



LOVELY
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**DECOLOURIZATION OF TRIPHENYLMETHANE DYE BY
GREEN SYNTHESIS OF NANOPARTICLE**

**SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF**

**MASTER OF TECHNOLOGY
IN
BIOTECHNOLOGY**

SUBMITTED BY:

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UNDER THE SUPERVISION OF

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2. Isolation and characterization of amylase producing microbes from waste water
3. Green synthesis of d-block ^{metal} nanoparticles

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PAC Remarks:

- OK*
- Got Topic Copied ~~from~~
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*Supervisor should finally encircle one topic out of three proposed topics and put up for a approval before Project Approval Committee (PAC)

*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation final report.

*One copy to be submitted to Supervisor.

Abstract

Microorganism play very important role in bioremediation of toxic chemical by reduction of metal ions. In this study we have reported the decolourization of Triphenylmethane dye by silver nanoparticles synthesised using by *Pleurotus ostreatus* and its comparison with fungal culture. Studies were also carried out to evaluate the effect of pH, light intensity and incubation period on nanoparticle mediated decolourization of Triphenylmethane dyes. Affirmation of nanoparticles synthesis is given by the change in colour from milky white to dark brown. UV-visible analysis showed an absorbance at 440nm and 455nm for nanoparticles synthesised by mycelia and mycelia water extract respectively, thus confirming nanoparticle synthesis. Further characterization by FTIR, SEM-EDS and XRD revealed an average particle size of 58.8 nm, with the particles having spherical shape. Synthesized nanoparticles were further evaluated for the potential application of nanoparticles for decolorization of dye dyes namely against Malachite Green and Crystal Violet. Decolourization of crystal violet and malachite green by AgNP at optimized condition was 51.15% and 74.69% respectively after an incubation of 6 hours.

Keywords: FTIR, *Pleurotus Ostreatus*, Photocatalyst, Silver Nanoparticle, Triphenylmethane Dye, XRD

CERTIFICATE

This is to certify that **Naveen Kishan** bearing registration no. **11307903** have completed Dissertation - II project report (BTY 731), entitled “**Decolourization of Triphenylmethane Dye by Green Synthesis of Nanoparticle**” under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any university. The report is fit for submission and the partial fulfillment of the conditions for the award of **M. Tech. In Biotechnology**.

Er. Robinka Khajuria

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DECLARATION

I hereby certify that the work which is being presented in this report entitled **“Decolourization of Triphenylmethane Dye by Green Synthesis of Nanoparticle”** by Naveen Kishan in partial fulfillment of requirement for this award of degree of M.Tech. In Biotechnology submitted in Lovely Professional University, Punjab is an authentic record of my own work carried out under the supervision of Er. Robinka Khajuria. The matter presented in this report has not been submitted by me in any other University/Institute for the award of any Degree.

Naveen Kishan

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Date:

(Naveen Kishan)

Place:

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CHAPTER - 1

INTRODUCTION

Textiles dyes are intensely coloured chemical compounds which are used extensively in industrial sector such as textile industries for dyeing cotton, silk, nylon, in pharmaceutical industry and paper printing (US Environmental Protection agency, 2005). Since 1856, commercially worldwide, over 105 different dyes with annual production of more than 7×10^5 metric tons are produced and consumed (Ali 2010). Major class of chemical synthetic dyes used in textile industries includes azo, triphenylmethane and anthroquinone dyes and most of these dyes are xenobiotic in nature thus, recalcitrant to bio-degradation. Triphenylmethane dyes are among the most common synthetic compounds used in the textile industries comprising of 30-40% of the total dye usage. (Assadi *et al.*, 2003). These dyes eventually after use are released as effluent into water bodies thereby disturbing the natural flora and fauna of ecosystem. They affecting aquatic life by photosynthetic activity to reduce light penetration and toxic to organism cause detrimental effect in liver, intestine, kidney and gonads & in some cases like in rodent, it is highly toxic to mammalian cell and cause hepatic tumor formation (Chen *et al.* 2010; Fernandes *et al.* 1991). It is because of presence of metals, aromatics, and chlorides etc. (Kalyani *et al.* 2009).

Increase in the use of dyes and their rampant release in the water bodies, has led to a growing concern regarding the remediation of dye contaminated water. Removal of dyes from water bodies has been a big problem due to limited treatment strategies available that can effectively decolourise dyes. Various combination of biological, chemical and the physical methods have been reported for the treatment of contaminated water bodies. The physico-chemical methods employed for the removal of dye from effluent include methods such as adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction (Ansari & Thakur, 2006; Zhang *et al.*, 2002). Biological methods combined with chemical or physical methods are immensely effective in dye removal but high costs involved in these operations make them impractical for use on large scales.

Due to high costs, use of toxic chemicals and huge setup of machinery, biological methods are preferred over conventional methods. One of the strategies used in biological treatment of dyes involves the use of fungi specifically White-rot fungi. Wood degrading white-rot fungi has been reported to have the potential to degrade coloured industrial effluents. (Nilsson *et al.*, 2006; Palmieri *et al.*, 2005). These fungi have lignolytic properties which help in mineralize the dyes completely i.e. its major advantages. The first reported fungi are *Phanerochaete chrysosporium* used for crystal violet decolourization by bumpus

and Brock in 1988, *Trametes versicolor*, *Phanerochaete chrysosporium* and *Bjerkand raadusta* are able to completely decolorize the dye used by Heinfling *et al.* in 1997. The majority of experiments were done related to dye degradation by fungi but, some of them do not fulfill the culture condition of lignolytic treatment of dye-stuffs that's a major disadvantage.

An alternate strategy for remediation of dye contaminated water could be the use of nanoparticles as catalytic agents for the decolorization of dyes. Nanoparticles could impact water bodies' microbial structures to synthesize a new class of composites involving magnetic, metallic and molecular sieve. These have many applications as disinfectants, recyclable catalyst and sorbent for removal of pollution of environment. For dye removal use of metallic nanoparticles have good choice because of large surface area, anti-microbial agent surface disinfectant etc.

Several physical and chemical methods have been reported for the synthesis of nanoparticles. These methods suffer from major drawbacks, viz., high costs involved, intensive labour and time requirements, generation of large quantities of secondary waste (Nagarajan *et al.*, 2013). Because of these shortcomings, biosynthetic methods employing either microorganisms or plant extracts are rapidly emerging as a simple alternative for nanoparticle synthesis (Sindhur *et al.* 2013). Fungus mediated nanoparticles have advantage to others because of their property of degrading and easy to culture them in lab.

The aim of this study was to evaluate the potential of *Pleurotus ostreatus* for the synthesis of silver nanoparticle and their ability to decolorize triphenylmethane dyes.

CHAPTER - 2

TERMINOLOGY

2.1 Triphenylmethane dye

It is a class of synthetic dyes or compound which is used in the textile industries for dyeing clothes, used in paper mill and other industries.

2.2 Green Synthesis of Nanoparticles

Instead of using of chemical and physical process of production of nanoparticles using a biological process to produce it. By means of fungi, yeast, bacteria and plants.

2.3 Photo-catalysis

Photo-catalysis is increase of rate of photoreaction in the occurrence of a catalyst. In this process of photo-catalytic, light absorbed by an adsorbed substrate and increase the reaction.

CHAPTER - 3

REVIEW OF LITERATURE

Synthetic dyes are extensively used in the textile industries and significant proportion appears in the form of wastewater and is spilled into the environment. More than 10,000

synthetic dyes include the azo, anthroquinone and triphenylmethane dyes, all of which are generally considered as xenobiotic compounds, are released as water bodies (US Environmental Protection Agency, 2005). These dyes which are manufactured and used by textile, cosmetic, plastic, and printing industries are stable to light, chemicals and microbial degradation. (Kammoun, *et al.*, 2009; Yang, *et al.*, 2009). Up to 50% of the dyes are lost after dyeing process and about 10-15% of them discharged in the effluents of textile manufacturing industry (Sarayu, *et al.*, 2012). In the industrial wastewaters, dyestuffs from textile industries are most difficult to treat. As they usually have a synthetic origin and complex aromatic molecular structures which render them more stable and more difficult to be degraded (Fu & Viraraghavan, 2001).

Several methods including biological, physical, and chemical methods have been conventionally used to treat dye waste. Although, these conventional physico-chemical methods are versatile and useful, they also transfer the pollutant from one medium to another medium, and produce secondary waste products. Another innovative technology is based on bioremediation, which can serve inexpensive and eco-friendly way of degrading dyes from large volume of effluents (Asgher, *et al.*, 2012; Banerjee, *et al.*, 2007). For decolourization, microbial systems have great potential for achieving complete colour removal. The capacity of microorganisms, particularly white rot fungi (WRF), have a great potential to decolorize and remove a wide variety of structurally diverse pollutants including synthetic dyes. White rot fungi have an advantage over bacteria owing to their capability to degrade insoluble pollutants by producing extracellular ligninolytic enzymes such as laccase, lignin peroxidase, and manganese-dependent peroxidase (Elgueta, *et al.*, 2012; Noreen, *et al.*, 2011; Kumaran, *et al.*, 2011). Moore (2006) reported that, biodegradation of dyes by white rot fungi offers an advantage over other processes because of their ability to completely mineralize various dyes. There are majority of experiment were done related to dye degradation by fungi but, some of them do not fulfilled the culture condition of lignolytic treatment of dye-stuffs that's a major disadvantage.

Nanotechnology offers various approaches in the decolourization process. It involves development of metallic nanoparticles with novel and distinctive properties having wide range of application in different fields such as electronics, photonics and catalysis (Moore, 2006).

Synthesizing of different metallic nanoparticles, such as silver, gold, iron, zinc, copper etc., involves chemical reduction of metallic ions in aqueous solutions with or without stabilizing agents (Liz-Marzan and Lado-Touino, 1996), chemical reduction and photo reduction in reverse micelles (Sun *et al.*, 2001), thermal decomposition in organic solvents (Esumi *et al.*, 1990), and radiation chemical reduction (Henglein, 2001) have been reported. However, these methods suffer from several disadvantages such as they are expensive; use of toxic and hazardous substances and unique extreme physical condition (Jae & Beom, 2009). Therefore, there is a need to develop 'biogenic' process for nanoparticle synthesis that do not use toxic chemicals. Biological methods of preparation of nanoparticle synthesis using microorganism (Nair and Pradeep, 2002; Klaus *et al.*, 1999; Uruga & Konoshi, 2007), fungus (Vigneshwaran *et al.*, 2007), enzymes (Willner *et al.*, 2006), and plants or plant extracts (Chandran *et al.*, 2006; Shankar *et al.*, 2004; Jae & Beom, 2009) can serve as an alternatives to chemical and physical methods.

3.1 Need for dye removal from effluents

Due to their superior performance, synthetic dyes are preferred for use over natural dyes. Synthetic dye impart brighter colours, show better light-fastness and more resistant to washing, as compared to natural dyes. It also provides variety of colours. In industry, the presence of dyestuffs effluents is more than just an aesthetic problem. Dyes absorb sunlight strongly due to its chromophores (da Silva, *et al.*, 2010). These dye effluents effect the aquatic flora and photosynthesis. Presence of dyes in the water body increases the Chemical and Biological Oxygen Demand (Kagalkar, *et al.*, 2010).

Effluent containing dyestuffs have a large concentration of suspended solid. These factors upset the ecological balance of the receiving water body. Due to these reasons, it is necessary to remove colorants from the effluents before it is discharged into a water body.

3.2 Causes of recalcitrance of pollutants

In general, the chemical structure of dyes contains conjugated double bonds and aromatic rings. The removal of colouring matter from effluent is a major problem. Have a

characteristic azo (-N=N-) linkage which is electron withdrawing in nature. The presence of this linkage decreases the susceptibility of azo dyes to oxidative reactions. Many synthetic dyes tend to persist in the environment due to the inherent stability of their molecular structure (Maddhinni, *et al.*, 2006). The presence of tertiary and quaternary carbon atoms also contributes to recalcitrance. Recalcitrance of a given pollutant may sometimes be attributed to unusual substitutions with halides because of large molecular size, and presence of unusual bonds and highly condensed aromatic rings (Jogdand, 2006).

Triphenylmethane dyes eventually after use are released as effluent into water bodies thereby disturbing the natural flora and fauna of ecosystem. They affecting aquatic life by photosynthetic activity to reduce light penetration and toxic to organism cause detrimental effect in liver, intestine, kidney and gonads & in some cases like in rodent, it is highly toxic to mammalian cell and cause hepatic tumor formation (Chen *et al.* 2010; Fernandes *et al.* 1991). It is because of presence of metals, aromatics, and chlorides etc. (Kalyani *et al.* 2009).

3.3 Conventional processes for removal of dyes from effluent streams

In the textile industry conventional method used, for colour removal from effluent include physico-chemical method like coagulation/flocculation and activated carbon adsorption (da Silva, *et al.*, 2010). However, both flocculation and adsorption generate large amounts of sludge and waste, which require separate treatment before disposal.

However, two another methods known as advanced oxidation processes (AOP_s) is an effective means of decolourization of dye present in effluents. These processes involve the generation of highly reactive species like the hydroxyl radicals that have strong oxidative potential. In addition to these ozonation and fenton process is also a method used to treat dyes in effluents. But conventional process are very high running cost, low efficiency of removal, unsuitable for effluent containing high concentrations of pollutants (Farah, 2010). For example, for removing of colour activated charcoal is extremely effective, but can only be used for only small effluent volume involves high capital cost, and operates at low speeds.

Coagulation and Flocculation methods for treatment, are very fast and lower capital cost, but compared to them biological process have many advantages like degradation of molecules in form of CO₂, water and less sludge formation. It is eco-friendly. Activated sludge systems are used by the industry for treatment of effluent, but some dyes are toxic

to microbes. Table 1 enlist various techniques used for treatment of dye contaminated effluents.

Table 1: Different methods treatment of dye containing effluents (Reife, 1993).

Physical	Chemical	Biological
Adsorption	Neutralization	Stabilization Pond
Sedimentation	Reduction	Aerated lagoon
Flootation	Oxidation	Trickling Filter
Flocculation	Electrolysis	Activated Sludge
Coagulation	Ion-Exchange	Anaerobic Digestion
Foam Fractionation	Wet-Air Oxidation	Bio-augmentation
Polymer Flocculation	Ozonation	
Reverse Osmosis/Ultrafiltration		
Ionization Radiation		
Incineration		

3.4 Decolourization of Dyes

According to Zhou and Zimmermann (1993) decolourization of dyes may take place in two ways: either biodegradation of the dyes by the cells or adsorption on the microbial biomass. These dyes are decolourized and biodegraded into simple and smaller parts. For example, decolourization of triphenylmethane dye occurs when cleavage of chromophoriccenter of the dye occurs. It involves the various physic-chemical process and microorganism help in the decolourization of these complex structures.

3.5 Microbial Degradation of Dyes

Generally, white rot fungus was used for degradation of dyestuffs. The acclimatized microorganism show a positive and negative inhibition when dye concentration is either high or low respectively (Chen *et al.*, 2003). Different microorganism, including fungi, yeast, bacteria, and algae, have been used for decolourization degradation process of synthetic dyes. They show different capabilities of degrading different dyes. The effectiveness of microbial decolourization depends upon the activity and adaptability of selected microorganism. For development of better dye degradation, selected strain which is suitable for application in biotechnology, and in under favourable condition it is use for potential degradation (Novotny *et al.*, 2004b).

3.5.1 Degradation by Bacteria

Five cases have been reported, when it comes to the biodegradation of triphenylmethane dyes by bacteria. Biological degradation of triphenylmethane dye by bacterial strain 13 NA *Pseudomonas pseudomallei* was reported by Yatome *et.al.*, (1981). By the help of thin layer chromatography, reaction products of dye Methyl Violet and Crystal Violet are explained. Bacterial decolourization is faster in comparison to others (Kalyani *et al.* 2009). Bacteria decolourize azo dyes reductively in anaerobically condition to colourless aromatic amines.

This amine should be degraded because they may be mutagenic, carcinogenic and toxic to humans and animals (Chen 2006). *Bacillus sp.* VUS is used for navy blue (Dawkar *et al.* 2009), *Citrobacter sp.* used for crystal violet (An *et al.* 2002), *Enterobacter cloacae* is used for reactive black 5 (Wang *et al.* 2009), *Enterobacter sp.* Reactive red 195 (Jirasripongpun *et al.* 2007).

3.5.2 Degradation by actinomycetes

Yatome *et.al.*(1991) reported the biodegradation of dyes by actinomycetes. They reported in biodegradation of Crystal Violet by two strains of actinomycetes i.e. *Nocardia globberula* and *Nocardia corallina*. After 24 hr sit was observed that dyes had completely decolourized.

In 1993 again, Yatome *et al.*, publish another report related to degradation of triphenylmethane by actinomycetes. In this report *N.corallina* could not decolourize Auamine O, a example of typical triphenylmethane dye. *N.corallina* decolourized Ethyl violet, Methyl violet, Victoria blue and Basic fuchsin to a much greater extent.

3.5.3 Degradation by fungi

The class of fungi which is most efficient in breaking down of synthetic dyes by white-rot fungi (Couto, 2009). It is efficient because it produces an efficient enzyme which decomposes the dyes. They produce various oxidoreductases that degrade aromatic and lignin compounds (Nozaki *et al.*, 2008). The property of ligninolytic fungi and its enzyme to oxidize a wide variety of organic pollutants involving dyes is due to an extracellular and non-specific enzyme system consisting manganese peroxidase (MnP), lignin peroxidases (LiP), and laccases (Kuhad *et al.*, 2004; Couto, 2009; Enayatzamir *et al.*, 2009).

Bumpus and Brock, in 1988 reported biological degradation of triphenylmethane dye i.e. Crystal Violet by *Phanerochaete chrysosporium*. In the broth culture, by sequential N-demethylation the disappearances of Crystal violet were disappeared. In 1995, Yesilada reported use of hydrogen per-oxide system in the extracellular fluid to demethylate Crystal Violet.

Wood rotting micro-organisms are good in degradation process of synthetic dyes. This is so because of lignocellulose containing material contains complex molecules with similar structure as synthetic dyes, which might microorganism growing on these materials are adapted to these organic compounds. This white rot fungus and their excretion of extracellular enzymes are capable to degrade whole synthetic compound of dye (Fross and Welander, 2009).

3.5.4 Degradation of Dyes by Nanoparticles

Remediation of waste streams containing dyestuffs, cleaning up of heavy metals from contaminated soil and water by absorption and sequestration are possible using nanoparticles. Nanotechnology is fast gaining importance in wastewater treatment. As it can offer more effective methods to decontaminate xenobiotics in the environment. Nanoparticles have a very large surface area to volume ratio, high reactivity and sequestration properties all of which have immense potential for use in wastewater treatment. The use of nanoparticles in Reactive Remediation Technology is of great interest to wastewater treatment, since it involves the complete degradation of contaminants to harmless products such as carbon dioxide and water (Fulekar, 2010).

Another type of novel nanoparticle is nanosponges. These are materials containing microscopic particles with nano-sized cavities. These particles can encapsulate or can be embedded with many types of substances and are capable of transporting them through an aqueous medium. Novel nanoparticles such as these could be synthesized using enzymes such as peroxidase and laccase. Such nanosponges could find application in remediation of wash streams from dyeing and textile processing industries. At present, the utilization of nanopolymers in wastewater is primarily in the removal of heavy metals. In recent times, research on carbon based nanotechnology such as the carbon nanotube is gaining momentum. The potential of these particles for use in remediation of soil, water and air is being evaluated. Carbon nanotubes carrying immobilized enzymes have been synthesized and incorporated into latex paints. The resulting materials can detect and eliminate hazardous chemical and biological agents (Watlington, 2005). Similarly, nanotubes

carrying oxidative enzymes such as laccases or peroxidases could be synthesized for utilization in treatment of recalcitrant pollutants in wastewater. In the near future, carbon nanotubes are expected to be utilized to a large extent in water treatment (Theron, *et al.*, 2008).

3.6 Biological Synthesis of Nanoparticles by Microorganism

Metal nanoparticles are widely being applied to areas of human contact (Jae & Beom, 2009), therefore there is a for growing need to develop 'biogenic' process for nanoparticle synthesis that do not use toxic chemicals. Biological methods of preparation of nanoparticle synthesis using microorganism (Nair & Pradeep, 2002; Klaus *et al.*, 1999; Uruga & Konoshi , 2007), fungus (Vigneshwaran *et al.*, 2007), enzymes (Willner *et al.*, 2006), and plants or plant extracts (Chandran *et al.*, 2006; Shankar *et al.*, 2004; Jae & Beom, 2009) serve as alternatives to chemical and physical methods of nanoparticle synthesis. In case of biological synthesis of metal nanoparticle, the stabilizer and the reducing agent are substituted by molecules produces by living organisms. These stabilizing or reducing compounds can be used from fungi, bacteria, algae, yeasts, or plants (Sintubin *et al.* 2012).

Synthesizing of different metallic nanoparticles, such as silver, gold, iron, zinc, copper etc., involves chemical reduction of metallic ions in aqueous solutions with or without stabilizing agents (Liz-Marzan & Lado-Touino, 1996), chemical reduction and photo reduction in reverse micelles (Pileni, 2000; Sun *et al.*, 2001), thermal decomposition in organic solvents (Esumi *et al.*,1990), and radiation chemical reduction (Henglein, 1993, 1998, 2001) have been reported. Table 2 summarize microbial synthesis of nanoparticle.

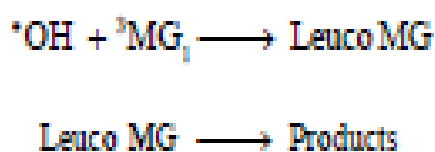
Table No. 2 Some typical metal nanoparticles produced by microorganisms

Microorganism	Products	Culturing temperature (°C)	Size (nm)	Shape	Location
<i>Sargassumwightii</i>	Au	Not available	8-12	Planar	Extracellular
<i>Plectonemaboryanum</i>	Au	25-100	<10-25	Cubic	Intracellular
<i>Plectonemaboryanum UTEX 485</i>	Au	25	10nm-6µm	Octahedral	Extracellular
<i>Candida utilis</i>	Au	37	Not available	Not available	Intracellular
<i>V. luteoalbum</i>	Au	37	Not available	Not available	Intracellular
<i>Escherichia coli</i>	Au	37	20-30	Triangles, hexagons	Extracellular
<i>Yarrowialipolytica</i>	Au	30	15	Triangles	Extracellular
<i>Pseudomonas aeruginosa</i>	Au	37	15-30	Not available	Extracellular
<i>Trichodermaviride</i>	Ag	27	5-40	Spherical	Extracellular
<i>Phaenerochaetechrysosporium</i>	Ag	37	50-200	Pyramidal	Extracellular
<i>Bacillus licheniformis</i>	Ag	37	50	Not available	Extracellular
<i>Escherichia coli</i>	Ag	37	50	Not available	Extracellular
<i>Corynebacteriumglutamicum</i>	Ag	30	5-50	Irregular	Extracellular
<i>Trichodermaviride</i>	Ag	10-40	2-4	Not available	Extracellular
<i>Aspergillusflavus</i>	Ag	25	8.29±40	Spherical	Extracellular
<i>Aspergillusfumigatus</i>	Ag	25	5-25	Spherical	Extracellular
<i>Verticillium sp.</i>	Ag	25	25±8	Spherical	Extracellular
<i>Fusariumoxysporum</i>	Ag	25	5-50	Spherical	Extracellular
<i>Neurosporacrassa</i>	Au, Au/Ag	28	32,20-50	Spherical	Extracellular
<i>Shewanella algae</i>	Pt	25	5	Not available	Intracellular
<i>Enterobactersp</i>	Hg	30	2-5	Spherical	Intracellular
<i>Shewanellasp</i>	Se	30	181±40	Spherical	Extracellular
<i>Escherichia coli</i>	CdTe	37	2.0-3.2	Spherical	Extracellular

3.7 Mechanisms of Biodegradation of Triphenylmethane Dye

From various studies conducted so far it becomes clear that a two-step mechanism viz. the physical adsorption and enzymatic degradation are involved in dye decolourization by the white rot fungus. The class of Fungi which is most efficient in breaking down of synthetic dyes by White-rot fungi (Couto, 2009). It is efficient because it produces an efficient enzyme which decomposes the dyes. They produce various oxidoreductases that degrade aromatic compound and lignin compounds (Nozaki *et al.*, 2008).The property of ligninolytic fungi to oxidize a wide variety of organic pollutants including dyes is due to an extracellular non-stereo selective and non-specific enzyme system consisting manganese peroxidase (MnP), lignin peroxidases (LiP), and laccases (Kuhad *et al.*, 2004; Couto, 2009; Enayatzamir *et al.*, 2009).

Use of many individual processes may not often sufficient to attain complete decolourization. So to do this new techniques focused that can complete the decolourization of dye molecules. Photocatalysis is one of the advanced oxidation process, which is new method actually (Perez *et al.*, 2006). Heterogeneous photocatalysis has the diverse phase from reactant. Ameta *et al.* reported in 2014 that using of lead chromate powder in photocatalytic decomposition of malachite green which is triphenylmethane dye is successfully observed. On their observations, tentative mechanism for degradation mechanism is shown in FigNo 1.



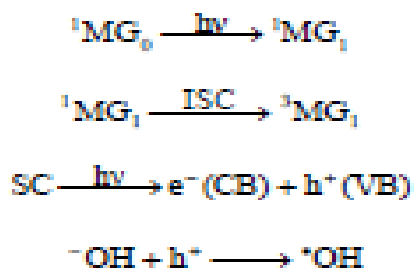


Fig No. 1 Mechanism of degradation of Malachite green by photocatalytic using lead chromate (Ameta *et al.*, 2014)

Malachite green absorbs radiations of preferred wavelength and it is excited giving 1st singlet state. Additionally, it undergoes intersystem crossing (ISC) to give stable triplet state. Laterally with this, semiconducting lead chromate (SC) utilizes this energy to stimulate its electron from valance band to conduction band. Electron can be distant from hydroxyl ion by hole (h^+) present in valance band of semiconductor producing $^*\text{OH}$ radical. This hydroxyl radical will react malachite green to its leuco form, which may eventually degrade to products. It was definite that $^*\text{OH}$ radical take part as an active oxidizing class in degradation of malachite green.

In 1995, Knapp and Newby observed that in many cases adsorption of dye in the microbial cell surface was primary mechanism of decolourization. In 1997, Young and Yu reported that by intra and extracellular enzymatic degradation is the reason for colour removal. The dye-saturated mycelium can be regenerated and used for repeated dye adsorption (Fig no 2) (C. Yatome *et al.*, 1993).

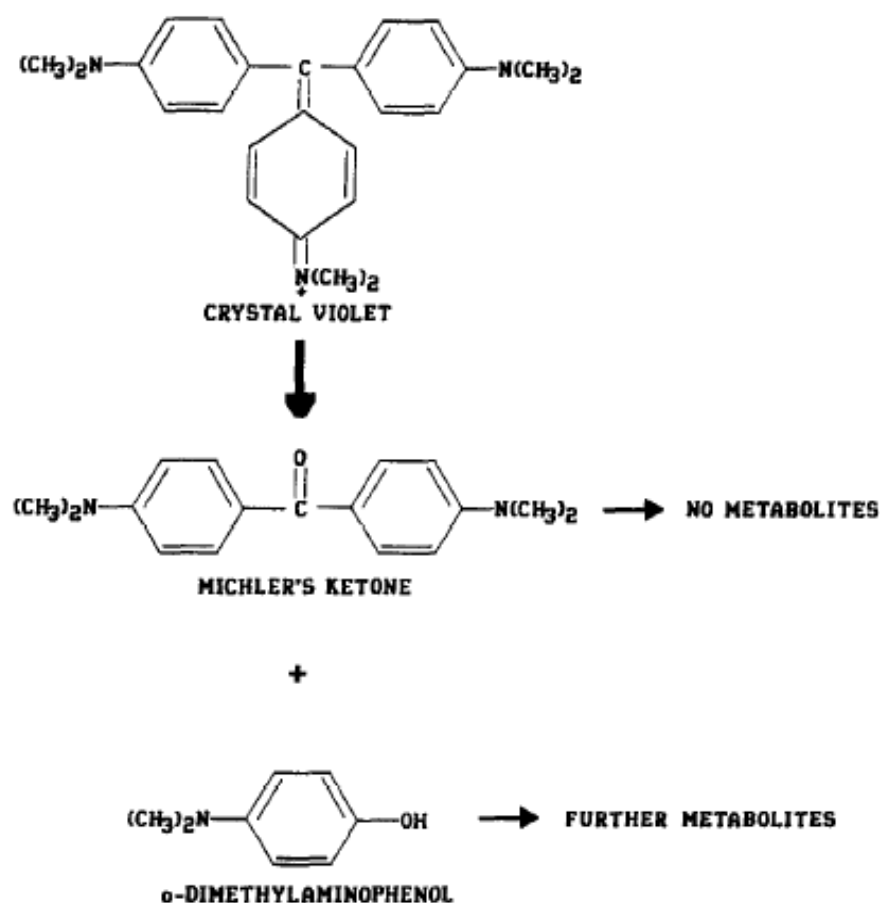


Fig No. 2 Degradative pathway of Crystal violet (Yatome *et al.*, 1993)

The early stages of triphenylmethane dye decolourization involves the breaking of trityl group as shown in fig., the ease of which has been found to be depending on the identity, number and position of functional group in the aromatic region and the resulting interaction with the trityl group. Further degradation involves aromatic cleavage which has also been found to be dependent on the identity of the ring substituent.

Limited number of studies attempted on molecular characterization of dye degradation (Kuhadet.*al.*2004). There is a gap in the bicolorization mechanisms of dyes by white-rot fungi and their activity of lignolytic enzymes (Couto, 2009). With new advanced analytical instrument techniques, study of various inter-mediate and degradational product of synthetic dyes can be achieved. These studies are very important value in many ways. Figure No 3 shows, proposed mechanism and degradative pathways of some dyes including triphenylmethane dye, azo dye and anthraquinone dye are presented below:-

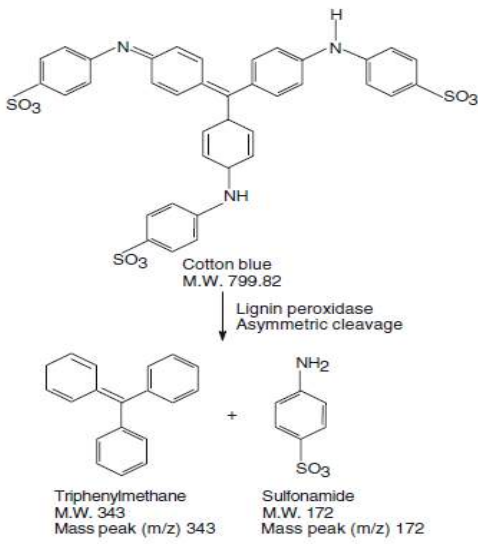
Name of synthetic dye	Proposed scheme of Degradation mechanism	Descriptions
Cotton Blue (Triphenyl methane Dye)	 <p style="text-align: center;">Cotton blue M.W. 799.82</p> <p style="text-align: center;">↓ Lignin peroxidase Asymmetric cleavage</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Triphenylmethane M.W. 343 Mass peak (m/z) 343</p> </div> <div style="text-align: center;"> <p>Sulfonamide M.W. 172 Mass peak (m/z) 172</p> </div> </div>	By <i>Penicillium ochrochloron</i> (Shedbalkar <i>et al.</i> , 2008)

Fig No. 3 Degradation mechanism of cotton blue (Shedbalkar *et al.*, 2008)

3.8 Characterization of Biodegradation Products

It is important to study the products of biodegradation of synthetic dyes and in order to know about the environmental fate of these pollutants. To analyze the treated water with regard to dye contents and its intermediates, especially aromatic amines, some are considered as carcinogenic in nature (Forss and Welander, 2009).

The aim of biodegradation of dyes is to remove the colour but, also eliminate and detoxify the dye constituents, a process called detoxification. For detailed characterization of the intermediates and produced metabolites during the degradation process, there must be assurance of the safety procedure (Kaushik and Malik, 2009; Couto, 2009).

There are various basic and advanced techniques available like chromatography and spectroscopy that can be used to characterize and isolate the products of degradative dyes. Thus, it gives the insight into the pathways and mechanism of biodegradation.

Relatively simple techniques like thin layer chromatography (TLC) and UV-visible spectrophotometer can be used. This techniques involves the absorption of dye particles on the microbial cell surface or by the breakdown of synthetic dye structure by the living microbial system or both. Till date, very few knowledge are available about the product of biodegradation or the intermediates of triphenylmethane dyes (Chen *et.al.* 2008).

3.9 Factors affecting Bio-degradation of dyes

3.9.1 pH

Generally, yeasts and fungi show good decolorization and degradation activities at neutral or acidic pH. But, bacteria at basic pH or neutral pH, Nozaki *et.al.* 2008, reported the decolorization of 27 dyes of different class, including diazo, monoazo, phthalocyanine, and triphenylmethane dyes, by using different 21 basidiomycetes. They found 3.0-5.0 is the optimum pH for decolorization of dyes.

For decolorization of synthetic dyes by white rot fungus *Lentinus polychrous* studied by Sarthimaet.al. 2009. They found 9.0, 3.0, 4.0, and 4.0-5.0 is the optimum pH for decolorization of Remazol Brilliant Blue R (RBBR), Methyl Red, Indigo Carmine, and Bromophenol Blue respectively. It has been detected that the rate of photocatalytic bicolourization of malachite green which is a triphenylmethane dye, increases as pH was increases and it attained optimum value at pH 7.5 to 9 (Ameta *et al.*, 2014)

3.9.2 Temperature

It is an important environmental factor and by changing in temperature biodegradation of microorganism is affected. That's why in summer, dead body decomposed fastely as compared to winter. It is because; the warmer condition is favourable for the multiplication and growth of the decomposers generally, in summer. Beside the optimum temperature degradation activities are decrease because of reproduction rate and slower growth and deactivation of degradative enzymes of microorganism. Thus, the performance of biodegradation by microorganism will be good at the optimum temperature needed for the reproduction, growth, and activities. Different optimum growth temperature is required for different fungi, mostly grown on 25-35⁰C (Fu and Viraraghavan, 2001).

Shedbalkar *et.al* .in 2008, studied that the suitable temperature for decolorization of Cotton Blue by MTCC 517 *P.ochrochloron* was at 25⁰C. Decolorization studied done by Jadhav *et.al.*, 2008 in which methyl red was used.

3.9.3 Initial Dye Concentration

On microbial decolorization, effect of initial dye concentration of synthetic dye is studied. On increasing dye concentration, there is decrease in dye decolorization. Some studies showing the effect of initial concentration of dye on microbial decolorization of synthetic dyes.

Gopinath *et. al.*,2009 reported, biodegradation of Congo Red by *Bacillus sp.*, obtained from effluent of tannery industry and it was observed on increasing the initial dye concentration there is decrease in decolorization rate at concentrations of 1,500 and 2,000 mg L⁻¹. Jirasripongpun *et.al.*, 2007, reported that *Enterobacter sp.* was unable to grow on higher concentration of dye, it was found to be dead when dye concentrations of 50and 100 mg L⁻¹ of Reactive Red 195 were test for decolorizing activity. At high dye concentrations it was toxic to the cells with to fungus.

3.9.4 Nitrogen Content in Medium

The Nitrogen content in medium for growth and decolorization of media of synthetic dyes may affect the microbial activity. It may affect the dye decolorization by altering production of enzyme by fungi; for different species of fungi, the lignolytic activity activity is suppressed by high concentration of nitrogen (25-60 mM) by Kaushik and Malik, 2009.

According to Bumpus and Tatarko in 1998, reported that addition of supplemental N only inhibited Bicolorization of Congo Red in high amounts of nutrient nitrogen containing plates.

3.9.5 Shaking

Many reports are available on the effect of agitation/shaking on decolorization of synthetic dyes by microbes. Some authors said, decolorization is increased by shaking while some said by static conditions. By Kalyani*et.al.*,2009 , in 24h *Pseudomonas sp.* SUK 1 agitated culture showed almost no decolorization and static culture showed more than 96% decolorization of the initial dye content of 300 mg L⁻¹ concentration of Reactive Red 2 in 6 h.

According to Husseiny (2008), decolorization study of Direct Red 81 and Reactive Red 120 by *Aspergillusniger*, by static conditions it were more efficient than shaking. In static conditions, higher enzymatic activities were observed (Kaushik and Malik 2009).

3.9.6 Aerobic/Anaerobic Culture Conditions

In number of cases, it was observed that the aerobic treatment efficiency is inferior to anaerobic condition of decolorization process (Forgacs *et.al.* 2004). Azo dyes were degraded by bacteria under aerobic conditions to toxic colourless aromatic amines, some of which are metabolized under aerobic conditions by Steffan *et.al.* in 2005.

3.9.7 Light Intensity

It is very important factor which affect the rate of degradation of dye molecules but after some point rise in light intensity there is no change observed. This is due to the difference of light intensity as light intensity rises number of photons increases to reach the active site of catalyst so amount of excited catalyst particles raises and subsequent rise the number of hydroxyl radicals then super oxide ions (O_2^-) and rate of degradation of dye particles also rise. This may reason that maximum number of photons which essential for excitation are available in fix series irradiating light intensity after it if we more raise light intensity no any extensive change observed in rate of degradation since there is no necessity of more photons for excitation (Swati *et al.* 2012).

CHAPTER - 4

SCOPE OF THE STUDY

In this topic of research, triphenylmethane dyes were taken for degradation process because these dye have aromatic region which is not degrade easily and are soluble in water. These dyes are toxic and carcinogenic in nature and are released in form of wastewater discharged from textile industries. That's why, it is necessary to develop strategies for degradation of these dyes. White-rot fungus is highly efficient in breaking down synthetic dyes as it produce sefficient enzymes capable of degrading dyes under aerobic conditions. However, fungus based remediation techniques suffer from several disadvantages. Therefore, there is a need to search for alternative methods for treatment of dyes contaminated effluent and photocatalytic degradation using nanoparticles may have the potential to remediate dye polluted water bodies and hence needs to be evaluated extensively.

CHAPTER - 5

AIMS & OBJECTIVE

The aim of this work was to investigate the potential application of green synthesized Silver nanoparticles using *Pleurotus ostreatus* for decolorisation of textile dyes namely Malachite Green and Crystal Violet. The following studies were carried out to meet objectives.

1. To maintain biomass of *Pleurotus ostreatus*.
2. To synthesise silver nanoparticles using mycelia and mycelia water extract.
3. To characterized the synthesised nanoparticles by UV-visible studies, FTIR, SEM - EDS analysis, and XRD measurement.
4. To evaluate nanoparticle mediated decolourization of Malachite Green and Crystal Violet.
5. To study the effect of light intensity, pH and incubation on dye decolourization.

CHAPTER - 6

RESEARCH METHODOLOGY

6.1 Microorganism

The organism used in this study was *Pleurotus ostreatus*, an edible fungus was taken from the Department of Microbiology, P.A.U (Punjab Agriculture University, Punjab) .The culture was maintained in Potato dextrose agar and incubated at 25°C until confluent growth was achieved. These plates were stored and 4°C maintained in the active stage by transferring Mycelia plugs aseptically on fresh plates of PDA from time to time.

6.2 Biomass production

P.ostreatus was inoculated in the potato dextrose broth for and incubation at 25°C in rotatory shaker (150 rpm) till confluent growth was achieved.

6.3 Synthesis of nanoparticles

Fungal mycelia was filtered, washed thrice with sterile deionised water to remove media and inoculated in sterile deionised. This was followed by incubation at 25°C for 3 days under stationary conditions. The biomass was filtered by whattman filter paper no.1. To the filtered biomass 4mM silver nitrate was added and incubated in dark conditions at 30°C under shaking conditions for 24 hours.

6.4 Characterization of the nanoparticles

6.4.1 UV-Visible Spectral Analysis

Conformation of fungus dependent bio-reduction of silver ions in aqueous solution to silver nanoparticles was carried out UV-Vis Spectroscopy at room temperature (Sun et al., 2001). The samples were subjected to a wavelength scan ranging between 280-800nm.

6.4.2 SEM-EDS studies

The data is collected by the UV-visible spectroscopy are then, confirm by the SEM-EDS images. Scanning electron microscopy is an instrument which help in the study of

nanoparticles, it give the images of nanoparticles and tells its shape, distribution, aggregation and size. Samples were dispersed in ethanol for 4hrs then after that sample were coated with Au for better resolution. After that sample is placed in SEM-JEOL for analysis at magnification rate of 30000x, 50000x.

6.4.3 FTIR

In Fourier transform infrared spectroscopy (FTIR) analysis, dried nanoparticles is further used in mixing with Potassium bromide at ratio 1:100 and spectrum will be recorded using a diffuse reflectance accessory.

6.4.4 XRD analysis:-

The vacuum-dried nanoparticles will be used for powder X-ray diffraction (XRD) analysis. The spectra recorded in aX'Pert Pro PAnalytical X-ray Diffractometer (Cu K α radiation, λ 1.54060) running at 45 kV and 30 mA. The diffracted intensities recorded from 20 degrees to 80 degrees 2θ angles. The scan axis used is Gonio and type is continuous.

.6.5 Decolourization of dyes by *Pleurotus ostreatus*

Two triphenylmethane dyes namely Crystal violet and Malachite Green with the concentration of 5, 10, 15mg/L respectively were added in growth media (Table No.3). Final pH of media was maintained at 7.3 ± 0.2 at 25°C . The media were inoculated with *P. Ostreatus* bits, followed by incubation under shaking condition (1500rpm) at 25°C for 1 week. Samples were regularly withdrawn after an interval of 24 hours and centrifuged at 4400rpm for 5mins and extent of dye decolourization was estimated using U.V- Visible spectroscopy at 590nm and 621nm for crystal violet & malachite green respectively. Media containing only dyes served as control and was subjected to similar treatments as the sample. The decolourization efficiency was expressed by following equations given by Nithya and Rangunathan, 2011:

$$\text{Decolourization (\%)} = \frac{[(\text{Initial Absorbance} - \text{Final Absorbance}) / \text{Initial Absorbance}] \times 100}$$

Table No. 3 Growth media for *Pleurotus ostreatus*

Components	Amount (in g/l)
Sucrose	30
NaNO ₃	3
KCl	0.5
MgSO ₄ .7H ₂ O	0.5
FeSO ₄ .7H ₂ O	0.01
K ₂ HPO ₄	1

6.6 Decolouration by Silver Nanoparticles

6.6.1 Effect of Light intensity on Nanoparticles mediated Decolourization

50ppm silver nanoparticles were added to 10mg/L Crystal violet and Malachite Green, followed by incubation in sunlight. Samples were withdrawn after regular intervals of 2 hours and absorbance was taken at 590 and 621nm for crystal violet & malachite green respectively. 10mg/l dyes dissolved in water served as control. All the experiments were carried out in duplicates. The decolourization efficiency was expressed by following equations as per the method given by Nithya and Raguathan, 2011.

$$\text{Decolourization (\%)} = \left[\frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}} \right] \times 100$$

6.5.2 Effect of pH on Nanoparticles mediated Decolourization

For this study, 50ppm of silver nanoparticle were added to 10mg/l of crystal violet and malachite green, followed by incubation with light with range of pH from 5-11. One absorbance was taken immediately and after 6hr of incubation, samples were withdrawn and absorbance was taken at 590 and 621nm for crystal violet & malachite green respectively. 10mg/l dyes dissolved in water served as control. All the experiments were carried out in duplicates. The decolourization efficiency was expressed by following equations as per the method given by Nithya and Raguathan, 2011.

$$\text{Decolourization (\%)} = [(\text{Initial Absorbance} - \text{Final Absorbance}) / \text{Initial Absorbance}] \times 100$$

6.5.3 Decolourization study by incubation of light intensity

50ppm silver nanoparticles were added to 10mg/L Crystal violet and Malachite Green, followed by incubation in sunlight. Samples were withdrawn after regular intervals of 24 hours and absorbance was taken at 590 and 621nm for crystal violet & malachite green respectively. 10mg/l dyes dissolved in water served as control. All the experiments were carried out in duplicates. The decolourization efficiency was expressed by following equations as per the method given by Nithya and Raganathan, 2011.

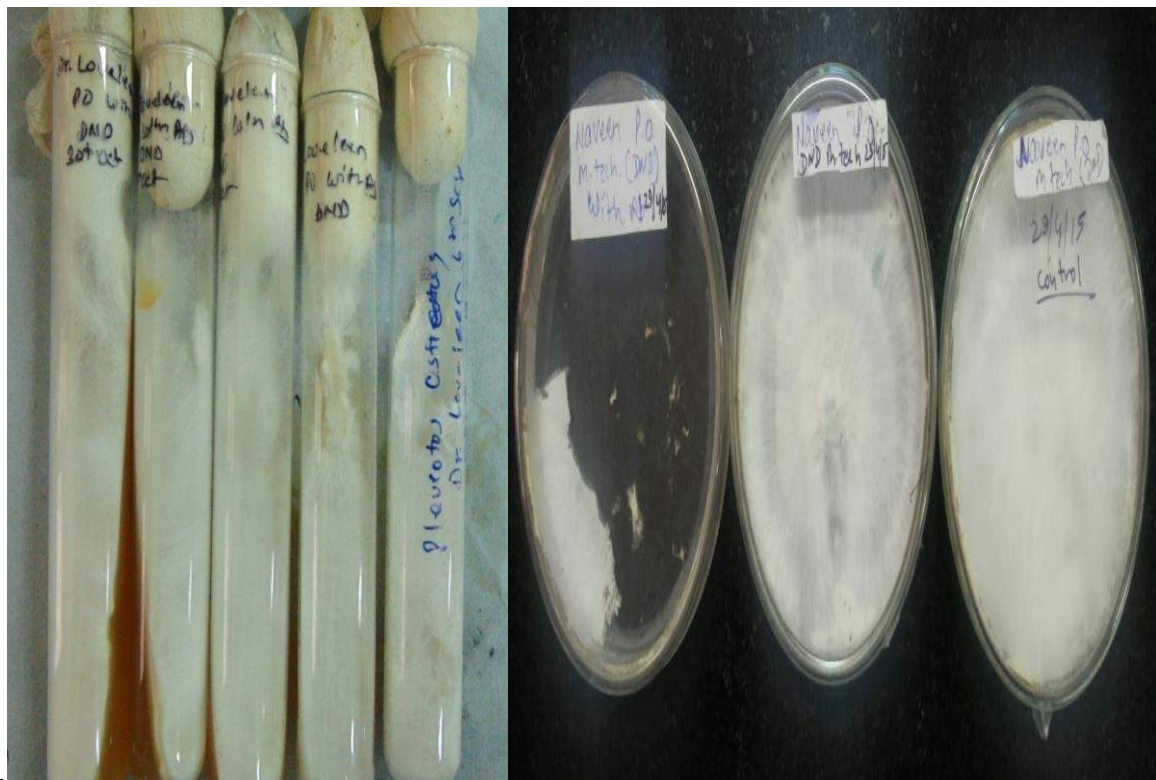
$$\text{Decolourization (\%)} = [(\text{Initial Absorbance} - \text{Final Absorbance}) / \text{Initial Absorbance}] \times 100$$

CHAPTER – 7

RESULT AND DISCUSSION

7.1 Maintenance of *Pleurotus ostreatus*:-

Pleurotus ostreatus was obtained from the Department of Microbiology, Punjab Agriculture University, Punjab and maintained on potato dextrose agar till confluent growth was observed. *Pleurotus ostreatus* forms white and fluffy colonies as shown in Fig 4(a and b)



(a)

(b)

Fig No. 4(a) Slants of *Pleurotus ostreatus* (b) Petri-Plates of *Pleurotus ostreatus*

7.2 Biomass production

P.ostreatus was inoculated in the potato dextrose broth for and incubation at 25⁰C in rotatory shaker (150 rpm) till confluent growth was achieved. Biomass of *Pleurotus ostreatus* achieved in form of mycelial pellets (Fig 5).

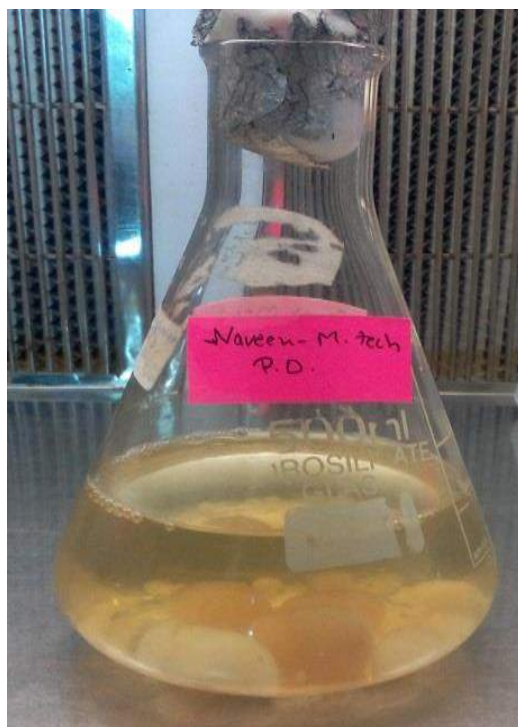
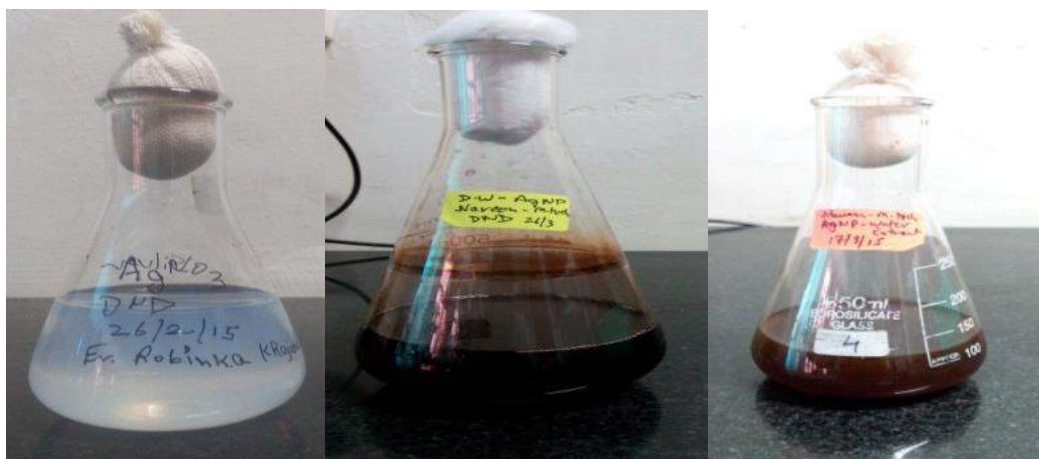


Fig No. 5 Biomass Production of *Pleurotus ostreatus*

7.3 Synthesis of silver nanoparticle

Fungal mycelia was filtered, washed thrice with sterile deionised water to remove media and inoculated in sterile deionised. To the filtered biomass 4mM silver nitrate was added and incubated in dark conditions at 30°C under shaking conditions for 24 hours. Preliminary indication of synthesis of silver nanoparticles is given by the change in the colour of the suspension to dark brown (Fig 6). Similar changes in the colour of the suspension have been reported by Forough *et al.* 2012 for the synthesis of silver nanoparticles using used aqueous extract of *Hedysarum* plant extracts. Change in colour from colour change milky white to dark brown on synthesis of silver nanoparticles *P.osteratus* has also been reported by Devika *et al.* 2012.



(a)

(b)

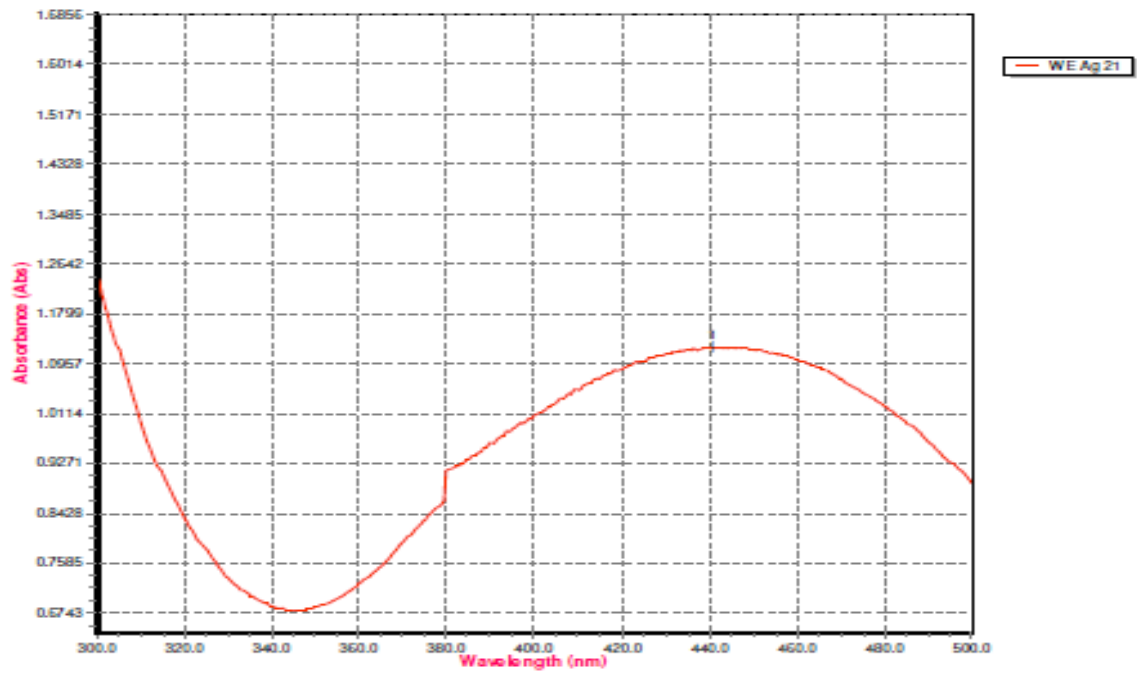
(c)

Fig. 6 Change in the colour of reaction mixture indicating Silver nanoparticles synthesis
(a) Reaction mixture before synthesis (b) Reaction mixture after synthesis using
Deionised water (mycelia) (c) Reaction mixture after synthesis using Water extract
(mycelia)

7.4 Characterization of Silver Nanoparticle

7.4.1 U.V Visible Spectroscopy

Silver nanoparticles were characterized by UV-Vis spectral analysis. The samples were subjected to a wavelength scan ranging between 280-800nm using distilled water as reference. Fig No. 7 (b) & (c) shows the U.V- Visible Spectra of silver nanoparticles after 72hrs of incubation. Synthesized silver nanoparticles from mycelia water extract exhibited an absorbance peak at 440nm while those synthesised from deionized water at 455nm, as silver nanoparticles are reported to exhibit a characteristic absorbance between 350-480nm (Kulkarni, 2009; Ahmad *et. al.* 2003). Nithya *et. al.* 2011 reported an absorbance peak of 381nm for silver nanoparticles synthesised using *Pleurotus caju* .Similar absorbance range has been reported for silver nanoparticles synthesized from *hedysarum* plant root extract (422nm) and *Fusarium oxyporum* (425nm) respectively (Forough *et. al.* 2010; Selvi *et. al.* 2012).



(a)



(b)

Fig. No.7 U.V- Visible spectral analysis of silver nanoparticles synthesised from (a) Mycelia water Extract (b) Deionised water (mycelia)

7.4.2 FTIR Analysis

FTIR analysis reveals possible interactions between silver and bioactive molecules, which are responsible for synthesis and stabilization of silver nanoparticles. The bands were seen at 3041.84 (cm^{-1}) shows N-H stretch bond with functional group of 1^o, 2^o amines & amides respectively, at 2823.88 (cm^{-1}) shows H-C=O: C-H stretch bond with functional group of aldehydes, at 2397.6 (cm^{-1}) shows, at 1585.54 shows N-H bend bond with functional group of 1^o amines, at 1033.88 (cm^{-1})-1381.08 (cm^{-1}) shows C-N stretch with functional group of aromatic and aliphatic amines, and 559.38 (cm^{-1}) shows C-Br stretch with functional group of alkyl halides. Two bands observed at 1033.88 (cm^{-1}) and 1381.08 (cm^{-1}) show N-H stretching vibrations of the aromatic, aliphatic amines respectively (Fig. No. 8). Selvi *et al*, (2012) reported the presence of peaks at 3302 & 2926 (cm^{-1}) indicating the presence and binding of enzymes with silver nanoparticles, corresponding to bending vibrations of amide I & amide II. Similar peaks at 3398 (cm^{-1}) showing bond (N-H) and vibrational peaks between 2899 and 2977 (cm^{-1}) showing (C-H) bond were reported for silver nanoparticles synthesised by leaf extract of *Morinda pubescens* (Mary *et al.*, 2012).

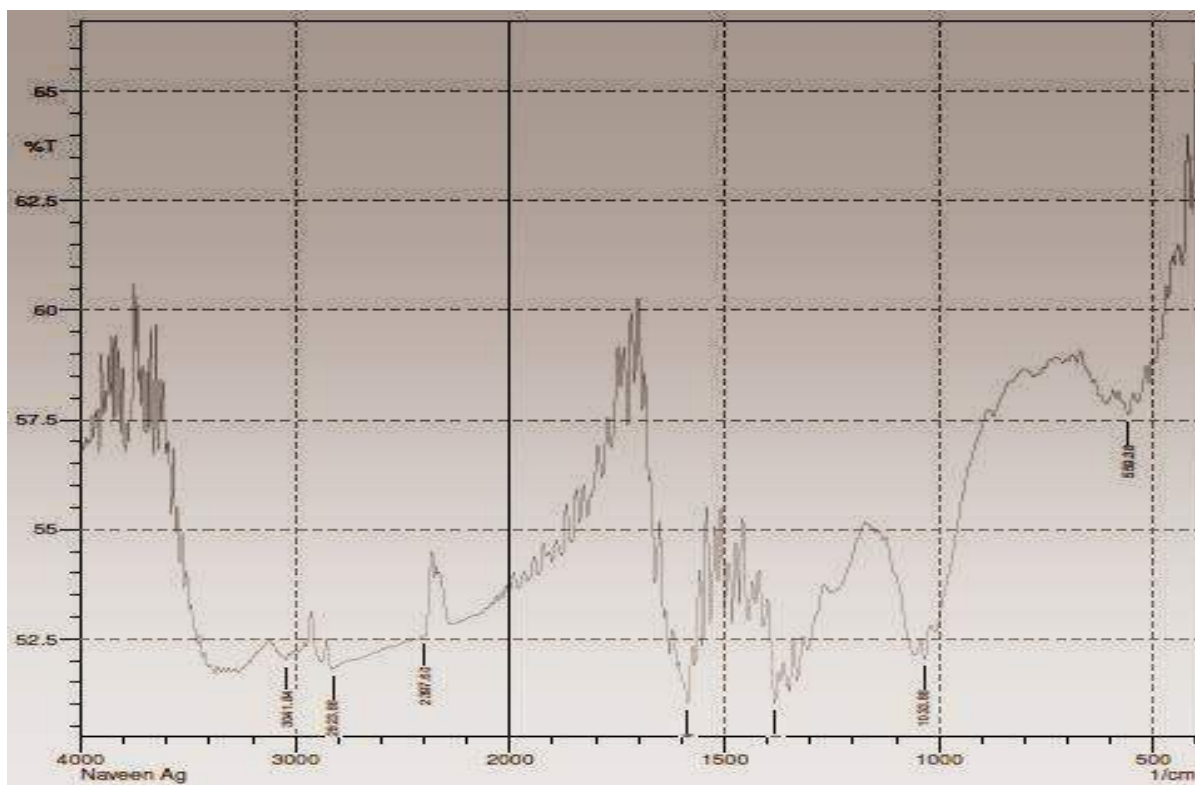


Fig. No. 8 FTIR spectra of nanoparticles synthesized from *Pleurotus ostreatus*

7.4.3 XRD Analysis

The vacuum-dried nanoparticles were used for powder X-ray diffraction (XRD) analysis. The spectra was recorded in aX'Pert-Pro PAnalytical X-ray Diffractometer (Cu K α radiation, λ 1.54060) running at 45 kV and 30 mA. The diffracted intensities recorded from 20 degrees to 80 degrees at 2θ angles. As shown in Fig 9, peaks intensities of 168.25, 304.84, 101.89, 173.46 were obtained at angles of 27.85°, 32.22°, 38.08°, 46.20° respectively with an average crystal size of 58.8 nm. Mary *et. al*, 2012 also reported similar peaks at planes of 111, 200, 220, 240 with 27.4°, 32°, 46.1°, 54.8° degree for silver nanoparticles synthesised by leaf extract of *Morinda pubescens*. Similar XRD patterns at angles of 38.33°, 37.76° and 37.69° have been reported by Kudle *et. al*, 2013 for silver nanoparticles synthesised using *Allmania nadiflora*.

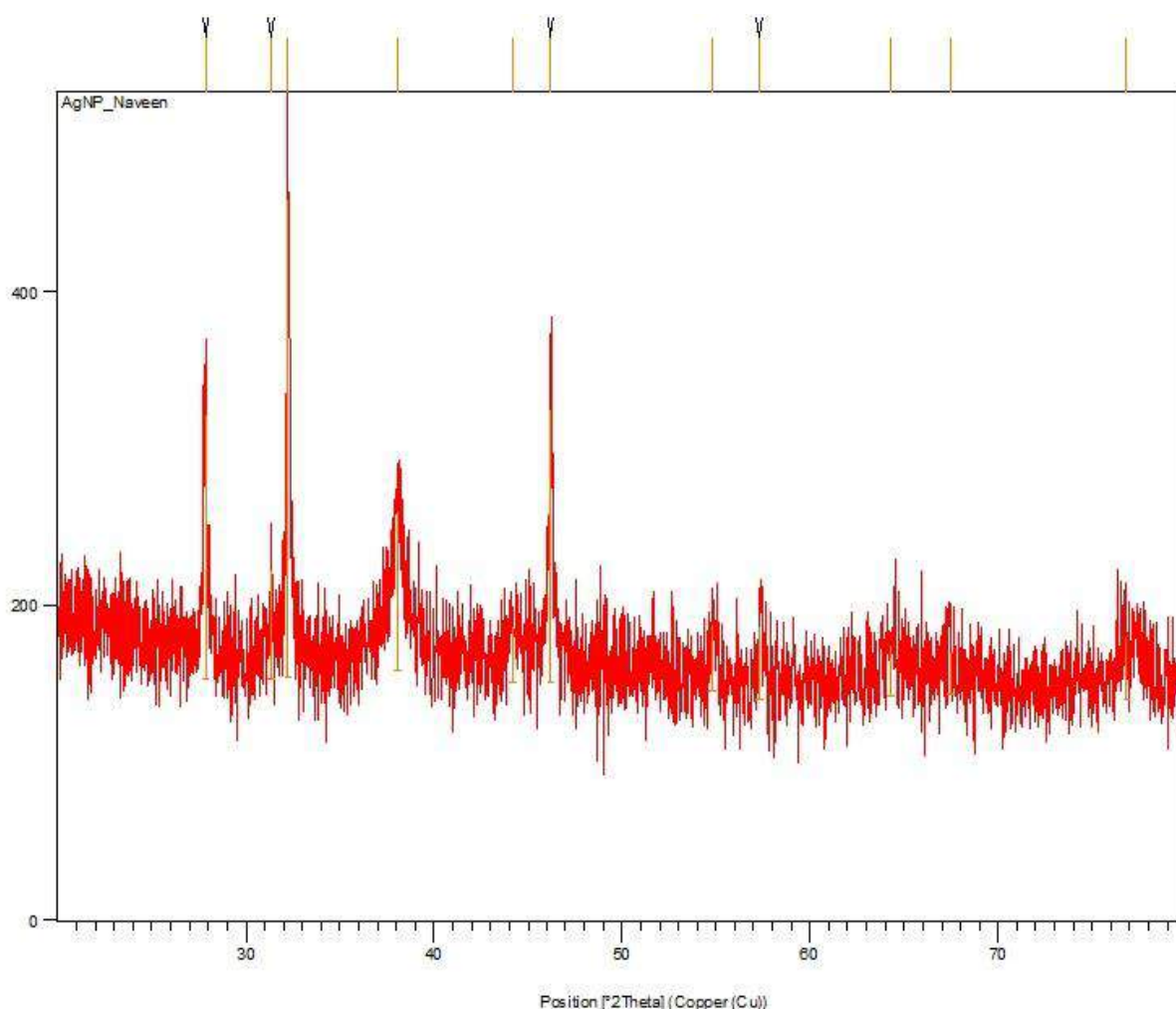


Fig. No. 9 X-Ray Diffraction pattern for silver nanoparticles

7.4.4 SEM

Samples were dispersed in ethanol for 4hrs then after that sample were coated with Au for better resolution. After that sample is placed in SEM-JEOL for analysis at magnification rate of 30000x, 50000x. As evident from SEM images (Fig. 10) silver nanoparticles synthesised were spherical in shape and highly aggregated. Yamini, 2011 reported spherical shape of silver nanoparticle synthesised using *Cleome viscosa*. Similarly, Selvi *et al.* 2012 reported that spherical shaped nanoparticles synthesized by *Fusarium oxyporum*. Kudle *et. al* , 2013 reported synthesis of spherical to oval shaped silver nanoparticle from *Allmania nadiflora*.

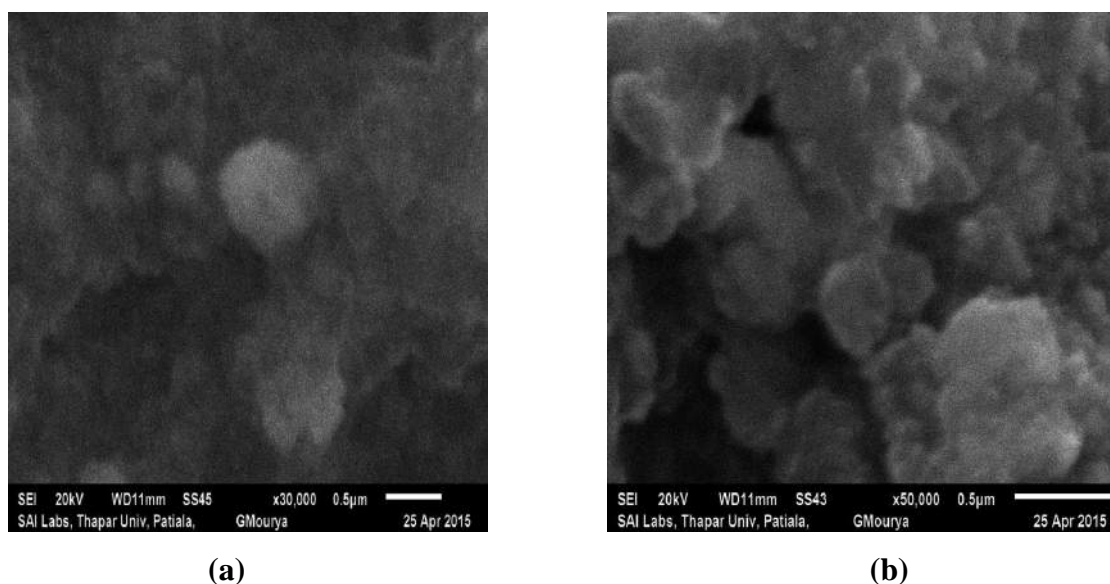


Fig No. 10 Silver nanoparticles (a) SEM magnification of 30000x, (b) SEM magnification of 50000x

7.4.5 EDX Studies

For Energy dispersive spectroscopy (EDS), samples were prepared with film of carbon coating of silver nanoparticles. Elemental study on single particle was done with using SEM equipment in-built in it with a thermo EDS attachment. Analysis shows the silver of about 83.79% with 16.21% impurities (Fig. No.11). Absorption peak observed at 3 kev that confirms the presence of metallic silver nano-crystals. This result is consistent with the report published by Sarvamangala *et. al.* 2013, confirming the presence of silver crystals by the presence of EDS absorption peak at 3kev EDS with elemental composition of silver which

indicates presence of silver nanoparticle. Similar, study done by Dimitrijević *et. al.* (2013) reported similar EDS patterns with absorbance peak at 3keV showing silver nanoparticles formation. Bhat *et. al.* (2010) reported similar pattern of silver nanoparticle using extract of fungi *Acremonium diospyri* at 3keV.

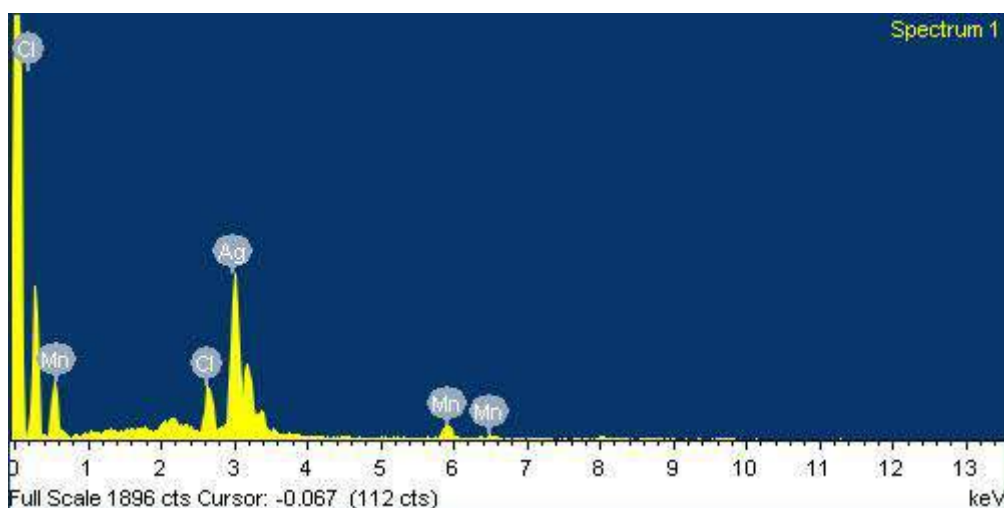


Fig. No. 11 EDS Analysis with peak at 3keV indicating the presence of silver nanoparticles

7.5 Decolourization of dyes by *Pleurotus ostreatus*

The extent of decolourization of dyes by *Pleurotus ostreatus* of Malachite green and Crystal violet was studied after regular interval of 24hrs for 1 week. It was observed that an increase in the incubation time led to an increase in the decolourization of the dyes. In case of Malachite green 75%, 73%, 59.50% decolourization was achieved with different concentration of 5, 10, 15mg/L respectively after an incubation of 1 week (Table 3) while in case of Crystal violet 42.5%, 35.55%, 28.35% decolourization was observed by *Pleurotus ostreatus* 5, 10, 15mg/L concentration respectively. At concentration of 5mg/L of Malachite green highest decolourization were observed about 75%. But in case of Crystal Violet at 5mg/L concentration, decolourization was observed about 42.5%. Comparatively better decolourization was seen for Malachite green than Crystal violet. This may be attributed to higher molecular weight and complex structure of Crystal violet as compared to Malachite green. Krishnaveni *et al.*, 2011, reported 100% and 90% decolorization of Malachite green and Crystal violet respectively by *Pleurotus florida* after an incubation of 14 days. Eichlerova

et.al., 2006, used Malachite green and Crystal violet for decolourization process by *Pleurotus calyptratus* and reported 27% and 5% decolorization after 14 days incubation.

Table No. 3 - Decolourization of Malachite Green (MG) and Crystal Violet (CV)

Incubation Time	% Dye Decolourization of Malachite Green (MG)		
	5mg/l	10mg/l	15mg/l
48hrs	53.19%	52%	48.52%
96hrs	75%	73%	59.50%
	% Dye Decolourization of Crystal Violet (CV)		
48hrs	25.15%	20.22%	18.30%
96hrs	42.5%	35.55%	28.35%

7.6 Decolourization by Silver Nanoparticles

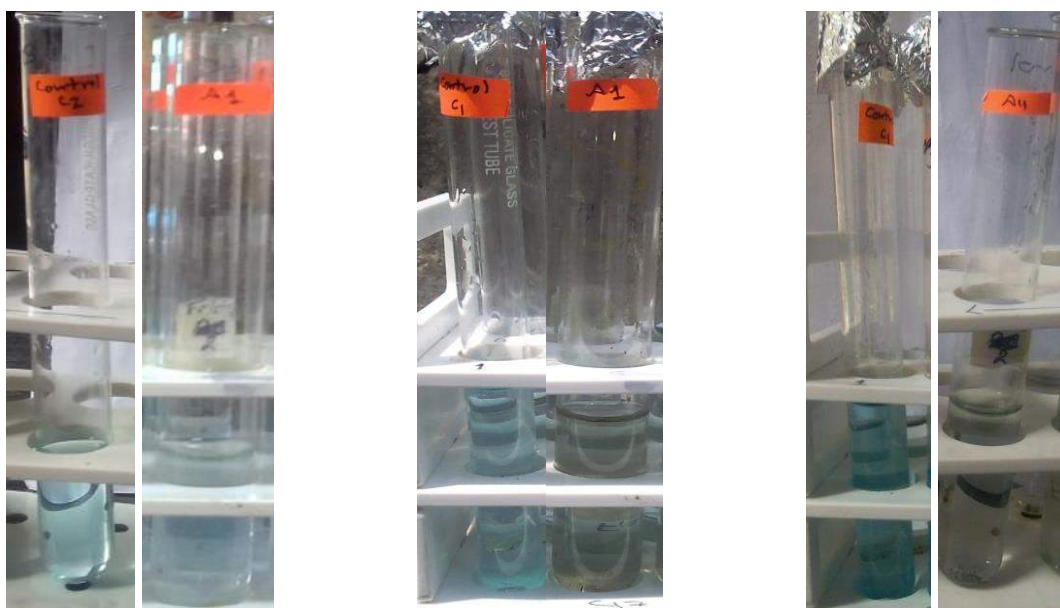
7.6.1 Effect of Light intensity on Nanoparticles mediated Decolourization

The extent of Photo-catalytic degradation of triphenylmethane dyes i.e Malachite green and Crystal violet was studied at regular intervals of two hours. It was observed that with the increase in incubation time, increase in per-cent decolourization was observed (Table No. 4). In case of Malachite green 82% decolourization was seen while in case of Crystal Violet 52.4% decolourization was achieved (Fig. No 12 and 13). Consistent with the results of decolourization by fungus only, in decolourization with nanoparticles also Malachite green showed maximum decolourization in comparison to Crystal violet. Maximum decolourization were achieved by Malachite Green by 74.69%, in comparison to 51.15%. This is due to the difference of light intensity, as light intensity rises number of photons increases to reach the active site of catalyst so amount of excited catalyst particles raises and subsequent raise the number of hydroxyl radicals then super oxide ions (O_2^-) and rate of degradation of dye particles also rise (Swati *et al.* 2012). Saha *et al.* in 2001, used TiO_2 saturated with silver nanoparticle to decolourize malachite green in aqueous medium, it observed that the presence of silver in TiO_2 increase the mineralization of malachite green completely in 1 hrs light radiation. Samira *et al.* in 2012, used crystal violet for photo-catalytic degradation process by

Nano TiO₂ containing anatase, showed solution of crystal violet in concentration of 2.5×10⁻⁵ mol/L was completely decolorize up to >99% after an radiation time of about 25 min.

Table No. 4 – Effect of Light intensity on Decolourization of dyes

Hours Dyes	% Decolourization	
	Malachite Green (MG)	Crystal Violet (CV)
2 hours	29.8% ± 1.331	23.85% ± 1.087
4 hours	52.43% ± 1.223	28.9% ± 1.198
6hours	74.69% ± 1.363	51.15% ± 1.556



Control Sample	Control Sample	Control Sample
2hours	4Hours	6 Hours

Fig. No. 12 Effect of light intensity on decolourization of Malachite green by fungus mediated nanoparticles

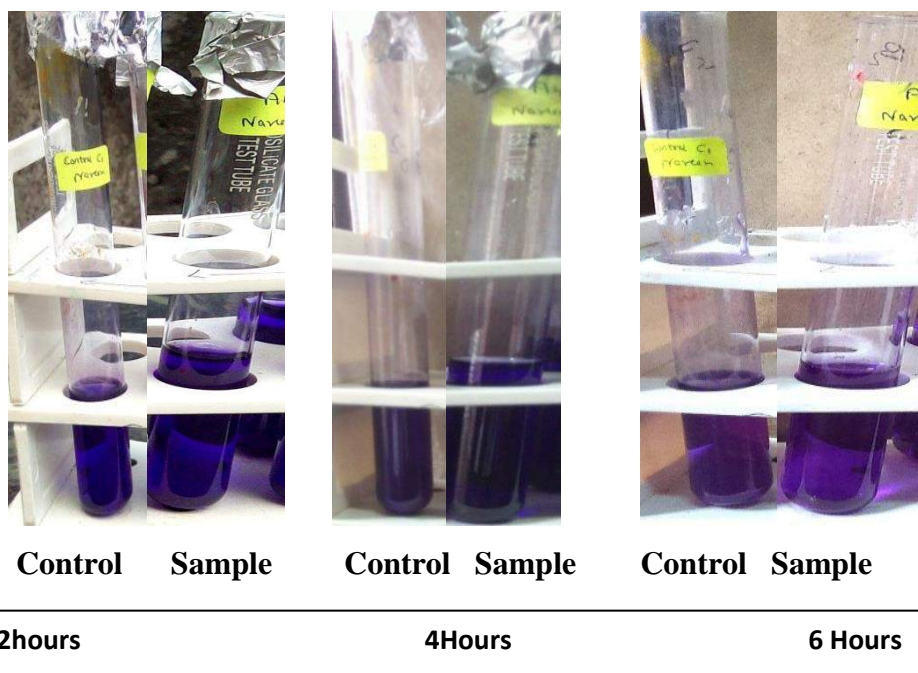


Fig. No. 13 Effect of light intensity on decolourization of Crystal Violet by fungus mediated nanoparticles

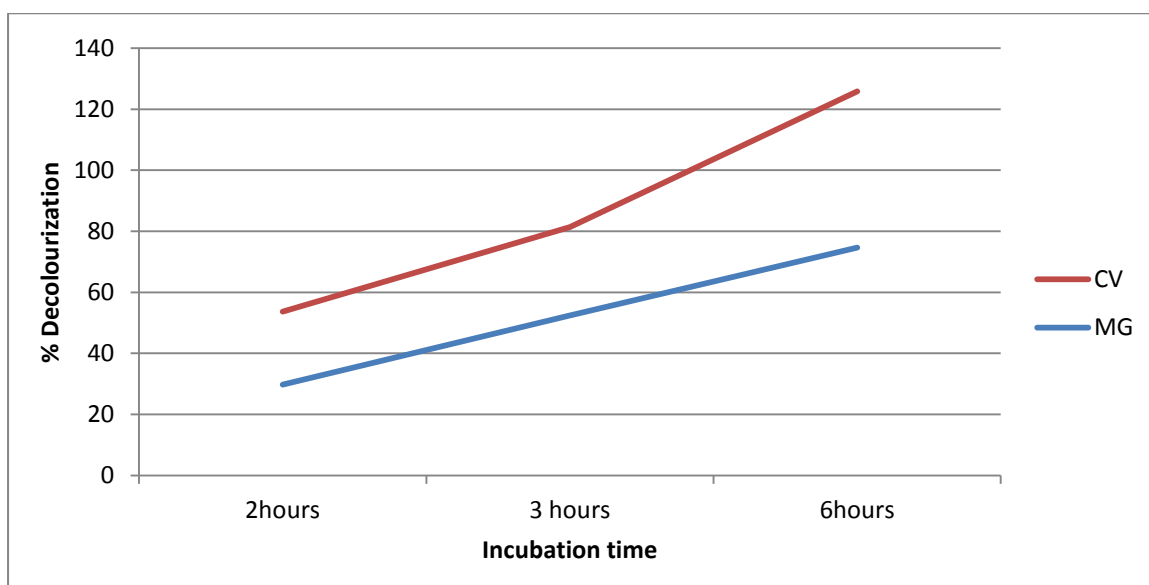


Fig. No. 14 Effect of light intensity on nanoparticles mediated decolourization of Malachite Green and Crystal Violet

7.6.2 Effect of pH on Nanoparticles mediated Decolourization

For this study, decolourization studies of dyes were done at pH range of 5-11 and it was observed that maximum decolourization occurred at pH-11 for Malachite green by silver nanoparticles is 83.3%, while in case of Crystal violet it was achieved at pH-11 by silver nanoparticle is 59% which is showed on Table No. 5. The efficiency of Malachite green is good in comparison to Crystal violet dye. The Effect of pH on nanoparticles mediated decolourization of malachite green and crystal violet is increasing on increasing the pH above 7. Maximum decolourization is shown by Malachite green by 83.3% at pH-11 and Crystal violet is by 59% at pH-11. This is because of nature of dye i.e. malachite green and crystal violet dye is basic in nature. So on increasing the pH, effect of nanoparticles mediated decolourization of malachite green increases. It has been detected that the rate of photo-catalytic decolourization of malachite green which is a triphenylmethane dye, increases as pH was increases and it attained optimum value at pH 7.5 to 9 (Ameta *et al*, 2014).

By Nozaki *et al.*, 2008 reported the decolourization of 27 dyes of different class, including diazo, monoazo, phthalocyanine, and triphenylmethane dyes, by using different 21 basidiomycetes. They found 5.0-10 is the optimum pH for decolourization of dyes. Ameta *et. al.* in 2014, reported that photo-catalytic breakdown of malachite green above lead chromate powder, tells if we increase the pH above 7.0 then the rate of degradation also increases up to 9.0 pH. Samira *et. al.* in 2012, used crystal violet for photo-catalytic degradation process by Nano TiO₂ containing anatase for effect of pH in decolourization process. It was observed that pH decreases, the photo-catalytic activity is also decreases. pH range were set at 3-13 and variation showed within 2% by pH change.

Table No. 5 Decolourization of Malachite Green and Crystal Violet

pH Dye	Effect of pH on Nanoparticles mediated Decolourization of Malachite Green Dye (MG) and Crystal Violet (CV)	
	MG	CV
5	38%±0.0058	27.9%±0.0012
6	58.8%±0.0017	32.9%±0.3412
7	59.48%±0.0012	34.7%±0.221
8	63%±0.1233	37.6%±0.0281
9	74.6%±0.1203	51.5%±0.1124
10	78.45%±0.0014	52.3%±0.0012
11	83.3%±0.0071	59%±0.002

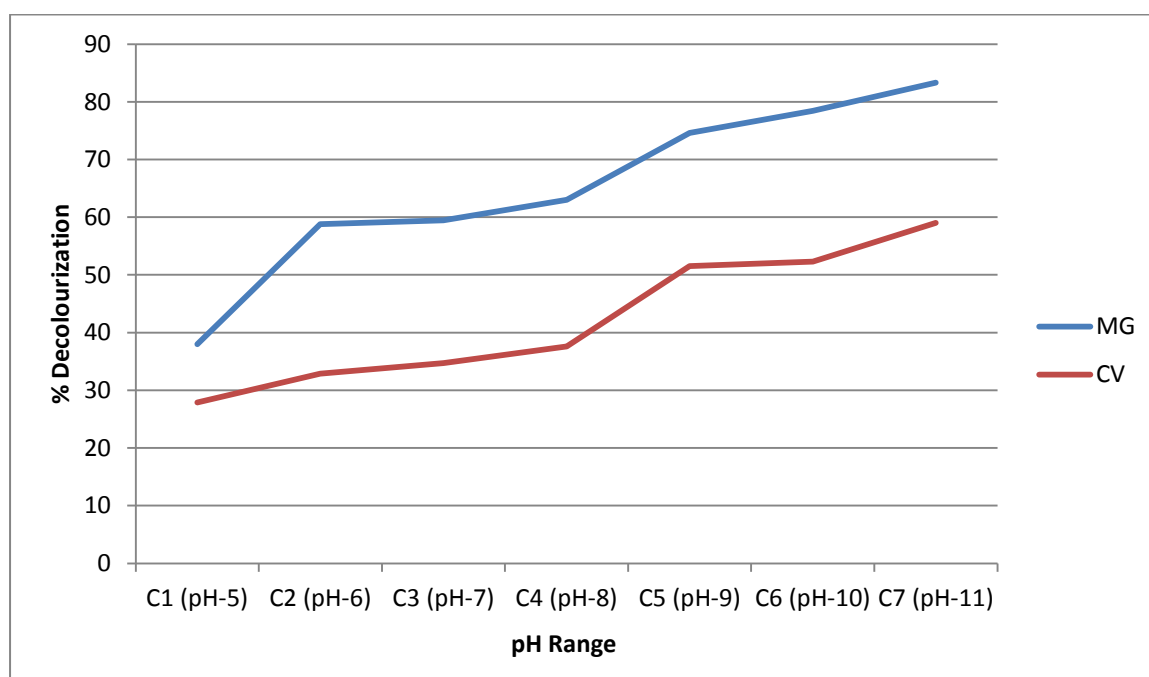


Fig. No. 15 Effect of pH on nanoparticles mediated decolourization of Malachite Green and Crystal Violet

7.6.3 Effect of incubation on Nanoparticles mediated Decolourization

The level of photo-catalytic degradation of triphenylmethane dyes i.e. Malachite green and Crystal violet dye was studied at regular interval of days. It was observed that with the increase in incubation time, degradation of dyes increases (Table 6). Decolourization in Malachite green dye by silver nanoparticle is 79% (Fig. No. 16), while in Crystal violet dye by 80.1% (Fig. No. 17). Maximum decolourizations were observed in Crystal Violet by 80.1% and in Malachite Green by 79%, which is less in comparison to Crystal Violet. The effect of light intensity and its photo-catalytic activity is done efficiently in every days of incubation. Effect of light intensity on fungus mediated nanoparticles is good in comparison to control which has only containing dye. This is due to the difference of light intensity as light intensity rises number of photons increases to reach the active site of catalyst so amount of exited catalyst particles raises and subsequent raise the number of hydroxyl radicals then super oxide ions (O_2^-) and rate of degradation of dye particles also rise (Swati *et al.* 2012).

Saha *et. al.* in 2001, used TiO_2 saturated with silver nanoparticle to decolorize malachite green in aqueous medium, it observed that the presence of silver in TiO_2 increase the mineralization of malachite green completely in 1 hrs light radiation. Samira *et. al.* in 2012, used crystal violet for photo-catalytic degradation process by Nano TiO_2 containing anatase, showed solution of crystal violet in concentration of 2.5×10^{-5} mol/L was completely decolorize up to >99% after an radiation time of about 25 min.

Table No. 6- Effect of Incubation Time Decolourization of Malachite Green and Crystal Violet

Hours Dyes	Percent Decolourization of dye	
	Malachite Green (MG)	Crystal Violet (CV)
24hrs	16.2% ± 0.0106	53.72% ± 1.0685
48 hrs	35.75% ± 0.037	74.55% ± 1.170
72 hrs	38.96% ± 0.337	76% ± 0.54006
96 hrs	79% ± 0.799	80.1% ± 0.6625



(a)



(b)

Fig. No. 16 (a) Effect of incubation on decolourization of Malachite Green a) At 00 hours (b) After 96 hours



(a)



(b)

Fig. No. 17 Effect of incubation on decolourization of Crystal Violet a) At 00 hours (b) After 96 hours

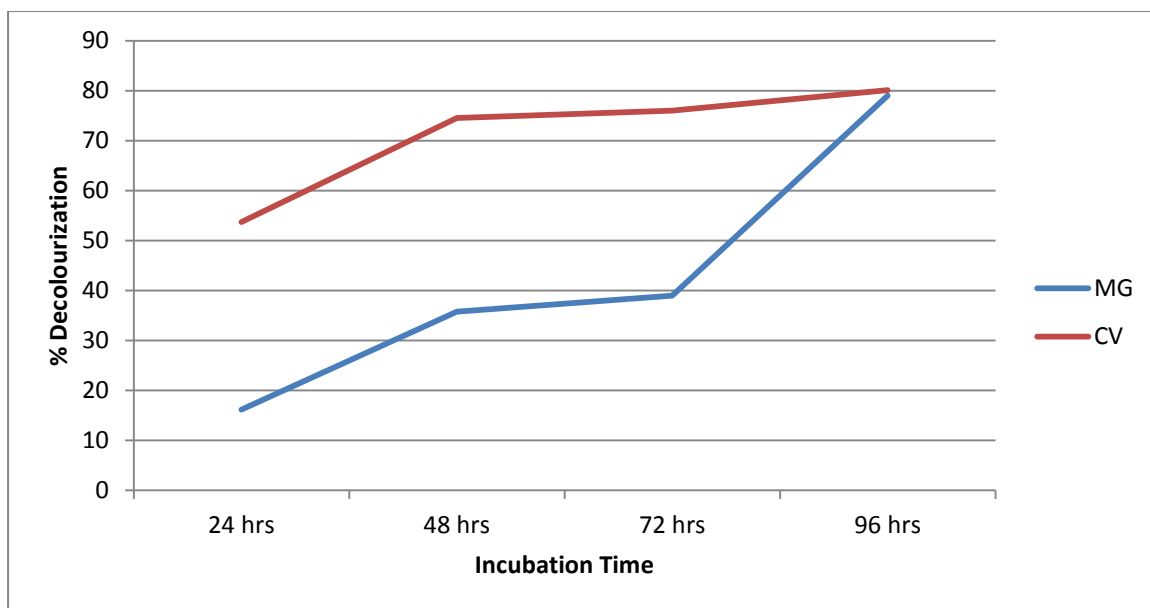


Fig. No. 18 Effect light intensity on nanoparticles mediated decolourization of Malachite Green and Crystal Violets

CHAPTER - 8

CONCLUSION

Dyes are the main pollutants present in waste waters released from textile and other industrial process. These dyes when mixed with fresh water resources cause several environmental problems such as increase in COD and decrease the intensity of light absorbed by the water plants and phytoplanktons, reducing photosynthesis. These dyes are highly dispersible, hard to treat, high in volume and hazardous in nature. The aim of this work was to synthesize silver using *P.ostreatus* as reducing/ capping agent. Characterization of nanoparticles was done with the help of XRD, SEM-EDX, and FTIR which revealed average particle size of 58.8nm for silver nanoparticles. Synthesized nanoparticles were further evaluated for the potential application of nanoparticles for decolorization of dye dyes namely against Malachite Green and Crystal Violet. Decolourization of crystal violet and malachite green by AgNP at optimized condition was 51.15% and 74.69% respectively after an incubation of 6 hours. Further optimizations need to be extensively studied to successfully use this technique for bioremediation of dye contaminated effluents.

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