Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 1692-1704
Received: 13-07-2019
Accepted: 17-08-2019

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Toxicity of TiO_2 nanoparticles resistance by waste water bacterial isolates with their consortium and evaluation of biofilm formation

G Arumugam, RR Thanighaiarassu and P Sandhya

Abstract
The TiO_2 toxicity resisted by waste water bacterial isolates. The waste water samples are collected and TiO_2 nanoparticles stability with waste water is studied by measuring hydrodynamic size by dynamic light scattering at different time intervals 0,6,12 and 24 hrs was carried out using 90 plus Particle Size Analyzer, Brookhaven Instruments, USA. Then from this waste water five bacterial pure culture isolated and five synergistic bacterial consortium also cultivated. The titanium nanoparticles showed its toxicity effect on the bacterial isolates *Bacillus flexus*, *Brevundimonas diminuta*, *Exiguobacterium indicum*, *Exiguobacterium acetylicum*, *Pseudomonas nitroreducens*) which are isolated from the waste water . Bacterial cell viability assay was proceded by standard plate count assay at 6, 12 and 24 hrs time intervals and 0.25, 0.5 and 1 µg/mL of titanium nanoparticles concentrations. Cell viability is statistically significant with respect to both dark and UVA conditions. The free radicals super oxide dismutase the reactive oxygen species level of treated (1µg/mL) of TiO_2 nanoparticles on all five bacterial species and consortium was found to increased in dark condition and increased UVA condition. The biofilm mass formed in the presence of TiO2 nanoparticles (1 µg/mL) of all five bacterial isolates and consortium is estimated to be increased in dark condition and UVA condition at optical density 590nm. Although biofilm formation in consortium was found to be higher when compared to individual isolates it was not significant when compared to UVA and dark condition. The extracted EPS from the TiO_2 nanoparticles (0.25, 0.50, 1µg/mL, 24 hrs) from individual five bacteria and the consortium was showed bacterial resistance against the toxicity produced. The cytotoxic effects of TiO_2 nanoparticles, morphology of cells, bio distribution of nanoparticles under UVA and dark conditions were observed by tem.

Keywords: TiO_2 nanoparticles, *Bacillus flexus*, biofilm formation, reactive oxygen species, bacterial tem image

Introduction
Nanotechnology is rapidly growing industry and steadily extending application of nano-enabled products reach from medical and research sectors, to wide range of consumer products. The production of engineered nanoparticles (ENPs) and nanomaterials is estimated to reach 58,000 tons within the next years [1]. Kiser and coworkers reported incomplete removal of TiO_2NP_S in wastewater treatment, with concentrations of TiO_2 in the effluents reaching from 10 to 100 µg L−1 [2]. Once released into the waste water environment, TiO_2NPs are expected to accumulate, with predicted environmental concentrations (PEC) ranging between 0.53 and 24 µg L−1 [3-5]. Previous studies reported adverse effects of TiO_2NP_S towards bacteria stream biofilms and soil bacterial communities [6, 7]. Toxic effects of TiO_2NP_S have also been reported for *Bacillus subtilis* and *Escherichia coli*. [8].
Over the last few decades, nanotechnology is emerging as a rapidly growing sector on knowledge based economy due to unique physiochemical properties of nanomaterial. This technology gained a tremendous impetus due to its capability of reformulating the particle of metals into new nano-sized form, with dimension less than 100 nm in size. Hence, it is used in manufacture of a wide range of products and in wastewater treatment [9-14]. Due to remarkable use of nanoparticles, wastewater treatment received considerable amount of nanoparticles such as TiO_2, with potential risk to environment [15-22].
Recently, implementation of nanotechnology in wastewater treatment enabled high performance, reasonable water and wastewater treatment solutions that less relies on large infrastructures. Wide range of nanomaterials tested regarding resistance of biofouling, elimination of toxic metals, organic and inorganic pollutants, pathogen detection as well as disinfection [23-26]. The economic view on nanotechnology allow for utilization of the most challenging water resources and energy conservation. Unfortunately, costs of this new technology should be properly managed due to competition with traditional waste water treatment technologies [27].

Titanium is seventh most abundant metal and ninth abundant element in the earth. The production of TiO_2 is 4.3 million tons [28]. TiO_2 nanoparticles has many applications like usage in cosmetics and other consumer products like paints and sunscreens. In food products it is used in cottage chesses, dressings and white sauce [29]. An average person take of TiO_2 is approximately 5.4mg/day. Ingested TiO_2 nanoparticles must be excreted from human body which in turn it is transported to waste water treatment. The usage of metal nanoparticles which has lead to direct or indirect release of nanoparticles in waste water treatment [30].

The titanium present in waste water treatment plants were found to be between 181 and 1233 µg/L. Various studies have been concluded that toxicity of TiO_2 depends on size, form and different concentration of nanoparticles. Different microorganisms reacted differently to the same toxicological conditions. Bacteria are used increasingly for nanotoxicological studies. The studies had proved that engineered nanoparticles like TiO_2 have strong antimicrobial properties and in water treatment process the biofilm formation can be stabilized or degraded by TiO_2. From all the recent studies it has been concluded that TiO_2 nanoparticles cause toxicity to the bacteria that there is minimal viability decrease and significant changes were found in biofilm formation after exposure to TiO_2 nanoparticles. The changes were not due to oxidative stress it is because of nanoparticles that had altered the gene expression. Since there is evidence that toxicity of nanoparticles was found to be lesser in consortia when compared to individual isolates isolated from freshwater and it is exposed to low doses of three different concentrations of titanium under two different irradiation (light and dark).

Membrane permeability showed significant results in dark when compared to light irradiation condition. Oxidative stress contributed considerably in both conditions. The biofilm and exo polysaccharides formation was found to be higher in the presence of nanoparticles. TEM and SEM images showed damaged cells and uptake of nanoparticles [31].

2. Materials and Methods

TiO_2 nanoparticles were procured from Sigma Aldrich, (dry titanium dioxide powder, 99.7% anatase, CAS no.637254). 2′, 7′-Dichloro fluorescein diacetate (DCFH-DA) was purchased from Sigma Aldrich. Analytical grade reagents and chemicals were used throughout the experiment.

Waste water was collected from VIT, Vellore. Waste water was filtered through whattman filter no.42 followed by filtration in whattman no.1 filter to remove large colloids from waste water and it is sterilized. The sterilized waste water matrix is used throughout the experiment. Secondary filtration was done prior to DLS analysis using 0.22µm membrane filter. The waste water matrix contains Total Organic Carbon TOC- 12±0.45, PH- 7.3, Dissolved Oxygen DO- 5.3mg/L, Total Dissolved Salts TDS-1.10±0.05µg/ml and its conductance was found to be 158±0.28.

2.1 Stability of Nanoparticles in Waste water by DLS

The stability of TiO_2 nanoparticles along with waste water is studied by measuring mean hydrodynamic size by dynamic light scattering at different time intervals 0,6,12 and 24 hrs was carried out using 90 plus Particle Size Analyzer, Brookhaven Instruments, USA. The 100µg/ml stock of TiO_2 nanoparticles was prepared in milliQ water and sonicated for 10 mins at 350W using an ultrasonic processor (Sonics, USA). Working concentration 0.25,0.50 and 1ppm was prepared in filtered waste water using working stock solution and their hydrodynamic size was measured by dynamic light scattering 90 plus Particle Size Analyzer, Brookhaven Instruments Corporations, USA.

2.2 Isolation and Identification of Pure Culture

The sample was collected from waste water VIT University, Vellore (India). The collected waste water was serially diluted and spread plated on nutrient agar plate and incubated for 24 hrs, 37 °C. The colonies were checked after 24 hrs and sub cultured to retrieve pure culture. Further, dominant colonies were selected for the study. The collected waste water was serially diluted and spread plated on nutrient agar plate and incubated for 24 hrs, 37 °C [32, 33]. The colonies were checked after 24 hrs and sub cultured to retrieve pure culture. Further, dominant colonies were selected for the study.

2.3 Consortium Development

The consortium was developed from five strains isolated from the same environment or niche was selected for the experiments. The strains used were (*Bacillus flexus*, *Brevundimonas diminuta*, *Exiguobacterium indicum*, *Exiguobacterium acetylicum*, *Pseudomonas nitroreducens*) of all these strains *Bacillus flexus* is a gram variable strain, *Brevundimonas diminuta* and *Pseudomonas nitroreducens* are gram negative strains, *Exiguobacterium indicum*, *Exiguobacterium acetylicum* were gram positive strains. For the consortium developmental, first a loop full of a strain is inoculated in 100ml nutrient broth and allowed to grow for 24 hrs at 30 degree Celsius at 120 RPM in incubator. 100µl of broth is taken and it is spread plated in nutrient agar using L rod. To this nutrient agar the second strain was streaked in centre of the plate. The same was followed for all the strains to check whether it is antagonistic/ synergistic. The plates were incubated at 34°C, 24 hrs and checked weather it is synergistic or antagonistic [34, 35]. All the 5 strains were synergistic and hence consortium was developed. To this toxicity of TiO2 nanoparticles were carried out.

2.4 Cytotoxicity Assessment

2.5 Experimental Setup

The bacterial isolates from waste water treatment plant (VIT waste water treatment plant, Vellore, India) were identified as *Bacillus flexus* is a gram variable strain, *Brevundimonas diminuta* and *Pseudomonas nitroreducens* are gram negative strains, *Exiguobacterium indicum*, *Exiguobacterium acetylicum* were gram positive strains. The experiment were carried on bacterial species *Bacillus flexus*, *Brevundimonas diminuta*, *Exiguobacterium indicum*, *Exiguobacterium acetylicum*, *Pseudomonas nitroreducens* and their consortia with 0.1 OD (Optical Density) is maintained throughout the experiment. Bacterial cells were inoculated and the pellet was harvested at exponential phase by centrifugation at 7000g for 10 mins. The cell pellet was washed with sterilized waste water to remove media components. For maintaining accuracy the experiments were performed in triplicates. Standard deviation and standard error were calculated. Individual strains and consortia were treated with different concentrations of TiO_2 (0.25, 0.50 and 1ppm) under two different conditions irradiation (dark) and radiation (UVA).

2.6 Cytotoxic assessment of TiO2 nanoparticles

Cell viability test was done to determine the toxicity of TiO_2 nanoparticles on individual isolates and their consortium. The control was contemplated to be 100% to calculate percentage

viability of the treated samples. Cell viability test was carried out by standard plate count assay at 6, 12 and 24 hrs time intervals and 0.25, 0.5 and 1 µg/mL of titanium nanoparticles concentration.

2.7 Oxidative stress assessment

2.8 Reactive oxygen species (ROS)

The free radicals like superoxide anion O_2^-, hydrogen peroxide H_2O_2 and hydroxyl groups OH^- are said to be reactive oxygen species. They can be measured using fluorescence method using 2', 7'-dichlorfluorescein-diacetate (DCFH-DA), Fluroscent probe. The non Fluroscent, 2',7'-dichlorfluorescein-diacetate (DCFH-DA) is first deacetylated by esterase in to 2', 7'-dichlorfluorescein (DCFH) which is non fluroscent and then it is converted to fluroscent DCF by peroxidase [36]. The pellet was collected for both control and treated samples and measured using following protocol of [37] and to the pellet 5µM DCFH-DA was added to the cell pellet and incubated for 30 mins. It is then centrifuged and the pellet was washed with buffer and suspended in buffer and sonicated. Fluorescence was measured in spectrofluorometer (SL174, ELICO) with (Excitation wavelength- 490nm and Emission wavelength-519nm).

2.9 Static Biofilm Formation

The 24 hrs culture of all 5 strains and their consortium was taken and was centrifuged to collect the pellet. The pellet were dissolved in waste water and set up to 0.1 OD dilutions. Biofilm formation assay was done in micro titre 96 well plates. For controls only the cultures were added to the wells whereas for treated 0.25, 0.50 and 1ppm of titanium nanoparticles were added along with the cultures and incubated at 30-37 °C for 24 hrs. The control blanks were carried out without adding cultures, to the wells only waste water was added. For treated blanks Tio2 nanoparticles were added to the waste water and incubated. After incubation wash the microtitre well plates with sterilized distilled water to remove all planktonic bacteria that adhere to the biofilm and non attached cells were also removed through this washing step. Add crystal violet 0.1% to all the wells and incubate it for 10 mins at room temperature and wash the wells with sterile distilled water to remove excess stain. Air dry the microtitre plate untill there is no moisture in the well plates and add 30% acetic acid to each well and incubate for 10-15 mins at room temperature and take OD at 500 nm [38, 39].

2.10 Microscopic Analysis-Transmission Electron Microscopy (TEM)

Cellular structural changes, Internalization and localization of nanoparticles in bacterial cells were analyzed using TEM. The consortia samples were interacted for 24hrs and the pellet was used for TEM analysis. The sectioned samples were observed under TEM (Philips CM12 Transmission Electron Microscope, Netherlands).

2.11 Statistical analysis

All *in vitro* toxicity tests were carried out in triplicates and the data are given as mean ±standard error. The data were processed using one-way ANOVA, followed by Dunnett's post-hoc test with ($p< 0.05$) for a standard plate count assay. The data for the ROS and SOD assay were processed through Student's t-test at $p< 0.05$.

3. Results

3.1 TiO^2 Stability in Waste water

To study aggregation behavior of TiO_2 nanoparticles (0.25, 0.50 and 1µg/mL) in waste water matrix, dynamic light scattering was done at 0, 6 and 24th hr time intervals. The effective diameter of TiO_2 nanoparticles in waste water matrix was estimated to be in the range of 500 to 600nm at 0th hr. At the highest concentration of TiO_2 nanoparticles (1µg/mL) the effective diameter, was found to be 625.20±60.06, 804.46± 73.7, 804.46± 73.7 at 0, 6 and 24th hrs.

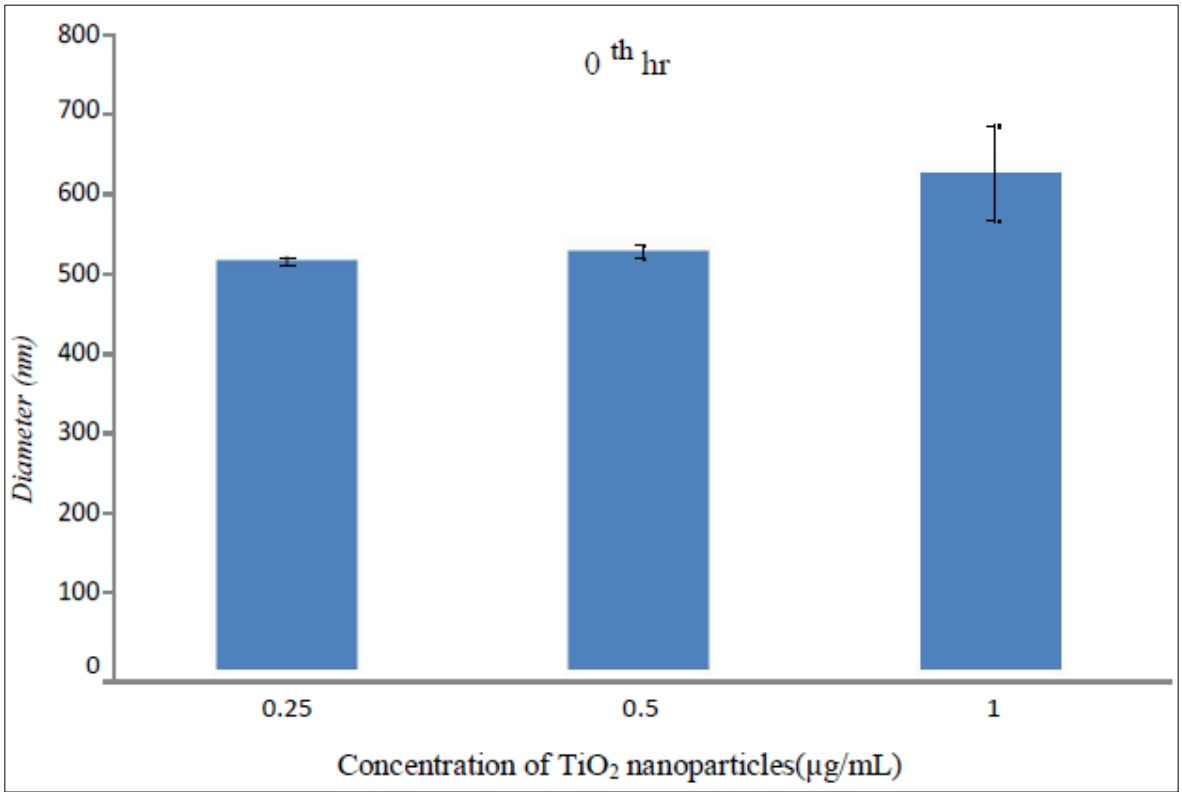

Fig 1: Effective diameter of TiO_2 nanoparticles in waste water matrix at 0th h

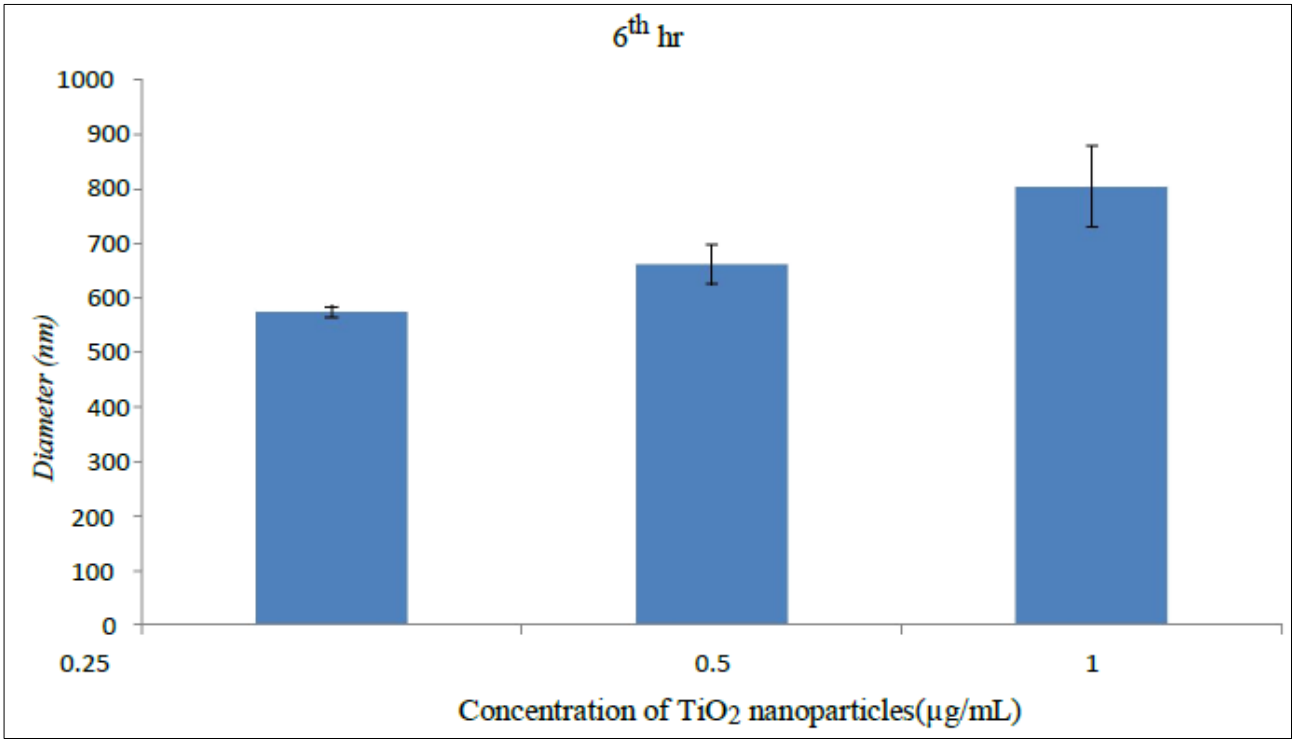

Fig 2: Effective diameter of TiO_2 nanoparticles in waste water matrix at 6th hr

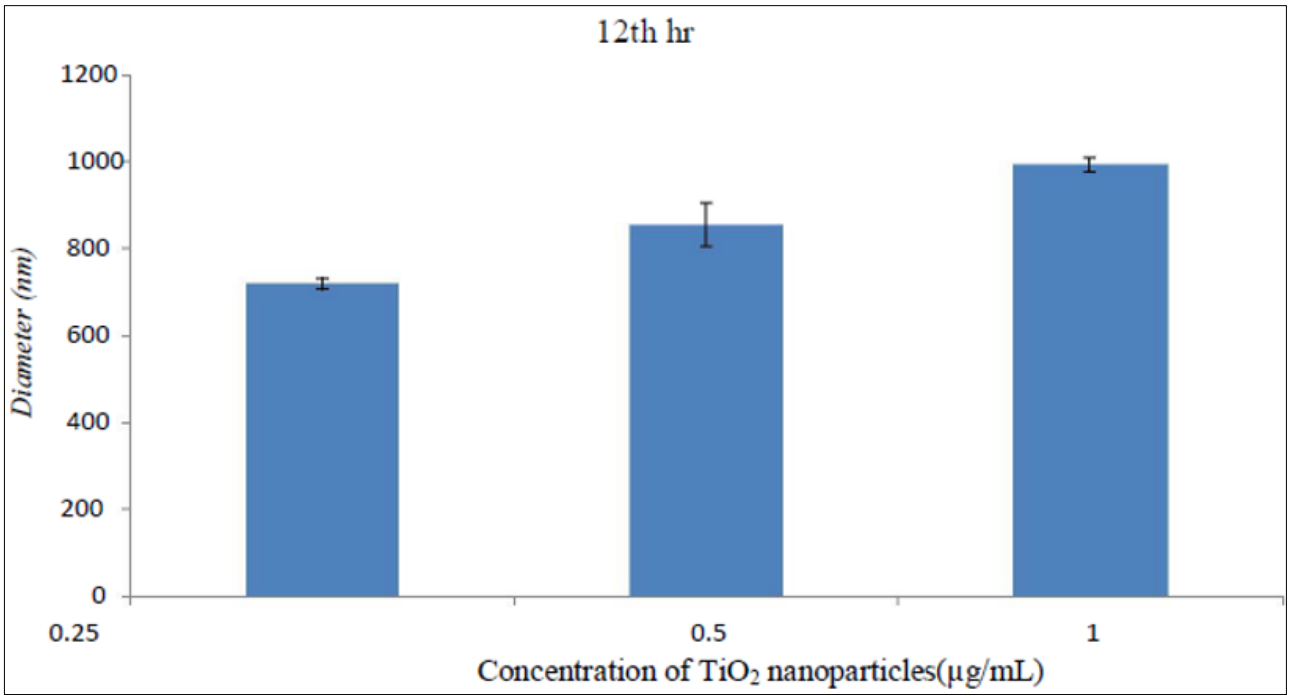

Fig 3: Effective diameter of TiO_2 nanoparticles in waste water matrix at 12th hr

3.2 Cytotoxicity of TiO_2 nanoparticles on waste water bacterial isolates

To determine toxicity of TiO_2 nanoparticles in waste water matrix, the cell viability for individual bacteria (*Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens*) and consortium were analyzed by standard plate count assay. The cell viability at 1µg/mL of TiO_2 nanoparticles was estimated to be 49.5±1.8, 55.1±2.5, 62.36±3.0, 70.37±2.728, 64.566±4.055 and 83.91±2.5 under dark condition and 48.95±1.8, 52.5±3.2, 58.62±3.0, 68.51±2.72, 61.90±1.45 and 75.18±2.9 under UVA condition for *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium respectively. The viability of all individual isolates and consortia was found to be significant ($p< 0.05$) with respect to control. Cell viability was found statistically significant with respect to both dark and UVA condition ($p< 0.05$).

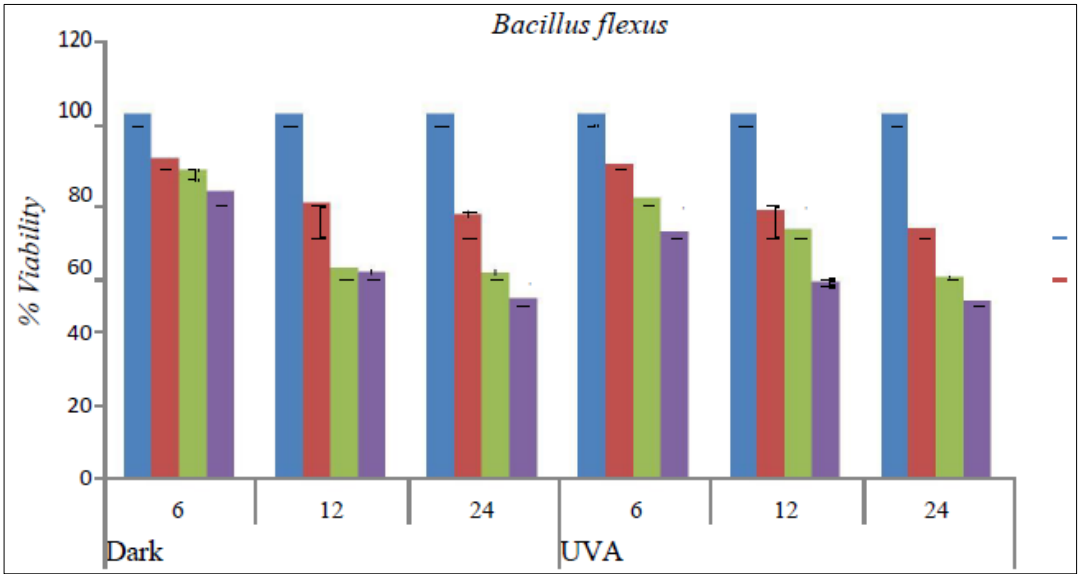

Fig 4: Viability studies of *Bacillus flexus* (6, 12 and 24 hrs) when exposed to TiO2 (0.25, 0.50 and 1ppm) nanoparticle concentration under UVA and dark condition.

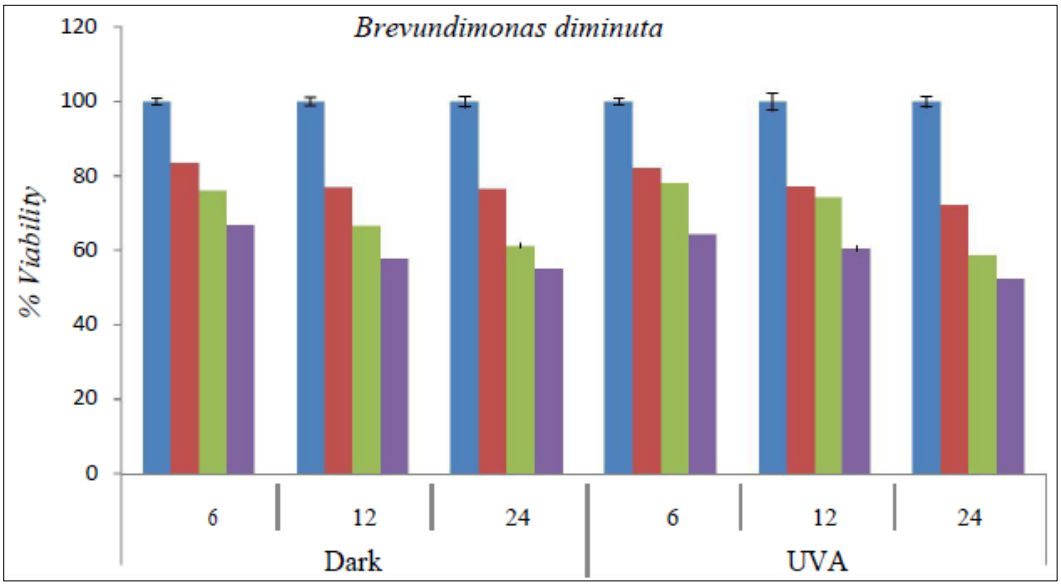

Fig 5: Viability studies of *Brevundimonas diminuta* (6, 12 and 24 hrs) when exposed to TiO_2 (0.25, 0.50 and 1ppm) nanoparticles concentration under UVA and dark condition.

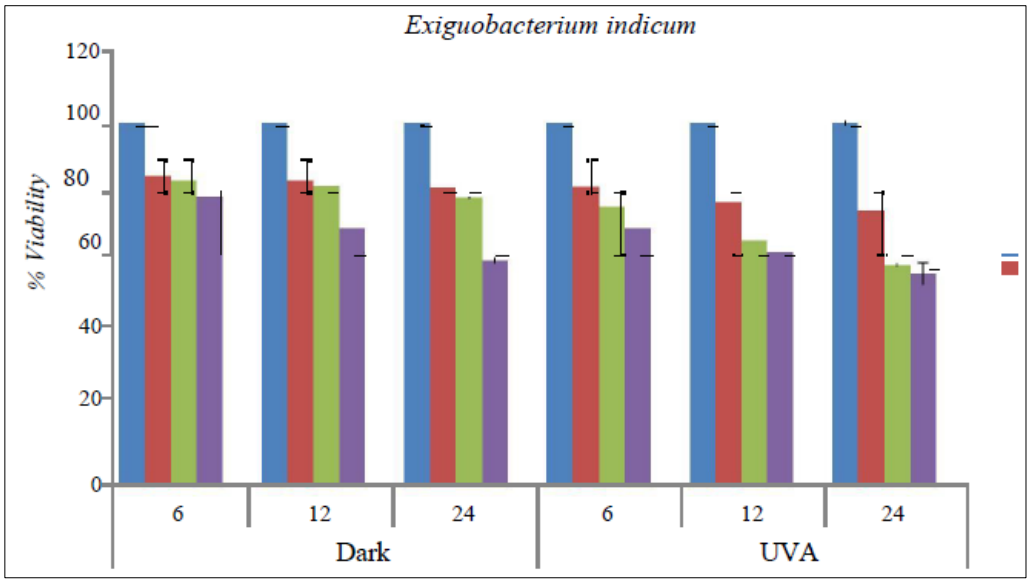

Fig 6: Viability studies of *Exiguobacterium indicum* at (6, 12 and 24 hrs) when exposed to TiO_2 (0.25, 0.50 and 1ppm) nanoparticles concentration under UVA and dark condition.

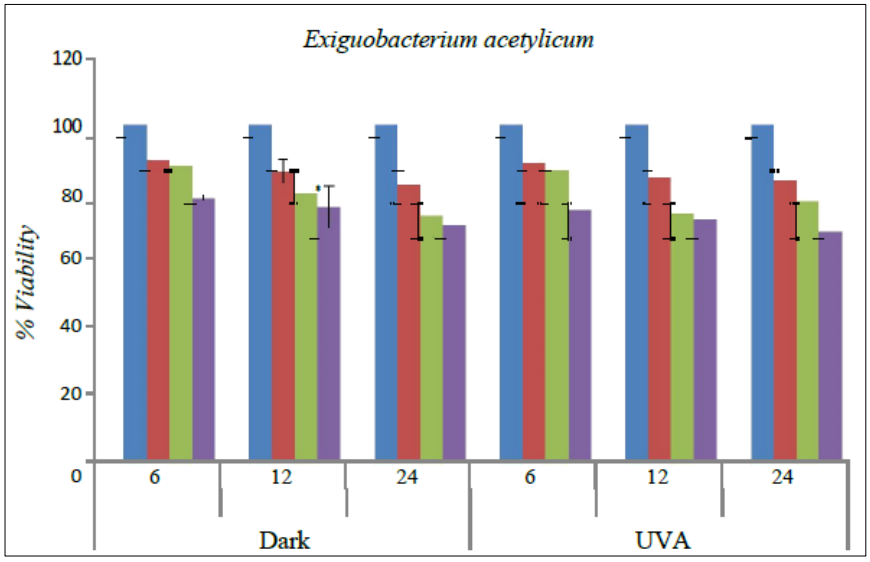

Fig 7: Viability studies of *Exiguobacterium acetylicum* (6, 12 and 24 hrs) when exposed to TiO_2 (0.25, 0.50 and 1ppm) nanoparticle concentration under UVA and dark condition.

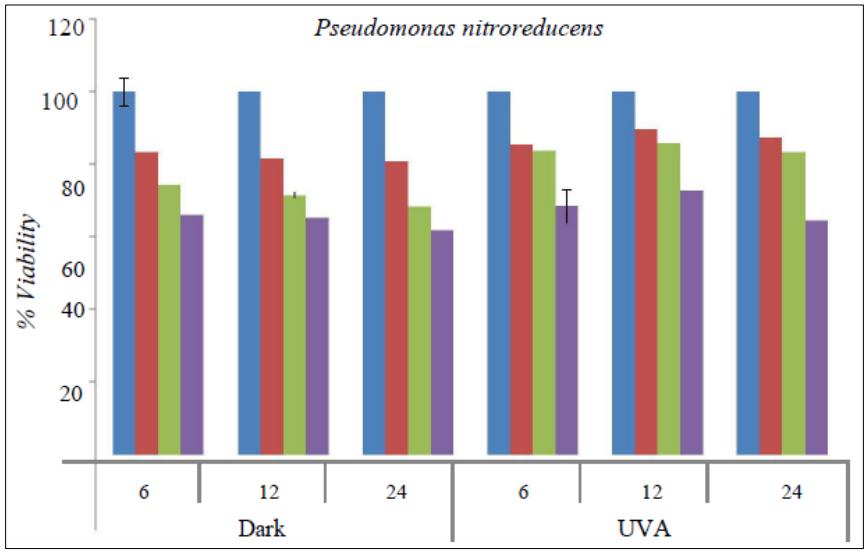

Fig 8: Viability studies of *Pseudomonas nitroreducens* (6, 12 and 24 hrs) when exposed to TiO_2 (0.25, 0.50 and 1ppm) nanoparticle concentration under dark and UVA condition

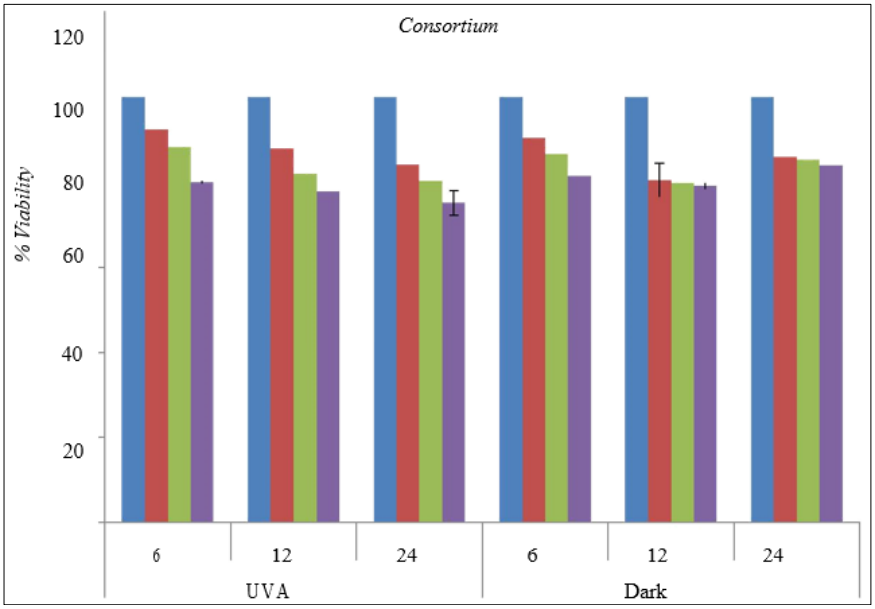

Fig 9: Viability studies of consortium (6, 12 and 24 hrs) when exposed to TiO_2 (0.25, 0.50 and 1ppm) nanoparticle concentration under UVA and dark condition

The viability results showed that viability decreased in *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium with respect to time (6,12 and 24 hrs) when exposed to TiO2 (0.25, 0.50 and 1ppm) nanoparticle concentration under UVA and dark condition (n=3). Significance with respect to control is represented by *.

3.3 Oxidative Stress Assessment

3.4 Reactive Oxygen Species (ROS)

A significant increase ($p < 0.05$) in the ROS for the treated *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium was observed with respect to control under UVA and dark condition. The ROS level of treated (1µg/mL) TiO_2 nanoparticles *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium was found to be 21.12±1.5, 21.06±1.3, 13.94±2.01, 9.28±12.005, 13.635±1.81, 8.81±0.70 in dark condition and 45.3±0.42, 37.22±1.4, 23.86±0.5, 20.71±0.14, 18.20±1.36, 14.92±0.72 in UVA condition. ROS generation was found to be significant ($p < 0.05$) when compared to dark and UVA condition.

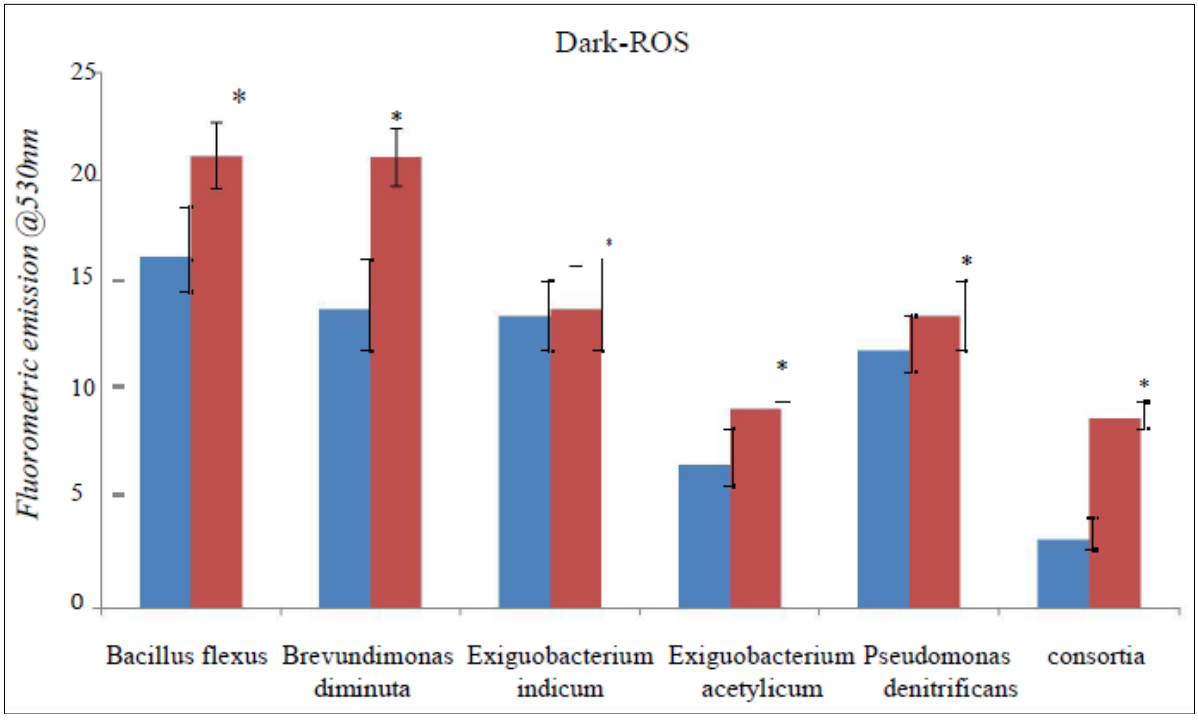

Fig 10: Shows Reactive Oxygen Species of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium of control and 1µg/mL of TiO_2 under dark condition at 24 hrs.

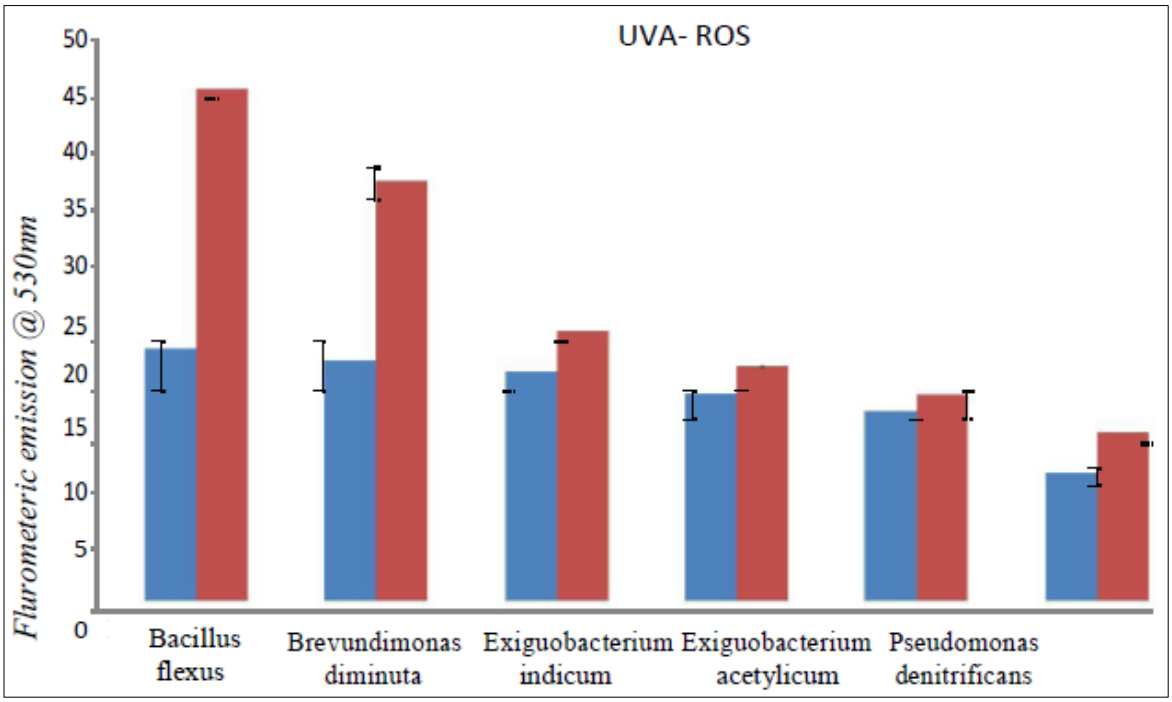

Fig 11: Shows Reactive Oxygen Species of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium of control and 1µg/mL of TiO2 under UVA condition at 24 hrs (n=3). Significance with respect to control is represented by *.

3.5 Super Oxide Dismutase (SOD)

The generation of superoxide dismutase was observed in both dark and UVA condition. The increase in SOD concentration upon TiO_2 nanoparticles upon treatment with 1µg/mL TiO_2 nanoparticles at 24hrs for both light and dark conditions compared to the control. It was found to be significant ($p< 0.05$) under both dark and UVA conditions with respect to control. The SOD level generated was estimated to be 0.006±0.0003, 0.00529±0.003, 0.00466±0.006, 0.00366±0.006, 0.004±0.00057 and 0.0046±0.001 in dark condition and 0.24±0.041, 0.044±0.014, 0.032±0.010, 0.017±0.005, 0.020±0.06 and 0.0123±0.004 in UVA condition at 1µg/mL TiO_2 nanoparticles for 24hrs. SOD generation was found to be significant ($p< 0.05$) when compared to both dark and UVA condition.

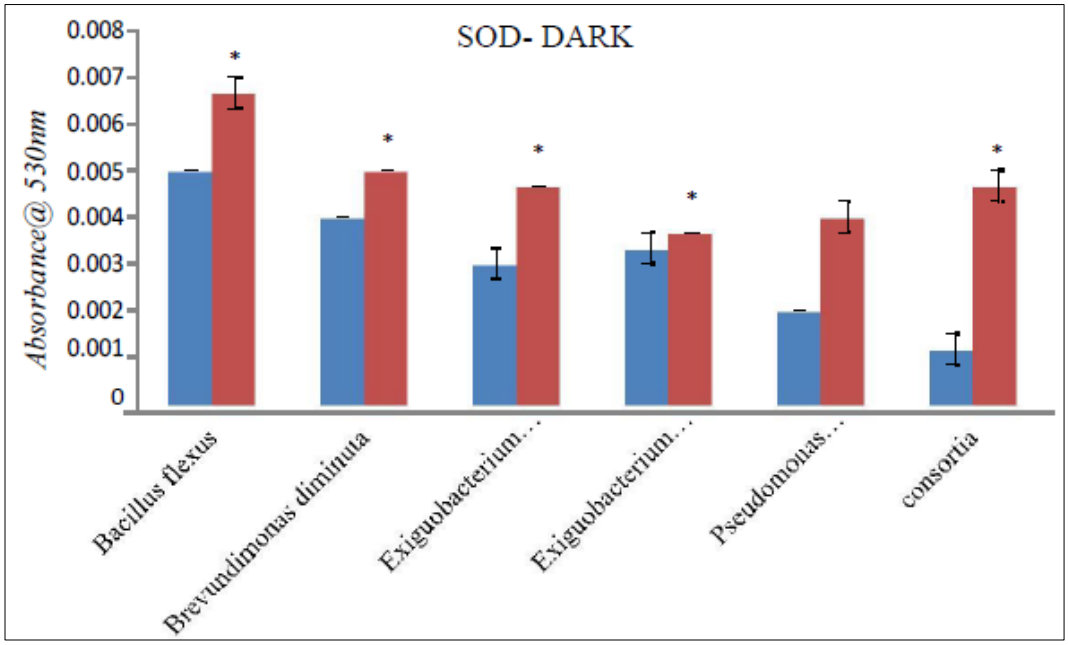

Fig 12: SOD generation of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium of control and 1µg/mL of TiO2 under dark condition at 24 hrs.

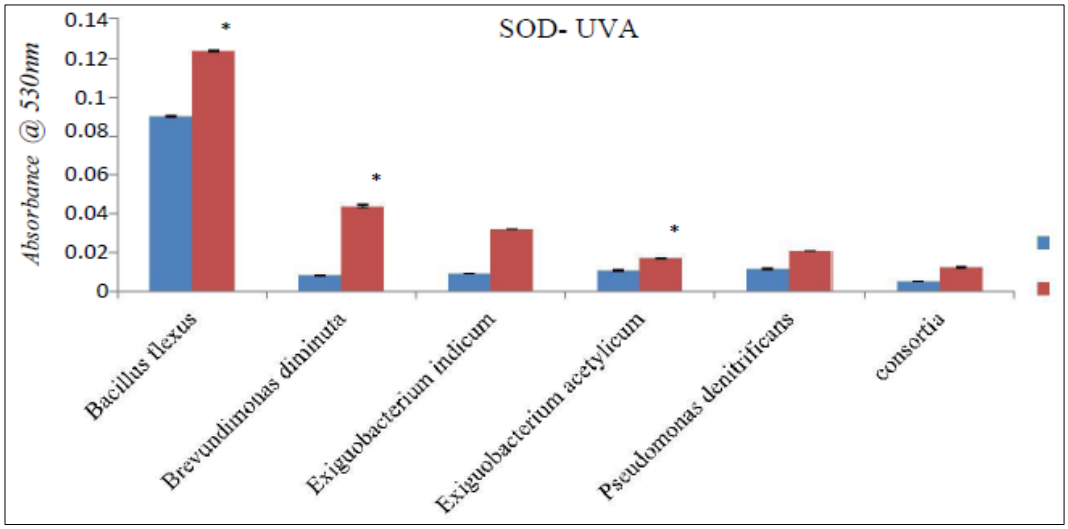

Fig 13: SOD generation of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium of control and 1µg/mL of TiO2 under UVA condition at 24 hrs. Significance with respect to control when compared with UVA and dark condition in the graph is represented by * in the graph.

3.6 Static Biofilm formation under Dark and UVA condition

The impact of TiO_2 nanoparticles on the capacities of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium strains to form biofilm was assessed under static conditions for 24 h incubations. The biofilm mass formed in the presence of TiO_2 nanoparticles (1 µg/mL) of *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium is estimated to be increased 1.35, 1.5, 1.827, 1.673, 2.17 and 2.98(dark); 3.432, 3.457, 4.96, 5.92, 6.6 and 7.6 (UVA condition) at optical density 590nm. Although biofilm formation in consortium was found to be higher when compared to individual isolates it was not significant when compared to UVA and dark condition. *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortium found to be significant ($p< 0.05$) at (1 µg/mL) of TiO2 nanoparticle with respect to control at both dark and UVA condition.

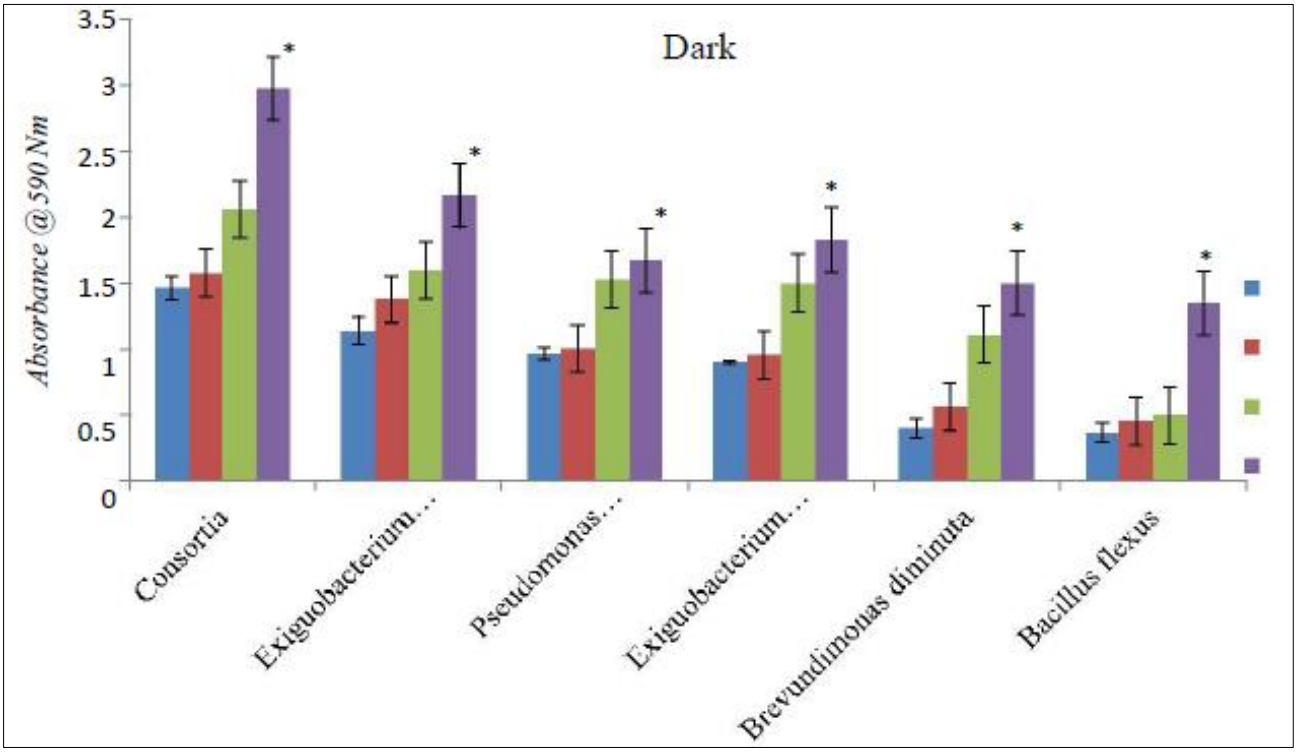

Fig 14: Biofilm formation at control and treated TiO2 (0.25, 0.50 and 1ppm) of individual isolates *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortia under dark condition.

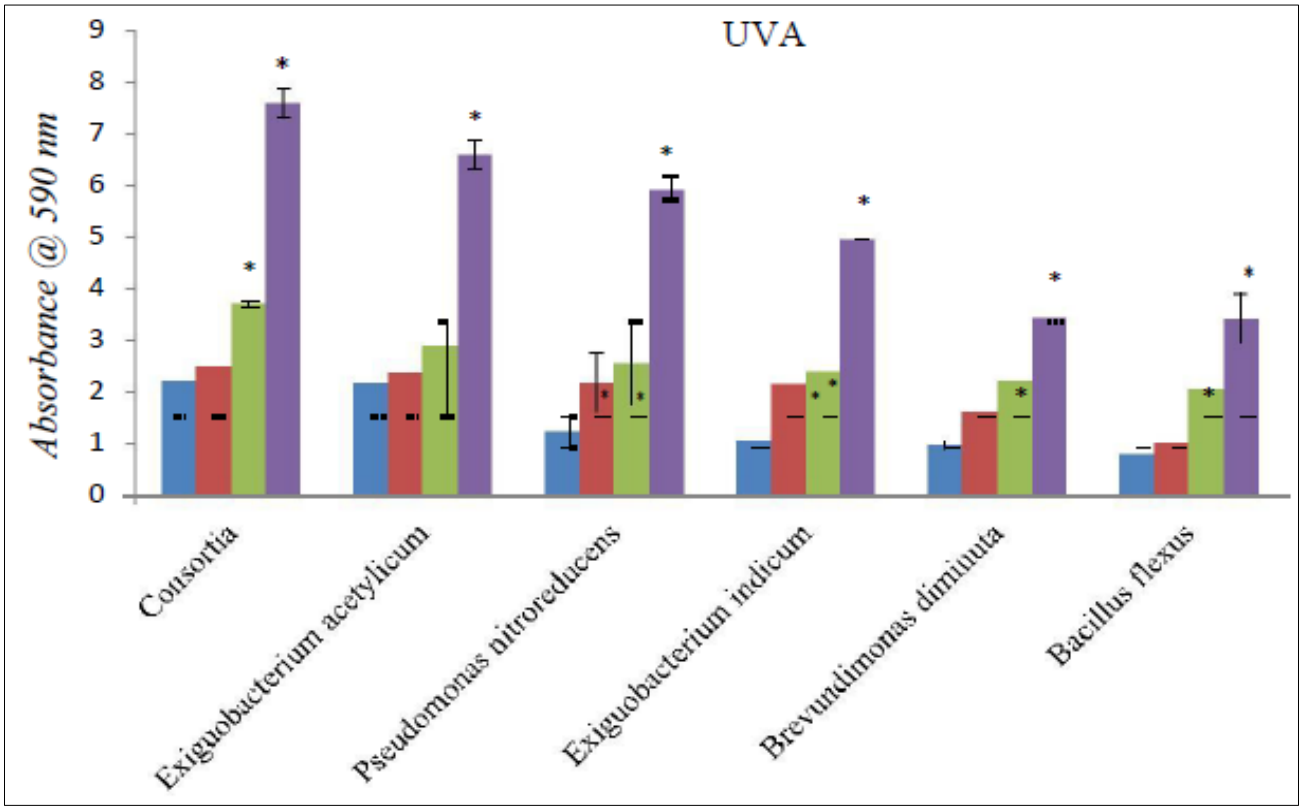

Fig 15: Biofilm formation of individual isolates *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and consortia interacted with TiO2 nanoparticles (0.25,0.50 and 1ppm) after 24 hrs incubation under UVA Experiments were done in triplicates (n=3). Significance with respect to control is represented by * symbol.

3.7 EPS production in Dark and UVA condition

The extracted EPS from the TiO2 nanoparticles (0.25, 0.50, 1µg/mL, 24 h) from individual bacteria *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and the consortium was quantified. To study the bacterial resistance against the toxicant produced 0.45± 0.0006, 0.545± 0.0003, 0.543±0.0006, 2.14±0.003, 2.73±0.005, 2.986±0.011µg/mL in dark and 0.566±0.0040, 0.58±0.0033, 0.59±0.005, 1.97±0.0057, 2.92±0.001, 3.06±0.001 µg/mL in UVA condition at 1µg/mL of TiO_2 nanoparticles concentration. It showed statistical significance ($p< 0.05$) with respect to control under both dark and UVA condition and found significant when compared to dark and UVA condition.

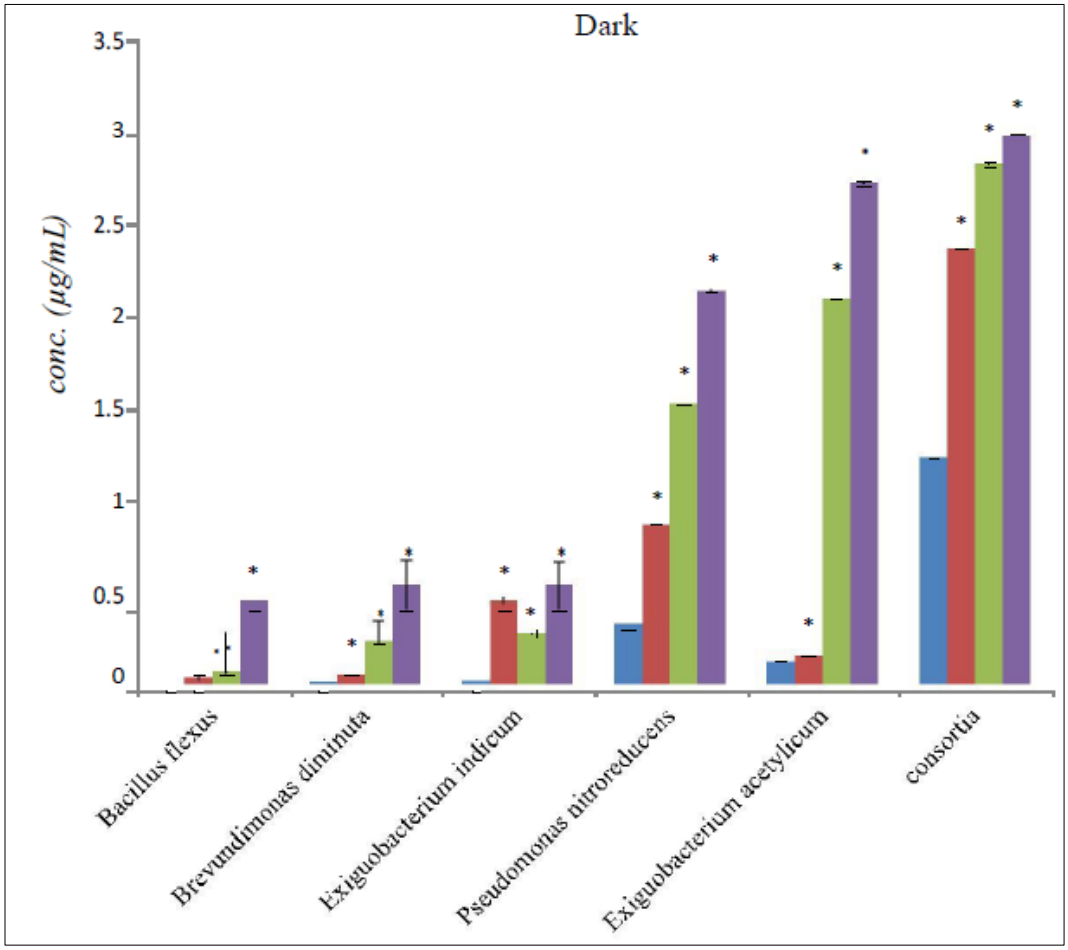

Fig 16: Shows EPS production at control and treated TiO2 (0.25, 0.50 and 1ppm) of individual isolates and consortia under dark condition.

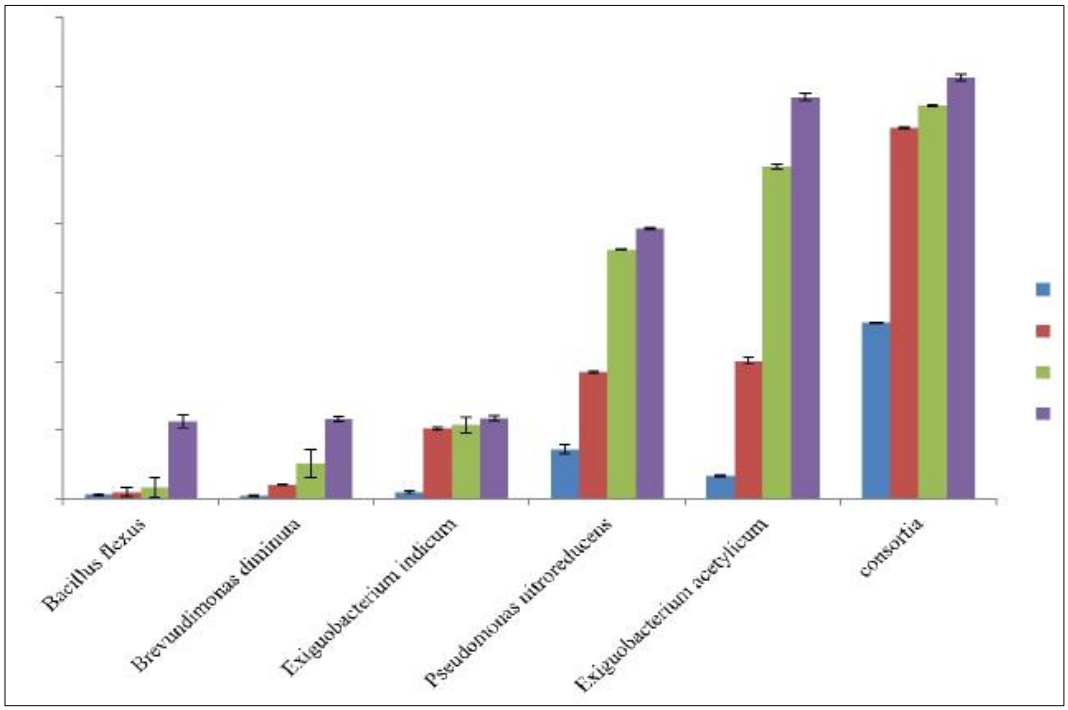

Fig 17: Shows EPS production at control and treated TiO_2 (0.25, 0.50 and 1ppm) of individual isolates and consortia under UVA condition. The significance difference between control and treated samples under both UVA and dark conditions is represented by

3.8 Microscopic analysis (TEM)

The cytotoxic effects of TiO_2 nanoparticles, changes in morphology of cells, and bio distribution of nanoparticles under UVA and dark conditions were observed by transmission electron microscopy. The typical appearance of the bacterial consortium before (1µg/mL) TiO_2 nanoparticles treatment, which is smooth and damage-free was seen in Fig 18. The disrupted cells observed when exposed to (1µg/mL) TiO_2 nanoparticles in the dark experiment, indicating a loss of cell integrity leading to the leakage of internal component, and therefore, the activation of the bacteria was visualized and small vacuoles were formed in fig 19.

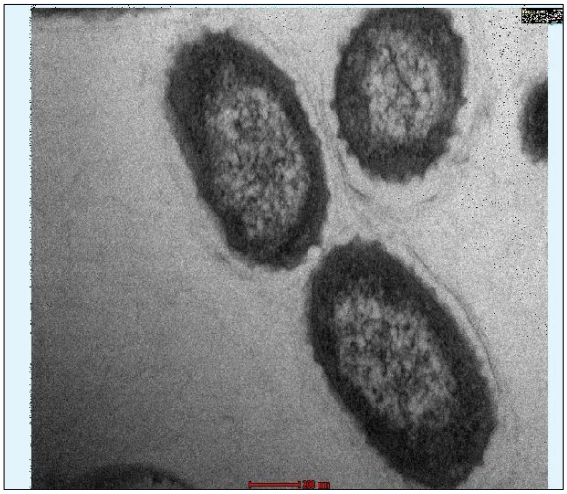

Fig 18: The typical appearance of the bacterial consortium before (1µg/mL) TiO_2 nanoparticles treatment, which is smooth and damage-free

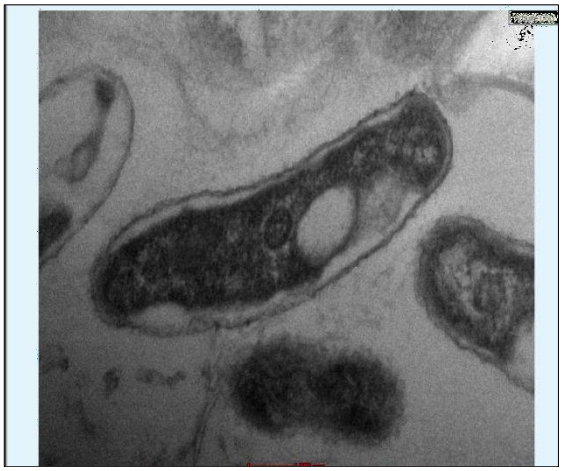

Fig 19: The activation of the bacteria was visualized and small vacuoles were formed

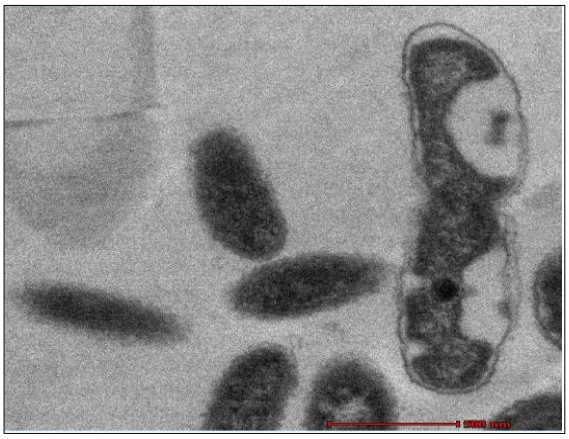

Fig 20: Shows disrupted morphology of cell membrane and presence of large vacuoles in UVA condition.

4. Discussion

The waste water plays crucial role in every Fact of human life, it is increasing in many parts of the world. It is recognized that nanotechnology and their applications play an important role in clearing issues relating to waste water treatment [40]. Owing to larger surface areas and size-dependent catalytic properties of TiO_2 nanomaterials, considerable efforts are being done to explore their application especially in wastewater treatment [41-44]. Moreover, TiO_2 nanomaterials can be ligand to many different chemical groups to increase affinity, recyclability, high capacity and selectivity. Although much attention focused on the development and potential benefits of TiO_2 nanomaterials in wastewater treatment processes, concerns raised regarding their potential human being as an environmental toxicity.

Due to emergency of many waterborne diseases and limited safe water resources, there is a great demand for improvement of water filtration system. The TiO_2 Nanofibers and nanobiocides can be useful solution to waste water treatment. Due to recent advances in nanotechnology, next generation of diagnostic methods for pathogen detection is started developing. However, some technical and practical problems need to be resolved before potential realization. This includes tight control over TiO_2 production and function. The sample processing, detection of multiple agents in a single sample, as well as improving sensitivity and selectivity of the assays for significant application to complex environmental samples is highly recommended.

Despite the reduction of bacterial abundance of nanoparticle exposure, total bacterial activity in many cases change significantly, which was due to a strong supporting activity per cell in the high TiO_2NPS exposure groups. This denotes the presence of bacterial groups which are very high resistant to TiO_2NP_S toxicity, or even stimulated in presence of TiO_2NP_S. This relative stimulation by TiO_2NPS may be based on removal of competitors from community; however studies investigating the effectiveness of TiO_2NPS exposure on bacterial community composition is necessary to understand this mechanisms. Changes in community structure are also observed from soil bacteria following exposures to TiO_2, nanoparticles [45].

5. Conclusion

The toxic effects of TiO_2 in waste water bacterial isolates *Bacillus flexus, Brevundimonas diminuta, Exiguobacterium indicum, Exiguobacterium acetylicum, Pseudomonas nitroreducens* and their consortium at low exposure concentrations of (0.25, 0.50 and 1µg/mL) were studied. The present study clearly supports the hypothesis that the consortium of the five bacterial species that were isolated from waste water might been less affected than that of individual species in the presence of TiO_2 nanoparticles in the environment. The results also suggest that toxic effect of TiO_2 NPs was strongly dependent on dose, duration, and the radiation conditions. Consortium produced higher level of biofilm and EPS matrix, such that it helps in protecting itself from TiO_2 nanoparticles effectively when compared to the individual isolates. However detailed study should be conducted to understand the wide range of environmental ultrafine sizes and its toxicity responses to different environmental microbes.

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