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Study on the UV screening Performance of Dip coated TiO_2 thin films on Agriculturally Beneficial *Rhizobium* and *Azotobacter*

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ABSTRACT

Rhizobium and *Azotobacter* are agriculturally beneficial living organisms present in biofertilizers which colonize the rhizosphere, fix atmospheric nitrogen into Ammonia and increases soil fertility thereby promote plant growth. Increased exposure of UV light has specific effect on various life forms. UV radiations kill or inactivate microorganism thereby prevent the growth and development of the plants. Titanium dioxide (TiO_2) is a wide band gap semiconductor, an efficient light harvester and has strong UV light absorbing capability. Here, we studied the UV screening effect of TiO_2 thin films as a protective layer in the enhancement of growth of *Rhizobium* and *Azotobacter*. TiO_2 thin films of different thicknesses were deposited on the glass substrates using TTIP as precursor by sol-gel dip coating technique by repeating the number of coating cycles which were annealed at 550^o C for one hour. The structural properties, surface morphology, stoichiometry and optical properties were characterized by XRD, SEM, EDAX, AFM and UV-Visible studies. The relation of survival rate of microorganism and thickness of the thin films was studied in detail. It is found that the crystallite size increases (21.3 nm-77.6 nm), band gap decreases (3.4eV-2.8eV) and transmittance decreases with increase in film thickness. We have observed that survival rate of organisms increases with increase in film thickness. This study showed that TiO_2 thin films act as protective layer against UV radiation and enhance the survival rate of organisms.

Introduction

In recent years, Titanium dioxide (TiO_2) has been extensively used because of its various qualities such as environment friendly nature (non toxicity), high photo catalytic activity, photo induced hydrophilicity, high refractive index, resistance to photo corrosion, chemical stability, low cost and high natural abundance [1,2]. TiO_2 films have wide applications such as optical coating, gas sensor, self cleaning, antireflection coating, antifogging, solar cells, biocompatibility, antibacterial activity [3] photo catalytic activity [4]*etc.*, TiO_2 belongs to the class of semiconductor with relatively wide band gap (3.2 eV for anatase and 3.0 eV for rutile structures). When exposed to the light of energy corresponds to its band gap, charge carriers such as electrons and holes are produced and oxidation and reduction reaction occurs on the TiO_2 surface. In comparison to the crystallite phase of TiO_2, anatase is most photoactive due to the difference in energy band structure [5] and the rutile protects the materials from ultraviolet radiations due to good scattering effect [6].

Ultraviolet light is one of the most important environmental factors has specific effects on human health, crop yield, terrestrial ecosystems, aquatic ecosystems, *etc.*, UV impairs photosynthesis in many species and reduces size, productivity, and quality in many of the crop plant species. Overexposure to UV radiations kills or inactivates the cells of living micro organisms. UV light restricts the growth of organisms by inhibiting conjugation, causing mutation and affects the metabolic pathways in it.

Biofertilizer contains living microorganisms such as *Rhizobium, Azotobacter, Azospirillum* and *blue green algae* (BGA) are extremely advantageous in enriching soil fertility and fulfilling plant nutrient. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR). *Rhizobium* and *Azotobacter* are microorganism functions in long duration, causing improvement of the soil fertility.TiO_2 thin films have UV resistant properties and efficiently transform destructive UV light energy into heat [7, 8]. This advantage enhances its ability to protect the micro organisms from ultraviolet light.

In our present work, we prepared nanocrystalline TiO_2 films using sol-gel dip coating method because it has many advantages such as simple, low cost, easy fabrication of large area film, excellent control over chemistry, homogeneity, purity and crystalline phase [9]. The structural properties, surface morphology, stoichiometry and optical properties were characterized by XRD, SEM, EDAX, AFM and UV-Visible studies. Here we studied the effect of film thickness of TiO_2 on the enhancement of survival rate of *Rhizobium* and *Azotobacter* as a protective layer against UV radiation.

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Experimental

Preparation of thin films

The TiO_2 sol was prepared by adding 6.3 ml of TTIP-Titanium Tetra Isopropoxide[Ti $(OC_3H_7)_4$], to a mixture of 5 ml acetic acid and 100 ml ethanol and stirring the mixture for 15 minutes using magnetic stirrer. The film was coated on ultra cleaned glass substrates by dipping it into the solution and pulling it up at a constant rate of 25 mm/min using a dip coating machine. The dip coated glass substrate was dried by heating at 150ºC for 5 minute andthe process was repeated for 4,6, 8 and 10 times to get four different thin films samples of various thicknesses. Finally all the samples were annealed at 550ºC for 1 hour in muffle furnace.

Material Characterization

The crystallite size of the TiO_2 thin films obtained from different coating cycles were characterized by X–Ray diffraction method (XRD) using X'PERT PRO X–ray diffractometer which was operated at 40 KV and 30 mA with CuKα_1 radiation of wavelength 1.5407Å. The thickness of the films has been measured using Stylus profilometer Surfest SJ -301. UV–Visible spectra were recorded in the range of 200 – 800 nm using the Schimadzu 1800 UV–VIS – NIR spectrophotometer. . The surface morphology observation and elemental analysis were done by Quanta SEG - 200 SEM and Bruker EDAX respectively. Surface roughness of the films was recorded using ND-MDT, NTEGRA Prima-Modular AFM in semi contact mode using SiN cantilever.

Bacteria preparation

Agriculturally beneficial microbe *Rhizobium* [10] and *Azotobacter* [11] was isolated from soil and grown in nutrient broth overnight at 37°C. Then 5 ml culture of *Rhizobium* was centrifuged at 3000rpm for 10 minutes using a centrifuge. The supernatant was discarded and the pellet was washed with 5 ml sterile water and again centrifuged. The same process was repeated for three times, and then the pellet was resuspended in 5 ml sterile water.The nutrient agar plates were prepared for the determination of bacterial count. The bacteria resuspended in 5 ml sterile water were diluted with suitable concentration of Ringer's solution and thus the culture was prepared.

UV screening performance of TiO_2 thin films

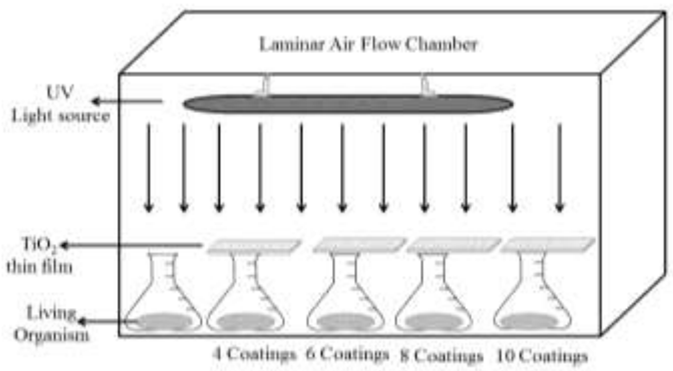

Fig 1. Schematic diagram of UV screening performance of TiO_2 thin films

A laminar air flow chamber was used in this experiment and the schematic diagram is shown in Fig 1. 5 ml of diluted culture of *Rhizobium* was equally taken in 5 different 100 ml conical flasks. One flask is completely exposed to UV light and the other 4 flasks were covered with our thin films samples of 4 different thicknesses coated for 4, 6, 8, and 10 times.The culture was exposed for 5 minutes with UV light of 260 nm at a distance of 40 cm. The UV irradiated culture spread uniformly on 5 different nutrient agar plates and one more agar plate with culture unexposed to UV light were incubated at 37°C for 24 hours. The experiment was repeated with *Azotobacter* microbes. After the incubation, the number of surviving microbes in each plate was counted by colony counter.

Results and Discussion

Structural properties

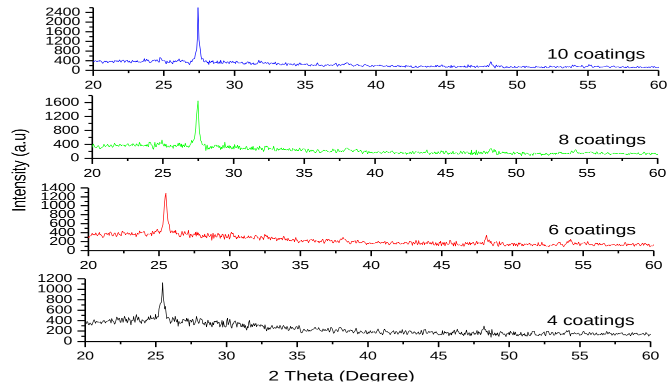

Fig 2. XRD patterns of TiO_2 thin films for different number of coatings

XRD patterns of the films annealed at 550°C for different number of coatings is displayed in Fig 2.The prominent peaks detected at 2θ = 25.41° and 25.44° can be attributed to the tetragonal (1 0 1) crystal structure with anatase phase for 4 and 6 coating films which is in agreement with standard JCPDS data (File No.21-1272). In 8 and 10 coatings of TiO_2 films the dominant peaks observed at 2θ = 27.42° and 27.61°and these films have tetragonal (1 1 0) crystal structure and rutile phase which are in agreement with standard JCPDS data (File No.21-1276). From the XRD pattern, it is observed that the width of the peak decreases and intensity of the peak increases with increase in number of coating cycles. The phase change from anatase to rutile in the TiO_2 film growth may be due to film thickness and composition in the film.

The crystallite size of the films were determined using the well-known Debye-Scherrer's formula,

$$D = \frac{k\lambda}{\beta\cos\theta}(nm) \quad (1)$$

Where k = 0.94, λ = 1.5407Å, β = Full Width Half Maximum (FWHM) and θ = Diffracting angle. We have found that the crystallite size increased from 21.3nm to 77.6 nm as the number of coatings increases and are shown in Table 1.

The origin of the strain is related to the lattice misfit, which in turn depends upon the deposition conditions. The micro strain (μ) developed in the spin coated TiO_2 thin films was calculated from the equation

$$\mu = \frac{\beta\cos\theta}{4} \quad (2)$$

Where'β' is full width half maximum and 'θ' is the Bragg angle. The calculated values are given in table1.It is observed that the microstrain exhibits a decreasing tendency with increase in number of coating cycles.This type of microstrain changes may be due to the crystallization process in crystalline thin films.

Table 1. Structural parameters of TiO_2 thin film for different coating cycles

Number of Coatings	Thickness (μm)	Crystallite size D (nm)	Dislocation density δ x 10^{15}(lines/m^2)	Microstrain μ x10^{-3}
4	0.64	21.3	2.208	0.0976
6	1.1	31.5	1.005	0.0658
8	1.8	42.7	0.548	0.0486
10	2.6	77.6	0.166	0.0267

The value of film thicknesses measured using Stylus profilometer is found to increase with number of coatings of the films [Table 1].The growth mechanism involving dislocation is a matter of great importance. Dislocations are imperfection in a crystal associated with the mis-match of the lattice in one part of the crystal with respect to another part. Dislocation density 'δ' was determined using the relation(3) and is given in Table 1.

$$\delta = \frac{1}{D^2} \qquad (3)$$

It is observed that 'δ' decreases with increasing number of coatings which imply decrease in lattice imperfection due to increase in crystallite size.

Surface morphology and Quantitative Analysis

SEM micrographs of the nanocrystalline TiO_2 thin films for 4 & 8 times coated films are shown in Fig 3 (a) & 3 (b).These images shows loosely agglomerated grains with inhomogeneous surface. In the EDAX spectra shown in Fig 3(c), characteristic peaks of Ti and O are observed which reveals that the obtained thin films are composed of Ti(39 wt%) and O (52%).

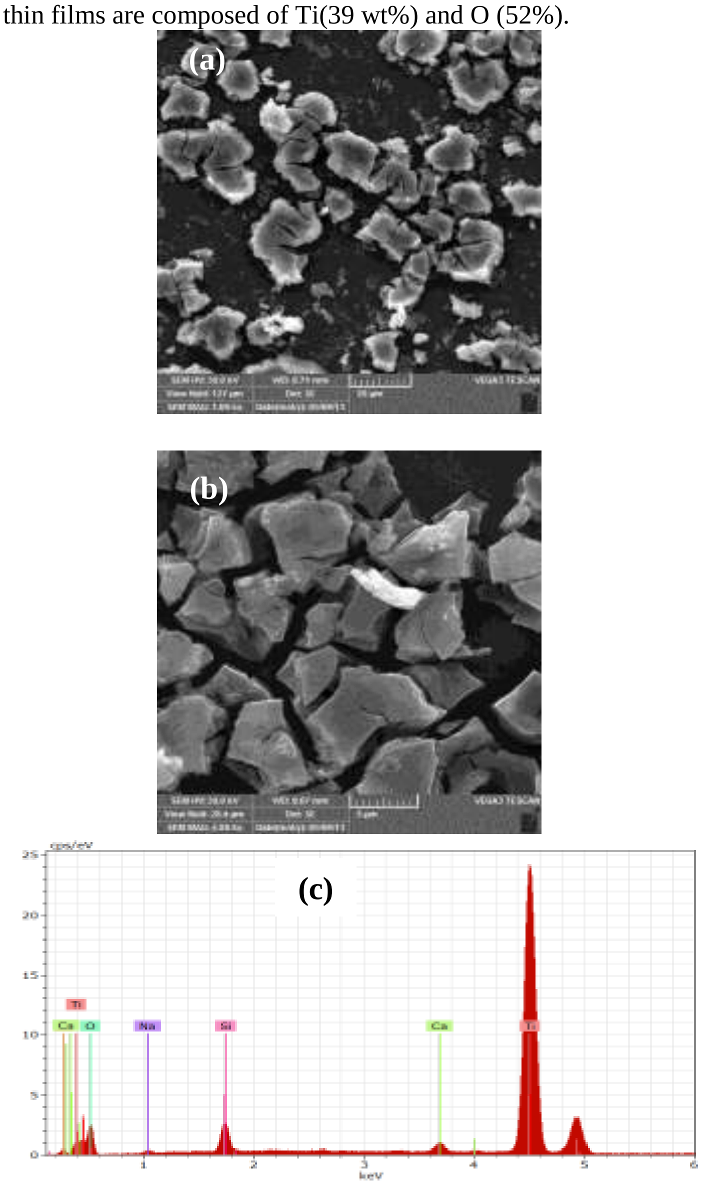

Fig 3. SEM images of the film deposited for (a) 4 coatings and (b) 8 coatings of TiO_2 thin films and (c) EDAX spectrum

AFM analysis

Fig.4 shows the 2D and 3D AFM images covering an area of 5μm x 5μm of TiO_2 thin films for 10 coatings. The surface plot reveals that the film is quite rough with average roughness (S_a) of 32.6314 nm and Root mean square roughness (S_q) of 46.471 nm.

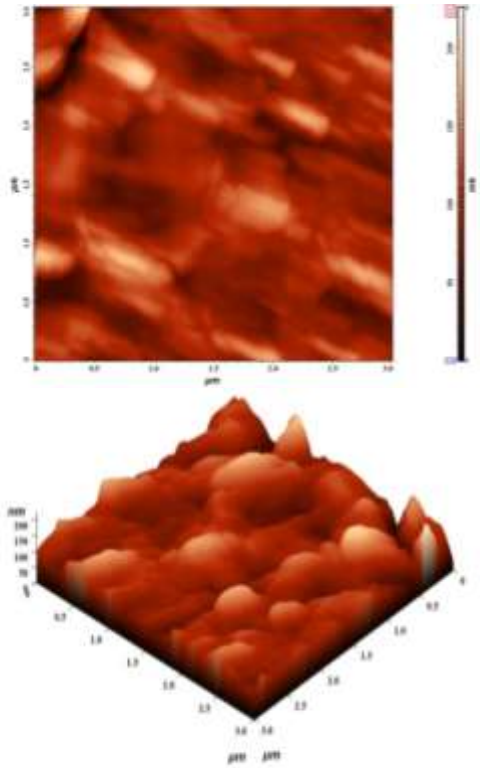

Fig 4. 2D and 3D AFM images of TiO_2 thin films for 10 coatings

As revealed by AFM microscopy, the films showed a high roughness resulting from the presence of important mountains and valleys. This allows a good ability to capture the incident photon energy since a larger surface favors multiple light reflections, thus considerably increasing the amount of absorbed photons. [12].

Optical analysis

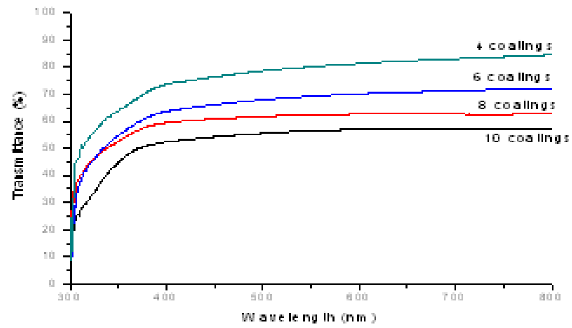

Fig 5 .Transmittance spectra of TiO_2 thin films for different number of coatings

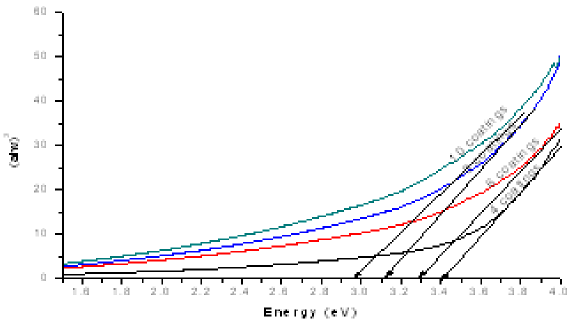

Fig 6. Direct band gap of TiO_2 thin films for different number of coatings

Fig. 5 shows the UV–Visible transmittance spectra of TiO_2 thin films for different number of coating cycles in the wavelength range 200 – 800 nm. The transmittance decreases when the number of coatings increases (Table 2) and it may be due to enhanced absorption of light by increase in film thickness and the scattering effect originating from increased crystallite size. Thicker layer of TiO_2 results in increased absorbance of the film in UV region [13]. Similar observations are made in our work also.

Bandgap energy (E_g) can be estimated from the optical absorption measurements. The plot of $(\alpha h\nu)^2$ with photon energy ($h\nu$) is shown in Figure.6

$$(\alpha h\nu) = A\left(h\nu - E_g\right)^n \qquad (4)$$

Where E_g is the separation gap between bottom of conduction band and top of the valence band, $h\nu$ is the photon energy and n is a constant.The value of n depends on the probability of transition;it takes values as 1/2, 3/2, 2,and 3 for direct allowed, direct forbidden, indirect allowed and indirect forbidden transition respectively. If plot of $(\alpha h\nu)^2$ vs $(h\nu)$ is linear, the transition is direct allowed and extrapolation ofthe straight line portion to zero absorption coefficient (α=0) leads to estimation of band gap energy (E_g) value. The direct band gap energy values of the TiO_2 thin films are found to decrease with increase in number of coating cycles which might be the result of the change in film density and increase in crystal size and it may be attributed to the quantum confinement limit of nanoparticles.

When a photon of energy higher or equal to the band gap value of the semiconductor is absorbed by a particle, an electron from valence band is promoted to conduction band with simultaneous generation of a photo generated hole (h_{vb}) in the valence band and photo generated electron (e_{cb}) in the conduction band given by the equation (5)

$$TiO_2 + h\nu \rightarrow TiO_2 + e_{cb} + h_{vb} \qquad (5)$$

Though, both anatase and rutile type of TiO_2 absorb UV radiation, rutile type can also absorb radiation that is nearer to visible light. The e_{cb-} and the h_{vb+} can recombine on the surface or in the bulk of the particle in a few nanoseconds and the energy is dissipated as heat or can be trapped in surface states and is given by the equation (6) [14]

$$e_{cb-} + h_{vb+} + TiO_2 \rightarrow TiO_2 + \text{heat}\ (\text{recombination}) \qquad (6)$$

Results of UV screening effect of TiO_2thin films

After incubation the organisms were counted using colony counter. The results were recorded in Table 2 and are represented in figure. 7(a) & 7(b) as the percentage of survival rate of organisms against TiO_2 thinfilms of different thicknesses. The survival rate of microbes unexposed to UV is 100% whereas completely exposed to UV radiation is 29% and 41% for *Rhizobium* and *Azotobacter* respectively. The percentage of survival rate increases with increase in number of coatings for both organisms .The highest thickness film (10 times coated film) is having less percentage of transmittance of UV radiation and high percentage of survival rate of microorganism. The present study show that UV screening performance of the films increases with increase in film thickness and the TiO_2 thin films protect the beneficial living microorganism by absorbing UV radiation.

Table 2. Optical parameters of TiO_2 thin film for different coating cycles

Number of coatings	Transmittance at 500 nm (%)	Bandgap (eV)	Survial rate of *Rhizobium* (%)	Survial rate of *Azotobacter* (%)
4	75	3.4	71	77
6	62	3.3	75	83
8	55	3.1	81	88
10	45	2.95	96	98
UV exposed	--	--	29	41
UV not exposed	--	--	100	100

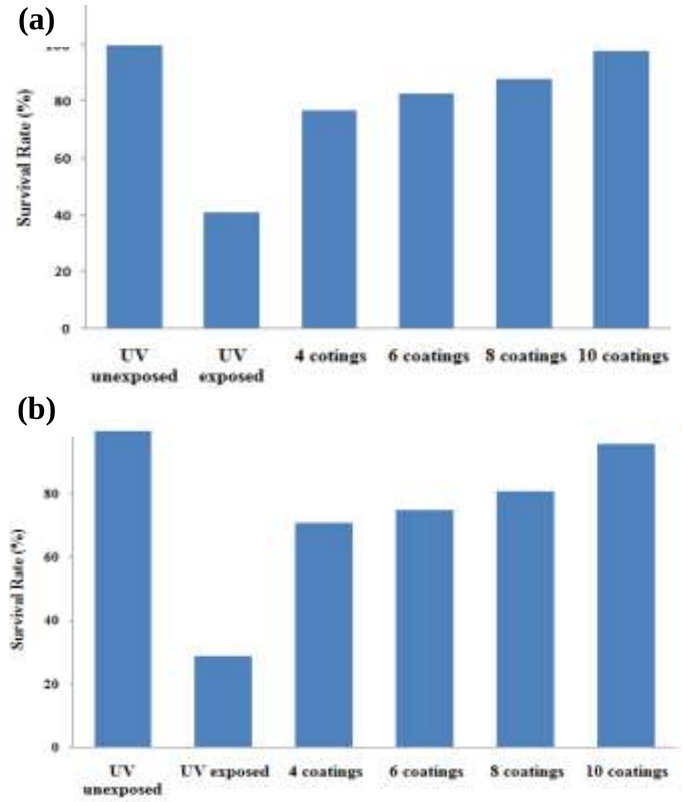

Fig 7. Comparison of Survival rate percent of (a) *Azotobacter* and b) *Rhizobium* for different number of coatings

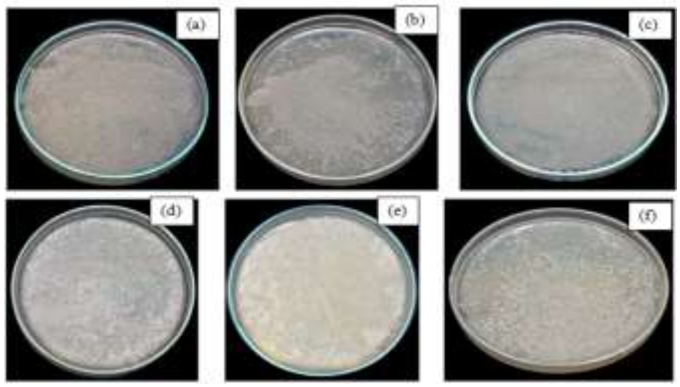

Fig 8. Growth of microbes on Nutrient agar plate (a) unexposed to UV light (b) completely exposed to UV light (c) protected by 4 times coated film (d) 6 times coated film (e) 8 times coated film (f) 10 times coated film.

Conclusion

TiO_2 thin films of various thicknesses have been deposited on glass substrate by sol – gel dip coating technique resulting in highly efficient UV absorbing film. The TiO_2 thin films coated for 4 and 6 times are in anatase phase and transform into the rutile phase for 8 and 10 coating cycles. The thickness of the film and crystallite size of the films increases and dislocation density and microstrain decreases with increase in the number of coatings. The transmittance and optical band gap of deposited thin films are reduced with increase in number of coating cycles. The surface morphology from SEM images reveals that the films have random crystalline growth with rough surface. The presence of Ti and O elements has been confirmed from EDAX measurements. AFM images confirm the roughness of the surface. The present study shows that the properties of TiO_2 thin films are considerably influenced by the film thickness. The percentage of survival rate increases with increase in number of coatings for both organisms. The highest thickness film (10 times coated film) is having less percentage of transmittance of UV radiation and high percentage of survival rate of microorganism. The TiO_2 thin film acts as protective layer by absorbing UV light and the survival rate of organism is enhanced with increase in film thickness.

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