sung vật liệu nano bạc ức chế sinh trưởng đối với vi khuẩn lam M. aeruginosa ở nồng độ 1mg/l. Hàm lượng bạc kim loại giảm từ 1 mg/l (ngày đầu tiên) xuống còn 0.8 mg/l (vào ngày cuối cùng). Sinh khối thực vật nổi trong đó có chi Microcystis trong mẫu xử lý với AgNPs đã giảm tương ứng từ 11.27 ± 0.56 µg/L (ngày đầu tiên, D0) xuống 1.98 ± 0.37 µg/L (ngày cuối cùng, D8). Các thông số môi trường của nước hồ đều nằm dưới giới hạn cho phép của QCVN 08:2015/BTNMT đối với chất lượng nước mặt.

Keywords: Cyanobacteria, *Microcystis aeruginosa*, effect, nanoparticles

1. Introduction

Nowadays, the rapidly industrialization and urbanization has significantly increased the amount of waste water

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from condominiums and urban areas, which contributes significantly to the amount of nutrients (mainly N and P) in the receiving resource systems. This process contributes significantly to improve the quality of human life, but also makes the problem of environmental pollution more difficult, especially the eutrophication in fresh waterbodies. Eutrophication is an ecological term used to describe the over-enrichment of inorganic nutrients in water, typically nitrate and phosphate salts lead to excessive growth of aquatic organisms in which the majority of Cyanobacteria species are potentially toxic (Smith, 1983; Lennevey, 2017). The initial cause was identified as a loss of nutrient balance in the ecosystem's input, thus creating a competitive advantage for one species compared to other organisms in the ecosystem. In recent years, harmful algal blooms have been occurring in both saltwater (red tide) and freshwater (water blooming) both in frequency, intensity and duration (Codd, 1997; Beversdorf et al 2015; Paerl, 2014; Wang, et al., 2016; Boopathi, et al., 2014; Drobac, 2013).

Until now, about 100 species of freshwater cyanobacteria have been discovered in 40 genera of which *Microcystis, Anabaena, Aphanizomenon, Oscillatoria, Nostoc* and *Cylindrospermopsis* are the most frequently encountered in water blooms (Dang et al. 2014). *Microcystis* genus is the most common cyanobacteria, it is toxic to humans, animals and other aquatic organisms. This is one of the main cyanobacteria cause to water blooming. *Microcystis* genus is a group of thousands of individual cells, ranging in size from 2 to 6 μm and each cell contains an stomata, extensively distributed from nutrient-poor to brackish water and seawater. They pose a serious threat to the quality of water resources, change aquatic ecosystems and cause water pollution problems such as "water blooming" around the world (Blahoslav et al., 2012). Therefore, prevention and reduction of the rapid development of cyanobacteria are important environmental issues.

Currently, nanotechnology is widely used in the removal of pollutants in water. The nanoscale materials tend to used in place of traditional chemicals have been observed in several countries around the world in recent years. Nanomaterials exist in the form of activated materials such as carbon, cellulose and aluminium with carriers such as zeolite, bentonite and compounds containing Fe that can be used in aquaculture to remove ammonia, nitrite and nitrate (Blahoslav, 2012; Drabkova, 2007). Because of many advantage features and high applicability over traditional materials so the production of NPs indicates that quantities produced globally will likely increase steadily. Application of nanomaterials to treat water pollution especially toxic algae treatment in Viet Nam and over the world has received much attention and obtained certain results (Gubbin et al., 2011). Silver nanoparticles with unique antibacterial properties have been used in many fields such as molecular diagnostics, medical, catalysing, electronics… and recently applied in the treatment of environmental pollution (Skamaran, 2012). There are some of documents reported about effect of silver nanoparticles on bacterial, fungal, and mammalian cells but the impact of silver nanoparticles on algal growth has not been studied extensively. In our study, we have selected the Tien lake water to conduct at laboratory scale to investigate the potential effects of environmental variables and the toxicity of silver material synthesized by chemical reduction method on growth and development of phytoplankton community (the *Microcystis* genus dominance) in the eutrophication Tien lake water, Hanoi city, Vietnam.

2. Material and methods

2.1. Experiment setup

Experimental setup is similar to the experiment with copper nanoparticles published by the authors (Tran TTH, et al., 2016). The experiment was performed in aquarium tank containing approximately 10 litters of water collected from Tien Lake. Silver nanoparticles (AgNPs) was added in aquariums with the concentration of 1 ppm. The experiment was performed in triplicate. The experiment was conducted in 14 days under room conditions without aeration process. During the experiment, physical variables (pH, Turbidity) chemical variables (NH_4^+, PO_4^{3-} and concentration copper) and phytoplankton biomass (Chlorophyll a) and density of cell were monitored at D0, 1, 2, 3, 4, 8 days.

2.2. Chemical synthesis of silver nanoparticles

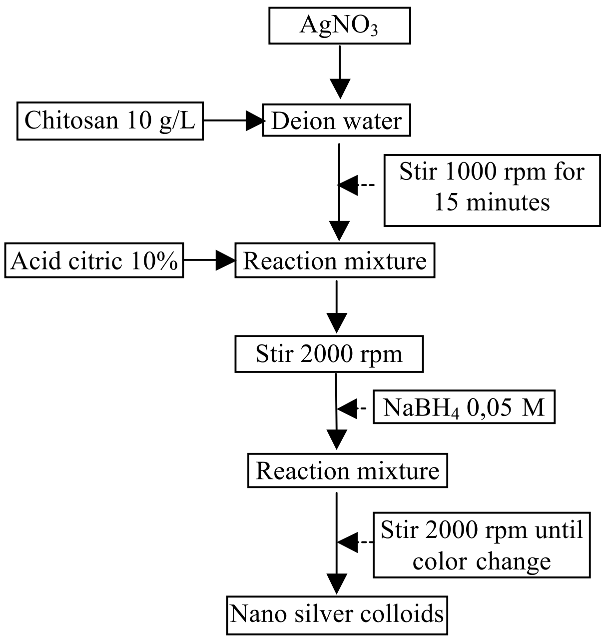

Figure 1. Overall procedure for preparing nanosilver colloids by chemical reduction method

The silver nanoparticles were synthesized by a chemical reduction method at room temperature (overall procedure showed in figure 1). Silver nitrate, $AgNO_3$ (>99%) as the precursor for the formation of Ag nanoparticles was purchased from the Merck Chemical Reagent Co. Sodium borohydride, $NaBH_4$ 98% purity used as the main reducing agent was purchased from the Scharlab (Sentmenat, Barcelona, Spain). Preparation of the stock solutions of $AgNO_3$, citric acid 1000 ppm and $NaBH_4$ 0.05M with deionic water. The 1000 ppm chitosan solution is dissolved in 10% acetic acid solution. To gain 100ppm silver nanoparticles, add the 200 mL of deionized water into glass beaker (500 mL) containing $AgNO_3$ and chitosan stabilizer. The mixture is stirred with IKA RW 20 digital stirrer with speed 1000 rpm for 15 minutes. Next, the citric acid solution is slowly added to the mixture according to the survey ratios. Then, slowly drop the solution $NaBH_4$ 0.05M into the mixture and continue to stir with 2000 rpm until the solution turns yellow. The obtained nanosilver colloids in the concentration 300 ppm were stored in hermetic flasks, dark colour.

Morphology and size of the nanoparticles were examined using a transmission electron microscope (TEM) (JEOL-JEM1010, Japan), a high-resolution transmission electron microscope (HR-TEM) (JEM 2100, Japan) and the UV-VIS 2450 (Shimadzu, Japan) methods.

2.3. Effect of nanoparticles on natural phytoplankton assemblage

These methods for assessment of effect of nanoparticles on natural phytoplankton assemblage such as: Cell counting by optical microscopy, Ammonium (NH_4^+) and Phosphate (PO_4^{3-}) measurement and the content of silver metal is similar to the experiment with copper nanoparticles published by the authors (Tran et al, 2016).

2.4. Statistical analysis

All experiments were done in triplicate and the data were calculated as mean ± SD (standard deviation) and drawn by the software GraphPad Prism 6. Statistical significance was accepted at a level of $p < 0.05$.

3. Results and discussion

3.1. Characteristics of silver nanoparticles

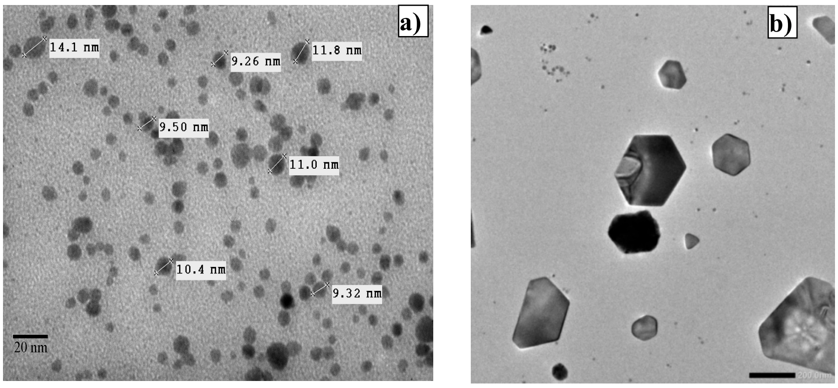

Figure 2. TEM (a) and HR-TEM (b) images of nanosilver colloids synthesized by chemical reduction method

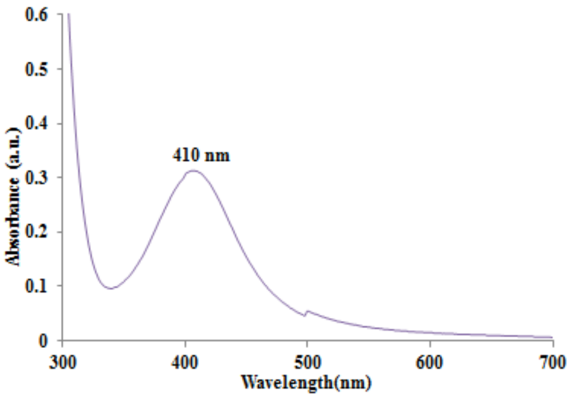

Figure 3. UV-VIS absorption spectrum of nanosilver colloid synthesized by chemical reduction method

Figure 2 and 3 shows the UV-Vis spectra, TEM and HR-TEM images of nanosilver colloid prepared by chemical reduction method under the following optimum reaction parameters: $[NaBH_4]/[AgNO_3]$ = 1 : 4; [chitosan] = 300 mg/L; [citric acid] = 3.0 mg/L and reductant drop rate = 10 drops/min. Figure 2a shows that the size of the synthesized silver is in the range of nanometre. The particles have spherical form and uniform size about 10-15 nm. The silver nanoparticle structure at the optimum ratio indicates that they have a hexagonal crystal structure typical of metallic nanoparticles (Figure 2b). The results of the HR-TEM imaging showed that the crystals are oriented along the Face centered cubic structure (Fcc) and matched the results of the previously published study (Kumar, 2000; Siwach et al., 2008). The UV–Vis absorption spectra of these nanosilver colloids revealed a high intense Plasmon absorption peak near 410 nm. Silver nanoparticles have a surface Plasmon absorption between 400 nm and 450 nm as reported in the previous literatures (Kumar, 2000; Siwachet al., 2008; Mithun, 2017). Optical absorption spectra of metal nanoparticles are dominated by Plasmon resonance surfaces. The position and shape of the absorption spectrum of the nanoparticles depends strongly on particle size, dielectric constant and absorption surface (Kholoud, 2010). This result demonstrates that silver nanoparticles are obtained with nanometre-sized.

3.2. Phytoplankton community under AgNPs exposure

In fact, phytoplankton communities in aquatic ecosystems are abundant and diverse with many different species and different morphologies. Qualitative analysis of the phytoplankton component in this experimental water showed that the cyanobacteria account for 90% of phytoplankton community. *Microcystis* genus and "water blooming" sample collected from the Tien Lake are mainly represented by species such as: *M. aeruginosa*, *M. wesenbergii* and *M. ichthyoblab*. Some other cyanobacteria species have also been found in the Tien Lake water samples such as: *Lyngbya, Aphanocapsa, Pseudanabaena, Anabaena*. In addition, some species of *Bacillariophyta, Chlorophyta, Dinophyta* were also recorded as: *S. gracile, S. paradoxum, A. granulata, N. placentula, N. gracillis, Ceratium* sp. In this study, the content of chlorophyll a and cell density were determined to assess the growth of phytoplankton communities in the lake. Based on the previous research results (Duong et al., 2016; Tran et al., 2016; Tran et al., 2015; Tran and Duong 2017), the nano silver concentration of 1 ppm was selected for the present study. Figure 4 shows a significant difference in the growth of phytoplankton communities in the Tien Lake between control sample (no silver nanoparticles) and the sample exposure with silver nanoparticles 1 ppm. In the control sample, the initial biomass was 11.42 ± 0.17 µg/L (D0) and increased slightly until the end of the experiment (D8) 12.6 ± 1.18 µg/L. In contrast, in the experimental sample exposed with silver nanoparticles, the biomass at the initial time (D0) was 11.27 ± 0.56 µg/L and then reduced to 1.98 ± 0.37 µg/L at the last day of experiment (D8).

Based on the results of cell density, we determined the effect of silver nanoparticles on the growth of both phytoplankton communities and *Microcystis* cyanobacteria in the Tien Lake. Figure 5 shows the variation of the phytoplankton and the *Microcystis* cyanobacteria cell density in the control sample and the experimental sample. In the control sample, the cell density of phytoplankton and the *Microcystis* cyanobacteria did not differ significantly between the first day (D0) and the last day (D8). In contrast, after exposure to silver nanoparticles with concentration 1 ppm, in the experimental sample the total cell density decreased compared to the control samplesat the last day of experiment. These results may confirm that silver nanoparticles are capable of controlling the growth of *Microcystis* cyanobacteria.

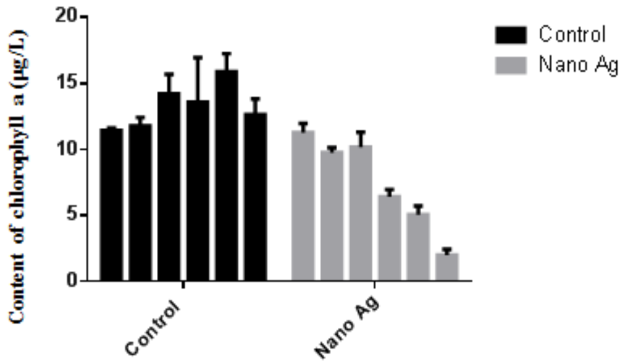

Figure 4. Variation of biomass (chla) between the control and the experimental sample were exposured with nanosilver colloids (1 ppm) after D0, D1, D2, D3, D4 and D8 days

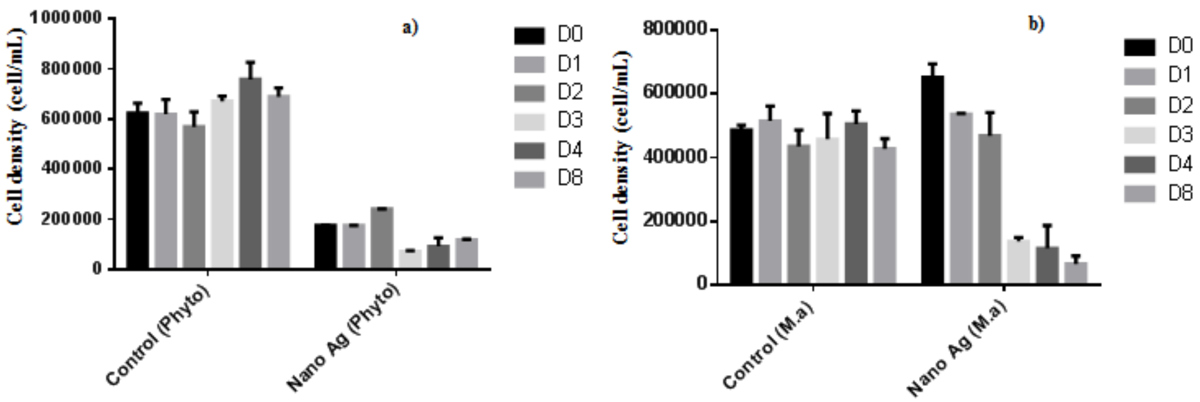

Figure 5. Variation of the cell density of phytoplankton (a) and Microcystis cyanobacteria genus (b) between the control sample and the experimental sample exposed with nanosilver colloids (1 ppm) after D0, D1, D2, D3, D4 and D8 days

To overall assess the effect of nanomaterials on the environment when applied, in addition to biological indicators, chemical and physcical variables such as pH, temperature, dissolved oxygen, turbidity... is also determined to assess the quality of the water environment before and after treatment with nanomaterials. Table 1 presents the average variation of the chemical and physical variables. The analysis results are showed in Table 1.

The water temperature in the experimental sample ranged from 23.7 - 23.8^{0}C, which is the temperature range suitable for growth of phytoplankton and cyanobacteria. The variables such as: electrical conductivity, total dissolved solids are measured quickly by using multi-indicator devices throughout the experiment period. These values are quite stable throughout the period and varied from 22.5 to 23.6 µS/cm and around 0.11 mg/L respectively. The values of the pH and dissolved oxygen (DO) varied from 7.41 to 7.92 and 1.4 to 1.7 mg/L respectively. The value of these variables did not differ significantly between the control and the experimental samples exposed with nanosilver colloids. The Ministry of Natural Resources and Environment has issued the Vietnam standard 08:2015/MONRE: national technical standard on surface water quality, including regulations on limit values of some indicators in the receiving water sources, especially the water used for irrigation and the activities for conservation of aquatic animals and plants. According to this standard, the permissible limit values of pH and DO in the lake water are 6-8.5 and ≥ 5 mg/L, respectively. The results in Table 1 show the variation in contents of ammonium and phosphorus which varied from 0.081 to 1.344

mg N/L and 0.011 to 0.056 mg P/L, respectively. These values are below the limit of the Vietnam Standard 08:2015/MONRE for surface water quality.

Table 1. Variation of the chemical and physical variables in experimental samples (exposed with 1 ppm nanosilver colloids) and control samples (Tien Lake water sample without nanosilver colloids)

Variables	Control sample	Experimental sample (add 1mg/L of nanosilver)
pH	8.8 (8.4 – 9.0)	7.74 (7.41 – 7.92)
Temperature (^{0}C)	21.4 (18.8 - 23)	23.7 (23.7 - 23.8)
Turbidity	139 (86.6 - 176)	80.3 (40.6 – 180)
DO (mg/L)	1.61 (1.4 - 1.7)	1.55 (1.4 - 1.7)
Conductivity (µS/cm)	19.4 (18.6 - 19.1)	23.1 (22.5 - 23.6)
TDS (mg/L)	0.11	0.10
NH_4^+-N (mg/L)	0.433 (0.081 – 1.344)	0.446 (0.446 – 0.078)
PO_4^{3-}-P (mg/L)	0.012 (0.003 - 0.050)	0.036 (0.011 – 0.056)
Ag (mg/L)	0	0.8

When the application of nanoparticles is more popular, the use of nanomaterials to control "water blooming" especially to cyanobacteria has obtained some of the certain results. The work of Park et al. (2010) has demonstrated that silver nanoparticles have effected and inhibited the growth of *Microcystis* cyanobacteria but do not affect other algal species (Park et al., 2010). According to Blahoslav et al. (2012), the ferric nanoparticle has the ability to removebioavailable phosphorus, to destructecyanobacteria cells, immobilizemicrocystins and prevent their release into the water column (Blahoslav et al., 2012). Furthermore, toxicological studies showed that the toxicity of iron nanoparticles to *M. aeruginosa* cyanobacteria was significantly higher than other experimental organism groups such as *Lemna minor, Daphnia magna*, plants and fish (Blahoslav et al., 2012). In another study, Sankar et al. (2014) showed that copper oxide synthesized from plant extract is an effective bactericidal active agent. This extract is capable of controlling the flowering of *M. aeruginosa* cyanobacteria at laboratory conditions (Sankar et al., 2014).

In addition, the physical and chemical properties of nanomaterials are influenced by many other factors in the environment in which they exist such as: the ability to release and dissolve ionic particles in the environment, pH, organic substances (humic acid), ionic intensity... (Wang et al., 2011; Huang et al., 2016). These factors can affect the antimicrobial toxicity of nanomaterials in water. Moreover, the release of metal ions from the nanoparticles is also affected by ambient environmental conditions (Wang et al., 2011). For example, the rate of metal ions released from the nanoparticles into the external environment will be limited by the organic polymers surrounding of the particles and causing the increase in water pH (Shamar et al., 2014; Liu et al., 2010). Huang et al. (2016) showed that humic acid reduced the toxicity of metal nanoparticles to cyanobacteria *M. aeruginosa* (Huang et al., 2016). Meanwhile, the presence of calcium and magnesium ions increased the toxicity of the metal nanoparticles for growth of these cyanobacteria species.

4. Conclusion

The silver nanoparticles were synthesized by chemical reduction method. The characteristic of nanomaterial was confirmed by using UV–visible spectrophotometer, TEM and HR-TEM methods. For AgNPs, their spherical form and uniform size varied from 10 to15 nm. The experimental results at laboratory scale show the clear effect of nanosilver colloids on inhibition of the growth and development of phytoplankton and *Microcystis* genus in aquarium tanks contained the Tien Lake water but no clear effect to water quality was observed. The environmental variables such as: pH, temperature, dissolved oxygen, turbidity, ammonium, phosphate... observed during the experiment were below the limit of the Vietnam Standard 08:2015/MONRE for surface water quality. Our results provide the possibility of application of silver nanoparticles for inhibition of toxic algae in fresh waterbody in Vietnam.

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