Toxic Effect of Nano-TiO_2 and Nano-Carbon on *Microcystis Aeruginosa*

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Abstract. To evaluate the environmental safety of the joint action of nano-TiO_2 and nano-carbon, *Microcystis aeruginosa* was used as the model organism to explore the effect of different concentration of nano- TiO_2 and nano-carbon on the growth of algae. This experiment suggested that the growth of algae will be changed under different nano-TiO_2 or nano-carbon concentrations and then affect the water environment. Therefore ecological and environmental effect of nano-materials should be considered.

Introduction

Because the development of nano technology, a large number of nanomaterials enter the environmental media through different pathways [1]. Since 2003, Service and Brumfiel discussed the biological effect of nanomaterials in *Science* and *Nature*, the environmental toxicity effect of artificial synthesis nano particles has become the research hotspot on international in recent years[2]. The present study evaluate the growth capacity of *Microcystis aeruginosa* at different concentrations of nano-TiO_2 and nano-carbon, with the aim to assess the possible toxic effects on *Microcystis aeruginosa*.

Experimental Set-up

Microcystis aeruginosa FACHB-1005 was purchased from freshwater algae culture collection at the institute of hydrobiology. Algae cultivated at the temperature of (26±1)℃, light dark ratio was 12 h:12 h, light intensity was 2000 lux. The culture medium was BG-11 and the solution of 1 $mol.L^{-1}$ HCl and 1$mol.L^{-1}$ NaOH were used to make sure the pH was 8. The water used in this experiment was distilled and the nano-TiO_2 and nano-carbon were prepared in the laboratory. Packed BG-11 in 250 mL sterile conical flask, 100 mL for each one. The logarithmic growth phase was used to make the initial algal density was 1×10^6 $cells.mL^{-1}$. Then cultivated algae and each concentration group set 2 parallel samples and took the average. Accurate weighed 0.0100 g nano-TiO_2(or nano-carbon) in 10 mL volumetric flask and constant the volume by distilled water. The addition amount of $CuSO_4\cdot5H_2O$ solution was 0, 20, 200, 400, 800, 1200 and 1600 $\mu g.L^{-1}$; nano-TiO_2 suspension and nano-carbon suspension added the same amount of $CuSO_4\cdot5H_2O$. The logarithmic growth phase was used to make the initial algal density was 1×10^6 $cells.mL^{-1}$. Then cultivated algae and each concentration group set 33 parallel samples and took the average.

Determination method

Took a certain amount of algae at the same time in each day and blood cell counting chamber was used to determine the biomass under the microscope. Calculate the inhibition ratio as following formula[3].

$$\text{Inhibition ratio (IR)}=(1-N / N_0)\times100\% \quad (1)$$

N: the cell number of experimental group ($cells.mL^{-1}$); N_0: the cell number of control group($cells.mL^{-1}$).

Data analysis

The experimental results were processed by Origin 8 to made the diagram.

Results and Discussion

The effect of nano-TiO_2 on the growth of *Microcystis aeruginosa*. Biomass of *Microcystis aeruginosa* in different concentrations of nano-TiO_2 solution showed in Fig. 1A. The algae cell number of experimental group and control group have been in a state of increase during the experiment but do not have so much difference before the eighth day. The most obvious effect at the concentration of TiO_2 was 800 $\mu g \cdot L^{-1}$ which had the maximum biomass 40.8×10^6cells.mL^{-1}. The concentration of 400 $\mu g.L^{-1}$, 20 $\mu g \cdot L^{-1}$ and 1200 $\mu g.L^{-1}$ had the similar biomass which was about 26×10^6 cells.mL^{-1}. The concentration of 1600 $\mu g.L^{-1}$, 200 $\mu g.L^{-1}$ had the least biomass but all the experimental groups' biomass were larger than control group. Inhibition ratio of *Microcystis aeruginosa* in different concentrations of nano-TiO_2 solution shown in Fig. 1B. The growth of algae was depressed under the concentration of 800 $\mu g.L^{-1}$ at the beginning 2 days. As time went on, TiO_2 played an increasingly significant role in the growth of algae and the maximum negative inhibition ratio was -1.32. All the experimental groups appeared negative inhibition from the third day except the concentration of 1600 $\mu g.L^{-1}$ which showed at the seventh day. Our experiment result is different from the previous research which pointed out that the TiO_2 can inhibitted the growth of the algae especially in the light[4]. It is possible that different species were used in the two experiments, and the concentration of the active oxygen stimulated the algae's self-defense, which increased the quantity of algal cell to resisted this adverse environment.

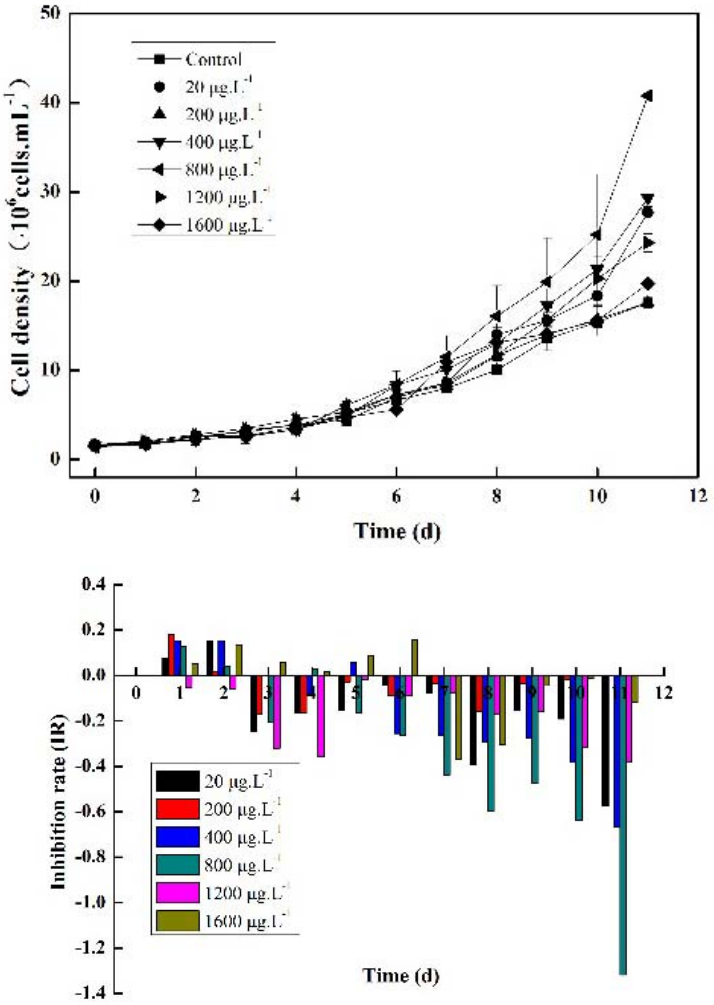

Fig. 1 The Biomass (A) and Inhibition ratio (B) of *Microcystis aeruginosa* indifferent concentrations of nano-TiO_2 solution (A).

The effect of nano-carbon on the growth of *Microcystis aeruginosa*. Biomass of *Microcystis aeruginosa* in different concentrations of nano-carbon solution showed in Fig. 2A. Nano-carbon and nano-TiO_2 had the similar effect on algae. The nano-carbon concentration was 1200 μg.L^{-1} had the maximum biomass of 49×10^6 cells.mL^{-1}. The experimental groups of 800 μg.L^{-1} and 400 μg.L^{-1} had the similar biomass which was about 35×10^6 cells.mL^{-1}.The concentration of 1600 μg·L^{-1} and 200 μg.L^{-1} had the least biomass but all the experimental groups' biomass were larger than control group. The inhibition ratio of *Microcystis aeruginosa* in different concentrations of nano-carbon solution showed in Fig.2B. The growth of algae was depressed under the concentration of 1200 μg.L^{-1} at the beginning 2 days. As time went on nano-carbon played an increasingly significant role in the growth of algae and the maximum negative inhibition ratio was -1.5. All the experimental groups appeared negative inhibition from the third day except the concentration of 200 μg.L^{-1} and 1600 μg.L^{-1} which showed at the seventh and eighth day. As the Shubbert[5] considered, one of the reaction of algae to pollution is stimulates growth at the low concentration and inhibit growth at the high concentration. The biomass has been increased in this experiment, maybe the pollutant activated the activity of enzymes in algae, so they chose blooms strive for living space to resisted the adverse survival environment. It's a kind of induced self-defense[6].

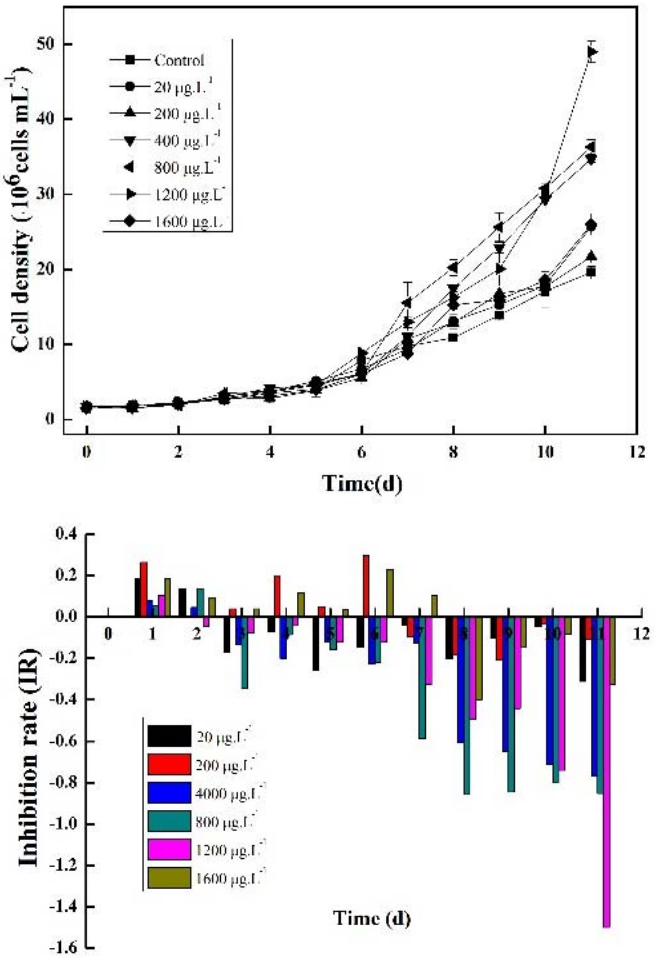

Fig. 2 Biomass (A) and Inhibition ratio (B) of *Microcystis aeruginosa* indifferent concentrations of nano-carbon solution.

Conclusion

Nano-TiO_2 is a kind of excellent photocatalysts and it's also a very promising environmental protection material. Because the active oxygen which produced under the photocatalysis have biological toxicity effect, we can consider how to use nano-TiO_2 to manage the blooms in the subsequent study. Algae will be affected by nano-carbon in the water environment but it can promoted the growth of *Microcystis aeruginosa* in this experiment. Maybe it's the self-defense of

Microcystis aeruginosa in the adversity under the pollution stress conditions and different species have different respond to it. So we can't ignore the ecological effect of nano-carbon.

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