BVG Trust J. Sci. Trans. Environ. Technov. 2017, 10(4) : 185 - 190

Scientific Transactions in Environment and Technovation

Characterization and application of Titanium dioxide (TiO_2) Nanoparticles synthesised by *Fusarium oxysporum*

https://doi.org/10.56343/STET.116.010.004.005
http://stetjournals.com

M. Kannan[1], S. Venkatesan[2], R. Ganesan[3], B. Dheeba[4] and B. Ramakrishnan[5]

[1] Research Department of Zoology, V.H.N.S.N. College (Autonomous), Virudhunagar - 626 001, Tamil Nadu, India
[2] Department of Environment Sciences, Periyar University, Salem, Tamil Nadu, India.
[3] School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai - 625 021, Tamil Nadu, India.
[4] Department of Chemistry and Biosciences, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam, Tamil Nadu, India.
[5] Mammalogy and Forest Ecology Lab, Department of Zoology and Wildlife Biology, Government Arts College, Udhagamandalam - 643 002, Tamil Nadu, India

Abstract

The nanotechnology and tools for the synthesis and characterization are developed in various research fields. The microbes were used for the synthesis of nanoparticles in life science studies. Biological approaches were done for the synthesis of TiO_2 (Titania) nanoparticles using *Fusarium oxysporum*. The biosynthesized nanoparticles were employed in the dye decolorization. The synthesized nanoparticle was characterized by techniques like UV-vis spectrophotometer, FTIR and SEM analysis. The process of TiO_2 nanoparticles synthesis was optimized for the pH, temperature, metal concentration and time of incubation.

The mycelium of *Fusarium oxysporum* with 0.025M TiO_2 solution was incubated at 27 °C for the synthesis of nanoparticles. The nanoparticles thus obtained were filtered and it was characterized by UV-vis spectrophotometer at 257nm. Further characterization by FTIR and SEM results confirmed the presence of nanoparticles. The optimum conditions for the development of TiO_2 nanoparticles were observed at pH 5, temperature 27 °C, metal concentration 10^{-3} and incubation time period 48 hours. Methyl orange dye was used with different concentrations of synthesized nanoparticles to investigate the decolourization. The samples were collected at various time intervals which showed the efficiency of nanoparticles in the decolourization process due to the broken down of functional group present in the azo dye.

Keywords: Titania, *Fusarium oxysporum*, azo dye, FTIR and SEM.

Received : February 2017 Revised and Accepted :April 2017

INTRODUCTION

Nanoparticles are viewed by many as fundamental building blocks of nanotechnology. Now a days Nanoparticles play an important role in a wide variety of fields including advanced materials, Pharmaceuticals and environmental detection and monitoring. A nanoparticle or nano powder is microscopic particles whose size is measured in nanometers (nm). 1nm=10^{-9} m depending on the application of interest, nanoparticles may be known by a number of alternatives and trade-specific names including particulate matter, aerosols, colloids, nanocomposites, nanopowders and nanoceramics. It is further classified according to size; In terms of diameter, fine particles cover a range between 100 and 2500 nanometres, while ultrafine particles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials (Buzea *et al.*, 2003).

Various chemical methods are used for the synthesis of nanoparticles. They are, Chemical vapour deposition (Klabunde *et al.*, 1991), vapor-phase synthesis, Hydrothermal synthesis (Komarneni *et al.*, 1992), Chemical reduction (Turkevich and Kim, 1970; Esumi *et al.*, 2000).

Various physical methods are used for the synthesis of nanoparticles. Titanium is suggested for use in desalinization plants because of its strong resistance to corrosion from sea water (particularly when coated with platinum). In medical applications titanium pins are used because of their non-reactive nature when contacting bone and flesh. Many surgical instruments, as well as body piercing are made up of titanium for this reason as well. In terms of a mechanism, Ti^{IV} binds well to transferrin in human serum, which could deliver it to the cancer cells. This further emphasizes their future role in cancer chemotherapy and gene delivery. TiO_2 has three crystal forms namely, anatase, rutile and bookite. The efficient photocatalytic activity

*Corresponding Author :
email: *microkannan@gmail.com*

of TiO_2 deeply depends on its crystallite size, surface area, and crystal structure (Yang *et al.*, 2002). As known, anatase or the mixture phase of anatase and rutile show the highest photocatalytic activity Anatase with large surface area, high crystallinity and nanoscaled crystallite size exhibits a high photocatalytic activity Semiconductor photocatalysis with a primary focus on TiO_2 as a durablephotocatalyst has been applied to a variety of problems of environmental interest in addition to water and air purification. It has been shown to be useful for the destruction of microorganism such as bacteria and viruses, for the inactivation of cancer cells, for odour control, for the photo splitting of water to produce hydrogen gas, for the fixation of nitrogen, and also for the cleanup of oil spills (Hoffman *et al.*, 1995). Titania particles have also been employed to remove organic pollutants and heavy metals in waste water (Wu *et al.*, 2008).

The synthesis of Titania nanoparticles was done by physical (Wu *et al.*, 1998; Watanabe *et al.*, 1977) and chemical method (Ayyub *et al.*, 1990; Boutonnet *et al.*, 1982). But limited works are available for the biosynthesis of Titania nanoparticles.(Nair and Pradeep, 2002). So in this work an attempt has been made to investigate the biosynthesis of Titania nanoparticles by using the fungus*Fusarium oxporum*.

The nanoparticles can be characterized by following the techniques, which provide important information for the understanding of different physicochemical features. The most extensively used techniques are Optical Spectroscopy, Ultraviolet-Visible (UV-Vis) Spectroscopy, Fluorescence Spectroscopy and Fourier Transform Infrared (FTIR) Spectroscopy.

MATERIALS AND METHODS

Collection of sample for isolation of fungi Fusarium *oxysporum*

For the fungal isolation, the roots and stems were aseptically collected from wilted tomato plants. The infected plants were certified by Department of Botany, V.H.N.S.N.College, Virudhunagar, Tamilnadu.

Surface sterilization of infected plant tissue

The infected part of the diseased leaves, stems and roots was cut with the help of sterilized scalpel, and kept in the first beaker containing saturated borax solution was 0.1%, for 10 to 15 minutes. Thereafter it was taken out of the first beaker with the help of glass rod, and thoroughly washed with mercuric chloride solution (0.1%) for 15 seconds. Then they were repeatedly washed with sterile distilled water. These sterilized inoculums were being transfered to sterilized Petri plate, and then inoculated on to acidified Potato Dextrose Agar (PDA). The plates were incubated at 22 °C for 5- 7days.The fungal hyphae were preserved in slant for further studies. (Smith *et al.*, 1985).

Fungal staining

The actively growing margin of the fungal isolates was cut and cleaned successively with antibiotic solution in Petri plate. The hyphae were stained with cotton blue and lactophenol, and observed under the microscope. Slide culture were prepared as described by Isaac (1956) and Hawksworth (1970), and identified on the basis of colony and conidial morphology, and on the identification key as proposed by (Booth, 1971).

Colony morphology

Colony morphology of the organisms was studied by growing them on Czapeks Dox agar (100 ml) composed of sucrose (3g), sodium nitrate (0.3g), dipotassium phosphate (0.1g), magnesium sulphate (0.05g), potassium chloride (0.05g), ferrous sulphate (1.0g), agar (1.5g); malt extract agar (100 ml) composed of maltose (1.2g), dextrin (0.27g), glycerol (0.23g), peptone (0.07g) and agar (1.5g); Oat Meal Agar (100 ml) composed of oat meal (6g) agar (1.2g); Potato Dextrose Agar (100 ml) composed of dextrose (2g), potatoes infusion (100 ml), agar (1.5g); Rose Bengal Agar (100 ml) composed of mycological peptone (0.5g) glucose (1g), potassium dihydragen phosphate (0.1g), magnesium sulphate (0.05g), rose Bengal (0.005g), agar (1.5g); and Sabouraud Dextrose Agar (100ml) composed of mycological peptone (1g), dextrose (4g) and agar (1g). Cultures were incubated at 22 °C for 5-7 days. The fungus colony diameter and morphological characters were recorded.

Biosynthesis of titanium nanoparticles by isolated *Fusarium oxysporum*

Fusarium oxysporum stock cultures were maintained by subculturing at monthly intervals. Growth medium used as was MGYP broth.

For the synthesis of nanoparticles, the fungus *Fusarium oxysporum*was grown in 500 ml Erlenmeyer flasks each containing MGYP medium (100 ml), composed of malt extract (0.3%), glucose (1.0%), yeast extract (0.3%) and peptone (0.5%) at 25–28 °C under shaking at 200 rpm for 96 h. The mycelial mass were then separated from the culture broth by centrifugation (5000 rpm) at 10 °C for 20 min and the settled mycelia were washed thrice with sterile distilled water. Some of the harvested mycelial mass (20g) was then used for the synthesis of Titania nanoparticles. This methodology was also followed by Ahmad *et al.* (2003) with small modification.

The harvested mycelia mass (20g wet weight) was then resuspended in 20 ml aqueous solution of 0.025(M) titanium dioxide (pH 3.5) was added separately in 500 ml Erlenmeyer flasks and kept on a shaker (200 rpm) at 27 °C. After incubation the reaction solution was observed. Nanoparticles containing fungal mycelia were filtered under laminar flow through Watman filter paper. The reaction solution was

removed and the absorption was measured by UV-vis spectrophotometer. Then allowed to calcinations at 180 °C for 5 hours has required for crystallization of Titania nanoparticles. Then the products were analysed by FTIR and SEM.

In control experiments, the fungal biomass was resuspended in double distilled water in the absence of aqueous solution and the filtrate obtained thereafter was characterized by UV-vis spectrophotometer and. This reaction did not result in the formation of Titania nanoparticles. In another control experiment, the hydrolysis of aqueous solution in double distilled water in the absence of fungal biomass was studied by UV-vis spectrophotometer and FTIR. This control experiment also was negative and no Titania nanoparticles could be detected.

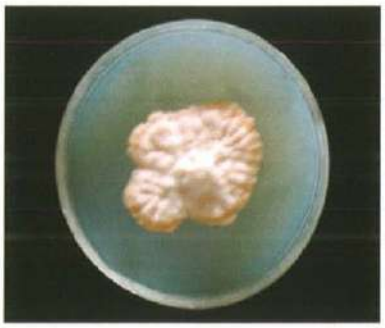


Fig. 1. Isolation of *Fusarium oxysporum* on Potato Dextrose Agar Medium and microscopic observation of the plate

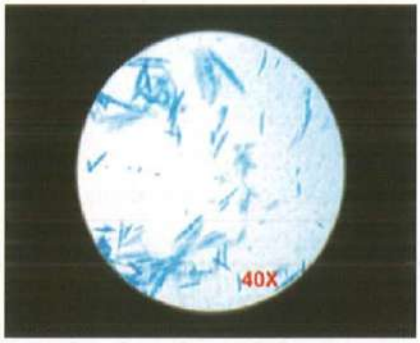
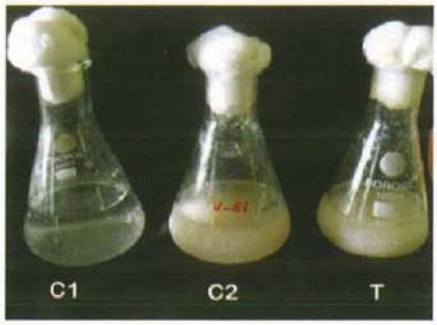


Fig. 2. Above the plate showed the orange coloured *Fusarium oxysporum* on Potato Dextrose Agar

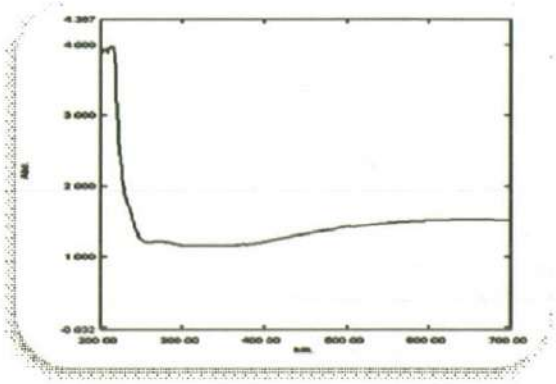


C1- Metal control, C2- Culture control and T- Biosynthesised nanoparticle

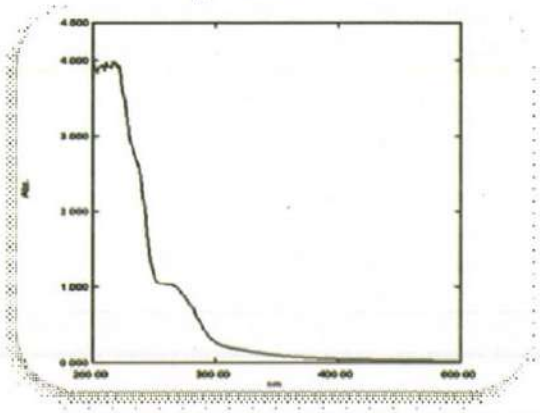
Fig. 3. Biosynthesis of Titania nanoparticles by *Fusarium oxysporum*

Optimization of biosynthesized nanoparticles

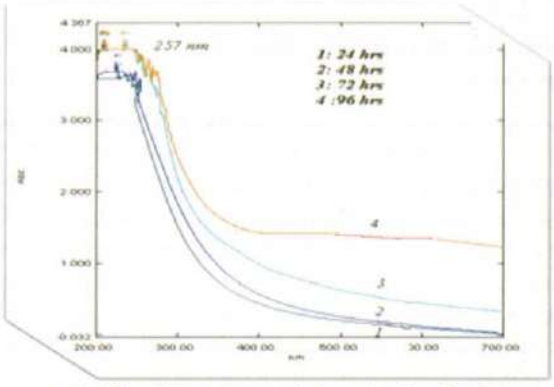
The biosynthesis of Titania nanoparticles were optimized for suitable pH (3 to 9), temperature (15,27,37 and 48 °C), metal concentration (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} mM) and time of incubation (24, 48, 72 and 96 h) for the synthesis of titania nanoparticles using *Fusarium oxysporum*. The optical density valueswere recorded by using the Uv-Vis spectrophotometer.



(a) Metal Control



(b) Culture Control



(c) Titania nanoparticles obtained with *Fusarium oxysporum*

Fig. 4. Uv-Vis spectra for Titania nanoparticles obtained by *Fusarium oxysporum*

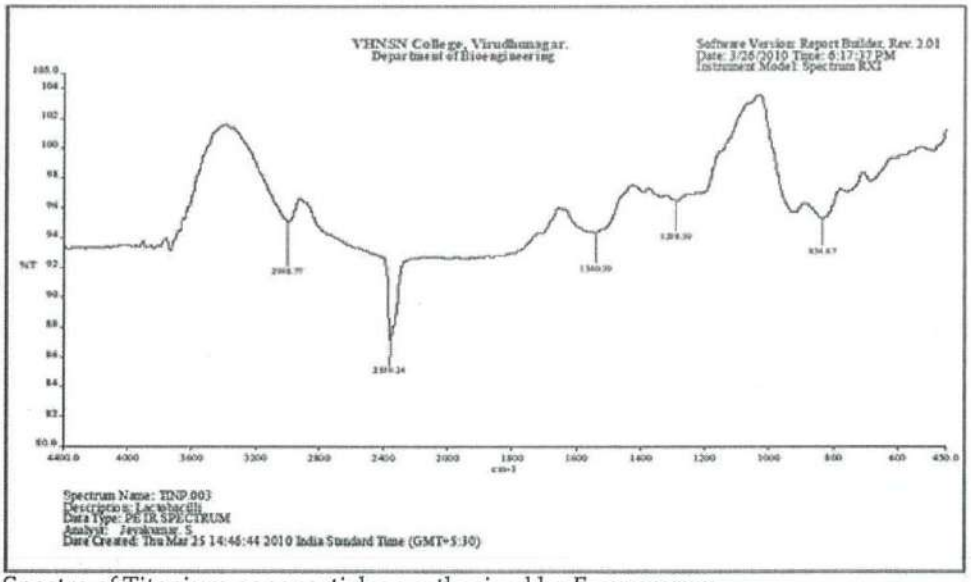


Fig. 5. FTIR Spectra of Titanium nanoparticles synthesized by *F. oxusporum*

TINP.003 1976 4400.00 450.00 87.08 103.57 4.00 %T 1 1.00

Fusarium

REF 4000 93.55 2000 92.64 600

2998.77 95.07 2359.24 87.07 1540.39 94.35 1288.39 96.53 834.67 95.31

END 5 PEAK(S) FOUND

Decolorization of dye

Dye solutions were prepared in double distilled water at 50 mgL^{-1} of Methyl orange. The 50 ml of dye solution was moved to 100 ml Erlenmeyer flask and then 0.25%, 0.5%, 1% (W/V) of biosynthesized titania nanoparticle was added. The flask was stirred at 150 rpm by shaker up to 300 min. The experiment was performed in triplicate and conducted at room temperature. Samples were collected in at 60,120,180,240,300 and minutes. The samples were centrifuged at 6,000 rpm for 5 minutes. The residual concentrations of dye were quantified by UV-Vis spectrophotometer at 461nm for methyl orange. Titania nanoparticles were also qualified by observing the change of UV-vis spectrum before and after treatment with methyl orange.

RESULTS

Isolation and characterization of *Fusarium oxysporum*

The plant pathogenic fungi were isolated from infected tomato plants. The infected parts were surface sterilized and a piece of the leaf was place on the Potato Dextrose Agar. After the incubation period the isolated fungi were observed under the microscope for morphological studies. The hyphae were septate and hyaline. Conidiophores were simple. Macroconidia were moderately curved, stout, thick-walled, have 3-5 septate, the aerial mycelium was white to orange in colour on Potato Dextrose Agar

The fungal morphology and mycelial growth of *Fusarium oxysporum* were observed PDA medium. Among these six media, mycelial growth of the *Fusarium oxysporum* on PD agar was greater than other media.

The biosynthesis of Titania nanoparticles was made by isolated *Fusariumoxysporum*.After 96 hrs of incubation period, the Titania nanoparticle was observed in the flask containing isolated culture with titanium dioxide. The deposits were observed at the bottom of the conical flask and the initial pH 3 was changed in to 6-7. In control flask, there was no deposition and a pH change was observed.

UV-Vis Spectrophotometer Analysis

The UV-Vis spectra were recorded from the aqueous potassium titanium dioxide with *Fusarium oxysporum*.Titania surface plasmon band occurs at ca. 257 nm. Steady increase in intensity was recorded due to the reaction of complete oxidation of the titanium dioxide by *F. oxysporum* that occured after nearly 96 hours of reaction. There was no observable result found in *Fusarium oxysporum* synthesized Titania nanoparticles.

FTIR analysis of Titania Nanoparticles

Fourier Transform Infrared (FTIR) analysis of Titania nanoparticles synthesized by fungai*Fusarium oxysporum*. Titanium dioxide reaction medium after 96 hrs of reaction showed the presence of a resonance at ca. 600cm^{-1}-1100cm^{-1} (Fig. 5), and these peaks correspond to exidation of Ti-O-Ti vibrational mode in the particles. A band at 834.67cm^{-1} peak corresponds to exidation of the Ti-O antisymmetric stretching of Ti-O-Ti bonds. The presence of protein in the Titania nanoparticles, the absorption bands at ca.1540.39cm^{-}

[1] indicated by the amide band in the particle, this bands absent in the Titanium dioxide.

Optimization of biosynthesized nanoparticles

The OD value obtained from Uv-Vis spectra was used to determine the optimum biosynthesis of selected nanopartices. Here the synthesis of Titania nanoparticles and yield due to *Fusarium oxysporum* was high at the optimum pH 5, temperature was 27 °C, and metal concentration 10^{-3}M. The suitable incubation period was 48 hours.

Decolourization of methyl orange dye by Titania nanoparticles

The absorbance peaks of the methyl orange in the visible region of 461nm were recorded . The UV-Vis spectra of dye methyl orange solutions were taken before and after decolorization with Titania nanoparticles. When compared with the control (untreated) the OD value was reduced more than 60%. This may resulted from the higher surface area of nanoparticles which provide more surface active sites for collision with dye molecules to accelerate the dye removal efficiencies. The decreas in absorbance indicated that chromophore group, the basic functional group of dyes, for its visible colour, was broken down.

The fungi and bacteria mediated green chemistry approach towards the synthesis of nanoparticles has many advantages such as ease with which the process can be scaled up, economic viability, possibility of easily covering large surface areas by suitable growth of the mycelia. *Fusarium oxysporum* was isolated from the wilted tomato plant. The cultural and the morphological characters of mycelium and conidial structures were identifical to that of the characters of *Fusarium* isolated from the tomato plant and described by Part *et al.* (1995). However *Fusarium oxysporum* on solid media such as Potato Dextrose Agar (PDA) showed different morphological forms. In general, the aerial mycelium first appeared white, and then may changed to a variety of colours- ranging from violet to dark purple depending on the strain (or special form) of *Fusarium oxysporum*. The culture appeared cream or orange in colour when sporodochia were abundantly produced.

In the present study the growth characters of *Fusarium oxysporum* on different solid media showed that the growth was maximum on Oat meal agar followed by Czapex's Dox Agar and Potato Dextrose Agar supported maximum growth of fungal colony. Margin was irregular on Potato Dextrose Agar and Saboroud Dextrose Agar.

Bansal (2005) reported that, *Fusarium oxysporum* with aqueous anionic complexes resulted in synthesis of crystalline Titania particles at the room temperature, while calcinations required 300 °C for crystallization of silica. The same trend was recorded in present study also. UV-Vis spectra of titanium nanoparticles in the present study showed that the band at 257 nm. Zecchina *et al.* (1996); Astorina *et al.* (1996) reported that the band was in the range of 230-280 nm. Thus the results of the present study also confirm the previous reports

In FTIR analysis, the standard wave number of si-o-si rocking and bending vibration bond occurred at about the 484 cm^{-1} position **[Feng and Wee]**. In this samples si-o-si rocking /bending vibration bond occurs at 480.14 cm^{-1}. The Si-H wagging modes occured at the wave number of 660cm^{-1} and 624 cm^{-1}. In this sample wagging modes occurred at 654.20 cm^{-1}.The strong bond of Si-O- Si asymmetric vibration was found in the standard wave number range 1000-1100cm^{-1} Norman *et al.* (1990). In the present study the second strongest bond is 1113cm^{-1}. The Si-Si vibration modes standard wave number was 740cm^{-1}. The observed wave number was 743.91cm^{-1}. The C-H stretching mode standard wave number range was 3400-3610 cm^{-1} and observed wave number was 3448.71cm^{-1}. The C=C stretching mode was absorbed at standard wave number range of 1590-1610cm^{-1} and observed wave number of 1623.62cm^{-1}. The CH_2 asymmetric stretching mode and symmetric stretching occurred at the standard wave number of 2960 cm^{-1} and 2853-2927cm^{-1} respectively (Norman *et al.*, 1990)The observed wave number was 2927.12cm^{-1}. The standard wave number of Si-CH=CH_2 and deformation mode occurred in the range of 1410-1590 $cm^{-1}$and observed wave number occurred at the 1412.04cm^{-1}.

The surface morphology of the Titania nanoparticles was studied using Scanning Electron Microscope. In the micrograph nanoparticles were observed in the size range between 20 and 50nm. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a protein capping agent.

The absorbance peaks of the methyl orange were in the visible region of 461 nm. Titania nanoparticles (TiNp) were much faster than those of potassium silico fluoride (K_2SiF_6) and titanium dioxide (TiO_2) under the same experimental condition. The UV-Vis spectra of dye methyl orange solutions before and after decolorization by titania nanoparticles showed the higher surface area of nanoparticles which provide more surface active sites for collision with dye molecules to accelerate the dye removal efficiencies. The decrease in the absorbance indicated that chromophore group, the basic functional group of dyes for its visible colour, was broken down.

REFERENCES

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R. and Sastry,M. 2003. Extracellular

biosynthesis of silver nanoparticles using the Fungus *Fusarium oxyporum*. Coll. Surf. B. 28(4): 313-318.

Astorino, E., Peri, J.B., Willey, R.J. and Busca, G. 1995. Spectroscopic characterization of Silicalite-1 and Titanium Silicalite -1. *J. catal.* 157(2) : 482-500.

Ayyub, P., Maitra, A.N. and Shah, D.O. 1990. Formation of theoretical denisty microhomogenous YBa2Cu307 - χ using a microemulsion-medicated process. *Physica. C. Superconductivity*, 168(5-6):571-579.

Buzea, C., Spalluto, G. and Proto, M. 2003. Nanomaterials and nanoparticles Source and Toxicity. Biointerphases. MR17-MR71.

Esumi, K., Suzuki, A., Yamahira, A. and Torigoe, K. 2000. Role of polt dendrimers for preparing nanoparticles of gold, platinum and silver. Langmuir. 16: 2604-2608.

Hoffman, M.R., Martin, S.T., Choi, W. and Bahnemann, D.W. 1995. Environmental application of semiconductor photpcatalysis. *Chem. Rev.*, 95:69-96.

Isaac, I. 1956. A comparative study of pathogenic isolates of *Verticillium* Trans. *Bri. Mycol. Soc.* 32: 137-157.

Klabunde, K.J., Li, Y.X. and Tan, B. J.1991. Solvated metal atom dispersed catalysts. *Chem. Materials.*, 3:30-39.

Komarneni, S., Roy, R. and Li, Q. H. 1992. Microwave-hydrothermal synthesis of ceramic powder. *Mater. Res. Bull.*, 27: 1393-1405.

Nair, B. and Pradeep, T. 2002. Coalescence of nanoclusters and formation of submicron crystallites assited by *Lactobacillus* strain. *Cryst. Growth. Des.*, 2: 293-298.

Norman, B., Lawrence, H. and Stehen, E. 1990. The infrared spectra of complex molecules 3rded. Newtork: Acabdemic press, Book of plant disease. Black well scientific publication: Oxford, P.583.

Park, Y.S., Kim, Y.H., Cho, M.R. and Kim, W.G. 1995. Tomato wilt cased by *Fusarium* sp., *J. Agri. Sci.*, 37: 303-307.

Smith, A.D. and Hunt, R.J. 1985. Solubilisation of gold by Chromobacterium violaceum. *J. Chem. Technol. Biotechnol.*, 35: 110

Smith, I.M., Dunez, J., Phillips, D.H., Lelliott, R. H. and Archer, S.A. editors1988. *European hand Chem. Mater.*, 3:30-39.

Turkevich, J. and Kim, G. 1970. Palladium: Preparation and catalytic properties of particles of uniform size. *Science*. 169: 873-879.

Wu, J., Doan, H. and Upreti, S. 2008. Decolorization of aqueous textile reactive dye by ozone. *J. Chem. Eng.*, 142:156-160.

Wu, T.X., Liu, G.M. and Zhao, J.C. 1998. Photoassisted degradation of dye pollutants V self photosensitized oxidative transformation of Rhodamine B under visible light irradiation in aqueous TiO_2 dispersion. *J. Phys. Chem. B.*, 102(30): 5845-5851.

Yang, J., Li, D., Wang, X., Yang, X. and Lu, L. 2002. Rapid synthesis of nanocrystalline TiO_2/SnO_2 binary oxides and their photoinduced decomposition of methyl orange. *Journal of Solid State Chemistry*. 165, 193-198.

Zecchina, A., Bordiga, S., Lamberti, C., Ricchiaridi, G., Scarano, D., Petrini, G., Leofanti, G. and Mantegazza, M. 1996. *Ctal.* Today. 32(9).