

# Toxicological Effects of Nanometer Titania on Chlorella

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**Abstract**—The aim of this work was to investigate the effect of titania nanoparticles (TiO<sub>2</sub> NPs) on chlorella cultivated in modified Blue-Green medium (BG-11). The chlorella species were exposed for 96 h with a daily reading of the quantity of chlorella cultivated in modified BG-11 medium with different concentrations (0, 10, 20, 50, 100 mg/L) of anatase and rutile, respectively. Experimental results revealed the growth of chlorella was promoted in BG-11 medium with anatase during 48 h exposing, while inhibited at 96 h. TiO<sub>2</sub> NPs in rutile crystal structure had little impact on the growth of chlorella in the entire set of tests conducted. At low concentration (<100 mg/L), the influence of titanium ion concentration on the growth of chlorella was not obvious.

**Keywords**—toxicological effects; chlorella; BG-11; anatase; rutile

## I. INTRODUCTION

Along with the widely application of nanomaterials in material science and nanotechnology, the risk of human exposure to nanometer materials is increasing, more and more importance is attached to the biological safety of them. The small particle sizes of nanomaterials make they have greater mobility and transmembrane abilities [1-2], which are more harmful. Many recent studies show that nanomaterials will induce the inflammation of target organ, prompt the oxidative stress reaction of body produces, and even enter the cell and the cell nucleus, and then exhibit the greater biological effect [3-4].

The algal cells are commonly used in experimental study of toxicity, because of the sensitivity to many poisons, a short growth period, which are easy to be isolated and can be observed directly on the cellular level of poisoning symptoms [5].

As an important industrial material, TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub> NPs) are widely used in numerous consumption products, such as sunscreens, cosmetics, paints and surface coatings [6-8], and in the environmental field: air soil and water decontamination processes [9-10]. Some of the toxicity studies were based on the evaluation of the

potential toxicity of TiO<sub>2</sub> NPs on human beings. It is found that TiO<sub>2</sub> NPs and other nanoparticles can lead to lipid peroxidation, cell growth inhibition and photosynthesis inhibition in algae [11-13], and the degree of toxicity is depend on concentration and particle size, even in bulkier inert material [14].

This paper takes TiO<sub>2</sub> NPs as the research object, and the aim is to investigate the biological toxicity of TiO<sub>2</sub> NPs on chlorella and provide data support for the biological toxicity evaluation of TiO<sub>2</sub> NPs, by testing the influence of anatase and rutile on the growth of chlorella, respectively.

## II. EXPERIMENTS

### 2.1 Materials and Instrument

Tetrabutyl titanate (CP), normal butyl alcohol (AR), ethanol (AR), Titanium tetrachloride (CP) and hydrochloric acid (AR) were purchased from commercial market. All reagents were used without further purification. Water was deionized and distilled before use. The Blue-Green medium (BG-11) was cultivated from our laboratory.

Nanometer titania (anatase and rutile) were prepared by the microwave digestion system (WX-4000 Shanghai Yiyao, China). The as-synthesis powders were characterized by X-ray diffractometer (D8 Advance, Bruker, Germany) and transmission electron microscopy (JEM-2010).

### 2.2. Preparation of TiO<sub>2</sub> NPs

TiO<sub>2</sub> NPs (anatase and rutile) were synthesized from our laboratory by microwave hydrothermal method, respectively. Single-phase and high purity nanocrystalline anatase was synthesized by hydrolysis of Ti(C<sub>4</sub>H<sub>9</sub>O)<sub>4</sub> at 120 °C for 1.5 h and then at 180 °C for 1 h. Well crystallized and single-phase rutile was obtained by hydrolysis of TiCl<sub>4</sub> first at 120 °C for 1.5 h and then at 180 °C for 1 h.

### 2.3 Toxicity Test of TiO<sub>2</sub> NPs to *Chlorella*

The TiO<sub>2</sub> NPs suspensions were prepared in 5000 mg/L stock solution with BG-11 medium prior to the experiments. Inoculums of *Chlorella* were grown in 250 ml glass flasks containing 100 ml of BG-11 medium at (25±1) °C. Five titanium ion concentrations (0, 10, 20, 50, 100 mg/L) of anatase and rutile were respectively added to the flasks from the stock solution of nanoparticle suspensions, which were treated with sonication after sterilization. The growth of *Chlorella* was assessed by numbering algal cells in haemocytometer after different exposing times every 24 hours.

## III. RESULTS AND DISCUSSION

### 3.1 Characterization of the as-Synthesized TiO<sub>2</sub> NPs

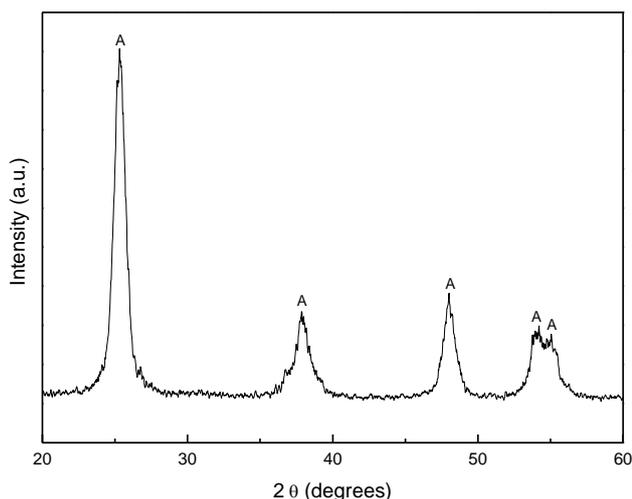


Figure 1. XRD spectrum of the as-synthesized TiO<sub>2</sub> powder by hydrolysis of Ti(C<sub>4</sub>H<sub>9</sub>O)<sub>4</sub>

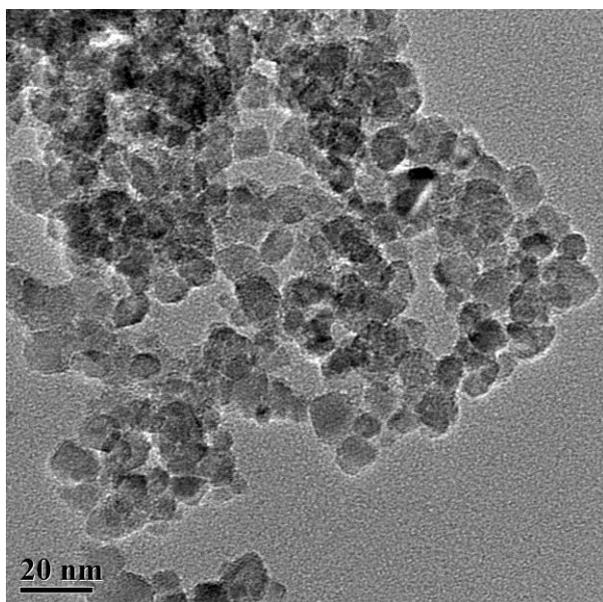


Figure 2. TEM photograph of the as-synthesized TiO<sub>2</sub> powder by hydrolysis of Ti(C<sub>4</sub>H<sub>9</sub>O)<sub>4</sub>.

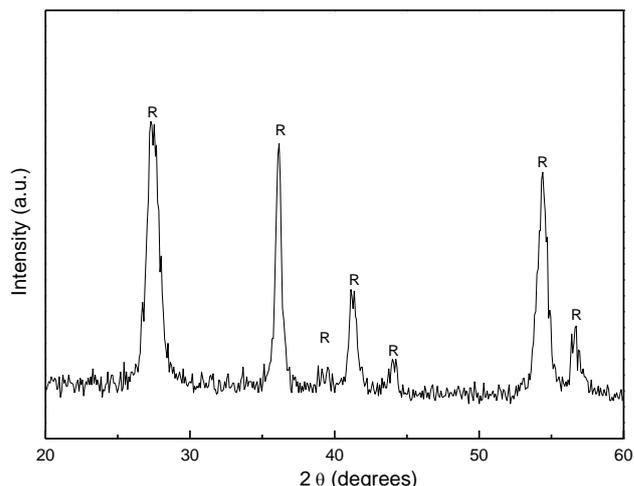


Figure 3. XRD spectrum of the as-synthesized TiO<sub>2</sub> powder by hydrolysis of TiCl<sub>4</sub>.

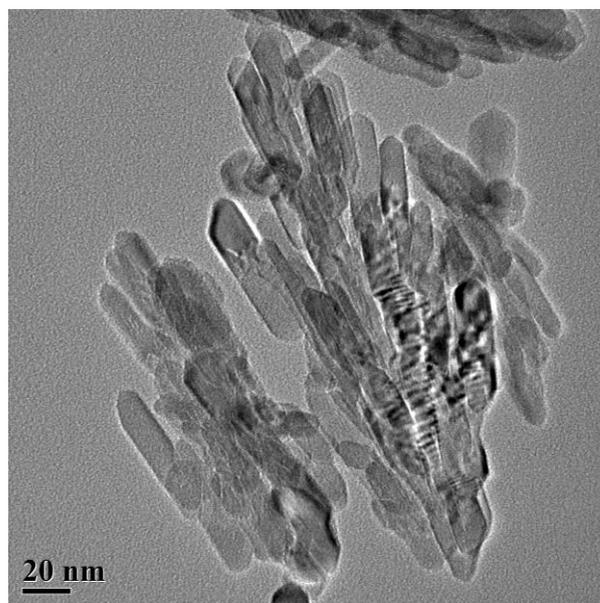


Figure 4. TEM photograph of the as-synthesized TiO<sub>2</sub> powder by hydrolysis of TiCl<sub>4</sub>.

The XRD spectrum in Fig. 1 indicated that the as-synthesized powder by hydrolysis of Ti(C<sub>4</sub>H<sub>9</sub>O)<sub>4</sub> was single-phase and high purity nanocrystalline anatase. Fig. 2 showed the TEM photographs of anatase exhibited granulous and uniformly dispersed, with the average crystallite size of 10 nm.

Fig. 3 showed the result of XRD analysis of the as-synthesized powder by hydrolysis of TiCl<sub>4</sub>. The XRD spectrum indicated the as-synthesized powder was well crystallized pure rutile. As shown in Fig. 4, the as-synthesized rutile was observed to be uniformly dispersed and exhibited shuttle-like morphology. The average diameter of rutile was about 10 nm.

### 3.2 Toxicological Effects of TiO<sub>2</sub> NPs on *Chlorella*

The growth of *Chlorella* was assessed by numbering algal cells in haemocytometer. The *Chlorella* species were

exposed for 96 h with a daily reading of the quantity of chlorella cultivated in modified BG-11 medium with different concentrations (0, 10, 20, 50, 100 mg/L) of anatase and rutile, respectively. The quantity of chlorella cultivated in BG-11 medium with different concentrations of anatase and rutile every 24 hours were listed in Table I and Table II, respectively.

TABLE I. QUANTITY OF CHLORELLA CULTIVATED IN BG-11 MEDIUM WITH DIFFERENT CONCENTRATIONS OF ANATASE

Time (h)	1 0mg/L 个数 (10 <sup>4</sup> )	2 10mg/L 个数 (10 <sup>4</sup> )	3 20mg/L 个数 (10 <sup>4</sup> )	4 50mg/L 个数 (10 <sup>4</sup> )	5 100mg/L 个数 (10 <sup>4</sup> )
0	278	278	278	278	278
24	2745	2290	2910	2438	2195
48	4470	9810	10995	7680	12345
72	10560	9300	11970	9450	8400
96	12000	11280	11355	12465	8715

TABLE II. QUANTITY OF CHLORELLA CULTIVATED IN BG-11 MEDIUM WITH DIFFERENT CONCENTRATIONS OF RUTILE

Time (h)	1 0mg/L 个数 (10 <sup>4</sup> )	2 10mg/L 个数 (10 <sup>4</sup> )	3 20mg/L 个数 (10 <sup>4</sup> )	4 50mg/L 个数 (10 <sup>4</sup> )	5 100mg/L 个数 (10 <sup>4</sup> )
0	205	205	205	205	205
24	1995	2433	2035	2353	2725
48	11715	12120	12030	10848	14895
72	9120	9120	10030	13650	9390
96	9060	13590	8490	13320	11055

The growth curve of chlorella cultivated in BG-11 medium with different concentrations (0, 10, 20, 50, 100 mg/L) of anatase and rutile were shown in Fig.5 and Fig.6, respectively.

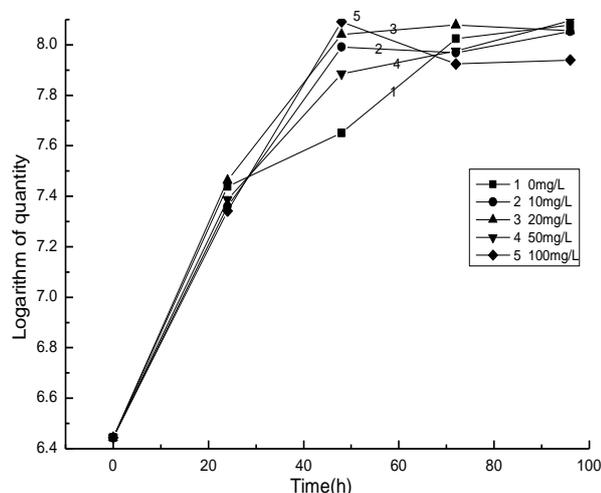


Figure 5. The growth of chlorella cultivated in BG-11 medium with different concentrations of anatase.

As shown in Fig.5, TiO<sub>2</sub> NPs in anatase crystal structure accelerated the growth of chlorella during 48 h exposing. By stressing chlorella cell in favor of the activity of some enzymes, nanocrystalline anatase promoted the growth of chlorella. However, at 96 h, the growth of chlorella cultivated in BG-11 medium with anatase was inhibited. The titanium ion concentration on the growth of chlorella is not obvious.

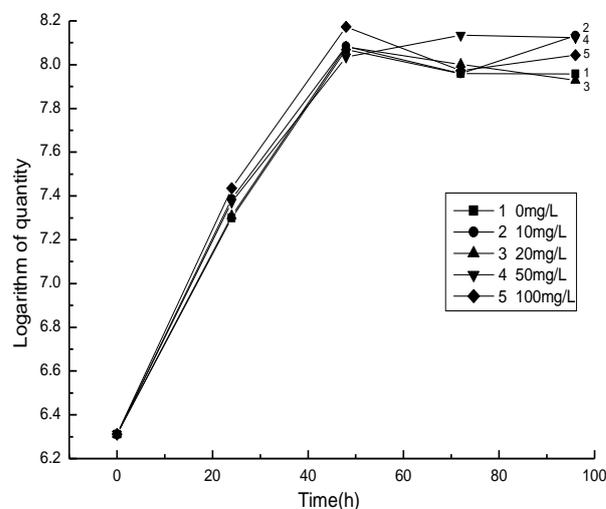


Figure 6. The growth of chlorella cultivated in BG-11 medium with different concentrations of rutile.

Fig.6 indicated that there were no significant differences among five growth curves, which showed that TiO<sub>2</sub> NPs in rutile crystal structure have little impact on the growth of chlorella in the entire set of tests conducted. Because of its lipophilicity, the rutile crystalline structure of TiO<sub>2</sub> NPs formed larger aggregates in aqueous medium; then they had less effect on biological organisms, and thus a lower toxicity than the anatase crystalline form of TiO<sub>2</sub>.

#### IV. CONCLUSIONS

At low concentration (<100 mg/L), the influence of titanium ion concentration on the growth of chlorella is not obvious. The growth of chlorella was promoted in BG-11 medium with anatase during 48 h exposing, while inhibited at 96 h. TiO<sub>2</sub> NPs in rutile crystal structure have little impact on the growth of chlorella in the entire set of tests conducted.

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