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Bio-Synthesis, Characterization and Application of Titanium Oxide Nanoparticles by *Fusarium oxysporum*

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Abstract: **The unique property of the titanium oxide nanoparticles to characterize the major attention towards the present nanotechnology. The environmentally nontoxic, ecofriendly, and cost-effective method that has been developed for the synthesis of titanium oxide nanoparticles using fungi creates the major research interest in the field of nanobiotechnology. So in this work we are planned to investigate the biosynthesis of titania nanoparticles by isolated fungi *Fusarium oxporum*. The nano particles can be characterized by following 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, Flurosence Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy.**

Keywords: **BIO-SYNTHESIS, titanium oxide, nano particles, Spectroscopy, *Fusarium oxporum*.**

1. 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 nanoparticles 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, collides, nanocomposites, nanopowers 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 vapor 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 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 durable photocatalyst 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).

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2. METHODOLOGY

Collection of sample for isolation of fungi Fusarium *oxysporum:*

For fungal isolation, the roots and stems were aseptically collected from wilted tomato plants. The infected plants were certified by Department of Biotechnology, Sri kaliswari College, Virudhunagar, Tamilnadu.

Surface sterilization of infected plant tissue:

The infected part of the diseased leaf, stems and roots was cut with the help of sterilized scalpel, and kept in the first beaker containing saturated borax solution 0.1%, for 10 to 15 minutes. Thereafter it is taken out of the first beaker with the help of glass rod, and thoroughly washed the second beaker containing mercuric chloride solutions 0.1% only for to 15 seconds. Once again the infected materials is thoroughly washed with in the third beaker contains distilled water. Now, these sterilized inoculums are being transformed in a sterilized petriplate, and then inoculated in acidified Potato Dextrose Agar (PDA) plates. The plates were incubated at 22^0C for 5- 7days.The fungal hyphae were preserved in slant for further studies. (Smith *et al.,* 1985)

Fungal staining:

The fungal isolates were cleaned up by subculture successively on antibiotic plates from the edge of actively growing colonies and single spores were then isolated. Microscopic cultures of *Fusarium oxysporum* were identified by their resting-structure morphology, by means of the slide culture technique presented by (Isaac, 1956) and the description of (Hawksworth, 1970).

Colony morphology:

Colony morphology of the organisms were studied by growing them on Czapek's 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 glucose (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), agar (1g). Cultures were incubated at 22^0C for 5-7 days. The fungus colony diameter, morphology were recorded.

Biosynthesis of titania 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 media (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 followed by Ahmad *et al.,* 2003 with small modification.

The harvested mycelia mass (20g wet weight) was then resuspended in 20 ml aqueous solutions of 0.025(M) titanium dioxide solution (pH-3.5) was added separately in 500 ml Erlenmeyer flasks and kept on a shaker (200 rpm) at 27^0C. 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 spec. Then allowed to calcinations at 180^0C for 5 hrs is required for crystallization of titania nanoparticles. After 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 spec 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 at was studied by UV-vis spectrophotometer and FTIR. This control experiment also was negative and no titania nanoparticles could be detected.

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3. RESULTS

Isolation and characterization of fungi *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 microscopy for morphological studies. The hyphae were septate and hyaline. Conidiophores were simple. Macroconidia was moderately curved, stout, thick-walled, have 3-5 septate, the aerial mycelium were white to orange in colour on Potato Dextrose Agar

The fungal morphology and mycelia growth of *Fusarium oxysporum* (Figure.1)observed PDA agar Medium.Among these six media, mycelial growth of the *Fusarium oxysporum* on PDA agar were greater then other media. The microscopic observation of fusarium oxysporum (Figure.2) is indicated that blue colonies which is viewed under 40x of the magnification.

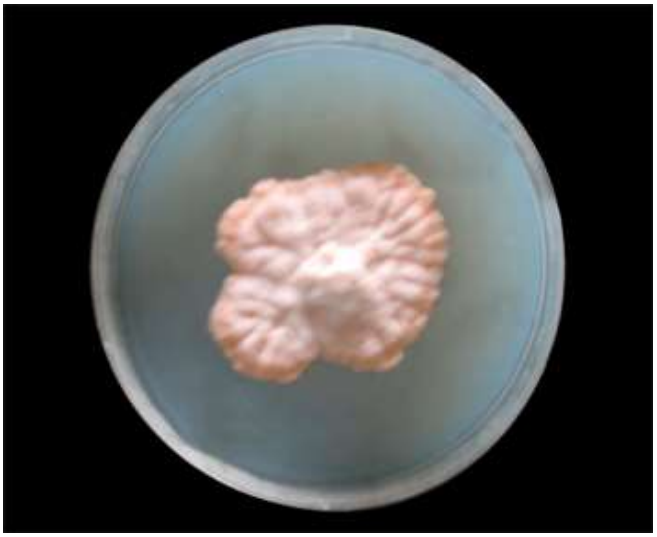

Figure.1: Isolation of *Fusarium oxysporum* on Potato Dextrose Agar Medium of the plate

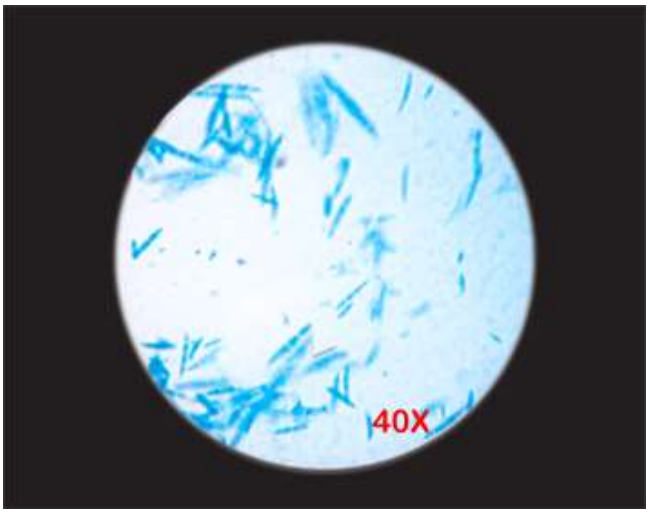

Figure.2: Microscopic observation of *Fusarium oxysporum* at 40X Concentration

The biosynthesis of titania nanoparticles was done by isolated *Fusarium oxysporum*.(Figure.3)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.(Figure4.A) consist of biosynthesized silica nanoparticle powder and (Figure4.B) contains the biosynthesized titania nanoparticle powder.

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Biosynthesis of titania nanoparticles by *Fusarium oxysporum:*

Figure.3: C1- Metal control, C2- Culture control and T- Biosynthesised nanoparticle

Biosynthesized nanoparticle powder:

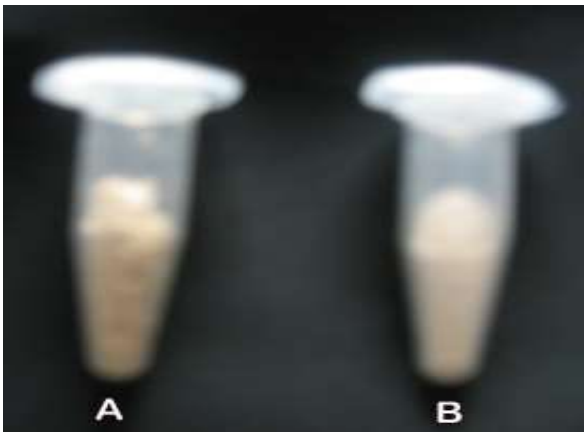

Figure.4: A - silica nanoparticle, B - titania nanpaticle

UV-Vis Spec Analysis:

The UV-Vis spectra recorded from the aqueous potassium titanium dioxide for *Fusarium oxysporum.* reaction medium as a function of the time of reaction.(Figure.5,6,7) Titania surface plasmon band occurs at ca. 257 nm. Steadily increases in intensity as a function of the time of reaction complete oxidation of the titanium dioxide by Fusarium oxysporum occurs after nearly 96 hours of reaction. There was no observable results were found in *Fusarium oxysporum* synthesized titania nanoparticles.

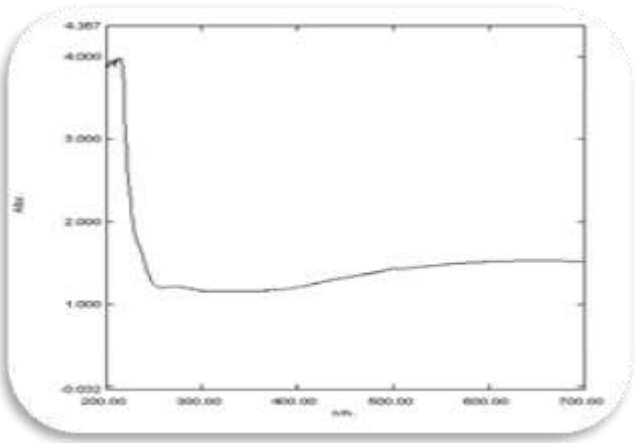

Figure.5: Metal Control

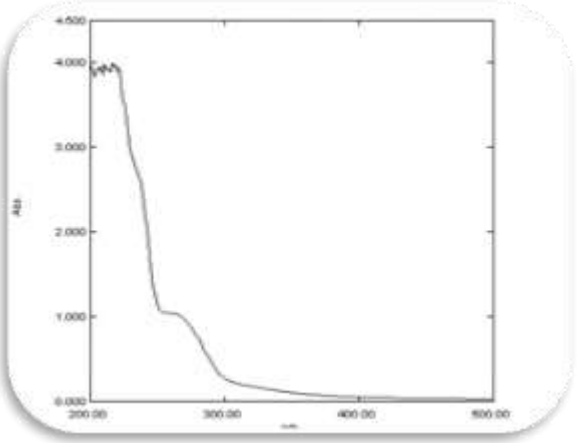

Figure.6: Culture Control

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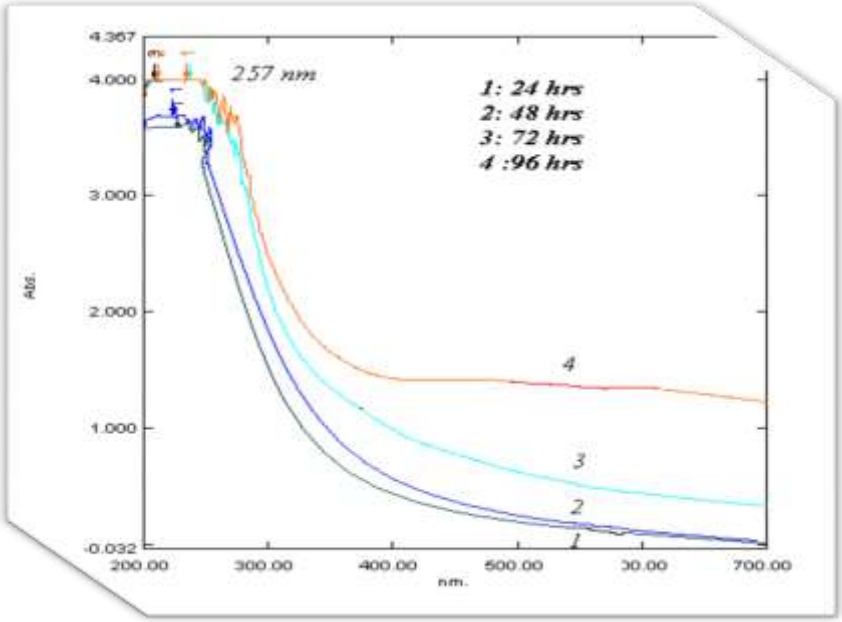

Figure.7: Uv-Vis spectra for titania nanoparticles obtained by *Fusarium oxysporum*

FTIR analysis of Titania Nanoparticles:

Fourier Transform Infrared (FTIR) analysis of titania nanoparticles from the fungus *Fusarium oxysporum*. titaniun dioxide reaction medium after 96 hrs of reaction showed the presence of a resonance at ca. 600cm^{-1}-1100cm^{-1} that are (Fig .8) these peaks correspond to exidation of Ti-O-Ti vibrational mode in the particles. A band at 834.67cm^{-1} weak 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.

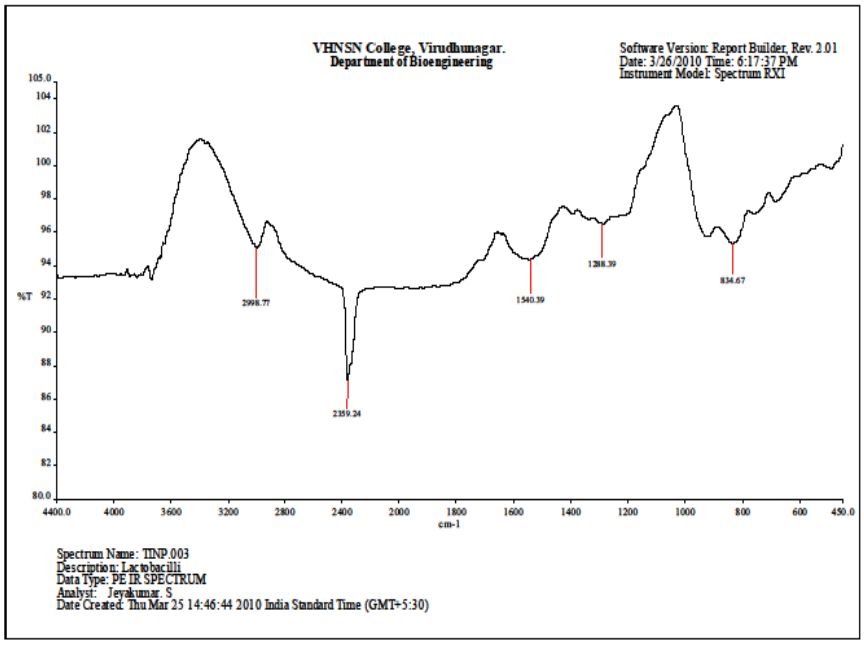

Figure.8: TINP.003 1976 4400.00 450.00 87.08 103.57 4.00 %T 1 1.00

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

4. DISCUSSION

The fungal 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. According to Park *et al.,* 1995 *Fusarium oxysporum* was isolated from tomato wilt plant. The morphological characters also identified similar finding was observed in present studies.In solid media culture, such as Potato Dextrose Agar (PDA), the different special forms of *Fusarium oxysporum* can have varying appearances. In general. The aerial mycelium first appears White, and then may change to a variety of colours- ranging from violet to dark purple- according to the strain (or special form) of *Fusarium oxysporum*. If sporodochia are abundant, the culture may appear cream or orange in colour

In present studies the growth characters of *Fusarium oxysporum* studied on different solid media indicated 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 in Potato Dextrose Agar and Saboroud Dextrose Agar.

Bansal, 2005 reported that, the fungus *Fusarium oxysporum* with aqueous anionic complexes results in the facile room temperature synthesis of crystalline titania particles while calcinations at 300°C required for crystallization of silica. The same report was observed in present study.In UV-Vis spectra of titanium nanoparticles, the band is 257 nm. Zecchina *et al.,* 1996; Astorina *et al.,* 1996 reported that the band in the range of 230-280 nm.

In FTIR analysis, the standard wave number of si-o-si rocking and bending vibration bond occurs about the 484cm^{-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 occur at the wave number of 660cm^{-1} and 624 cm^{-1}. In this sample wagging modes occur 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 present study the second strongest bond is 1113cm^{-1}. The Si-Si vibration modes standard wave number range 740cm^{-1}. The observed wave number was 743.91cm^{-1}. The C-H stretching mode standard wave number was 3400-3610cm^{-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 occurs at the standard wave number of 2960 cm^{-1} and 2853-2927cm^{-1} respectively (Norman *et al.,* 1990)The observed wave number of 2927.12cm^{-1}. The standard wave number of Si-CH=CH_2 Deformation mode occurs in the range of 1410-1590 $cm^{-1}$and observed wave number occur the 1412.04cm^{-1}.

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