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Inactivation of Microcystis aeruginosa by undoped and cobalt-doped copper oxide nanoparticles: towards sustainable water treatment

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Abstract: One of the major challenges in reducing the risk of cyanobacteria to environmental health is employing safe and effective techniques to treat and remove their cells from water sources. Nano-materials such as copper oxide nanoparticles (CuO NPs) have drawn a lot of interest as a sustainable way to inactivate microorganisms and adsorb contaminants in wastewater because of their catalytic activity, low cost and low cytotoxicity. Herein, we investigated the inhibitory effects of undoped and cobalt-doped copper oxide nanoparticles (Co-CuO NPs) on Microcystis aeruginosa, a common toxic cyanobacterium found in water sources. The results showed that both undoped and Co-doped CuO NPs inactivated M. aeruginosa growth, with doped NPs having a higher effectiveness (100%) than undoped ones (99%) at 25 mg/L. According to regression Probit analysis, the medium effective concentrations (EC50) of these NPs varied with exposure time, reaching their lowest values (0.8 and 0.3 mg/L, respectively) at 96 hours and their greatest values (5.6 and 4.6 mg/L, respectively) at 24 hours. Additionally, the results showed that these NPs can concurrently adsorb microcystin (MC) toxins that are released into the medium upon cell lysis. Since nanoparticles are firmly bound to the surfaces of cyanobacterial cells, they settle as flocs and are easily eliminated with the sludge. Therefore, both undoped and doped CuO NPs could be employed as coagulants in drinking water treatment plants to remove microcystin toxins and cyanobacterial cells at the same time.
Keywords: Microcystis aeruginosa; microcystin; coagulant; Nano-CuO.

1. Introduction

In recent decades, the growing frequency of nutrient enrichment in water bodies, especially nitrogen and phosphorus, coupled with climate change has made cyanobacteria bloom a major environmental concern worldwide [1-3]. One of the most common toxic cyanobacteria species, M. aeruginosa outgrows under eutrophic water bodies and forms harmful blooms, and thus represents a serious threat to the aquatic ecosystem, fishery, tourism industry, and drinking water sources through production of potent toxins called microcystins (MCs) [4-5].

Many strategies including physical, chemical and biological methods have been proposed to control or remove cyanobacterial blooms from water bodies [6-7]. However, physical methods are expensive and time-consuming [8], while biological methods using microorganisms are not specific for killing cyanobacteria but also can destroy other beneficial algae. Additionally, chemical inactivation of cyanobacterial cells (e.g., by CuSO4, as well as Cu2+-based algaecide) is efficient strategy for mitigation of cyanobacteria bloom and widely applied in lakes and reservoirs [9]. However, it affects the integrity of the cyanobacterial cells causing massive cell lysis and release of microcystin toxin in the water, which are hard to be removed by conventional treatment processes [10-11]. Therefore, it is necessary to look for a cost-effective and efficient cyanobacterial inactivator without secondary pollution and toxin release in the natural environment.

Recently, previous investigations have shown that some nanoparticles could efficiently inactivate the growth of algae and cyanobacteria [12-15]. However, the mechanism of all these studies was based on the damage of algae cells, which would induce the release of intracellular compounds including toxins and other substances serves as precursors for disinfection byproducts [16]. Therefore, the ideal inactivating chemical for algae cells should boost the efficiency of algae removal while avoiding cell damage. Among metal oxide nanoparticles, CuO NPs have garnered special attention because of their unique physical and chemical characteristics, as well as their antibacterial, antioxidant, and antifungal qualities, which have allowed them to be used in a wide range of biomedical, industrial, agricultural, electronic, and environmental applications [17-18]. In fact, CuO nanoparticles biosynthesized from plant extracts have advantages over those that are chemically synthesized because the bioactive metabolites in the extracts (such as phenols, flavonoids, carboxylic acids, terpenoids, and tannins) can influence the physico-chemical properties, stability, and toxicity of the nanoparticles, as well as

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enhance their affectivity and biocompatibility [19- 21]. Therefore, the present study aimed to investigate the ability of biosynthesized CuO and Co-doped CuO nanoparticles to inactivate the growth of M. aeruginosa and eliminate microcystin toxins.

2. Materials and Methods

2.1 Culturing of Microcystis aeruginosa

Microcystis aeruginosa used in the experiments was obtained from from culture collection at Department of Botany & Microbiology, Sohag University, Egypt. This cyanobacterium was originally isolated from freshwater fishpond and found to produce three MC variants (MC-LR, -RR, -YR), with cell quota of 160,140 and 58 pg cell-1, respectively [22]. M. aeruginosa was grown in BG-11 medium at 25˚C under cool white fluorescent lights at an intensity of 50 µmol m−2 s−1 (14h light/10h dark) for 14 days (i.e., the exponential growth phase). The cells were harvested at exponential stage (cell density=2.1 ×107 cells mL−1) and centrifuged at 6000 x g for 15 min.

2.2 CuO nanoparticles

Both undoped and doped nanoparticles used in our adsorption experiments were obtained from Biophysics laboratory, Physics department, Sohag University. These nanoparticles were previously biosynthesized using sugarcane juice [23] and characterized by the following features. CuO and 9% Co-doped CuO nanoparticles are composed of roughly spherical particles with average size of 9.3 and 13nm, respectively. The X-ray diffraction (XRD) pattern of these biosynthesized nanoparticles showed their highly crystalline structure with average crystallite size of 11.8 and 8.3 nm, respectively.

FTIR spectra for ZnO NPs showed broadband at ~3409, 2079, 1632, and 694 cm-1, corresponding to vibration hydroxyl group O-H, the isothiocyanate stretching C=O, stretching amide group, C=N, and characteristic ZnO stretching, respectively .FTIR spectra for CuO and Co-doped CuO nanoparticles showed broadband at 3290, 1639, 1060, and 586 cm-1, corresponding to hydroxyl group O-H, C=O stretch group, O-C=O, and bending vibration =C–H, respectively. Stock solution of CuO and Co-doped CuO nanoparticles was prepared in deionized water, and dispersed by sonication for 45 min at 40 Hz, using Probe sonicator (Q-500, Qsonica, USA). From the stock solution, working solutions were made in BG-11 medium for cell removal experiments.

2.3 Growth inhibition of Microcystis aeruginosa by nanoparticles

This experiment was conducted in 1000 ml- beakers containing BG-11 medium (pH8, close to that of the natural freshwater), exponentially growing cells of M. aeruginosa (initial cell density= 1x107 cells/mL) and different concentrations of either CuO or Co-doped CuO nanoparticles (5,10, 20 and 25 mg/L). Beakers containing only BG-11 medium and M. aeruginosa cells but without nanoparticles were used as controls. Both control and treated cultures were incubated for 96 hours at the same conditions as described above in Culturing of Microcystis aeruginosa section, while stirred by a magnetic stirrer at 250 rpm to simulate turbulence in natural water bodies. Cell densities were counted every 24 h using hemocytometer under light microscope. The algae growth inhibition rate (%) was calculated according to the following equation

$$\text{Growth Inhibition}(\%) = \frac{C_0 - C_t}{C_0} \times 100$$

where C0 and Ct are the cell number (cells/mL) at 0 h and t h, respectively. The inactivation efficiencies were evaluated as medium effective concentration (EC50), which was determined using Probit regression analysis in Excel software.

To study the potential release of microcystin toxin into the medium under the effect of CuO nanoparticles, an aliquot of control and treated cultures was aseptically drawn every 24 hours. The samples were filtered via GF/C filters, and microcystin concentrations were then determined directly in the filtrate using ELISA kits (Abraxis, Warminster, PA, USA) according to Carmichael and An et. al. [24]

2.4 Statistical analysis

The differences in removal percentages of cyanobacterial cells and microcytsin concentrations between treated and control groups were estimated by One-way Analysis of Variance (ANOVA) ($P < 0.05$) using SPSS 22.0 (SPSS Inc., USA). All Data were expressed as average ± standard deviation.

3. Results and discussions

The results of the present study shown in Fig.1A and B, showed that the growth inhibition rate of M. aeruginosa increased gradually with increasing the concentrations of either pure CuO NPs or Co-doped CuO NPs, and with the exposure time as well. Undoped CuO NPs exhibited highest inhibition rate (99%) at a concentration of 25 mg L-1 after 96 hours, with lowest inhibition rate (46%) obtained at a concentration of 5mg/L after 24 hours (Fig.1A). In comparison, Co-doped CuO NPs showed stronger growth inhibition against M. aeruginosa (Fig. 1B). The two highest concentrations (20 and 25 mg L-1) of Co-doped CuO NPs caused complete growth inhibition (100%), while the lowest inhibition rate (51%) was recorded after 24 hours.

These results agree with those of previous studies demonstrating that nano-copper carbon composite and CuO NPs could have severe inhibitory effects against microalgae and cyanobacteria [13, 25- 26].

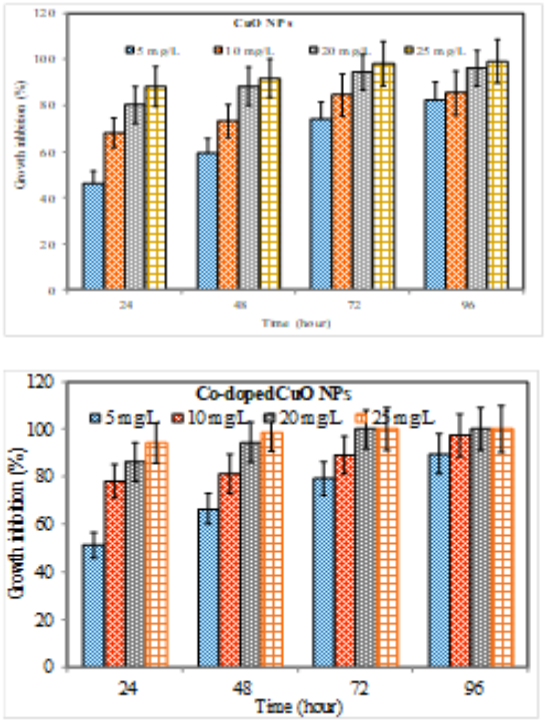

Fig. 1.: Microcystis aeruginosa growth inhibition by CuO NPs (a) and Co-doped CuO NPs) at different concentrations for 96 hours.

These studies attributed the inhibitory effect of these NPs to the mechanical action of CuO NPs, which aggregate on algal cell wall and damages cell membranes. This could be the inactivation mechanism of M. aeruginosa in our study, where NP aggregates were observed on the sheath and cells of this cyanobacterium (Fig. 2).

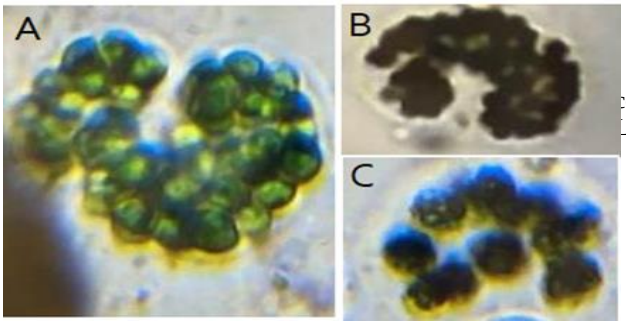

Fig. 2: Micrographs of M. aeruginosa cells in control cultures (A) and treated cultures with with 20 mg/L CuO NPs (B and C)

NPs adsorption is caused by electrostatic interaction between positively charged NPs and negatively charged surfaces of algal cells [27]. It is typical for M. aeruginosa [8,15, 28] and many other species of microalgae [29- 30] to exhibit this response to metallic nanoparticles.

Additionally, the linear regression of Probit analysis showed strong correlation between growth inhibition rate and the concentrations of either undoped or Co-doped CuO NPs (R2= 0.956-1). We also used the dose-response probit model to determine the median inhibitory concentration (IC50) for translating experimental findings into ecological context [31]. Based on the linear regression of Probit analysis. The results indicated that Co-doped CuO NPs exhibited higher efficiency on cyanobacterial growth inhibition with low IC50 values (0.3-4.6 mg/L) compared to undoped CuO NPs that showed higher IC50 values (0.8-5.6 mg/L). Also, the results revealed that IC50 values varied significantly ($P<0.05$) with the exposure time of M. aeruginoa to NPs. The highest the exposure time the lowest the IC50 (Table 1).

Table1: EC50 values estimated by Probit analysis for CuO Nps and Co-doped CuO NPs against Microcystis aeruginosa growth

Exposure time (hour)	IC_{50} (mg/L)	Regression Equation	R^2
CuO NPs			
24	5.63±1.1	y = 1.719x + 3.7106	0.9812
48	4.5±0.7	y = 1.7526x + 3.8574	0.8962
72	2.3±0.4	y = 1.7198x + 4.3968	0.9721
96	0.8±0.2	y = 1.0697x + 5.1075	0.8942
Co-doped CuO NPs			
24	4.6±1.2	y = 1.9882x + 3.6812	0.9562
48	2.8±0.5	y = 1.6528x + 4.2549	0.9953
72	1.3±0.3	y = 1.3786x + 4.8533	1
96	0.3±0.1	y = 1.0492x + 5.5313	1

The higher anti-cyanobacterial efficiency of Co-doped CuO NPs than undoped ones agree with the findings of earlier studies, is consistent with previous studies showing that co-doped CuO NPs exhibited better antibacterial activity than undoped ones [32- 33]. Those authors explained this by the fact that cobalt doping regulates the bandgap of CuO nanoparticles, increases surface area, reduces agglomeration, increases magnetism and enhances catalytic activity.

In addition to inactivation of cyanobacterial growth, both undoped and Co-doped CuO NPs showed a decrease in microcystin concentrations released into the culture medium upon cell lysis caused by these nanoparticles. As shown in Fig.3A, during the first 24 hours of the experiment, MC concentrations in the medium of CuO NP-treated cultures rose above those released in control cultures. Thereafter, they began to progressively decline over the course of the remaining experiment until they reached undatable levels for the highest two NP concentrations within 72 hours. Similarly, in M. aeruginosa cultures treated with Co-doped CuO NPs, MC concentrations increased throughout the first 24 hours and then gradually decreased. However, Co-doped CuO NPs showed a greater reduction in extracellular MCs than undoped ones, at 72 hours, MCs vanished entirely for all NP concentrations (Fig. 3B). These results indicate that undoped and Co-doped CuO NPs not only inactivated the

cyanobacterial growth and caused cell lysis of M. aeruginosa, but also may be able to degrade or adsorb MC toxins released into the medium upon cell rupture. These findings corroborate those of earlier studies, demonstraing that CuO NPs and other nano-compoistes act as excellent photocatalytic and adsorption materials for the effective removal of cyanobacterial cells and MC toxins in practical applications [8, 15]. However, further in situ studies are needed to employ photocatalysis by undoped and doped CuO NPs to enhance the removal of algae in the coagulation phase in drinking water treatment plants.

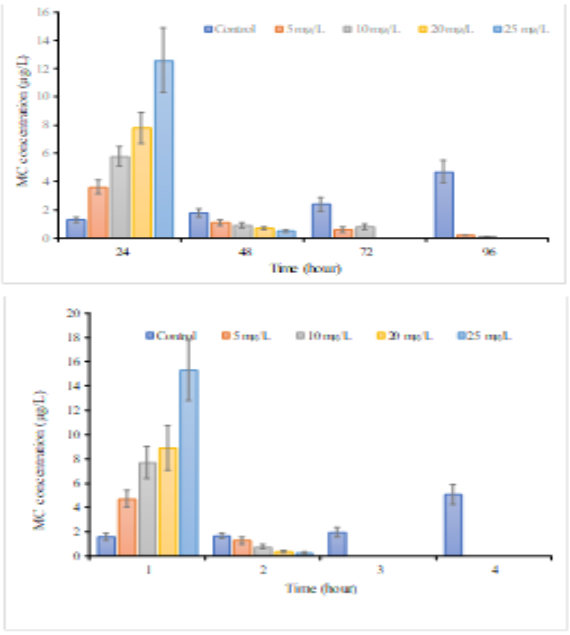

Fig. 3.: Effect of CuO NPs (a) and Co-doped CuO NPs (b) on microcystin concentrations released into the medium of Microcystis aeruginosa cultures for 96 hours.

4. Conclusion

In conclusion, our study clearly demonstrated a significant inhibitory effect of undoped and Co-doped CuO biosynthesized nanoparticles on the growth of Microcystis aeruginosa, with removal efficiency of 99-100% at a dose of 25 mg/L, respectively. This indicates that undoped CuO NPs are more effective for inactivation of cyanobacterial cells than Co-doped CuO NPs. Meanwhile, both undoped and doped NPs were also able to adsorb and remove MC toxins released upon cell lysis into medium. Because of their strong electrostatic attraction to the surfaces of cyanobacterial cells, these NPs will form nanoparticles-cyanobacteria -flocs, which settle in the sedimentation tank and are then easily removed with the sludge. Therefore, the study suggests using undoped and Co-doped CuO NPs as coagulants for simultaneous removal of cyanobacterial cells and microcystin toxins in drinking water treatment plants.

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