

# Toxicity of Titanium Dioxide Nanoparticles on Growth Kinetics of Activated Biomass

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## ABSTRACT

This study focused on effect of titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) on growth kinetic parameters of aerobic sludge bacteria and its associated toxicity to the activated biomass of a typical sewage treatment plant. Initially no difference in lag periods or exponential growth periods was found due to presence of nanoparticles. Also, a considerable amount of decrement in the absorbance values of the bacterial growth phase due to the toxicity of the NPs with the highest toxicity obtained during the stationary phase after 72 hours with a reduction of almost 52% in presence of 2 mg/L and 72% in presence of 20 mg/L of TiO<sub>2</sub> NPs. Further, the amount of culturable bacteria also was reduced to almost 50% due to the effect of 2 mg/L and 20% due to the presence of 20 mg/L of TiO<sub>2</sub> NPs, indicating effect of NP on culturability of bacteria. More kinetic studies using TiO<sub>2</sub> concentrations till 20 mg/L are required to understand its toxicity to activated biomass.

**Keywords:** Bacteria; Titanium dioxide nanoparticles; Toxicity; Wastewater

## 1. INTRODUCTION

Nanoparticles come in to existence with the advent of complex molecular processing and nanotechnology in modern ages. A nanoparticle can be defined as the smallest particle that behaves as a whole element in terms of its properties. Usually a nanoparticle ranges between the sizes of 1 nm to 100 nm. The word nano derives from the Greek word 'nanos' which means dwarf or extremely small.

The sources of nanoparticles in day to day life are mainly consumer products like sunscreen and deodorants used frequently in modern life. Other than this paints, varnishes etc add to a considerable amount to the nanoparticle bulk amount ejected in the environmental media.

The production and use of titanium nanomaterial in different consumer and health products has increased in day to day life, thus it was necessary to put stress on the monitoring and assessment of toxicity posed by the titanium nanoparticles to the environment. About 90,000 tonnes of titanium and about 4.3 million tonnes of titanium dioxide metal are being manufactured yearly [3]

This leads to the involvement of many potential hazards posed by the nanoparticles to the living systems present in the environment. Silver nanoparticles are found in many cases accumulated in the blood of the human or In the colon of the person causing colon cancer [3]. Silver nanoparticles are found to be accumulated even in the heart, liver and kidneys of a person [1]. These engineered nanoparticles

ultimately find its way through different transport routes and ultimately end up in the waste water and waste water sludge [2].

The accumulation of nanoparticle in the waste water sludge can lead to potential threat to the mix consortia of bacteria present in the activated sludge. The nanoparticles are either adhere to the microorganisms or taken up by the microorganisms creating a potential gradient of a nanoparticle based layer in and outside the microbial cells. The carbon nanotubes pose a potential threat to sludge bacteria in [7] with the decrement in the respiration rates in the presence different concentration of nanomaterials and also attachment of the nanomaterials on microbial cells through scanning electron microscope (SEM) analysis.

Monitoring of the titanium nanoparticles in the waste water treatment plant (WWTP) shows the accumulation of TiO<sub>2</sub> nanomaterials in the bio solids ranges from 1-6 µg/mg and that in the raw sewage ranging from 100 to 3000 µg/ L [6]. The value was much higher than the effective concentration level of 1 µg /L in the waste water influent. Thus it is evident that the presence of titanium metallic nanoparticles might threat the existence of mix consortia of bacteria present in the waste water treatment sludge. This may lead to the decrease in the treatment efficiency of the WWTP.

The objective of this study was to focus on the microbial growth kinetics of the mix culture of bacteria present in the domestic sewage water in presence and absence of titanium di-oxide nanoparticles and see the effect of toxicity of nanoparticles on the microbial growth at different time interval. TiO<sub>2</sub> was selected due to increased usage and this there is high possibility of it coming in wastewater. The amount of bacterial colonies presence after interaction with nanoparticles after incubation on nutrient agar plates for 24 hours was also studied. After the exposure of nanoparticles to the mix culture of bacteria on agar plates for 24 hours the morphology of the bacteria present in absence and presence of nanoparticles was studied by light microscope in order to assess the type of bacteria resistant to nanometallic stress.

This study will give an overview of the toxicity of the nanoparticle on mixed culture of bacteria present in the waste water. All the experiments were done in duplicates if not mentioned otherwise.

## 2. MATERIALS AND METHODS

### 2.1 Generation of mixed culture of biomass from domestic sewage

Domestic sewage water was collected from the internal drainage system, in the campus at Indian Institute of Technology, Delhi, India. A biomass acclimatization reactor of 1 litre was set up for generation of appropriate amount of biomass containing 0.5% of glucose, 0.5% of potassium dihydrogen phosphate and 0.5% of peptone and 100 ml of domestic sewage water collected freshly and was aerated for 48 hours. The generation of mixed culture of microbial population was visible from the appearance of turbidity inside the reactor. The mixed liquor was centrifuged, the supernatant was discarded and the cell pellets was collected. This cell pellets were re suspended in nutrient media and the growth of the microbes was studied.

### 2.2 Growth kinetics study of the mixed culture of microorganisms

Three different semi-batch type bioreactors was set up containing 3.75 g of glucose, 1.14 g of ammonium chloride and 7.5 ml of 0.2 M of phosphate buffer per 1000 ml of the reactors and containing 2000 mg of the biomass generated from the acclimatization reactor. The three reactors were containing 2 mg. and 20 mg of titanium dioxide nanoparticles and one reactor without nanoparticle was kept as control. The pH of 7-7.4 and a temperature of 36-37°C were maintained inside the reactor for the optimum growth of the microbes. The growth of the microbes was measured by the UV-Vis spectrophotometer at 600 nm [8] in presence and absence of nanoparticles. Separate reactors were also put up containing media and only 2 mg and 20 mg per litre of nanoparticles and the value was subtracted from the absorbance values of the reactors containing both nanoparticle and bacteria to observe the actual growth of the bacteria due to the toxic effect of the nanoparticles for 7 days at different time intervals of growth.

### 2.3 Toxicity of NPs on bacteria in solid media

Samples were collected from the aeration tank from the sewage treatment plant (STP) in New Delhi (India), diluted to  $10^3$  times and were mixed with a nanoparticle concentration of 2 and 20 mg/L in and 100  $\mu$ l of this was spread on the Petri dishes containing nutrient agar. They were kept in the incubator for incubation at about 37°C for 24 hour. After the stipulated time of incubation the number of bacterial colonies was counted [5]. A nutrient agar plate containing only bacteria was studied as a control. All the results were expressed in terms of CFU (colony forming unit) and were multiplied by the dilution factor to get the original population of the bacteria per ml of the sample.

### 2.4 Morphology Study of the Nano particle resistant bacteria

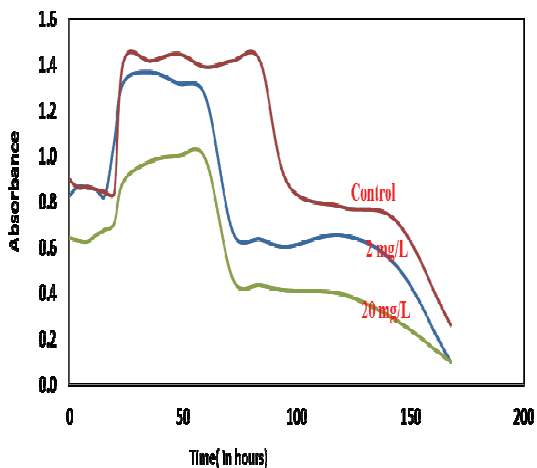
Two glass slides were washed properly and cleaned with 95% pure ethanol in order to make the surface free from any kind of contamination. A loop of autoclaved water was put on the glass slide in front of the Bunsen burner inside the laminar air flow (LAF) and bacterial collected from bacterial colonies grown in absence and presence of 20mg/L of nanoparticle on the nutrient agar plates was thoroughly mixed together. A smear of bacterial cells was thus formed on the glass slide. The cells were heat fixed by taking the opposite side of the glass slide in front of the Bunsen burner.

The crystal violet stain was put on the samples as a primary stain and was kept for 1 minute. After that excess unbound crystal violet stain was washed by drop wise flow of water for 15 seconds. Gram's iodine was added as a mordant for fixing crystal violet to the bacterial cells and it was kept for 1 minute. It was also washed by drop wise flow of water. The glass slides was then rinse with ethyl alcohol for 5 seconds to remove the excess stain and was again washed by drop wise flow of water. Lastly the safranin was added to the sample as a secondary stain and was kept for 1 minute and was then washed by gentle flow of water for 5 seconds. (Hans Christian Gram, 1884).The glass slides were then observed under the light microscope at 100x zoom under oil immersion.

## 3. RESULTS AND DISCUSSIONS

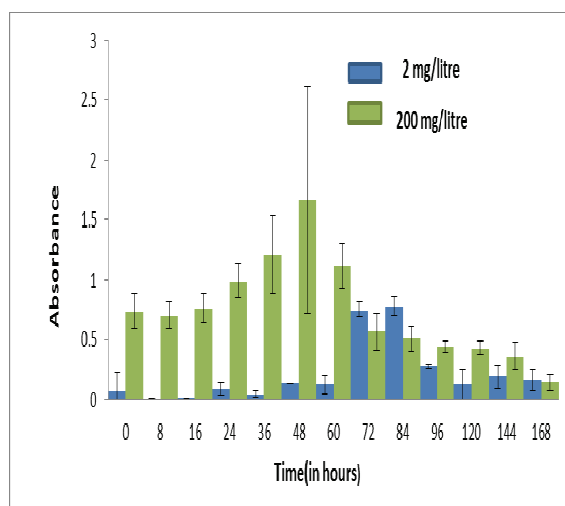
### 3.1 Effect of titanium NP on growth kinetics of the bacteria

It was seen that the titanium nanoparticles are having immense toxic effect of the different phase of growth kinetics of the bacterial population. Figure 1 indicates that the maximum effect control reactor is having normal absorbance values in the absence of the nanoparticles showing distinguish phases (lag, log, stationery and decay) of the growth of the bacterial population.



**Figure 1: Microbial growth kinetics with and without titanium dioxide nanoparticles**

The absorbance values of the bacterial population in presence of 2mg /L of nanoparticle was lesser than that of the absorbance values of the control reactor in absence of nanoparticles. The most toxic effect on the microbial growth kinetics was seen in the presence of 20 mg/L of TiO<sub>2</sub>NP.

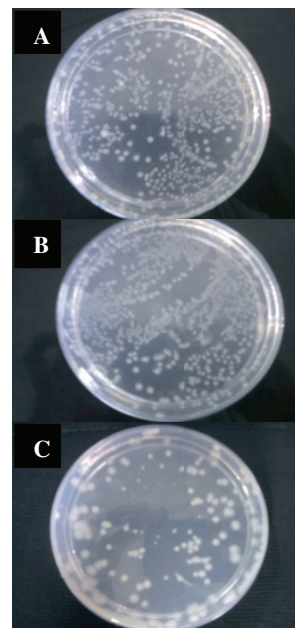


**Figure 2: TiO<sub>2</sub> NP-associated bacterial toxicity (in terms of reduction of absorbance compared to that of control) at different time intervals**

High NP concentration was found to impart more toxicity to bacteria than lower NP concentration for all observation periods (Figures 1 & 2), with highest difference obtained at 48 hours of time interval from start (Figure 2). These results clearly indicate the threat pose by the metallic NPs to the consortium of mixed bacteria present in waste water.

### 3.2 Effect of NPs on sludge bacteria on agar plates

Figure 3 shows culturability of bacteria after NP exposures. In nutrient agar plates, bacterial colonies were observed to 460×10<sup>4</sup>/ml (without NP exposure) compared to 206×10<sup>4</sup>/ml and 84×10<sup>3</sup>/ml during exposures of 2 mg/L and 20 mg/L TiO<sub>2</sub> NPs.

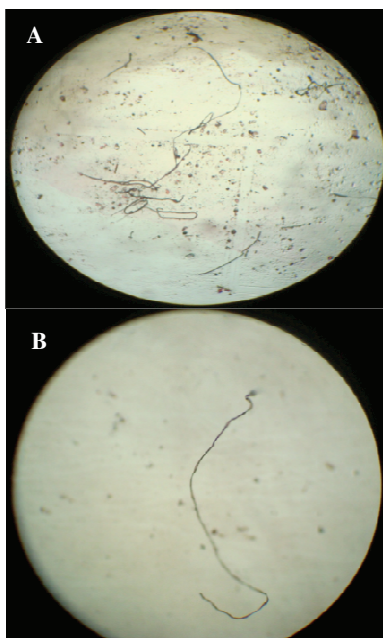


**Figure3: Growth of microbial colonies in absence (A) and presence of 2 mg/L (B) and 20 mg/L(C) TiO<sub>2</sub> NPs at zeroth hour of NP exposures**

This result indicates the effect of NP concentration on culturability of bacteria after exposure.

### 3.4 Morphology study of the nanoparticle resistant bacteria

The morphology study and type of bacteria which is resistant to nanoparticle was viewed under light microscope after staining. Figure 4 shows the microscopic view of the morphology of the different types of bacteria present in the activated sludge as well as that of which are resistant to titanium di oxide nanoparticle.



**Figure 4: Morphology of mixed culture of bacteria presents in the original sludge (A) and after the exposure to 20 mg/L TiO<sub>2</sub> NPs (B)**

It is clear that among many gram positive and gram negative bacteria which is present in the original sludge present only few are left after the exposure to metallic oxide NPs. The titanium dioxide nanoparticle resistant bacteria can be either filamentous or round shaped. This may be either gram positive *bacillus* (cell wall violet colored) or gram negative *coccus* (cell wall pink colored).

#### 4. CONCLUSIONS

1. There was a decrement in the absorbance values of the bacterial growth in presence of nanoparticles with 6% reduction after 12 hour and 24 hours and 8% reduction after 48 hours in presence of 2 mg/L TiO<sub>2</sub> NPs and 23% after 12 hours, 37% after 24 hours and 30% after 48 hours in presence of 20 mg/L of TiO<sub>2</sub> NPs.
2. Results indicated that the TiO<sub>2</sub> NPs posed maximum threat to bacteria during their stationary phase of growth which might affect the flocculation process of sludge bacteria during biological wastewater treatment.
3. The plate count method also revealed the considerable decrease in the growth of the microbial colonies due to the presence of nanoparticles with a reduction of almost 50% due to the effect of 2 mg/L and 20% due to the presence of 20 mg/L of TiO<sub>2</sub> NPs.
4. In the microscopic analysis it is clear that the microbial population decreased to a considerable amount in presence of metallic oxide nanoparticles.
5. These findings indicate that initially TiO<sub>2</sub> imparts high toxicity to biomass, which could be used in deciding

hydraulic retention times in WWTP activated sludge processes. Future detailed studies are required to study TiO<sub>2</sub>-bacteria interaction during activated sludge operations to understand long-term impact of NP exposures to bacteria and to reduce NP toxicity-associated uncertainties for bacteria.

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#### REFERENCES

1. Ahamed, M., Mohamad, S. A., Siddiqui, M. K. J., Silver nanoparticle applications and human health, *Clinica Chimica Acta* 411 (2010) 1841–1848
2. Brar, S. K., Verma, M., Tyagi, R. D., Surampalli, R. Y., Engineered nanoparticles in wastewater and wastewater sludge – Evidence and impacts, *Waste Management* 30 (2010) 504–520
3. Emsley, J. *Nature’s Building Blocks: An A-Z Guide to the Elements* (2001), Oxford University Press: Oxford.
4. Gatti, A. M., Montanari, M., Monari, E., Gambarelli, A., Capitani, F., Parisini, B., Detection of micro- and nano-sized biocompatible particles in the blood, *J. Mater. Sci. Mater. Med.*, 15(2004), 469–472
5. Khan, S., Mukherjee, A., Chandrasekaran, N., Silver nanoparticles tolerant bacteria from sewage environment, *Journal of Environmental Sciences* 2011, 23(2) 346–352
6. Kiser, M. A., Westerhoff, P., Benn, T., Wang, G., Perez-rivera, J., Hristovski, K., Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants, *Environ. Sci. Technol.* 2009, 43, 6757–6763
7. Luongo, Zhang, Toxicity of carbon nanotubes to the activated sludge process, *Journal of Hazardous Materials* 178 (2010) 356–362
8. Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., Mukherji, S., Strain specificity in antimicrobial activity of silver and copper Nanoparticles, *Acta Biomaterialia* 4 (2008) 707–716