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Photocatalytic and Antimicrobial Properties of Titanium Dioxide Micro-particles under UV Irradiation on Escherichia coli Strains

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Abstract: *This study investigates the antimicrobial properties of Titanium Dioxide (TiO2) micro-particles in suspension under UV irradiation on Escherichia coli strains obtained from soil and sewage samples. The aim of this research is to explore the potential applications of TiO2 as a non-toxic and effective agent to eliminate microorganisms in areas where traditional chemical cleaning agents or biocides have proven to be ineffective. The methodology involved the collection of samples from sewage and soil, isolation of E.coli from the samples, preparation of TiO2, illuminating E.coli under UV light, and performing plate count. The observations showed a significant decrease in the viable count of E.coli under UV illumination, with a higher reduction observed for the E.coli strain from soil samples than the sewage sample. TiO2 particles, when phagocytosed by the microbial cell, cause rapid cell injury, resulting in various damages to the microbes, and act as a strong oxidant that causes various damages to bacteria. The study concludes that TiO2 exhibits a high rate of bactericidal activity and can be used for various antimicrobial applications. However, a more comprehensive study would be required to ascertain a more critical concentration of TiO2 to be used as an anti-microbial. The findings of this study have the potential to contribute to the development of eco-friendly and non-toxic antimicrobial agents.*

Keywords: Titanium Dioxide, Photocatalysis, Antimicrobial, Escherichia coli, UV Irradiation

INTRODUCTION:

TiO_2 is a white powder, insoluble in water & sensitive as a photo catalyst to ultraviolet light. TiO_2 photo-catalysis under UV irradiation has attracted a large attention as a promising method of water and air cleaning. Highly reactive radicals are formed from TiO_2 under UV irradiation. Hydroxyl radicals are generated by photo-holes from the TiO_2 valence band and superoxide ions are formed due to the interaction of photo-electrons from conduction band with molecular oxygen. These radicals participate in the series of oxidation reactions resulting in the destruction of various organic components. These radicals being strong oxidants cause various damages to microbes. Along with this, TiO_2 particles when phagocytosed by the microbial cell cause rapid cell injury by reacting with various cellular components. This mechanism first involves the degradation of outer lipopolysaccharide layer which acts as a blocker for radicals. These radicals then penetrate the inner peptidoglycan layer and degrade the cytoplasmic membrane. Thus, in this way photo-killing of microbial cells takes place by using TiO_2. A high rate of bactericidal activity has been observed.
TiO_2 can be used for elimination of microorganisms in the areas where the use of chemical cleaning agents or biocides has proven to be ineffective, as it is non-toxic and can be used as a food additive.

Materials And Methods:

1. Collection of sample from sewage and soil source
2. Preparation of MacConkey agar, EMB agar, Luria bertani broth and nutrient agar.
3. Isolation of E.coli from samples
4. Preparation of TiO_2
5. Illumination of E.coli under U.V

6. Plate count

Collection of sample from sewage and soil-

▫ Soil sample was collected from VIT - hexagon ground floor.
▫ Sewage sample was collected from VIT- water treatment plant near technology tower.
▫ After collecting, samples were stored in refrigerator.

Preparation of media:

Preparation of LB broth:

▫ 950 ml of LB broth was prepared by adding 23.75gm of LB broth powder to 950 ml of distilled water.
▫ 120 ml for serial dilution.
▫ 400 ml for inoculating colonies from MacConkey agar plate.
▫ 400 ml for inoculating colonies from EMB agar plate.

Preparation of MacConkey agar

▫ 100 ml of MacConkey agar was prepared by adding 4.953 gm of MacConkey agar powder in 100 ml of distilled water.
▫ 4 plates of MacConkey agar was prepared and kept for refrigeration.

Preparation of EMB agar:

▫ 100 ml of EMB broth was prepared by adding 2.25 gm of EMB powder in 100 ml of distilled water.
▫ 1.5 ml of agar powder was added to solidify the broth.
▫ 4 agar plates were prepared and then kept for refrigeration.

Preparation of nutrient agar :

▫ 1100 ml of nutrient agar was prepared by adding 30.8 gm of nutrient agar powder in distilled water.
▫ 2gm /100 ml of agar powder was added to make 54 plates of solidified nutrient agar media. Then these plates were kept for refrigeration.

Isolation of pure E.coli culture from sample:

Serial dilution :

Both sewage and soil samples were serially diluted in LB broth up to 10^-5 dilution.
For sewage – 1 ml of sample was added to 9 ml of distilled water to make dilution of 10^0. Further serial dilution was done up to 10^-5 dilution.
For soil- 1gm of sample soil was added to 10 ml of distilled water to make 10^0 dilution. Further dilution was done up to 10^-5 dilution.

Streaking of culture on MacConkey plates:

A loop full of 10^-5 dilution of both sewage and soil cultures were streaked on MacConkey agar plates. Then the plates were kept for incubation at 37 degree Celsius for 16 hours. As MacConkey agar acts as selective media for E.coli we obtain two different coloured colonies. Pink colonies are lactose fermenting and those of white /pale color are non-fermenting. Two different pink colonies were picked from both sewage and soil sample, as these might be E.coli and then each colony was inoculated in 100 ml of LB broth. This broth is then kept in shaker to allow these colonies to grow.

Streaking on EMB agar plates:

The colonies which were collected from MacConkey agar were then streaked on EMB agar plates. A loop full of culture was taken and streaked then on EMB agar. These plates were then kept for incubation at 37 degree Celsius for 16 hours. EMB agar is the differential media for E.coli. So after incubation metallic green colonies of E.coli were seen on EMB agar. Two colonies were then picked from each plate and each colony was inoculated in 100 ml of LB broth. Colonies were allowed to grow till the O.D. value reaches nearly 1.

Preparation of TiO_2:

0.25 g of TiO_2 powder was added to distilled water to make the solution to 10 ml ($25x10^{-3}$). The concentration is lowered to 25 ppm by progressive serial dilutions. It should be noted that because of very low solubility of TiO_2 in water, these steps are achieved with the help of an ultra-sonicator. Similarly, 50 ppm, 75 ppm and 100 ppm concentrations were prepared. **Illumination of E.coli under U.V. light:**

E.coli pure cultures obtained by isolating them from sewage and soil sample were kept under U.V illumination. Before illumination, 1ml each of E.coli culture was taken in 9 eppendorfs. Pellet of E.coli cells was obtained by centrifugation at 10,000 rpm for 10 minutes. The pellet was re-suspended in 0.9% saline and again centrifuged at 10,000 rpm for 10 minutes.

Add 1ml each of 25 ppm concentration of TiO_2 to 2 eppendorfs and re-suspend the pellet. Keep one under UV light and other in dark.

Repeat the above step for other concentrations as well.

A control was prepared with 0.9% saline (no TiO_2) and kept in UV.

Illumination was done for 2 hours. And after every 2 hours illumination, spread plate was performed on nutrient agar plates. 20 microlitres from each eppendorf was taken for spread plate. These plates were then kept for incubation at 37 degree Celsius for 12 hours. This illumination was done for 6 hours and for each illumination, plating was done. These plates were then kept for incubation.

Cultures which were kept in dark were similarly plated after every 2 hours. 20 microlitre of culture was taken for plating. Then plates were kept for incubation at 37 degree Celsius for 12 hours.

Plate count:

After incubation of plates for 16 hours, colonies of E.coli were obtained. Number of colonies was counted by using colony counter. After counting, result was inferred.

OBSERVATIONS:

PLATE-COUNT READINGS FOR SOIL SAMPLE 1ST ILLUMINATION:

CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	179	25	192
50	158	50	152
75	145	75	171
100	112	100	177
Control	**250**		

2nd ILLUMINATION:

CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	160	25	212
50	102	50	190
75	50	75	218
100	59	100	200
Control	**222**		

3rd ILLUMINATION:

CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	84	25	190
50	50	50	100
75	44	75	202
100	35	100	147
Control	**220**		

PLATE-COUNT READINGS FOR SEWAGE SAMPLE

1ST ILLUMINATION:

CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	195	25	204
50	200	50	216
75	170	75	240
100	125	100	197
Control	**266**		

2nd ILLUMINATION:

CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	175	25	196
50	144	50	244
75	100	75	198
100	97	100	201
Control	**245**		

3rd ILLUMINATION:

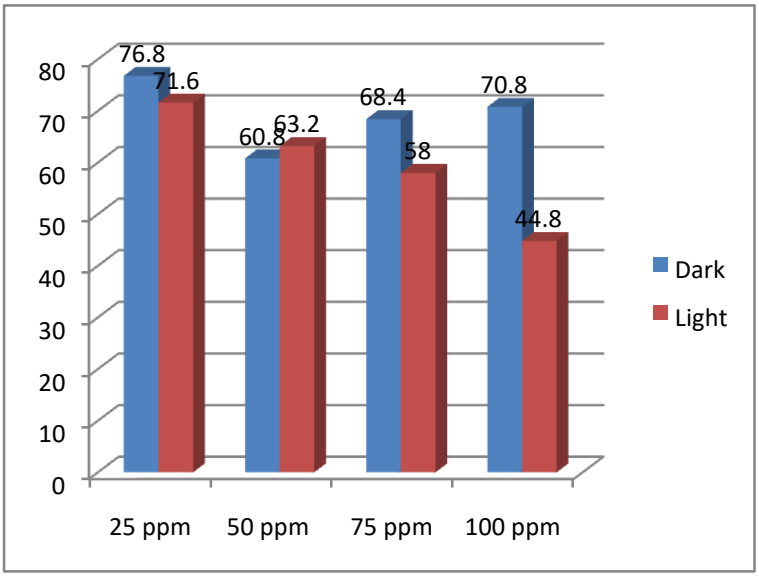
CULTURE UNDER UV LIGHT **CULTURE PLACED IN DARK**

Concentration of TiO_2 (in ppm)	**Number of colonies**	**Concentration of TiO_2 (in ppm)**	**Number of colonies**
25	122	25	180
50	116	50	175
75	90	75	182
100	88	100	190
Control	**202**		

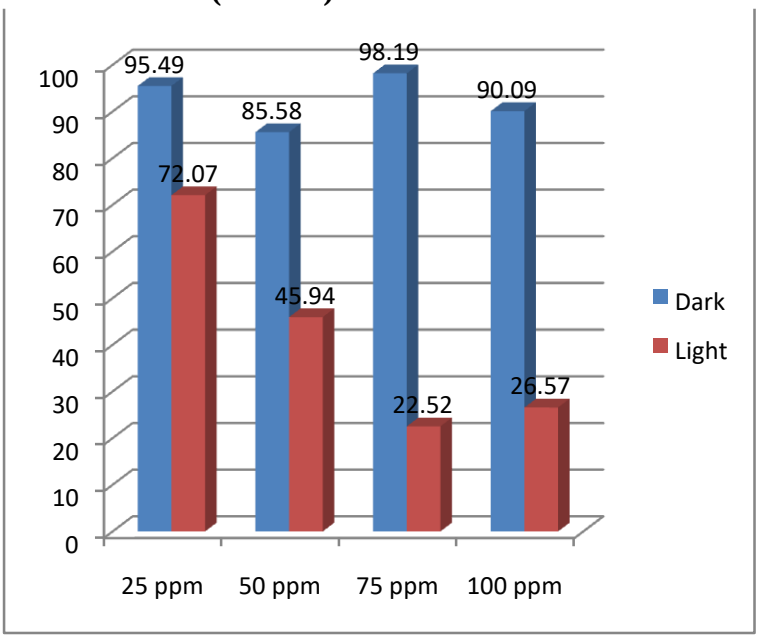
RESULTS:

% Viability = (Number of colonies in a plate/ Number of colonies in control plate) X 100 colonies in control plate) X 100.

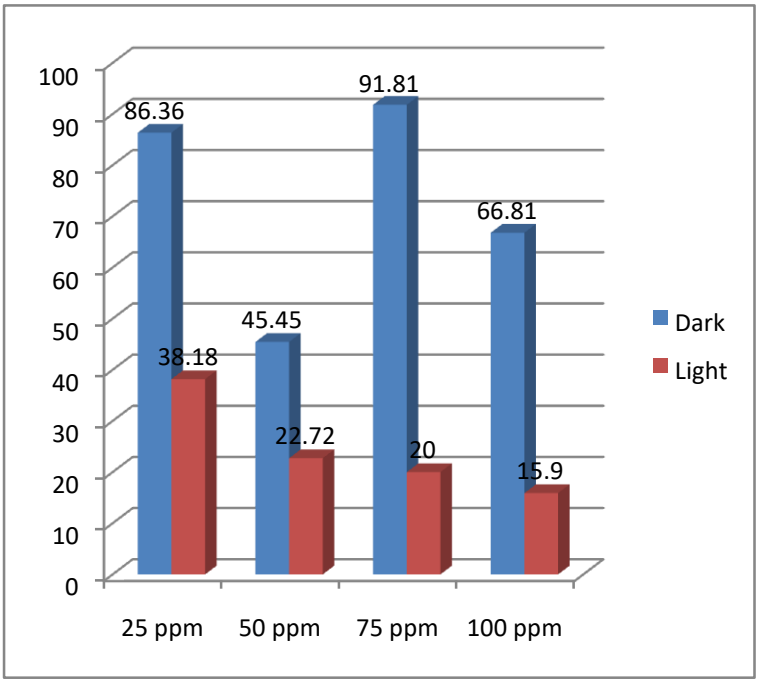
SOIL SAMPLE:



1st illumination (2 hours)

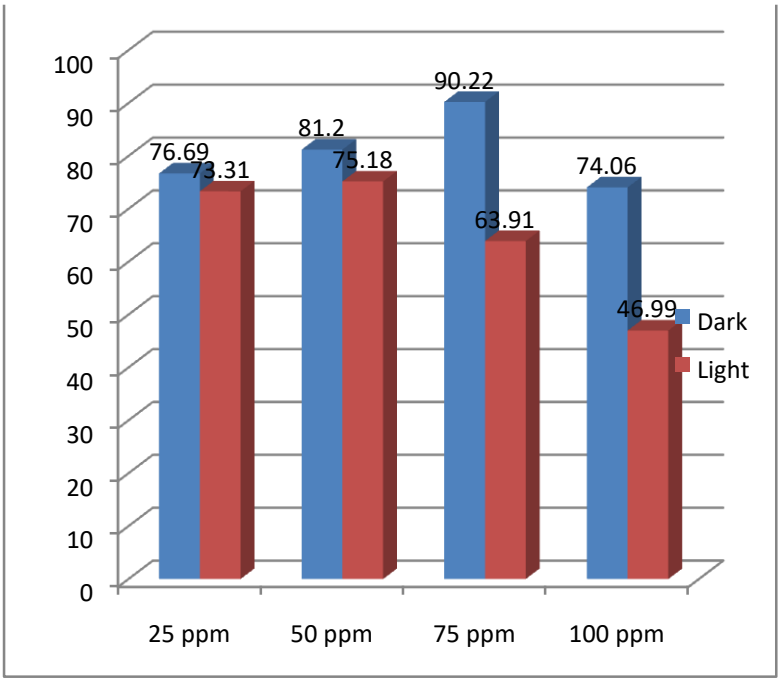


2nd Illumination (4 hours)

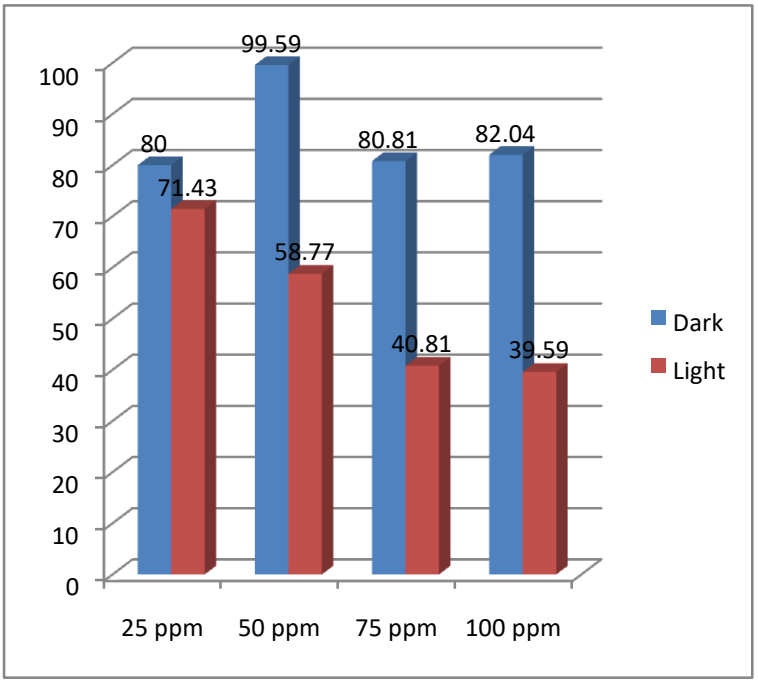


3rd Illumination (6 hours)

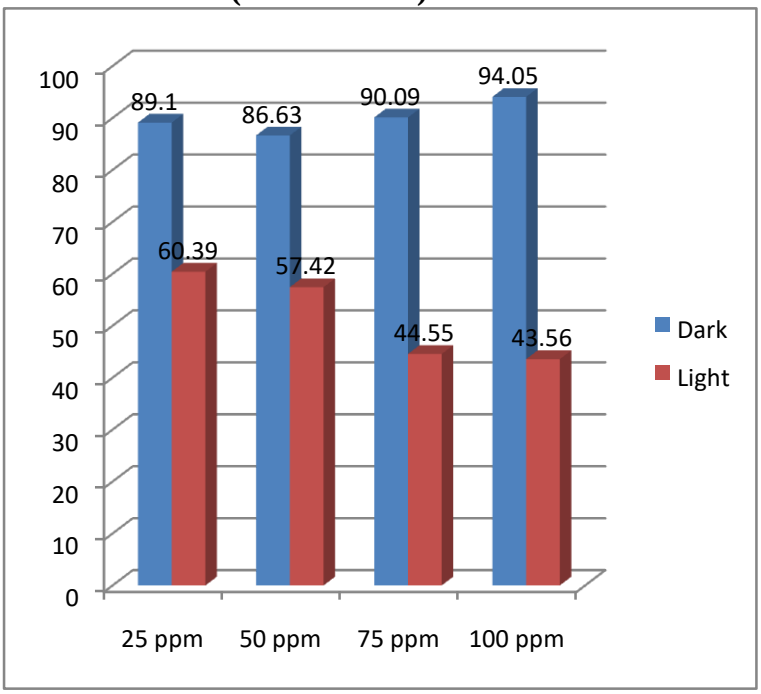
SEWAGE SAMPLE:



1st Illumination. (after 2 hours)



2nd Illumination (after 4 hours)

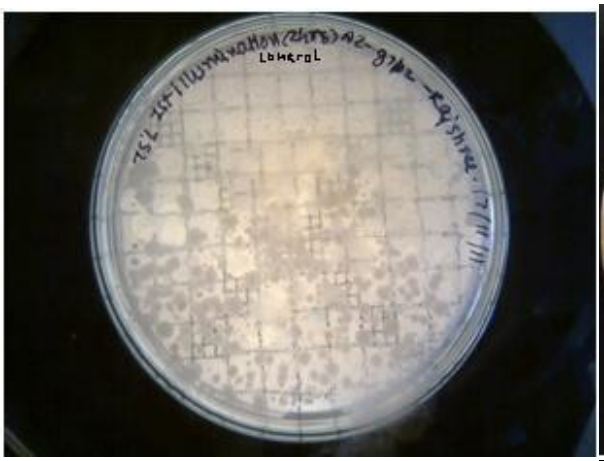


3rd Illumination (after 6 hours)

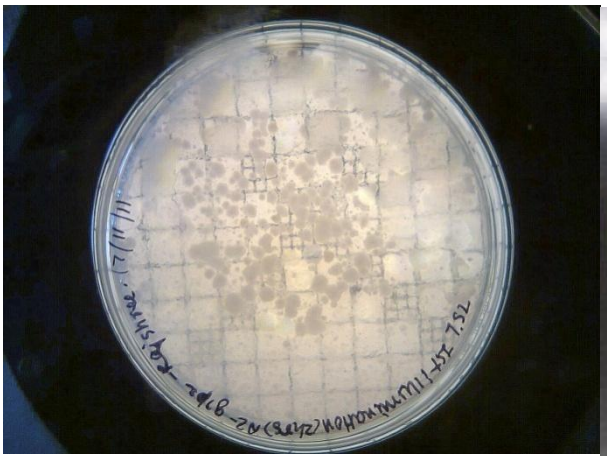
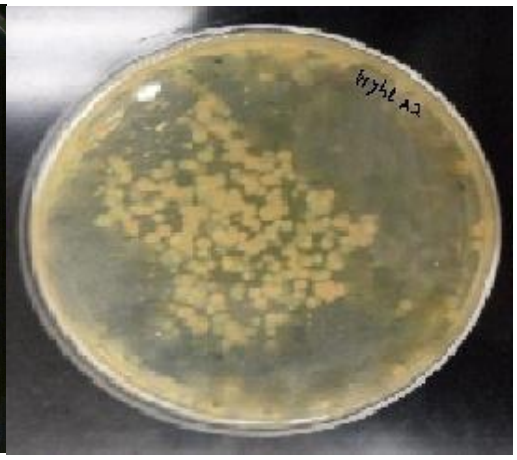
1. As compared to the cell count in Control plates, lowering in number is observed in the plates illuminated with UV light.
2. A significant drop in % viability is observed in Soil samples at 50 ppm concentration, whereas a significant drop in % viability in Sewage samples is observed mostly at 75 ppm.
3. For all respective illuminations, the %viability is lower for soil samples as compared to sewage samples.

1st Illumination(2 hrs):

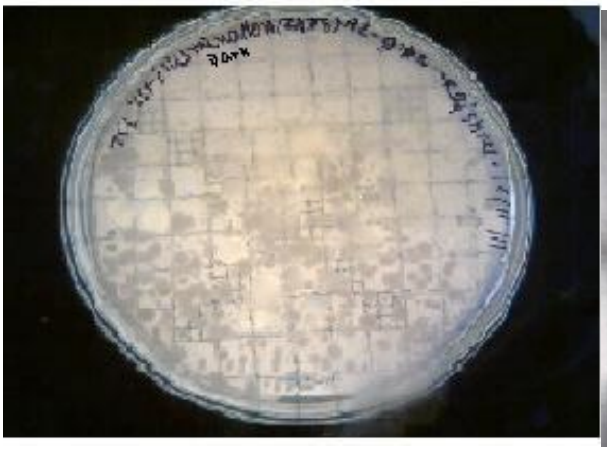
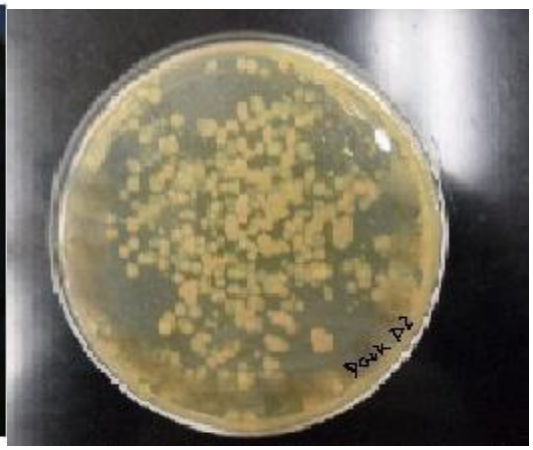
E.coli from soil sample **E.coli from sewage sample**




Control Plate Control Plate

UV illuminated Plate UV illuminated Plate

Dark Plate Dark Plate

DISCUSSIONS :

TiO_2 shows the property of photo catalysis when exposed to UV light. When present in aqueous medium, it oxidizes water to create hydroxyl radicals. This process is observed even in visible light, albeit at a lower rate. Hence, it can be considered as a potential anti-microbial, when applied in an aqueous medium. Almost all the plates have shown a consistent lowering in cell viability when TiO_2 is added, and UV light is present.

Even though both are showing lowering of viability, colonies from sewage prove to be relatively more viable compared to their soil counterpart. This may be the result of one of the favorable mutations in sewage

organisms which enable them to survive higher concentrations of potentially harmful substances in sewage effluents.

A direct consequence of this can be seen as the significant reduce in soil populations at 50 ppm, compared to the changes in sewage sample at 75 ppm. Hence, the anti-bacterial action of TiO_2 may vary from organism to organism.

CONCLUSIONS:

U.V.-induced bactericidal activity of Titanium Dioxide (TiO_2) in suspension was successfully analysed. As expected sewage sample could handle a higher concentration of TiO_2 as compared to the soil sample. However, a more comprehensive study would be required to ascertain a more critical concentration of TiO_2 to be used as an anti-microbial.

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