EFFECT OF ENGINEERED NANOPARTICLES ON CYANOBACTERIA STRAIN

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Abstract: The aim of the present study was to investigate the effect of three engineered nanoparticles (silver, copper and titan oxide) on Cyanobacteria strain (*Microcystis aeruginosa*). The silver, copper nanoparticles were produced by reduction methods and titan oxide was synthesized by using sol-gel method. The silver and copper nanomaterials showed growth inhibition against *M.aeruginosa* at three concentrations 3, 5 and 10 ppm after 6 days. Nano titan oxide had no toxic effect on *M. aeruginosa*

Keywords: Cyanobacteria, *Microcystis aeruginosa*, inhibition, engineered nanoparticles.

I. INTRODUCTION

Cyanobacteria are a group of microorganisms with a broad distribution in the marine, freshwater, terrestrial, and lithoidal environment. The increasing intensity of the mass occurrence of cyanobacteria in eutrophic to hypertrophic water bodies can cause a variety of water quality problems. Cyanobacteria blooms cause a variety of water quality problems including unpleasant odors, dissolved oxygen depletion, increased pH… Cyanobacteria can produce several potent toxins and the presence of these compounds in drinking and bathing water supplies poses a serious hazard to human health.

Various methods have been developed and applied to dealing with cyanobacteria bloom problems including reduction of external and internal phosphorus, UV radiation, biological control and chemical, physical and mechanical methods… Nanotechnology had recognized as an effective method for water treatment.

Nano-materials including natural and engineered materials are applied in many different industries and fields such as medical devices, cosmetics, electronics, chemical catalysis, environment treatment [9]... They have more advantage characteristics than other materials because their specific size is smaller than 100 nm, larger surface to volume ratio, crystalline structure, high reactivity potential, creating the effect of resonance Plasmon surface [8, 17]... and especially, for treatment of sewage contaminated such as using nano-iron to remove phosphorus in water, destroying algae cells to prevent the development of cyanobacteria [1] or taking advantage of the presence of dissolved organic matter in water to increase the toxicity of copper nano to kill algae [23].

The aim of current study was to assess the effect of different concentrations of the three engineered nanoparticles (silver, copper and titan oxide) on the growth and development of *M.aeruginosa* strains.

II. EXPERIMENTS

2.1. Materials and methods

2.1.1. Algal cultures

M.aeruginosa was obtained from Department of Environmental Hydrobiology collection (IET, VAST). It was isolated from eutrophication water in Ke Go Lake, Ha Tinh province, Viet Nam and cultured in CB medium at 25 ± 2^0C under fluorescent light (1000 lux, 14 h light/8 h dark). The CB medium was composed of 0.15 g/l Ca $(NO_3)_2$. $4H_2O$, 0.1 g/l KNO_3, 0.04 g/l $MgSO_4$, 0.05 g/l β - sodium glycerophotphate and 00001 mg/l Bicine. It was controlled pH = 7.3 before autoclaving by adding NaOH 0,1M or HCl 0.1 M solutions. All experiments were carried out in this medium.

2.1.2. Nano-Cu treating method

Copper nanomaterial powder was synthesized by reduction method with reducing agent $NaBH_4$ (Cica-Japan) and precursor $CuSO_4$ (Merck, Germany). Take 300 ml of distilled water into the beaker (500 ml) containing 7.5 g $CuSO_4$ power; stir the mixture by IKA RW 20 digital stirrer with stirring speed of 1500 r/min for 15 minutes. Then, gradually pour $NaBH_4$ (in ratio $CuSO_4$:$NaBH_4$ = 2:1) into the mixture, stir until the solution turns black color. Wash the solution by alcohol 96^oC for 5 times to remove residual ions. Nano copper powder was obtained by drying in 70°C vacuum oven for 24 hours then stored in the dark [19, 24].

2.1.3. Nano-Ag treating method

In this study, the reduction method has been used to fabricate silver nanomaterial. Chemicals used include natri borohydrit (Cica - Japan) as reducing agent, Chitosan as Stabilizer (Sigma) and silver nitrate (Merck) as precursor agent. 0.93 mmol $AgNO_3$ and 15 mmol $NaBH_4$ are prepared with deion water and chitosan 500 ppm solution is prepared by 10% acetic acid. Stir the silver nitrate solution with chitosan (pH 5-6) and add $NaBH_4$ solution by drops until the solution turns yellow color, we obtain silver nanoparticle solution.

2.1.4. Nano-titan treating method

Nano-titan material using in this study was received from the Department of Environmental Physico-Chemistry - IET, VAST and manufactured by sol - gel method [21].

2.1.5. Determined Characteristics of synthesized nanoparticles

The morphological features of Ag, Cu and TiO_2 nanoparticles were further characterized by SEM, TEM, and XRD system. Similarly, the preliminary characteristics of nanoparticles were determined by measuring the UV-VIS spectra at 200-800 nm wavelengths.

2.2. The effect of nanoparticles on growth of cyanobacteria *Mycrocystis aeruginosa KG*

M.aeruginosa KG was cultured in CB medium [20]. Growth of cyanobacteria strain *M.aeruginosa KG* is assessed by optical density (OD) at 680 nm wavelength using a UV-VIS spectrophotometer (Simadzu) [2] and the efficiency of growth inhibition is calculated by the following formula [17]:

Cyanobacteria inhibition efficiency (%) = [(control - treatment)/control] x 100.

Briefly, varied concentrations of copper and silver nanoparticles [3, 5 and 10 ppm] were added into flask containing 97 ml CB medium and 3ml biomass of *M.aeruginos*a. Concentrations of titan added were [50, 100 and 150 ppm]. The growth dynamics of cyanobacteria strain *M.aeruginosa KG* is monitored at T0, T2 and T6 of experiment period.

2.3. Statistical analysis

All experiments were done in triplicate and the data were analyzed and drawn by the software GraphPad Prism 6 (one-way ANOVA). Statistical significance was accepted at a level of $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1. Characteristics of synthesized nanoparticles

3.1.1. Characteristics of copper and silver nanoparticles

Both silver and copper nanoparticles using in this study are synthesized by chemical reduction method. The results of SEM and TEM images are shown in Fig. 1. The synthesized copper nanoparticles show to be spherical, non homogeneous in distribution and have an average particle size of 50 - 80 nm (Fig. 1a, b). Similarly, the TEM image has showed Ag-nanoparticles have also spherical shape and size smaller than 10 nm (Fig. 1c). Silver nanoparticles are distributed more homogeneously than Cu-nano and no cluster.

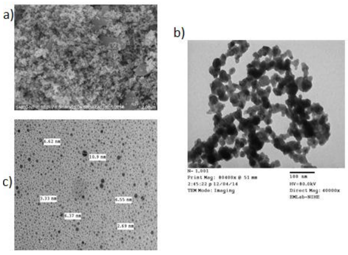


Figure 1. The SEM, TEM images of copper and silver nanoparticles.

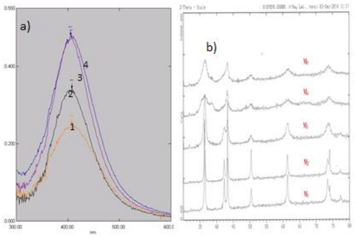


Figure 2. a) The UV - VIS with concentration ratio BH_4^-/Ag^+: 0,2 (1); 0,4 (2); 0,6 (3); 1 (4); b) The XRD image of copper nanomaterial with concentration ratio BH_4^-/Cu^{2+} M_1-M_5: 1:1; 1,5:1; 2:1; 3:1; 4:1.

The UV-VIS and XRD results are shown in Fig. 2. The UV - VIS measurement results have showed the silver nano solution is absorbed in 402 - 408 nm wavelengths. The ratio BH_4^-/Ag^+ is investigated in 0.2 and 1.0 range. From 0.2 to 0.6, the maximum absorption peak is quite acute, wide range of the absorption peak is relatively narrow. However, with the concentration ratio $NaBH_4/Ag^+$ of 1.0, the maximum adsorption peak magnitude is low and relatively obtuse; it proves that size of Ag nanoparticles is distributed in a wide range (Fig. 2a). The XRD results of copper nanomaterial have shown the characteristic peaks in the diagram are intense, sharp and wide range of the absorption peak is narrow, indicating high level of crystallization of the materials (Fig 2b).

3.1.2. Characteristics of titan dioxide nanoparticles

TiO_2 nanomaterial used in this study was synthesized by sol-gel method at room temperature and using tetra-n-butyl orthotitanate $Ti(OBu)_4$ (99% Merck, Germany) as precursor. SEM and TEM images are shown in Figure 3. The nanoparticles show to be spherical, homogeneous and have an average size of 15-20 nm.

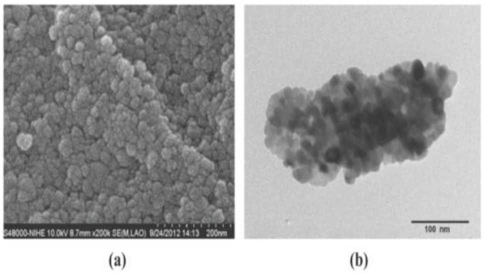


Figure 3. SEM (a) and TEM (b) images of TiO_2 nanomaterial

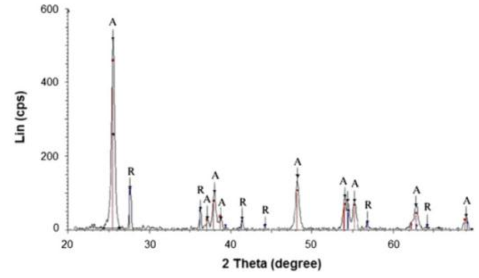
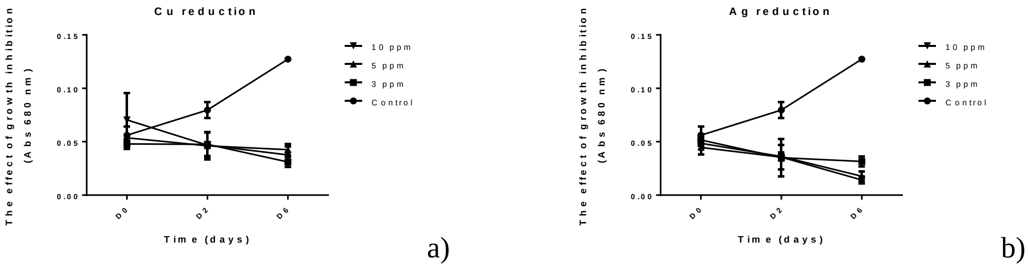


Figure 4. The XRD images of TiO_2 nanomaterial synthesized by sol-gel method. R: rutile, A: anatase

The XRD results of nanomaterial are shown in Figure 4, the maximum spectral peaks of anatase phase and rutile phase are at $2\theta = 25.3°$ and $2\theta = 27.4°$, respectively. The average crystal size of TiO_2 nanoparticle calculated from XRD data with Scherrer equation is 15.84 nm. The percentage of anatase, A (%), was determined by the IR peak intensity of rutile at $2\theta = 27.4°$ and IA of anatase at $2\theta = 25.3°$. The calculation rate for anatase and rutile phases are 90.68% and 9.32%, respectively.

3.2. The effect of silver, copper and TiO_2 nanomaterials on the growth of cyanobacteria strain *Microcystis aeruginosa KG.*

The initial investigated results are shown in Figure 5. Both silver and copper metal nanomaterials influence the growth and development of *M.aeruginosa KG* at all three concentrations 3, 5 and 10 ppm after 6 days. OD values of silver nano at initial time (T0) and at the end of the experiments (T6) were significantly different, OD (T0) was 0.04 ± 0.005 and OD (T6) 0.02 ± 0.008, respectively. For copper nano, OD value changed less, OD (T0) was 0,06 ± 0,02, after 6 days OD (T6) was fell to 0,03 ± 0,006.



a) b)

Figure 5. The effeciency of nanomaterials on growth and development of cyanobacteria strain M. aeruginosa KG at different concentrations (3, 5 and 10 ppm): a) nano Cu; b) nano Ag

The growth inhibition efficiency of Ag and Cu nanomaterial concentrations on growth of *M.aeruginosa KG* after 48h and 6 days are shown in Fig. 6. For the silver nanomaterial, the inhibition > 70% was observed in both 3 experiment concentrations after 6 days, at the time of 48h this result only reached to be 50%. Meanwhile, the inhibition > 55% of copper nanomaterial was only observed after 6 days at all three concentrations.

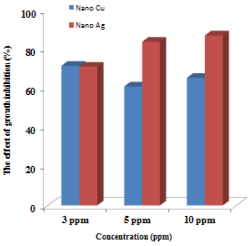


Figure 6. The growth inhibition efficiency of concentrations of silver and copper solution (3; 5, 10 ppm) after 48 h and 6 days on M. aeruginosa KG

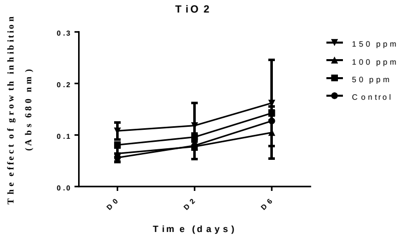


Figure 7. The efficiency of titan nanomaterial on growth and development of cyanobacteria strain M. aeruginosa KG at different concentrations (50, 100, 150 ppm)

The results in Figure 7 shows that the concentration of the solution of nano titanium added to the culture medium not only do not affect the growth and development of the algae population but also because of photochemical catalytic properties of the material, spur algae grow better. This is evident at all three concentrations, measured OD optical density increased linearly with control sample, the lowest OD value was 0.08 (50 ppm) and in the highest was 0.17 (150 ppm); the OD of control samples were 0.05 and 0,11, respectively. The average OD value at the initial time (T0) was 0.08 ± 0.02 and after six days of exposure OD (T6) increased to 0.13 ± 0.05. These results proved at all concentrations, titanium nanomaterial cannot be toxic against *M.aeruginosa KG* algae.

Different nanoparticles exhibited differently the growth against *M.aeruginosa*. Two silver and copper metal nanomaterials influenced cyanobacteria strain *M.aeruginosa* but TiO_2 nanoparticle failed to inhibit *M.aeruginosa* at all tested concentrations. Some reports published recently have also the same results [12, 13, 16, 19, 25]. Semiconducting properties of titanium materials play a major role in the process of removing organic contaminants, rapid recombination of photochemical electron pairs and no selectivity of the system are the main reasons for application restrictions of photochemical catalytic process [18]. According to Konrad et al (2012), at concentrations 50, 100, 200 and 300 ppm, titan-nanomaterial does not affect the growth of *M.aeruginosa*. On the contrary, TiO_2 was found to be aggregated, forming a change of the cyanobacterial cell, reducing their biomass [12]. This is explained that the titanium nanoparticles in liquid media has surrounded the cell surface, reducing surface activity, preventing photosynthesis and metabolism between cells and their surroundings, resulting in inhibition of the growth of algae [13, 19]. Titan nanomaterial was significantly less sensitive to *M.aeruginosa* than the four algal species tested [5]. Therefore, the mechanism of action of titanium nanomaterials to prokaryotes still needs to be investigated more.

Cu and Ag nanomaterials influence on growth of *M.aeruginosa* at all three concentrations. However, the efficiency of growth inhibition of copper nano is lower than Ag nano. According to Renu Sankar et al, (2014) copper dioxide nanoparticle inhibited the growth of *M.aeruginosa* at all three concentrations 12,5; 25 and 50 ppm, the efficiency reached to 31,8% (at 12,5 ppm) and 89,7% (50 ppm). In addition, nano oxides (TiO_2 and CuO) were less toxic than their formulations (Ti and Cu) through measuring EC50 index [22]. On the other hand, the presence of dissolved organic matter also increases toxicity of CuO against *M.aeruginosa* [25]. Nano silver is very sensitive to bacteria; at very low concentrations they also have the ability to inhibit the growth of cyanobacteria. At a concentration of 1 mg/l, silver nanomaterial inhibited the growth of *M.aeruginosa* population to 87% compared with control sample [15]. Besides, the nano-Ag^+ synthesized by in vivo method is capable of inhibiting cancer promoters when it decreases cell count and total chlorophyll content of *M.aeruginosa* [14]. Until now, the mechanism of action of nanomaterials for prokaryotic cells has remained controversial. These outstanding features of nanomaterials are increasingly being studied but the interaction of nanomaterials with aquatic organisms like algae, phytoplankton remains limited.

IV. CONCLUSIONS

The initial results have showed the silver and copper nanomaterials synthesized by reduction method affect the growth and development of *M.aeruginosa KG* population, the efficiency of growth inhibition of copper nano is lower than that of silver nano. Titanium nanomaterial synthesized by sol - gel method has no effect on the growth of this cyanobacteria strain. However, this is only a range of preliminary concentrations investigated; the study should be carried out at the different concentrations to find the most appropriate concentration range which has the ability to influence and the greatest inhibition against *M.aeruginosa KG* cyanobacteria population.

V. ACKNOWLEDGMENT

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