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BIOSYNTHESIS OF TITANIUM-DIOXIDE NANOPARTICLES USING SACCHAROMYCES CEREVISIAE MTCC 463 AND ITS APPLICATION IN WASTE WATER TREATMENT

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ABSTRACT

Nanoparticles are the perfect materials for wastewater treatment and efficient in removal of pathogens, dye decolourization and organic compound removal and the biological synthesis of nanoparticles is an ecofriendly process. Hence the present study trends in about the biosynthesis of Titanium dioxide nanoparticles and evaluation of its ability in waste water treatment. The biologically synthesized Titanium-dioxide nanoparticles were characterized by UV-Vis, SEM-EDAX, TEM, XRD and FTIR. The antibacterial activity was carried out by disc diffusion method against *Escherichia coli, Shigella* sp., *Salmonella* sp., *Pseudomonas* sp., *Proteus* sp, *Streptococcus* sp., *Bacillus* sp. and *Staphylococcus* sp., isolated from the tannery effluent. The antifungal activity was done against *Aspergillus niger, Aspergillus fumigatus, Mucor* sp., *Rhizopus* sp. and Yeast. The photocatalytic activity of Titanium-dioxide was highly effective in organic compounds removal and dye decolorization.

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INTRODUCTION

Nanotechnology, the deliberate manipulation of matter at size scales of less than 100 nm, holds the promise of creating new materials and devices due to their high reactivity by having large surface to volume ratio (Dharmendra et al., 2008). The nanoparticles possess unique physio-chemical, optical and biological properties which can be manipulated suitably for desired applications in the field of electronics, pharmaceuticals, cosmetics, processed food, optics, high performance materials, chemical engineering, energy production and environmental sciences (Logothetidis, 2012). Nanoparticles have demonstrated antimicrobial activities; the development of novel applications in this field makes them an attractive alternative to antibiotics. The recent discovery of the biosynthesis of metal nanoparticles point towards new biotechnological methods in material science (Vishnu et al., 2012). Nanotechnology holds great potential in advancing water and wastewater treatment to improve treatment efficiency as well as to augment water supply through safe use of unconventional water sources (Ana et al., 2012; Xiaolei et al., 2012).

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The properties of nanoparticles have been applied in removing pathogenic bacteria and harmful organic materials from water and air, as well as in self-cleaning or self sterilizing surfaces for places such as medical centers (Wong et al., 2008). Nano titanium-dioxide is a more effective catalyst than microscale Titanium-dioxide and can be used to treat water by chemically degrading organic pollutants that are harmful to the environment (Tahira et al., 2013). Nanomaterials such as Titanium-dioxide nanoparticles (TiO₂NPs), less than 100 nm in diameter, have become a new generation advanced materials due to their brilliant and interesting optical, dielectric, and photo-catalytic characteristics from size quantization (Morteza et al., 2012). Titanium-dioxide is a semiconductor material with a wide variety of applications; ranging from catalysis and dye sensitized solar cells to cosmetics. Among other factors, this variety of applications of Titanium-dioxide is possible because of properties like high stability, low cost and nontoxicity. Catalytic applications of Titanium-dioxide have been studied during decades for the elimination of environmental pollutants (Vetrivel et al., 2015). Nanostructured materials are attracting a great deal of consideration because of their capability and selectivity, particularly in biological and pharmaceutical applications (Wu et al., 2003; Fortner et al., 2005; Li et al., 2005). Nanoremediation methods entail the application of reactive nanomaterials for transformation and

detoxification of pollutants. These nanomaterials have properties that enable both chemical reduction and catalysis to mitigate the pollutants of concern (Barbara et al., 2009). Antimicrobial nanomaterials are envisaged to find their applications in three critical challenges in water/wastewater systems: disinfection, membrane biofouling control, and biofilm control on other relevant surfaces (Xiaolei et al., 2012; Sudarsan et al., 2015). The antimicrobial activity of the nanoparticles showed that the Zinc-oxide and TiO₂ NPs have great potential to be used as antimicrobial agents against microorganisms (Jesline et al., 2014). Photo catalysis based on nano-catalysts is a very promising method for the treatment of contaminated or waste water. There are two different types of photocatalytic application in water treatment: solar photocatalysis and photocatalytic systems equipped with artificial ultraviolet light (Ijadpanah et al., 2014; Ahmed et al., 2015). The present study is aimed to biosynthesis Titaniumdioxide nanoparticles using Saccharomyces cerevisiae MTCC 463 and evaluating their ability of removing pathogenic bacteria and fungi isolated from tannery effluent and dye decolorization efficiency in textile effluent by photocatalysis.

MATERIALS AND METHODS

All the chemicals used in the present study were analytical grade and used as such without further purification. Standard laboratory glasswares made of borosil were used. Saccharomyces cerevisiae MTCC 463 was purchased from MTCC, Chandigarh, India. The lyophilized culture was revived in Yeast extract Peptone D-glucose slant (Yeast Extract-5g; Peptone - 10.0g; 20% D-Glucose-50mg/l; Agar-7.5g; Distilled water-500ml; pH-6.5 ± 0.2). Tannery Effluent was collected near Nagelkeni, Chrompet, Chennai, India. The bacteria were isolated from tannery effluent by serial dilution using sterile Nutrient agar plates (Peptone-5.0g; Beef extract-0.75g; Yeast extract -0.8g; Nacl-0.25g; Distilled Water-500ml; Agar-7.5g; pH-7.0) by incubating at 37[°] C for 24 h. The well isolated typical colonies were pure cultured and maintained in Nutrient agar slants at 4°C. Identification of the bacterial isolate was performed based on their morphological, cultural characteristics and biochemical reactions using Bergey's Manual of Systematic Bacteriology (1984). The fungi were isolated by serial dilution technique using Poatato Dextrose agar (PDA- Potato starch -2.0g; Dextrose-10.0g; Distilled water-500ml; Agar-7.5g; pH-3.5) by incubating for 7 days in RT. The well isolated typical colonies were pure cultured and maintained in PDA slants at 4°C. The fungal isolates were identified based on their macroscopic morphology and microscopic features.

Biosynthesis of Titanium-dioxide Nanoparticles using Saccharomyces cerevisiae MTCC 463

The pure culture of *Saccharomyces cerevisiae* MTCC 463 inoculated in Yeast extract Peptone D-glucose broth for 24 h incubation was used for the biosynthesis of Titanium-dioxide nanoparticles. The broth was filtered using What man filter paper No. 1 and diluted four times using 30% Ethyl alcohol and incubated at RT for 24 h until it attains straw yellow color. 20 ml of 0.05g of Titanium-dioxide solution was added to culture broth and heated by steam bath at 60°C for 10-20 minutes. A white deposit was obtained in the flask. It was cooled and incubated at RT. After 12-48 h, cluster of Titanium-dioxide nanoparticles was obtained at the bottom of the flask. The obtained deposit was further centrifuged at 6000

rpm for 10 min and washed thrice with 30% ethyl alcohol. Then it was dried and stored at 20° C for further study.

Characterization of Titanium-dioxide Nanoparticles

The biologically synthesized nanoparticles were analyzed for its composition, structure, and various properties like physical, electrical, magnetic, etc. The Titanium-dioxide nanoparticles were characterized by UV-Visible Spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray (EDX) spectroscopy, X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR) Spectroscopy and Transmission Electron Microscopy (TEM).

UV-visible Spectroscopy analysis of Titanium-dioxide Nanoparticles

UV-visible spectroscopy analysis was carried out with a resolution of 2.0 nm between 200 to 800 nm using ELCO SL159 UV-Visible absorption spectrophotometer at SRM University, kattankulathur, Chennai, India.

Scanning Electron Microscopy (SEM) analysis of Titaniumdioxide Nanoparticles

Scanning Electron Microscopy analysis for the analysis of the surface morphology with magnification of 12X to greater than 10,000X was carried out in FEI Quanta FEG200, Netherland at Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of technology (IIT), Chennai, India.

Energy Dispersive X-Ray (EDAX) analysis of Titaniumdioxide Nanoparticles

Energy-dispersive X-Ray (EDAX) Spectroscopy analysis for the determination of elemental composition was carried out in a JEOL JSM-6400 Microscope fitted with Oxford -6506 in EDAX analyzer at SAIF, IIT, Chennai, India.

Transmission Electron Microscopy (TEM) analysis of Titanium-dioxide Nanoparticles

TEM study was done to determine the size and shape with a lattice resolution of 0.14 nm and a point to point resolution of 0.12 nm which have a standard probe and variable temperature probe (100 to 500 k) to find out exact particle size of synthesized Titanium-dioxide nanoparticles was carried out in JEOL 3010 instrument with a UHR pole piece at National Center for Catalytic Research, IIT, Chennai, India.

Fourier Transmission Infrared Spectroscopy (FTIR) analysis of Titanium-dioxide Nanoparticles

FTIR analysis was done by mixing synthesized Titaniumdioxide Nano particles with potassium bromide and made to a pellet and analyzed with a scan of MIR 50-4000 cm⁻¹ with the resolution of 2.0cm⁻¹ was carried out in BRUKER RFS system in SAIF, IIT, Chennai, India.

X-ray Diffraction (XRD) analysis of Titanium-dioxide Nanoparticles

The powder X-ray diffraction technique was done to analyze the structure and composition of Titanium-dioxide Nanoparticles by Cu-K X-rays of wavelength ()=1.5406 Å

and data was taken at 2 range using a Bruker make diffractometer in the Department of Chemistry, IIT, Chennai, India.

Evaluation of antimicrobial activity of Titanium-dioxide Nanoparticles

The antibacterial activity of Titanium nanoparticles against pathogenic bacteria isolated from tannery effluent was studied by standard Disc diffusion Method. The bacterial isolates were inoculated in test tubes containing 2ml of Nutrient broth separately and the turbidity was compared to 0.5 Mac Farland Standard. The tubes were incubated at 37°C for 24 h. The isolates were swabbed on Muller Hinton Agar plates (MHA) (Beef infusion form-150g; Casein hydrolysate-8.7g; Starch-7.5g; Agar-8.5g; Distilled Water-500ml; pH-7.3)and dried for 5 minutes. Sterile discs of 5mm in diameter were loaded with 10µl of Titanium-dioxide Nanoparticles with different concentrations such as 60µg, 80µg, 100µg, 120µg, 140µg prepared in 1 ml of the solvent and negative control of sterile disc was loaded. All the plates were incubated at 37°C for 24h. The evaluation of antibacterial activity of Titanium-dioxide Nanoparticles was determined based on the size of the inhibition zone around the disc. The antifungal activity of Titanium nanoparticles was studied by standard Well diffusion Method. PDA plates were prepared and stored at 4°C. The fungal isolates were inoculated onto the test tubes containing 2ml of Potato Dextrose broth separately and incubated at 28°C for 48h. The isolates were inoculated on PDA plate by swabbing over the entire surface of the plates and dried for 5 min. Six wells were made, each 4mm in dia, cut out of agar (Magaldi et al., 2004) and loaded with 10µl of Titaniumdioxide Nanoparticles with different concentrations like 60µg, 80µg, 100µg, 120µg, 140µg prepared in 1 ml of the solvent and negative control of sterile disc was loaded. All the plates were incubated at 37°C for 24h. The evaluation of antifungal activity of Titanium-dioxide Nanoparticles was determined based on the size of the inhibition zone around the disc.

Removal of organic compounds in Tannery effluent by Photocatalysis using Titanium Nanoparticles

100mg Titanium nanoparticles was added to 10ml of tannery effluent taken in two beakers were treated under illumination and ultraviolet light separately for the removal of organic compounds from the tannery effluent. The solutions were examined at fixed intervals of time in colorimeter and optical density of the samples was noted at 400nm upto 120min. Initial OD value was taken at 0 h and then four readings were taken for every 30 minutes. The values were recorded. Percentage of organic compounds removal efficiency is then calculated according to the following formula.

degradation =
$$\frac{C_0 - C_e (\%)}{C_0} \times 100$$

Where,

 C_0 – Initial Concentration of Effluent before photocatalysis C_e – Final Concentration of Effluent after photocatalysis

Decolorization of Textile dyes by Photocatalysis using Titanium Nanoparticles: Textile dyes like methyl orange, crystal violet, congo red and reactive black N dye were used. About 10 ml of textile dye was taken in a sterile glass beaker and added with 100mg of titanium nanoparticles and mixed well. The beakers were kept under illumination and ultraviolet light separately for dye decolorization. The solutions were examined at fixed intervals of time in colorimeter and optical density of the samples were noted at 420nm. Initial OD value was taken at 0 h and readings taken at every 30 minutes upto 120min. The values were recorded. Percentage of degradation is then calculated according to the following formula.

(%)degradation =
$$\frac{C_0 - C_e}{C_0} \times 100$$

Where,

 C_0 – Initial Concentration before photocatalysis C_e – Final Concentration after photocatalysis

RESULTS AND DISCUSSION

The tannery effluent sample collected was dark brown in color and eight bacteria pathogenic to human beings such as *Escherichia coli, Shigella* sp., *Salmonella* sp., *Pseudomonas* sp., *Proteus* sp, *Streptococcus* sp., *Bacillus* sp. and *Staphylococcus* sp. and five fungi *Aspergillus niger*, *Aspergillus fumigatus, Mucor* sp., *Rhizopus* sp. and Yeas tsp. were isolated (Tamilselvi *et al.*, 2012; Akpomie, 2016; Sivakumar, 2016).

Characterization study of Titanium-dioxide Nanoparticles

UV-Visible Spectroscopy analysis

The formation of titanium nanoparticles was confirmed by color changes (Figure 1) followed by UV-Vis spectrophotometric analysis. The UV-Vis spectrum obtained exhibited peak at 203 nm (Figure 2) (Ashraful *et al.*,2012; Vetrivel *et al.*,2015).

Scanning Electron Microscopy (SEM) analysis

The SEM micrographs of biologically synthesized Titanium nanoparticles were characterized for their structure and morphology by Scanning Electron Microscopy are shown in Figure 3. The SEM analysis revealed that the shapes of the particles were hexagonal. The average size of the Titanium nanoparticles was measured as 100 nm. The pure TiO₂nanoparticles exhibited irregular morphology due to the agglomeration of primary particles and with an average diameter of 100 nm (Kavitha *et al.*, 2013; Vetrivel *et al.*, 2015). The EDAX results revealed sharp signals indicated the presence of the elements Titanium and Oxygen (Balachandran *et al.*, 2010) in Titanium nanoparticles (Figure 4).

TEM analysis of Titanium-dioxide Nanoparticles

The morphology of Titanium-dioxide nanoparticles were observed using TEM are shown in Figure 5. The results clearly depicted hexagonal forms of nanoparticles having the size of approximately 100 nm (Sangaraju *et al.*, 2006; Muneer *et al.*, 2012).

FTIR analysis of Titanium-dioxide Nanoparticles

FTIR analysis showed a strong absorption between 800 and 450 cm⁻¹ attributed to the formion of TiO_2 nanoparticles (Figure 6). The peaks at 1641 cm⁻¹ corresponds to stretching

and bending vibrations of the –OH group. The peaks at 1408 cm⁻¹ showed stretching vibrations of Ti-O-Ti. The peaks between 2854 and 2962 cm⁻¹ corresponds to the C-H stretching vibrations (Hema *et al.*, 2013; Vetrivel *et al.*, 2015). The peaks at 3400 cm⁻¹ and 1650 cm⁻¹ in the spectra belongs to stretching and bending vibrations of the –OH group (Kavitha *et al.*, 2013).

X-ray Diffraction (XRD) analysis of Titanium-dioxide nanoparticles

The XRD patterns of the Titanium nanoparticles were shown in Figure 7 and the peaks are in the whole spectrum of 2 values ranging from 10-70. The XRD pattern of the prepared Titanium nanoparticles showed the diffraction peaks at 2 of

Table 1. Antibacterial activity of Ti	itanium- dioxide Nanoparticles by disc diffusion method

Activity of Isolated Bacterial Strains	Co					
	60µg/ml	80µg/ml	100µg/ml	120µg/ml	140µg/ml	Control
Escherichia coli	9	18	20	22	24	Nil
Streptococcus sp.	Nil	12	15	16	16	Nil
Bacillus sp.	10	14	16	18	25	Nil
Shigella sp.	11	18	19	19	19	Nil
Salmonella sp.	Nil	10	11	11	12	Nil
Pseudomonas sp.	6	11	12	14	15	Nil
Staphylococcus sp.	11	17	18	20	19	Nil
Proteus sp.	7	10	11	13	14	Nil

Table 2. Antifungal activity of Titanium-dioxide Nanoparticles by well diffusion method

Activity of Isolated	Activity of Isolated Concentration of Titanium-dioxide Nanoparticles							
Fungal Strains		Zone of Inhibition (mm)						
	60µg/ml	80µg/ml	100µg/ml	120µg/ml	140µg/ml	Control		
Aspergillus niger	Nil	Nil	Nil	Nil	4	Nil		
Aspergillus fumigatus	Nil	Nil	Nil	Nil	2	Nil		
Mucor sp.	Nil	Nil	Nil	Nil	Nil	Nil		
Rhizopus sp.	14	20	21	23	25	Nil		
Yeast	8	15	17	19	19	Nil		

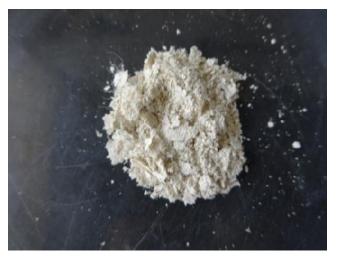


Figure 1. Biologically synthesized Titanium-dioxide Nanoparticles

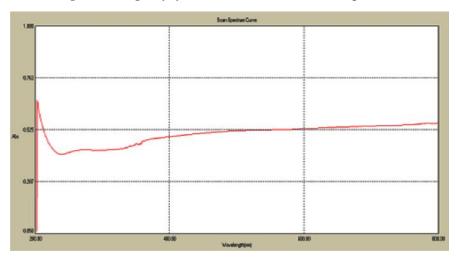


Figure 2. UV-Visible spectroscopy analysis of Titanium-dioxide Nanoparticles

25.5°, 27.5°, 36°, 39°, 41.5°, 44°, 48°, 54.5°, 57°, 62.8°, 64.2° and 69° were associated with (101), (110), (101), (200), (111), (210), (200), (211), (220), (002), (310) and (301) respectively based on the Debye-Scherrer's formula given by equation (D=K / cos ; where Dis the crystal size; is the wavelength of the X-Ray radiation for Cuk ; is the line width at half maximum height) (Vetrivel *et al.*,2015). Strong diffraction peaks were obtained at 25.5° and 48° 2 peaks confirmed the crystal structure of Titanium nanoparticles (Swayamprava *et al.*, 2012).

Antibacterial activity of Titanium-dioxide Nanoparticles

From the tannery effluent five Gram negative bacteria such as *Escherichia coli, Shigella* sp., *Salmonella* sp., *Pseudomonas* sp. *Proteus* sp., and three Gram positive bacteria such as *Streptococcus* sp., *Bacillus* sp., and *Staphylococcus* sp. were isolated. In the study of antibacterial activity of Titanium-dioxide nanoparticles, all the isolates showed inhibition of growth and greater inhibition exhibited (Table 1 & Figure 8).

Thus Titanium dioxide Nanoparticles posses noteworthy antibacterial activity against the pathogenic microbes present in the tannery effluent. Inhibition zone measurements indicated that by increasing concentration of TiO₂, the zone of inhibition also increased (Morteza *et al.*, 2012; Jesline *et al.*, 2015). From the results it is evident that Tio₂ nanoparticles have greater activity in inhibiting the growth of microorganisms and thus can be used in the treatment of waste water (Anitha, 2014)

Antifungal activity of Titanium dioxide Nanoparticles

Five fungi such as *Aspergillus niger, Aspergillus fumigatus, Mucor* sp, *Rhizopus* sp. and Yeast were isolated from the tannery effluent and the antifungal activity of Titaniumdioxide nanoparticles was evaluated by well diffusion method. All fungal isolates of showed greater inhibition at 140μ g concentration than at other concentrations tested. The results shown by the fungal strains are shown in Table 2 (Young *et al.*, 2009).

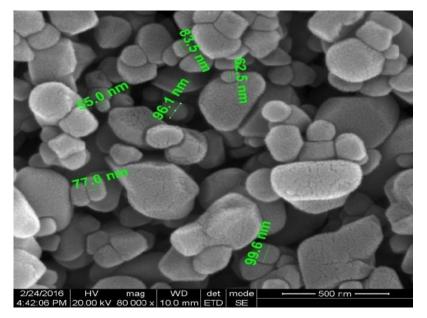


Figure 3. SEM analysis of Titanium-dioxide Nanoparticles

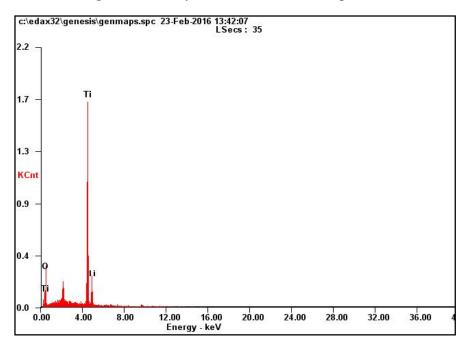


Figure 4. Energy dispersive X-Ray spectroscopy analysis of Titanium-dioxide Nanoparticles

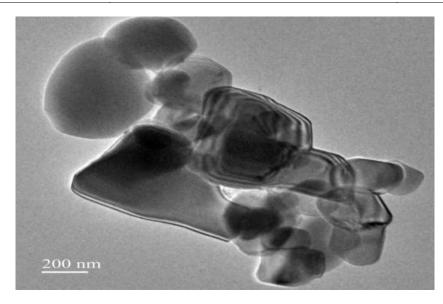


Figure 5. Transmission Electron Microscopy analysis of Titanium-dioxide Nanoparticles

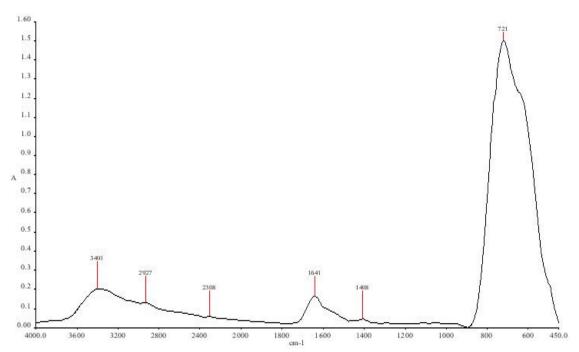


Figure 6. Fourier transmission Infrared spectroscopy analysis of Titanium-dioxide Nanoparticles

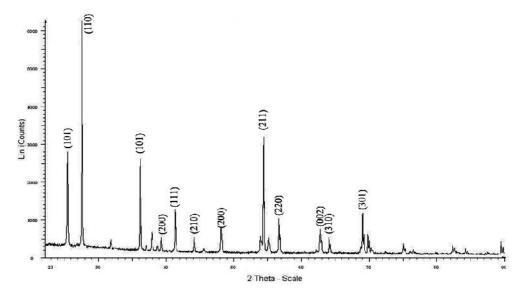
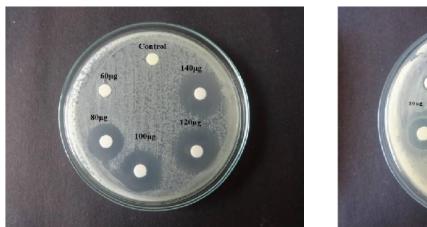
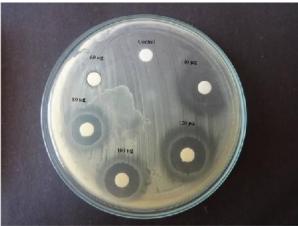


Figure 7. XRD analysis of Titanium-dioxide Nanoparticles



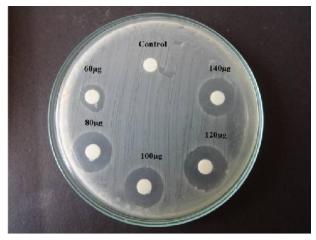
Escherichia coli



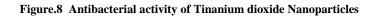
Bacillus sp.



Shigella sp.



Staphylococcus sp.



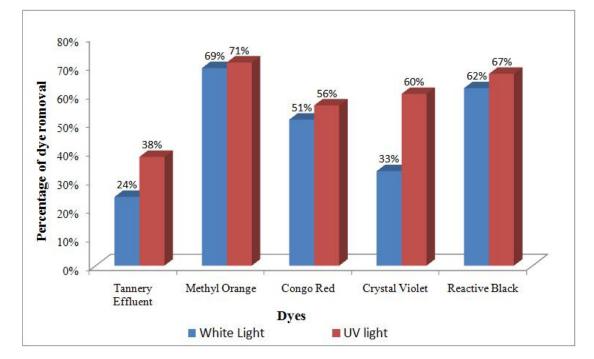


Figure 9. Organic compounds removal efficiency and dye decolorization efficiency of nanoparticles under different illumination sources

 TiO_2 nanoparticles can able to kill yeast cells by producing intracellular reactive oxygen species (Haghighi *et al.*, 2013).

Removal of organic compounds and Dyes in effluent by Titanium-dioxide nanoparticles

The removal of organic compounds in the tannery effluent was efficiently carried out through the process of photocatalysis. Notable results were obtained in the UV light (38%) than in white light which showed only 24% removal. Photocatalytic oxidation is an advanced oxidation process for removal of trace contaminants (Medanna et al., 2011; Xiaolei et al., 2013). When the light passes into the effluent containing nanoparticles, the TiO2 which acts as a catalysts through excitation. The manipulation of materials in nanometric scale led to a new generation of photocatalysts, which, often under visible light, are significantly more stable and active. TiO₂based materials represent the major photocatalysts, and their reactivity is strictly related to their nanostructure (Cargnello, 2012). Titanium nanoparticles are used as biocatalysts because of its because of its strong oxidizing abilities, Super hydrophilicity, chemical stability, long durability, nontoxicity, low cost, and transparency to visible light. The titanium dioxide is an important wide band gap semiconductor in the field of photocatalysis, dye-sensitized solar cells, self-cleaning, photolysis of water and energy storage (Pihosh 2014). Dye decolorization ability of Titanium-dioxide nanoparticles was tested in various commonly used textile dyes under white light and UV light. The prominent results were observed in ultraviolet radiations than in the illumination and the percentage removal of dyes were noted (Figure 9) as methyl Orange (71%), congo red (56%), crystal violet (60%) and reactive black (67%). The notable dye decolorizationability of Titanium-dioxide nanoparticles was reported many researchers (Jessica et al., 2008; Oman et al., 2013; Prateeksha et al., 2014).

Conclusion

In the present world Environmental Nanotechnology plays a vital role in the remediation process. Thus the water purification is necessary to treat the industrial effluent and to eliminate pathogens present in it. Nanotechnology based remediation is very effective, eco-friendly and low cost approach. Hence the Titanium-dioxide nanoparticles synthesized using *Saccharomyces cerevisiae* MTCC 463 could be used in the treatment of tannery effluent for the removal of pathogenic microbes, reduction of organic compounds and also in dye deocolorization in textile effluent.

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