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RESEARCH ARTICLE

BIOACCUMULATION AND CHARACTERIZATION OF TITANIUM NANOPARTICLE CONTAINING *ASPERGILLUS WENTII* BIOMASS AS A CHALLENGING PHOTOCATALYSTS

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Abstract

In current situation most of the water bodies and ground water was contaminated due to the seepage of the textile dye, paper & plastic industries and there by contaminate the drinking and irrigating water. Effort should be made to increase the treatment efficiencies of waste water generated. This is on only through eco-friendly microorganisms that can degrade and have a significant result. In recent year's application of nanoparticles to the environmental cleanup have been one of the most promising area. In the field of nano particle preparation researchers are now looking at biological system for their inspiration and in turn looking at microorganisms as eco-friendly nano factories. Among metal nano particles the Titanium nano particle act as best catalyst when microbial accumulated titanium nanoparticle activate photochemical reaction in order to reduce synthetic dyes. The present study was planned to determine the titanium nanoparticle accumulating activity of *A. wentii* and its photo catalytic effect on fast green dye degradation. The TiO_2 bio accumulating ability of *A. wentii* was determined and characterized using bright field microscopy, followed by scanning electron microscopy, EDAX spectral analysis and FTIR. The results revealed that the TiO_2 biomass exhibited higher significant level than that of untreated biomass. The photo catalysis removed 98.99%, 96.77%, 93.85%, 90.46% and 83.35% at a concentration of 500, 1000, 1500, 2000 and 3000 mg/L of fast green dye using TiO_2 treated biomass. By this one can indirectly enrich the soil fertility by reclamation of polluted water and soil.

1. Introduction

Minimum time, miniaturization and non-hazardous processes are key parameters for any kind of technology acceptance in Nano sciences. The use of microorganisms for the biosynthesis of metal nanoparticles has recently developed. In that aspect the fungal mediated green chemistry approach towards the synthesis of nanoparticle is potentially exciting since they secrete large amounts of enzymes, good mono dispersity, well defined dimension, easy scale up process, economic viability, possibility of easily covering large surface areas by suitable growth of the mycelia and this will play a challenging role in the process of synthetic dye degradation. Compared to bacterial fermentations, in which the process technology involves the use of sophisticated equipment for getting clear filtrates from the colloidal broths, fungal broth have a dual benefit, first it will try to degrade or bio accumulate the toxic dye substrate and secondly the broth can be easily filtered by filter press of similar simple equipment, thus saving considerable investment costs for equipment. Moreover added advantage in fungal mediated synthesis of nanoparticles would be of importance in catalysis as nanoparticles can be immobilized in different matrices and used for different catalytic processes. The only main disadvantage is that the genetic manipulation of eukaryotic organisms as a means of overexpressing specific enzymes identified in nanomaterial synthesis would be much more difficult than that in prokaryotes.

1.1. Biosynthesis of Nanoparticle Using Fungi : The exposure of *Verticillium sp*. to silver ions resulted in intracellular growth of silver nanoparticles (**Mukherjee P, et al., 2001)**.The exact mechanism leading to the intracellular formation of gold and silver nanoparticles by *Verticillium* is not fully understood at the moment. Since the nanoparticles are formed on the surface of the mycelia as a thin bio flim and not in the solution, it is thought that the first step involves the trapping of the metal ions on the surface of the fungal cells possibly via electrostatic interaction between the ions and the negatively charged carboxylate groups in the enzymes present in the cell wall of the mycelia. Thereafter the ions are reduced by enzymes present in the cell wall leading to the formation of the nuclei, which subsequently grow through the further reduction of metal ions and accumulation of these nuclei. The ability of *Verticillium* cells to multiply after exposing to metal ions proves the capability of using microorganisms in the synthesis of nanomaterials. Quite surprisingly, the plant pathogenic fungal strain *Fusarium oxysporum* behaved considerably differently; the reduction of the metal ions occurred extracellularly, resulting in the rapid formation of highly stable gold (**Mukherjee P, et,al.,**2002) and silver (**Ahmad A, et.al.,2003)** nanoparticles of 2- to 50-nm dimensions. Moreover, the aqueous extract of the fungal biomass can reduce gold and silver ions to the corresponding nanoparticles. Most probably, the reduction of the $AuCl4^-$ and Ag^+ ions occurs due to reductases released by the fungus into the solution, thus opening up a novel fungal/enzyme-based in vitro approach to nanomaterials. Similar to *Verticillum sp, A.wenti* also have the potential in degrading process of synthetic dye through photolytic activity by bio accmulation and adsorption of titanium particle. By considering this the current challenging work has been taken.

1.2. Microbial Uptake and Accumulation : Microorganisms possess mechanisms by which metal cations can be taken up and accumulated from their environment. There appear to be two main types of metal uptake by organisms. The first involves nonspecific binding of the metal to cell surfaces, slime layers, extracellular matrices, etc., where most heavy metals can be adsorbed onto the surface of microbial cells, both living and dead, and, in fact, the addition of dead bacterial cells to copper-inhibited laboratory cultures of bacteria is effective in reducing toxicity (**Griffiths A. J., et.al., 1975).**In yeasts, metabolism-independent surface binding is often to anionic groups of two species, polyphosphate and carboxyl, and such binding is rapid and reversible. Isolated cell walls of *S. cerevisiae* have been shown to bind their own weight of mercury to "high affinity" sites (**Murray A. D., et.al.,1975).**The second type of metal uptake, metabolism-dependent transport, has been studied in various algae and yeasts **(Broda E**. 1972, **Fuhrman G. F., et.al.,**1968, **Norris P. R., et.al.,**1977), bacteria (**Bucheder F., et.al.,**1974, **Doyle J. J., et.al.,** 1975, **Norris P. R., et.al.,** 1976)and fungi. At higher concentrations, intracellular precipitation of the metal may occur after uptake. This itself can be a means of detoxification since the metal is compartmentalized and may be converted to another more innocuous form. For example, certain yeasts are capable of precipitating thallium within the mitochondria as thallium oxide. The oxide may subsequently be discharged from the mitochondria and excreted from the protoplast. This is termed oxidative detoxification. There is also evidence for the intracellular deposition of iron, as ferrous sulfide, within the sulfate-reducing bacteria *Desulfo vibrio* and *Desulfo tomaculum(***Jones H. E., et.al.,** 1976).

1.3. Application of Titania Nanoparticles in photo catalytic degradation : In recent years, applications of titania nanoparticles to the environmental clean-up have been one of the most active areas in photo catalysis. This is inspired by the potential application of TiO2-based photo catalysts for the destruction of organic compounds in polluted air and wastewaters (**Ollis D.F, et.al., 1993)**because TiO_2 nanoparticles have high photo activity on the decomposition of organic materials, and chemically stable properties, etc. Titanium dioxide powders, added to organic contaminated water and illuminated by mild UV light, works as a photo catalyst, oxidizing dissolved toxic organic compounds into relatively benign species. Whereas organic compounds are not fully decomposed by conventional technology they can be completely decomposed to H_2O and CO_2 by photo catalysis. In addition, no secondary pollutants are generated in the latter process. There are many variables that affect the photo activity such as particle size, crystal structure, incident light intensity, pH of solution, and preparation method of particles. Crystal structure and particle size are considered as important factors that determine photo activity.

1.4. Mechanism Of Photo catalysis : When a photo catalyst is illuminated by the light stronger than its band gab energy, electron-hole pairs diffuse out to the surface of the photo catalyst and participates in the chemical reaction with electron donor and acceptor. To achieve a higher photo activity, it is essential to suppress the subsequent recombination process and to increase the lifetime of separated electron-hole pairs, so that fast electron transfer occurs from the surface to the adsorbed intermediates (**Hoffman M.R., et.al.,**1995, **Kominami K., et.al.,** 1997). Titanium dioxide, in the presence of UV light, generates reactive metabolic substances such as hydroxyl and superoxide radicals that cause degradation of organic compounds and, potentially, bacteria. During the irradiation

of TiO_2 with ultraviolet (UV) light, a UV photon is absorbed by a TiO_2 particle, and an electron (e–)-hole (h+) pair is generated. The e– and h+ may migrate to the surface of the photo catalyst particle and react with adsorbed reactants resulting in the desired process, or they may undergo undesired recombination by suitable titanium bioaccumulated microorganisms.

2. MATERIALS AND METHODS :

2.1. B**ioaccumulation of Titanium Nanoparticles by *A.wentii* :** The fungi *A.wentii* biomass was inoculated in Czapek Dox broth and after 24 h varying concentration of titanium namely 0.3mM. 0.5mM and 0.7mM were added to the broth and incubated in a rotary shaker at 27 ± 2 °Cat 125 rpm. The organism was allowed to grow in the presence of titanium for 3-5 days and the color difference was noted. The cells were harvested by centrifugation and the obtained biomass was washed generously with sterile distilled water to remove adsorbed titanium particles onto the surface. The biomass was dried at room temperature and was used for characterization studies.

2.2. Characterization of Titanium Nanoparticle Containing Biomass : The dried biomass was subjected to bright field microscopic studies, Scanning electron microscopic studies, EDAX spectral analysis and FTIR analysis. Bright field observations were made with the help of Nikon microscope with photographic attachment. Scanning electron microscopy was carried out using FEI- Scanning electron microscope equipped with EDAX spectral library. The FTIR analysis was carried out using Jasco model FTIR**.** FTIR spectra were obtained at a scanning speed of 2mm sec^{-1} at a resolution of 4 cm^{-1}. The completely dried samples were treated with spectral grade KBr for pelleting.

2.3. Photo Catalysis of Fast Green Dye : The photo catalysis experiments were carried out using various concentration of dye (500 to 3000 mg/L). 100 mg of untreated biomass and titanium nanoparticle containing biomass was added to 50 mL of fast green solution in a conical flask and kept in the direct sunlight for various time interval of 30 min up to 180 min. Appropriate controls were maintained for comparison of photo catalysis by titanium nanoparticle.

3. RESULTS :

3.1. Bioaccumulation of Titanium Nano particles by *A. wentii* : In the present study, *A.wentii* grown in Chepek Dox broth for 24 h was subjected to 0.3, 0.5 and 0.7mM of TiO_2 to augment titanium nanoparticle accumulation. The culture was visually observed for color change if any nanoparticles were formed or not. After 5^{th} day the broth containing 0.3mM of titanium remained colorless whereas 0.5mM and 0.7mM titanium containing broth changes color from colorless to orange indicating that some transformation of titanium has taken place (Figure 1) the biomass in all the flasks were impregnated with titanium which could not be observed in the control flask lacking titanium (Figure 1). *A. wentii* biomass grown with titanium (0.7mM) was harvested washed several times with distilled water to remove any unbound or untransformed titanium particles and were used for further studies.

3.2. Microscopic Observations of Biomass: The control and the TiO2 exposed biomass were microscopically analysed at 100X magnification using Nikon microscope with photographic attachment. The control image showed the presence of discrete septum in the hyphae along with prominent spores. Whereas when TiO2 biomass was imaged it showed the presence of dots onto the surface of the hyphae which were not present in the control (Figure 2). Hence these observations confirm the presence of titanium particles onto the surface of the TiO2 treated biomass.

3.3. SEM and EDAX Analysis : The control and the Ti treated biomass were subjected to SEM analysis at 3000X and 6000X using 20KV energy source. The control samples were smooth as observed in the bright field microscope whereas the titanium containing biomass showed precipitates of titanium on the surface. These images show that the titanium has been accumulated onto the surface and there is a chance for intracellular accumulation of titanium (Figure 3). The SEM images were subjected to energy dispersive analysis of X-rays (EDAX) spectrum to determine whether the precipitates present in the surface is titanium. The figure 3 EDAX spectrum confirms that the precipitates on the surface of the cells are titanium particles.

3.4. FTIR Analysis : IR analysis permits spectrophotometric observation of the adsorbent surface in the range of 400–4000 cm−1 and serves as a direct means for the identification of organic functional groups on the surface. An examination of the adsorbent before and after adsorption reaction possibly provides information regarding the surface groups that might have participated in the adsorption reaction and also indicates the surface sites on which

adsorption have taken place. The control biomass when subjected to FTIR analysis showed multiple sharp peaks indicating the presence of diverse surface functional groups. Sharp peaks at 3563.81 cm^{-1} and 3457.74 cm^{-1} indicated the presence of NH and OH group in the biomass surface. However in TiO_2 treated biomass the band shift has been noted from 3563.81 cm^{-1} to 3555.13 cm^{-1} indicating the presence of OH group. 3349.75 cm^{-1} to 2457 cm^{-1} and 3370. cm^{-1} to 2612.11 cm^{-1} in control and TiO2 treated biomass respectively indicates the presence of carboxylic group. The control spectra of untreated biomass showed sharp peaks and shoulder peaks in the range of 400 to 1000 cm^{-1} indicating the absence of Ti-O-Ti symmetry, however the broad peak observed at the range of 400 to 1000 cm^{-1} indicate the doping of titanium nanoparticles by the biomass.

3.5. Photolysis of Fast Green Dye : Nano-sized titanium dioxide (TiO_2) is an excellent candidate for a multi-purpose photo catalyst, because of its optical properties, including a high refractive index leading to a hiding power and whiteness, as well as it chemical stability and relatively low production cost. Along with appropriate control, TiO_2 containing biomass was used to analyze its photo catalytic degradation of fast green. The control biomass without TiO2 nano particles showed comparatively lower photo catalytic activity when compared to TiO_2 containing biomass (Figure 4). The photo catalytic profile of TiO_2 containing biomass showed increased dye removal/degradation. The photo catalysis removed 98.99%, 96.77%, 93.85%, 90.46% and 83.35% at a concentration of 500, 1000, 1500, 2000 and 3000 mg/L of fast green using TiO^2 treated biomass.

3.6. Photocatalytic Isotherms : Langmuir and Freundlich, are used to describe the adsorption data for a range of adsorbate concentrations. These isotherms relate adsorption density q_e (uptake of adsorbate per unit weight of adsorbent) to equilibrium adsorbate concentration in the ulk fluid phase, C_e. The Langmuir treats surface sites analogues to dissolved complexing ligands. It is derived by combining sorption equilibrium constant with a mass balance on the total number of adsorption sites. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. The model assumes uniform energies onto the surface and no transmigration of adsorbate in the plane of surface (**Langmuir I.,**1918.)
The isotherm is represented by the following equation.

$$q_e = Q_o b C_e / 1 + bC_e \quad (1)$$

where $C_e$is the equilibrium concentration (mg adsorbate per liter of solution) and q_e is the amount adsorbed (mg adsorbate per g of adsorbent) at equilibrium. The constant Q_o signifies the monolayer adsorption capacity (mg/g) and b is related to the energy of adsorption (L/mg). A linear expression for the Langmuir equation is

$$C_e/q_e = 1/Q_o b + C_e/Q_o \quad (2)$$

Plots of q_e vs C_e show the agreement of experimental data with Langmuir plots for fast green removal using untreated fungal biomass and titanium nanoparticle containing biomass. Langmuir constants, Q_o and b were determined from the slope and intercept of the respective plots. The values of correlation coefficient (r^2), which is a measure of the goodness of fit, confirms Langmuir equation results in closure prediction of the isotherm as compared to the experimental data. The Langmuir Q_o and b values for adsorption of fast green by untreated fungal biomass and titanium nanoparticle containing biomass are presented (Table 1)

TABLE 1: Langmuir and Freundlich plots for adsorption of fast green

Adsorbent	**Langmuir plot**			**Freundlich plot**		
	Q_o (mg/g)	**b (L/mg)**	**R^2**	**K_F ($mg^{1-1/n}$ $L^{1/n}$ g^{-1})**	**n**	**R^2**
Untreated biomass	1735.58	0.006	0.9916	86.616	2.322	0.8076
Titanium treated biomass	2722.90	0.016	0.9889	282.23	2.832	0.9998

Figure 1: BIOACCUMULATION OF TITANIUM NANOPARTICLE BY *A. wentii*

CONTROL

0.3mM of Ti

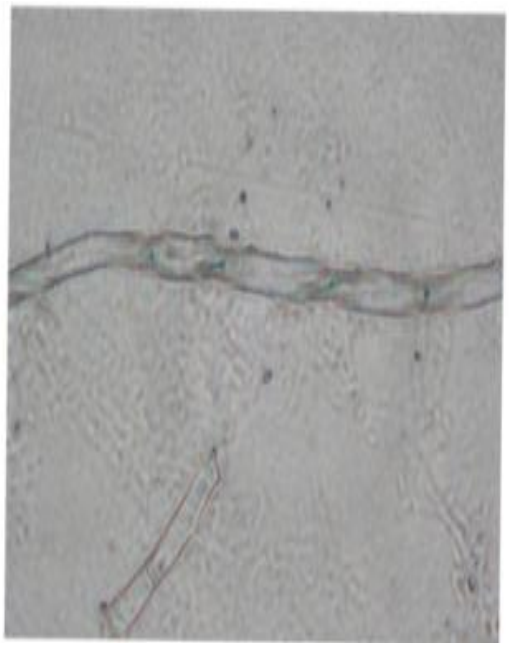
0.5mM of Ti

0.7 Mm of Ti

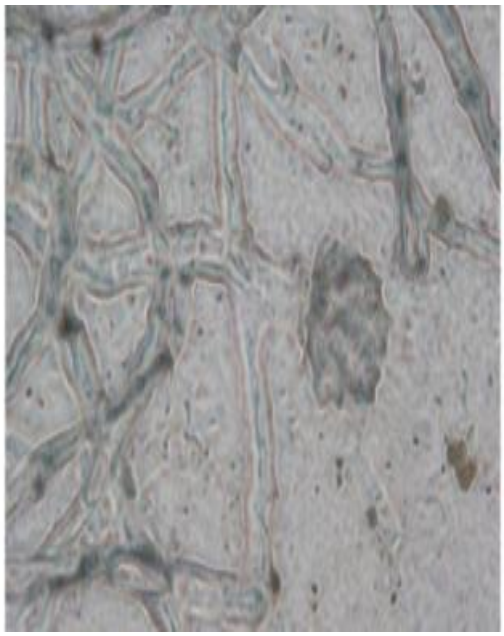
UNWASHED AND WASHED BIOMASS

Figure 2 : MICROSCOPIC CHARACTERISATION OF *A. wentii* BIOMASS

CONTROL (100 X)



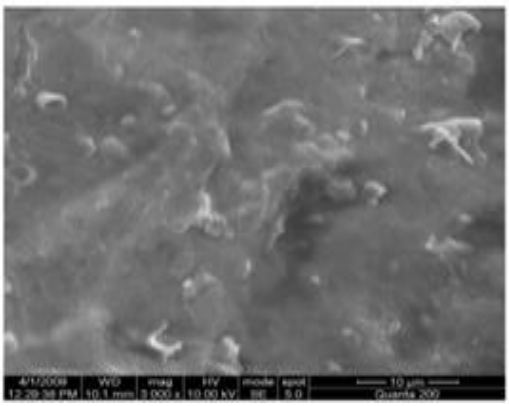
CONTROL (100 X)



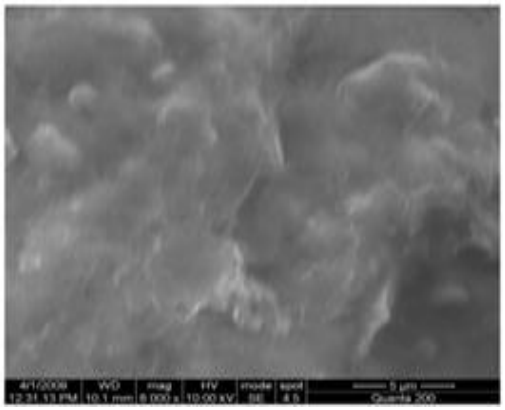
TI TREATED (100 X)

TI TREATED (100 X)

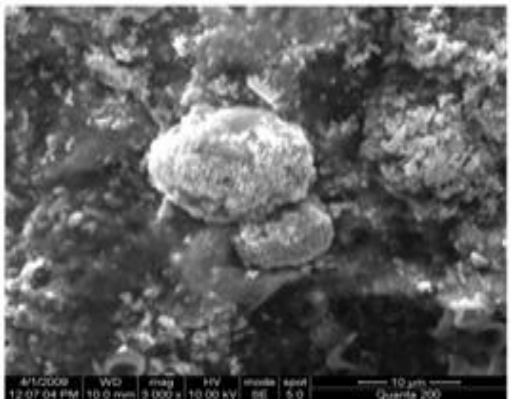
Figure 3 : SEM AND EDAX OF *A. wentii* BIOMASS



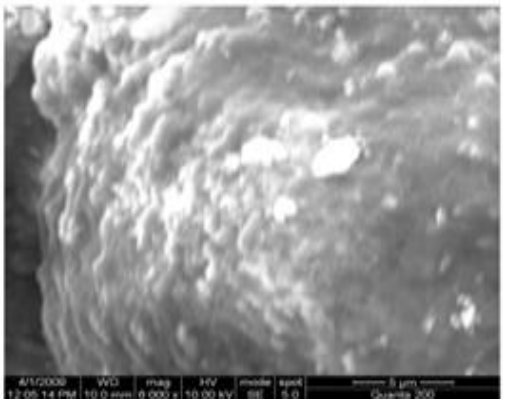
CONTROL (3000 X)



COONTROL (6000 X)



TI TREATED (3000 X)



TI TREATED (6000 X)

EDAX SPECTRA OF SEM

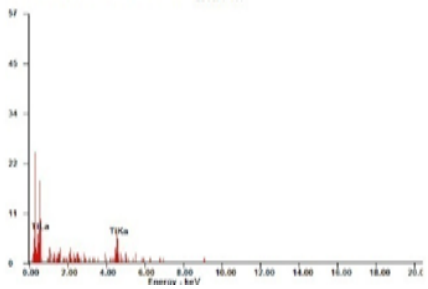


Figure 4 : ADSORPTION OF FAST GREEN DYE BY *A. wentii* BIOMASS

CONTROL LIVE AND Ti TREATED BIOMASS

4. DISCUSSION : The data presented in the table revealed that, the titanium nanoparticle containing biomass of *A. wentii* had a higher photocatalytic capacity for fast green when compared to the native fungal biomass. The photocatalysis was almost double that of the control biomass indicating that titanium treatment has resulted in the enhancement degradation of fast green dye removal. From this it is clear that a very small amount of microorganism in the presence of titanium nanoparticle can able to degrade detoriate and decolourise the textile industry waste effluent. This is one of the friendly approaches to get rid or degrade the synthetic dyes present in the waste water during the time of discharge. In the present study the TiO_2 bio accumulating ability of *A.wentii* was determined and characterized using bright field microscopy, followed by scanning electron microscopy, EDAX spectral analysis and FTIR. The surface functional groups present in the surface of TiO_2 treated biomass and control were compared and analysed. Based on the result it is clear that the titanium nanoparticles were accumulated on the inner surface of A. wenti biomass as a process of detoxification. The TiO_2 treated biomass was used for photo catalytic degradation of fast green dye. The results were compared with control biomass lacking TiO_2 treatment. The results revealed that the TiO_2 biomass exhibited higher significant level than that of untreated biomass. The photo catalysis removed 98.99%, 96.77%, 93.85%, 90.46% and 83.35% at a concentration of 500, 1000, 1500, 2000 and 3000 mg/L of fast green dye using TiO_2 treated biomass. By this one can indirectly enrich the soil fertility by reclamation of polluted water and soil. The bio treated industrial waste water is now safer to discharge. The photolytic activity was supported by S. Rengaraj, X.Z. Li(2005), in Enhanced photocatalytic activity of TiO_2 by doping with Ag for degradation of 2,4,6-trichlorophenol in aqueous suspension research work.

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