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The Effect of Titanium Dioxide Nanoparticles Against Cyanobacterial Microorganisms

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) present a high interest as an excellent antibacterial agent against pathogenic microorganisms. Currently, the massive proliferation of potentially toxic cyanobacteria in the water of dams presents a threat to the public health of these water consumers. This study was maintained to evaluate the antibacterial activity of TiO_2 NPs against the cyanobacteria « *Microcystis* sp.» responsible for toxic blooms in the freshwater reservoirs intended for drinking water production. Three concentrations of TiO_2 NPs (150 mg.l^{-1}, 300 mg.l^{-1} and 600 mg.l^{-1}) were tested in the laboratory on two strains of *Microcystis* sp. (S1، S2) over a period of eight days. In parallel, three biotic variables were measured: *Microcystis* sp. cells density (cell.ml^{-1}), Chlorophyll (a) (µg.l^{-1}), and Phycocyanin (µg.l^{-1}) content every 48 hours all along the experimental period. A remarkable decrease in cell densities was registered, recording decreased values from 77490 to 39091 cell.ml^{-1} for S1 and from 2801 to 0 cell.ml^{-1} for S2 with the concentration of 600 mg.l^{-1}. Additionally, the chlorophyll (a) content fell from 45 to 8 µg.l^{-1} for S1, and from 34 to 0 µg.l^{-1} for S2. Similarly, treatment with TiO_2 NPs caused the degradation of phycocyanin from 200 to 65 µg.l^{-1} for S1 and from 200 to 0 µg.l^{-1} for S2 with the highest concentration of these nanoparticles (600 mg.l^{-1}). TiO_2 NPs exhibit a very important antibacterial effect against *Microcystis*. sp. Thus, TiO_2 NPs can be used in the future to reduce the high biomasses of cyanobacteria during the process of tap water production.

INTRODUCTION

The innovative nanotechnology applications and their nanomaterials are primarily used in biomedical، environmental and electronic fields. The group of metal oxides nanoparticles is the largest group of nanomaterials that received a significant attention being, hence, applied in a plenty of products.

Obeizi *et al.* (2020) and **Obeizi *et al*. (2021)** confirmed that, several metal oxides might show bacteriostatic or bactericidal effect.This antibacterial activity differs according to the type and number of the tested bacteria, depending on the physicochemical properties, the concentration and the experimental conditions of the used nano-molecules **(Vojislav *et al*., 2020)**.

Titanium dioxide nanoparticles (TiO_2 NPs) are among the most globally produced nanomaterials **(Piccinno *et al*., 2012)** due to their high refractive index and resistance to discolouration , especially to stability, photocatalytic¸ depolluting and antibacterial properties. **Kahru and Dubourguier (2010)** classified those NPs as harmful to many water organisms such as crustaceans and nematodes. Moreover, they may harm microorganisms as yeast, micro-algae and bacteria. Considerably, NPs are used as catalyst for water purification **(Botelho *et al*., 2014; Janer *et al*., 2014)** and as an antimicrobial agent as well **(Benbouzid *et al*., 2019)**.

On the other hand, the increasing cyanobacterial bloom occurrences around the world has introduced a considerable research subject for scientists **(Merel *et al*., 2013)** to identify the responsible factors for their proliferation. Additionally, this may draw scientist to think carefuly and place in action an arranged and well structured surveillance system that can prevent and reduce the cyanobacterial bloom occurrence. Such a concern is due to the toxicological risks of those microorganisms and the impact of which that uncontrolled cyanobacterial blooms may have on aquatic ecosystems **(Carman & Tomevska, 2019)**.

It is worth mentioning that, the cyanobacteria degrade water quality **(Fleming *et al*., 2002)** and produce several cyanotoxins as neurotoxins, hepatotoxins, and dermatotoxins that can affect both animals and humans **(Lance *et al*., 2010a, b; Carmichael & Boyer, 2016 ; Metcalf *et al*., 2020)**. In this context, the frequent consumption and/or the exposure to the contaminated drinking water or aquatic foods can lead to a serious threat to public health safety **(Agasild *et al*., 2019)**.

Consequently, the main aim of this study was to evaluate the antibacterial effect of TiO_2 NPs on a potentially toxic cyanobacteria on basis of the probability of using those NPs in water treatment processes in the future. The present test was achieved via the exposure of two freshwater strains *Microcystis* sp. on an increasing concentrations of TiO_2 NPs suspension, where three biotic parameters were measured (*Microcystis* sp. cells density (cell.ml^{-1}), Chlorophyll (a) (µg.l^{-1}) and Phycocyanin (µg.l^{-1}) contents) in a period of eight days.

MATERIALS AND METHODS

The preparation of TiO_2 NPs suspension

Three concentrations of TiO_2 NPs (150 mg.l^{-1}, 300 mg.l^{-1} and 600 mg.l^{-1}) were prepared in BG11 medium (particules were kindly donated by the Surfaces and Solids

Interfaces Laboratory Badji mokhtar-Annaba University), then the solutions were sonicated with pulsatile movements for 5min to insure the dispersion of the nanomolecules.

Cyanobacteria culture and cells growth testing

The growth of cyanobacterial cells culture was maintained in BG11 medium, pH = 7 at 25 °C with a photoperiodic cycle of 12h: 12h light and dark. Each culture contained 1g of *Microcystis* sp. cells (*Microcystis* sp. strains were collected from Algerian freshwaters in 2019) then the prepared concentrations were added (150 $mg.l^{-1}$, 300 $mg.l^{-1}$ and 600 $mg.l^{-1}$ of TiO_2 NPs) to the *Microcystis* sp. Cultures. A frequent agitation was realized to avoid precipitation of any cell or molecule in the flasks. Each prepared serie contained a control sample without NPs. The experience extended for eight days successively.

Cells densities was counted with "Nageotte" counting cell using a Carl Zeiss model (Axiostar plus) microscope equipped with digital uEye32 camera and according to the technique of **Brient *et al.* (2001)**. The aforementioned work was achieved beginning from day 0, every 48 hours all along the experiment. To minimize error, the counting operation was performed in triplicate. The cell densities were expressed in ($cells.ml^{-1}$)

Photosynthetic pigments measurement

The chlorophyll (a) (Chl a) pigments were measured using chlorophyll TRIOS MicroFlu-Chl probe; which uses a fluorimeter equipped with a blue diode.The excitation wave length filter is at 470 nm and the emission is at 685 nm, with a range from 0 to 100 $\mu g.l^{-1}$ and a sensitivity = 0.1 $\mu g.l^{-1}$. The results were expressed in $\mu g.l^{-1}$.

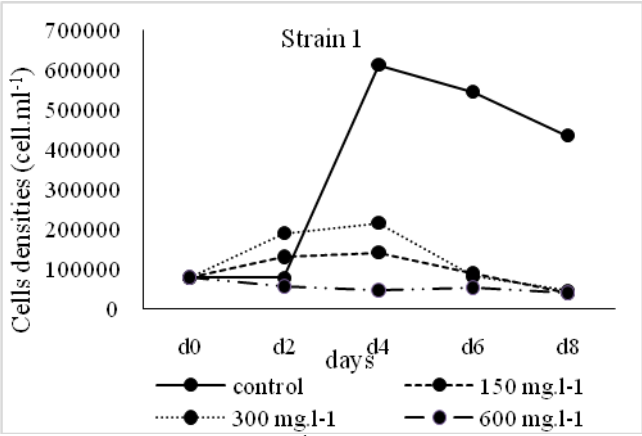
Phycocyanin (PC) pigments (pigment characteristic of cyanobacteria) measurement was carried out using a sensitive and high selective probe (TRIOS MicroFlu-blue phycocyanin) which uses a fluorimeter, submersible and miniaturized (diameter of 48 mm and 200 mm length), equipped with an ultra-brightred LEDs, and provided with excitation wave length filter at 620 nm and emission at 655 nm with a band width of 10 nm. This probe gives a linear response to phycocyanin concentration up to 200 $\mu g.l^{-1}$ and a precision of 0.02 $\mu g.l^{-1}$ .The results were expressed in $\mu g.l^{-1}$.

RESULTS

***Microcystis* sp. densities variation**

The contact between *Microcystis* sp. strains and the TiO_2 NPs allowed a reduction in the densities which varies from 213926 $cells.ml^{-1}$to 38441 $cells.ml^{-1}$ for S1 and from 15959 $cells.ml^{-1}$ to 0 $cells.ml^{-1}$ for S2. Fig.(1) shows a decreasing variation in the function of TiO_2 NPs concentrations and the exposure time to those nanoparticules, with a remarkable reduction in S2 from the second day comparing to S1. The highest TiO_2 NPs concentration gave the highest rate of cell decrease of S1 after 8 days of contact, however, the S2 was eliminated by the 150 $mg.l^{-1}$ dose from the second day. Those results

show that the TiO_2 NPs exerts a good bactericidal activity against the two strains of *Microcystis* sp., and that the antibacterial activity is directly linked to the concentration of TiO_2 and the contact time. Furthermore, it was noticed that the S2 was more sensitive than the S1.



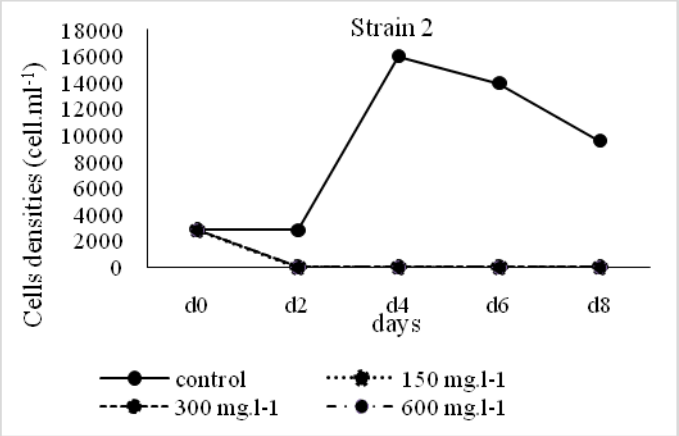


Fig. 1. *Microcystis* sp. cells density (cells.ml^{-1}) with the three TiO_2 NPs concentrations during the experimental period.

Fig.(2) shows a representative explanation for the damages that TiO_2 NPs caused to the cells of *Microcystis* sp. in particular, and to the colonies in general. The mucilageneuse membrane is deformed and the molecules of TiO_2 NPs are clearly covering the colonies.

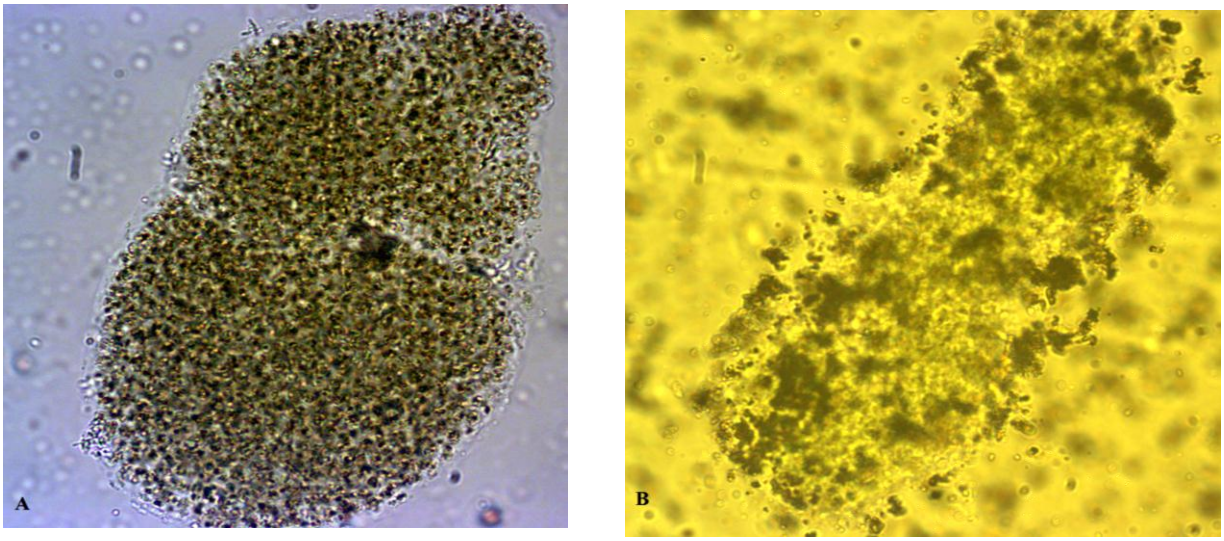


Fig. 2. *Microcystis* sp. colonies appariation under (Axiostar plus) microscope GRx400, A : Control solution, B : Presence of TiO_2 NPs (2019).

Photosynthetic pigments variation

The results of the chlorophyll (CHl) (a) and phycocyanin content in the two strains; S1 and S2, represented in Fig. (3 & 4) show a decrease that is proportional to the three concentrations of TiO_2 NPs and the contact time. The lowest content of the two pigments were observed in S1, with the concentration of 600 $mg.l^{-1}$ after eight days of contact. However, the lowest values for S2 were observed with the concentration of 150 $mg.l^{-1}$ beginning from the second day.The maximum concentration of Chl(a) content was registered with the S1 (45 $\mu g.l^{-1}$), however the minimum concentration was 0 $\mu g.l^{-1}$ for the second one. Thus, the levels of PC varied from200 $\mu g.l^{-1}$ to 65 $\mu g.l^{-1}$ for S1 while for the second strain, they ranged from 200 $\mu g.l^{-1}$ to 0 $\mu g.l^{-1}$.

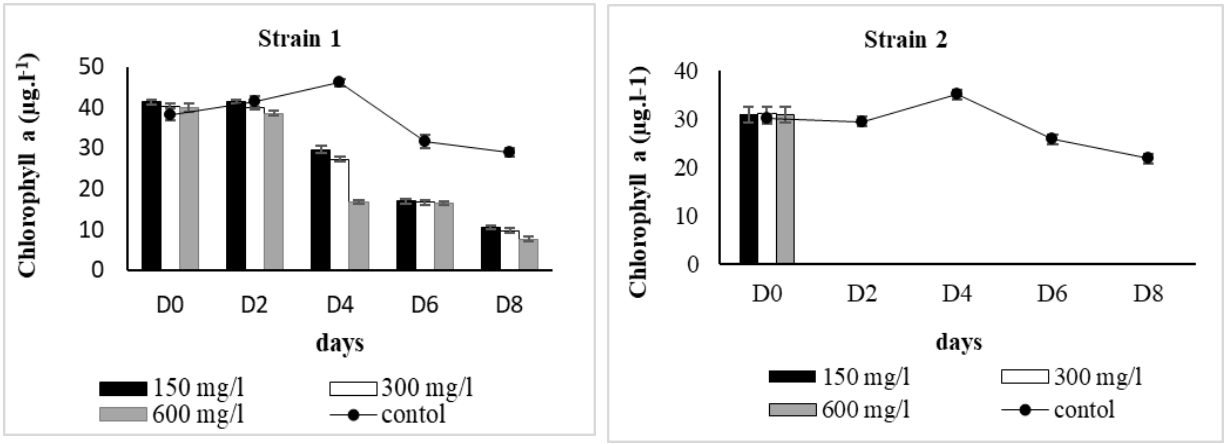


Fig. 3. Chlorophyll (a) concentrations ($\mu g.l^{-1}$) on the function of the TiO_2 NPs concentrations along the eight days.

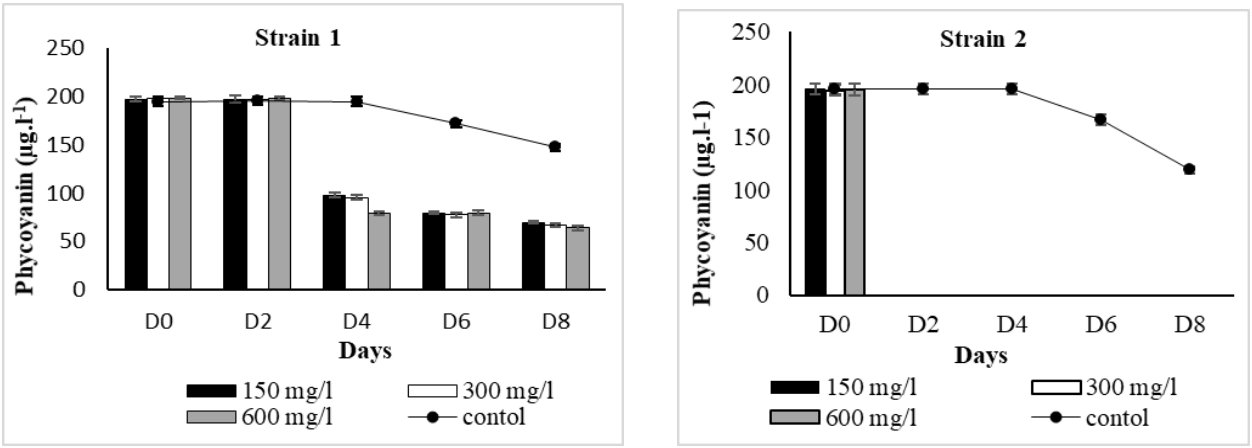


Fig. 4. Phycocyanin pigments concentration ($\mu g.l^{-1}$) on the function of the TiO_2 NPs concentrations along the eight days.

DISCUSSION

The results relating to both of the cyanobacterial strains densities showed a proportional decrease with the tested concentrations of TiO_2 NPs. The lowest densities were observed with the highest concentration (600 $mg.l^{-1}$). Microscopic examination allowed researchers to see the antibacterial effect of TiO_2 NPs on *Microcystis* sp. through the deformation in the morphology of cell walls and colonies, besides the presence of a large number of destroyed cells in the slide (Fig. 2).

The membrane of cyanobacteria is charged negatively, it is composed of peptidoglycan, glycopeptide and polysaccharide which offers many binding sites for TiO_2 NPs since it is charged positively via non-specific interactions ; hydrogen bonding, hydroliptic or electrostatic **(Rai *et al*., 2009; Liu *et al.*, 2018)**. Those bonds can perforate cells membrane and allow the accumulation of those nanoparticles inside the cell. The nanoparticules accumulation would, hence, generate oxidative stress **(Tran *et al*., 2016; Hu *et al*., 2018)**. Consequently, the intensive presence of radical molecules (OH^-, O_2^-) would cause non reversible damage that leads to cell death.

Rai *et al*. (2009) reported that the smallest nanoparticles have the strongest bactericidal effect. Thus, the antibacterial effect of TiO_2 as a metal oxide nanoparticules on *Microcystis* sp. strains is probably due to the small size that allows their penetration inside the cell causing oxidative stress via over production of reactive oxygen species « ROS ».

In addition, **Miller *et al*. (2012)** reported that, the « ROS » high production is due to protein oxidation, unsaturated lipids or even nucleicacids, which can cause serious DNA damage and may lead to bacterial cell destruction. Noticeably,TiO_2 dissolution to Ti^{+2} and OH^-, in the presence of water and oxygen, can lead to the formation of a highly reactive hydroxyl radical which can react with 2-deoxyribose of DNA and generate a modified nucleic bases. The generated element is one of the predominant lesions generated by the hydroxyl radical in the body that corresponds to the 8-oxo-7,8-dilydroguanine resulting from the oxidation of guanine by TiO_2 NPs.

For the two photosynthetic pigments: the results of both Chlorophyll (a) and phycocyanin showed a decrease with the nano-concentrations, in a way that coincides with results of **Liu *et al*. (2018)** and **Tran *et al*. (2016)**. Chl (a) reduction can be explained by the presence of the O_2^- radical which destroys that pigment. Based on those parameters, it was found that, TiO_2 NPs exerted an antibacterial effect against *Microcystis* sp. with respect to the two strains S1 and S2.

CONCLUSION

It can be concluded from the results of the present study that TiO_2 NPs have an antibacterial effect on *Microcystis* sp. and that this effect is directly dependent on the concentrations of TiO_2 NPs, the contact time with *Microcystis* sp. and susceptibility or resistance of the strains. Therefore, TiO_2 NPs can be offered as an antibacterial agent against *Microcystis* sp.with doses that do not exceed 600 $mg.l^{-1}$.

With a view to reduce health and economic risks associated with the presence of *Microcystis* sp. and its toxins (Microcystin LR) in the majority of blooms produced in drinking water production, further investment is recommended to know at which stage of water treatment it should be introduced. Nevertheless, the current study should be supplemented by eco-toxicological studies on TiO_2 NPs to prevent their approximate risks on environmental and public health.

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