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GREEN SYNTHESIS OF SILVER NANOPARTICLES AND APPLICATION IN DYE DECOLORIZATION BY *PLEUROTUS OSTREATUS* (MH591763)

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ABSTRACT
Green synthesis of silver nanoparticles (AgNPs) that have environmentally acceptable solvent systems and eco-friendly, reducing agents is of great importance and its application in industries and bioremediation. The aim of this work was to synthesis of AgNPs using cell free filtrate of *Pleurotus osteratus* (MH591763) as reducing agents for silver ions as well as stabilizing agents for the synthesized AgNPs were confirmed by UV–Vis spectroscopy, FTIR analysis XRD and SEM. The orange dye decolorization was carried out by laccase enzyme and AgNPs. The AgNPs biosynthesis were confirmed with solution color change, yellow color into brown reddish and characterized using UV–vis spectroscopy, FT-IR, XRD and SEM. The orange dye decolorization by laccase showed 82% followed by AgNPs 39%. The AgNPs treated paper pulp Kappa number showed as 56.36% followed by laccase enzyme 46.32%. The paper pulp brightness AgNPs showed as 66.32% followed by laccase enzyme 64.32% that indicates the residual lignin was removed by the fungal treatment

KEY WORDS: Silver nanoparticles, Orange dye, *Pleurotus ostreatus*, Laccase

INTRODUCTION

Textile industry is one of the greatest consumption of dyes around $7x10^5$ tones worldwide and generates deep color of the effluent, which contain more than 10,000 different synthetic dyes. (Karthikeyan *et al*., 2010). Based on the chromophores the dyes are classified into groups such as azo, anthraquinone, sulfur, indigo, triphenylmethyl and phthalocyanine derivative. These dyes are highly carcinogenic to human beings (Chung, 1983). The various physicochemical methods are available to remove those dyes, such as coagulation, flocculation, membrane filtration, and activated carbon adsorptions are carried out before discharge into the environment. But these methods have high operating cost of large scale level (Robinson *et al*., 2001). Hence the alternative bioconversion method as considered for it's cheaper, highly efficient and ecofriendly manner for the partial or completes removal of those pollutants (Kuhad *et al*., 2004).

Laccases (benzenediol :oxygen oxidoreductases, (EC:1. 10.3.2) are a diverse group of tetrameric glycoprotein, which contain four copper atoms per monomers. They have three functional binding sites with low substrate specificity. Hence, they oxidize wide range of substrate such as diphenols, polyphenols, substituted phenols, diamines and aromatic amines, with simultaneous a reduction of molecular oxygen to water (Thurston, 1994). These characteristics feathers are attracted by many research to efficient cleanup those environmental pollutants such as highly toxic industrial recalcitrant synthetic dyes, Industrial effluents, toxic pesticides (Wang *et al*., 2018; Buchicchio *et al*., 2016; River Hoyos *et al*., 2013: Ottoni *et al*., 2013; Hou *et al*., 2004; Cerniglia and Sutherland 2001; Knapp *et al*., 2001: Leonowicz *et al*., 2001; Yaropolov *et al*., 1994). Laccases are mostly found in plants, fungi and bacteria (Alexandre and Zhulin, 2000; Claus, 2004) which has been mostly produced by white rot fungi belonging to basidiomycetes (Ranocha *et al*., 2002). The laccase produced by *Pleurotus sp* has the ability to oxidize several dyes such as amaranth, coccine, orange G, tartrazine (Chagas and Durrant, 2001), Remazol Brilliant blue R and Congo red (Lim, Lee, & Kang, 2013).

AgNPs have high photo catalyst properties are depends on the size and shape of the AgNPs. The methods to produce AgNPs are reported mainly concentrated on green synthesis. However, a green synthesis is proposed as more eco-friendly aspects and cost effective method. For safe operation, energy saving and avoiding the use of organic solvents, the suitable green synthesis processes of AgNPs for the degradation of organic dyes in aqueous solutions under mild condition is still in demand both industrially and environmentally. The biological treatment of dye degradation has been studied in recent years. Roy *et al*. (2015) reported that the biological mediated synthesis of AgNPs shows effective photocatalytic property to degrade methyl orange under sunlight irradiation. Edison and Sethuraman (2012) reported the synthesis of AgNPs by using water extracts of *Terminalia chebula* fruit which shows good catalytic activity on the reduction of methylene blue. Vidhu and Philip (2014) reported the dye degradation of synthesized AgNPs by using *Trigonella foenum-graecum* seeds against methyl orange, methylene blue and eosin Y by $NaBH_4$. In the present study laccase production of *Pleurotus ostreatus*, to evaluate the industrial application of laccase enzymes in the dye decolorization and synthesis of AgNPs.

MATERIALS & METHODS

Chemicals and Reagents

The reagent grade chemicals Potato Dextrose Broth (PDB), Silver Nitrate was purchased from Sigma-Aldrich Mumbai India.

Strain and Culture Condition

The white rot fungi was isolated from tree bark from Yelagiri Hills (Vellore, Tamil Nadu, India) and identified as *Pleurotus osteratus* by DNA amplification and sequencing the ITS regions using ITS1 and ITS4 (Gene Bank Accession number MH591763) according to the previous report**.** Stock culture of *P. ostreatus* was maintained in PDA slant at 4^{o}C in the dark. The fungal mycelium from the slant was transferred to PDA plates and incubated at 27^{o}C for 7 days. Actively growing mycelium culture was used as inoculum preparation.

Synthesis of silver nanoparticles (AgNPs)

For the synthesis of AgNPs, 2 ml of the purified laccase enzyme of *P. ostreatus* (MH591763) was added into 60 ml of 1mM silver nitrate ($AgNO_3$) solution and incubation at 37^{o}C for 2hrs. The complete reaction was done in dark condition to minimize the $AgNO_3$ photoactivation. After incubation, the solution color turns into a reddish brown indicates the synthesis of AgNPs then the solution was centrifuged at 15,000rpm for 20min. The transparent solution was discarded and the pellets of AgNPs were collected and dried in the oven at 45-50°C.

Characterization of AgNPs

UV-Visible spectrophotometer

The synthesised sample was primarily characterised by UV-Visible study, the synthesised sample was scanned under spectrophotometer in the wavelength of 200-600nm using Microprocessor Labtronics LT 291. The observed Plasmon peak was denoting the presence of silver nanoparticle.

FTIR

To identify the biomolecules responsible for the reduction of silver ions and stabilization of AgNPs solution was confirmed by using Fourier Transform Infrared Spectroscopy. SHIMADZU instrument from the range of 750 to 4000cm^{-1} was used for the laccase NPs**.**

XRD

Crystalline AgNPs were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu Kα radiation source using Ni as filter at a setting of 30 kV/30 mA. All XRD data were collected under the experimental conditions in the angular range $3° \leq 2\theta \leq 50°$

SEM

Scanning Electron Microscopy analysis was done for the identification of size and morphology of the NPs. The synthesised sample was converted to pellet and fine powder for the examination, using ZEISS

Application

Dye Decolorization

The dye decolorization ability of laccase enzyme of *P. ostreatus* was performed as described by El-Batal, *et al*., 2015 with minor modification. Orange dye was chosen to test the laccase enzyme ability to reduce their color. The reaction mixture contain 0.1ml orange dye (20ppm) was mixed with 2ml distilled water and 2ml of the partially purified enzyme extract, the color reduction was monitored for 3hrs and was determined by spectrophotometrically with absorbance of dye wavelength.

The decolorization efficiency (R%) was calculated as follows: Dye decolorization percentage= [(Initial absorbance -final absorbance)/(initial absorbance)] X100. Initial absorbance indicated absorbance of the untreated dye at the characteristic peak and the final absorbance indicated absorbance of dye after treatment with laccase at the same peak after 3hrs.

Biopulping of laccase and AgNPs

Kappa number was used as the criterion for the lignin content of pulps and it was determined as the volume of 0.1N potassium permanganate (mL) consumed by 1g of moisture free pulp. A portion of the cut piece of hand sheets that could consume approximately 50% potassium permanganate solution (0.1%) was weighted out and disintegrated in 500mL distilled water until it was free form of fiber clots or bundles. The disintegrated suspension was made up to 800mL. 100mL of (0.1N) $KMnO_4$ solution and 100ml of H_2SO_4 (4N) was added, cooled at 25^{o}C and immediately added to the disintegrated hand sheet suspension. After 10min, the reaction was stopped through addition of 20ml of potassium iodide solution (1N) and titrated against sodium thiosulphate solution (0.2N). Starch solution (0.2%) was used as indicator. A blank titration was carried out in the same manner but without the pulp.

The kappa number was calculated by the formula.

$K = p \times f / w$

$P = (b-a)\ N/0.1$

Where,

K = Kappa number

f= Factor for correction to the 50 per cent permanganate Consumption depending on the colume of P (TAPPI, 1993)

w =Weight of moisture free pulp sample used for estimation (g)

p= Amount of 0.1N permanganate consumed by the sample (mL)

b =Amount of thiosulphate consumed in blank determination (mL)

a = Amount of thiosulphate consumed by sample (mL)

Correction for reaction temperature

$K = \frac{pf}{} [1.0+0.013(25-t)]$

Where,

T = actual reaction temperature in degree Celsius

Biobleaching of laccase and AgNPs

Brightness of the hand sheets was measured at 457nm in a Perkin Emmer λ3B spectrophotometer equipped with a reflectance sphere.

Statistical analysis

All experiments were conducted in triplicate and comparisons made using two-way analysis of variance (ANOVA) using GraphPad Prism 6.0 (Graph Pad, San Diego, CA, USA).

RESULT AND DISCUSSION

Synthesis of AgNPs

AgNPs biosynthesis was confirmed with solution color change, yellow color into light brown, brown, and finally brown reddish (Fig 1). This color change indicates biosynthesis of AgNPs. The aqueous color change due to surface excitation of plasmon resonance phenomenon of

Ag^+ ions. In the previous studies the fungal strains *P. atropurpurascens, B. cinerea, P. expansum, P. ostreatus, P. chrysosporium, R. stolonifer, G. spectabilis*, and *G. frondosa* were found to reduce the Ag^+ ions to AgNPs. This was observed through a color change in the reaction mixtures (Sanguinedo *et al.*, 2018).

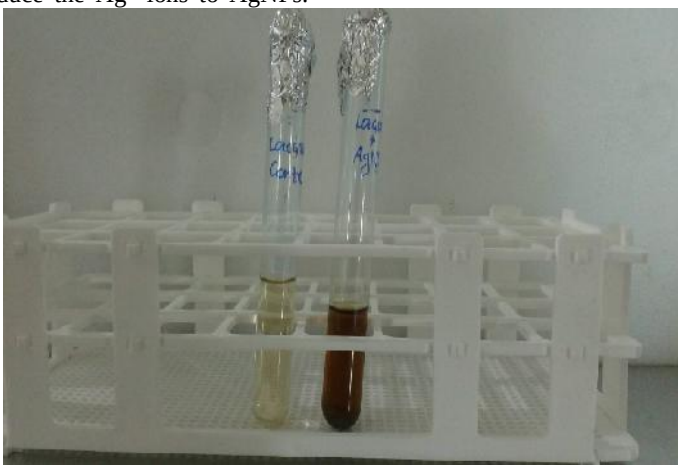

FIGURE 1. AgNPs synthesis using *P. ostreatus* (MH591763)

Characterization of AgNPs

UV spectrophotometer

Biosynthesis of AgNPs was confirmed by UV–vis spectroscopy such as their size, shape, and stability in aqueous suspensions. Fig. 2 shows the absorption spectra of AgNPs spanned a wide range from 300 to 550nm with a prominent peak at 430 nm. This peak indicates the formation of AgNPs because it is within the range of the surface plasmon resonance (SPR). In the previous studies, the color change was accounted for by SPR excitation in the collective oscillation of free conduction electrons provoked by an interacting electromagnetic field. A strong SPR band was noticed around 445nm, in the ideal wavelength range provided for Ag NP colloidal solutions (Kajani *et al*., 2014).

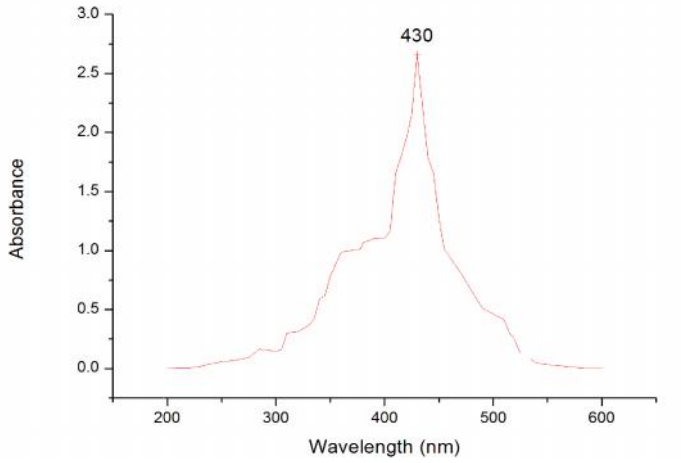

FIGURE 2. The UV spectrophotometer show the peak at 430nm indicates AgNPs formation

FTIR

FT-IR characterizes the biomolecules present in *P. ostreatus* mycelial extract were reduction Ag^+ ions into AgNPs formation. Fig. 3 shows FTIR absorption bands of biosynthesized AgNPs in the range of 400-4000cm^{-1}. A band at 3292cm^{-1} is assigned to the stretching vibration of the hydroxyl (-OH) group and a band at 2926cm^{-1} arises from the sp3 bond of aromatic C-H bending and a potent peak at 1690 cm^{-1} is due to C=O bond adsorption of double carbonyl group in keto mycelial extract. Two bands at 1614 and 1495cm-1 are because of C=C double bonds and aromatic C=C double bond in the heterocyclic ring of native extract respectively. The peaks at 1151 and 1004cm-1 are assigned to the absorption of C–O and C–O–C bonds, respectively Previous that demonstration of the IR spectrum of AgNPs of bands at 3415, 1630, 1460, 1376, and 1190cm^{-1} are, respectively, related to O-H stretching of H-bonded alcohols and phenols, carbonyl stretching, N-H bends primary amines, C-N stretching of the aromatic amino group and C-O stretching alcohols, and ethers. The IR spectrum of AgNPs shows that various organic molecules, that include ketones, aldehydes, terpenoids, alcohols, and carboxylic acid surround the AgNPs and cause their formation (Veisi *et al.*, 2018; Kanchana *et al*., 2011).

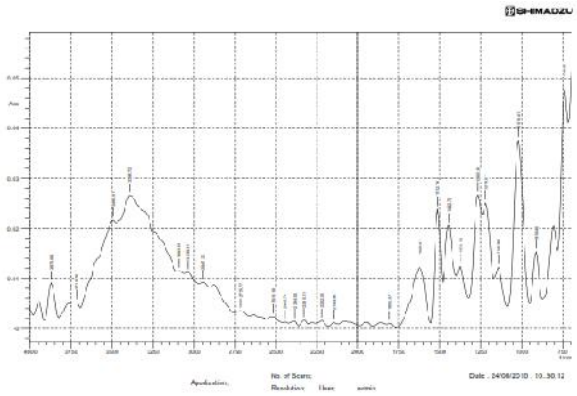

FIGURE 3. FTIR spectrum showed the organic molecules involved the AgNPs synthesis

XRD

The result showed various Bragg peaks (angle 2 θ), sets of lattice planes which could index to the 111, 200, 220, and 311 (Fig 4). The average crystallite size of AgNPs was found to be 10.41nm using Debye Scherrer formula. In the previous studies the XRD spectrum of AgNPs having five separate diffraction peaks at 38.28°, 44.40°, 64.57°, and 77.48°. However, the lattice plane value is noticed and indexed at 111, 200, 220, and 311 of the cubic silver (Veisi *et al.*, 2018).

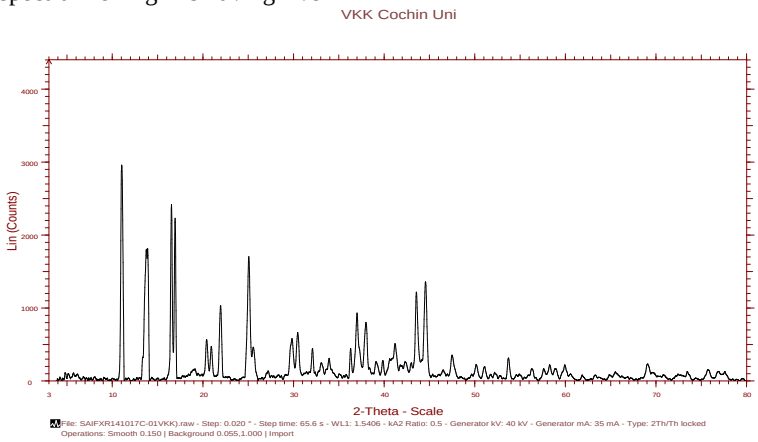

FIGURE 4. XRD peak of synthesized AgNPs

SEM

SEM analysis shows uniformly distributed AgNPs on the surfaces of the cells (Figure 5). The AgNPs were spherical in shape with particle size range from 5-40nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. In the previous studies the SEM image showed relatively spherical shape nanoparticle formed with a diameter range and providing the morphology, size details of AgNPs. The sizes of prepared AgNPs were more than the use of NPs which should be; *i.e.*; between 1-100nm. The size was more than the proteins were bound to surface of AgNPs and gave spherical shape (Chandirika and Annadurai, 2018).

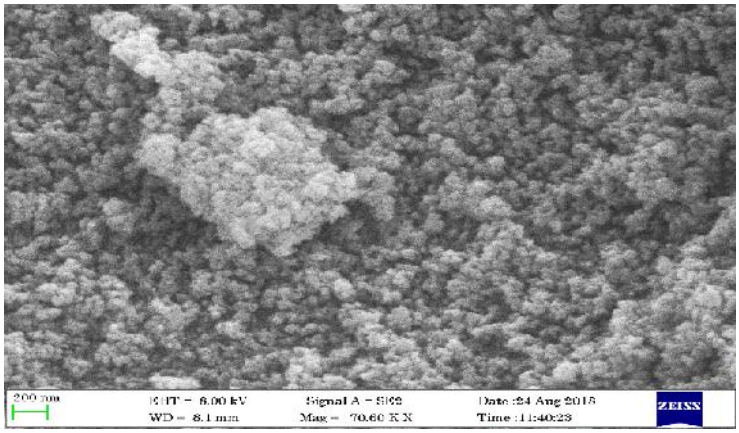

FIGURE 5. SEM result showed the size of AgNPs

Applications

In the present study, *P. ostreatus* and their enzymes were employed in various biotechnological processes Azo dyes Decolorization, Biopulping and Biobleaching

Azo Dye Degradation

The total orange dye was removal by absorbed by the laccase enzyme of *P. ostreatus* and AgNPs, the rate of absorption and the amount of dye degraded by the fungal enzyme were quantified. The AgNPs have photocatlytic property due to this the orange dye was decolorized. The *P. ostreatus* laccase shows more degradation of organ dye 82% followed by AgNPs 39% (Fig 6). In the previous studies that an enriched bacterial consortium can efficiently decolorize DB 151 and DR 31 up to 97.57% and 95.25%, respectively in 5 days. The individual isolates were also able to degrade the mixed dyes. A strain of *B. cereus* showed maximum decolorization ability of up to 93.37% in 5 days (Lalnunhlimi and Krishnaswamy, 2016) and the *P. sanguineus* laccase were partial decolorize azo dyes namely orange G and amaranth and complete decolorize triphenylmethane dyes namely bromophenol blue and malachite green was achieved in SMF (Pointing and Vrijmoed, 2000).

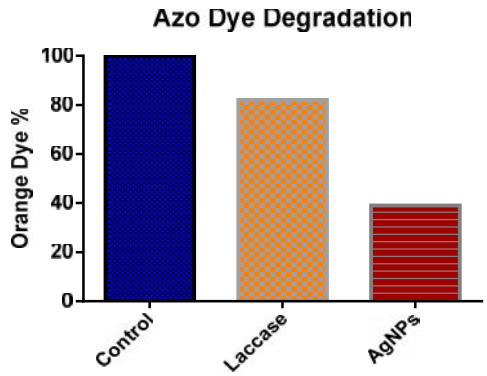

FIGURE 6. Dye Decolorization of Orange dye treated with both AgNPs and laccase enzyme

Biopulping of laccase and AgNPs

Kappa number and brightness of the paper pulp before and after laccase enzyme and AgNPs treatment was estimated. The AgNPs treated paper pulp Kappa number shows as 56.36% followed by laccase enzyme 46.32% (fig 7). In the previous studies Soliveri, 2003 reported that the new laccase produced by *Streptomyces cyaneus* showed its suitability for biobleaching of eucalyptus kraft pulps resulted in a significant decrease in kappa number. The laccase oxidize a nonphenolic compound, such as veratryl alcohol, in the presence of ABTS opens up new use of bacterial laccases in the pulp and paper industry.

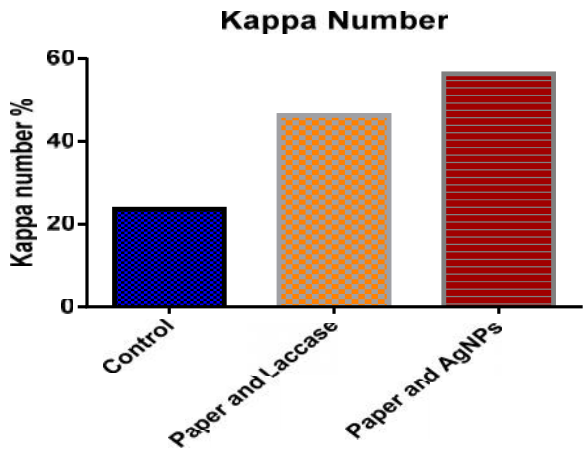

FIGURE 7. Kappa number of paper treated with AgNPs and laccase enzyme

Biobleaching of laccase and AgNPs

The AgNPs treated paper pulp brightness shows as 66.32% followed by laccase enzyme 64.32% that indicates the residual lignin was removed by the fungal treatment (Fig. 8). Sigoillot *et al.*, 2004 studied that the Laccase from *P. cinnabarinus* characterized in the paper pulp bleaching. Treatment of wheat straw Kraft pulp using laccases with 1-hydroxybenzotriazole as redox mediator achieved a 75% delignification pulp.

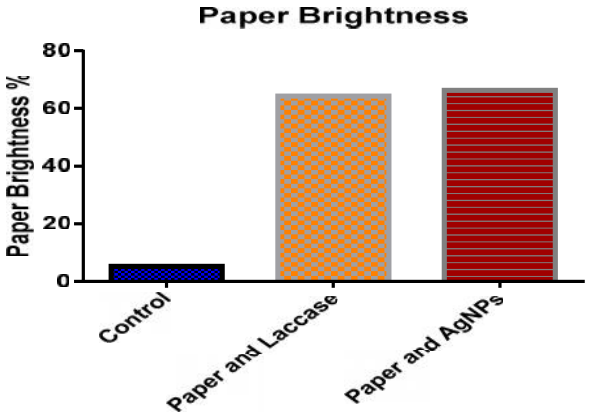

FIGURE 8. Paper pulp treated with AgNPs and laccase enzyme

CONCULSION

NPs are an emerging field that has made its impact to human life. There is an ever increasing need to develop ecologically benign methods in the synthetic and connecting toxic components. As a result researcher in the field of NPs synthesis and assembly focusing their attention on biological systems. Here we described a rapid biosynthesis of AgNPs and applied in dye decolorization and paper pulp treatment process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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