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MYCOBASED SYNTHESIS OF SILVER NANOPARTICLES AND ITS APPLICATION IN BIODEGRADATION OF A TEXTILE DYE ME$_4$BL

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ABSTRACT

Since the Bronze Age the synthetic dyes are prominent in the Indian textile industry. The uses of such synthetic dyes have many adverse effects in the environmental bodies. This study focuses on the ability of selected fungal isolates to decolorize and degrade ME$_4$BL a widely used synthetic textile dye. The textile effluent was collected from the industrial zones of Surat. The reactive dye ME^{4}BL was obtained from GIDC Ahmedabad. Various Fungi were isolated from textile effluent and screened by their capability to decolonize and decolorize the dye. Optimization of parameters for ME$_4$BL dye decolourization was studied under static as well as shaking conditions. The static condition was proved better than the shaking condition in the degradation activity. The optimum pH and temperature for A4 fungi were 4 and 37ºC respectively. The UV - visible absorbance spectra and the HPLC analysis of the decolorized ME$_4$BL significantly differed from those of the parent dye, indicating that the ME$_4$BL was degraded by the fungal isolate. The conjugation of Silver nanoparticle and fungal isolates were highly effective in removal of the dye from effluents within a short period of time. The 18 ITS gene sequencing method was carried out in which strain A4 was identified as *Aspergillus terrace*. The degradation rate was reported as 74.35 % in 72 hrs. in 100 mg/L dye concentration.

Key words: ME$_4$BL dye, effluent, degradation, nanoparticles

1. INTRODUCTION

Environmental pollution is one of the crucial dilemmas of this recent society. Perhaps industrial development is inevitable to gratify the necessity of the world's excess overcrowding population but at the same time, it endangers the various life forms on earth by contaminating the environment. Conventional wastewater processing technologies are proved to be for inefficient and incompetent in coping with the effluent of synthetic dyes due to the enduring nature the chemicals of such pollutants **[1**]. Decolorization process is reliable for color elimination though it does not eliminate the detrimental aromatic amines from effluent. The absolute evacuation of the achromatic aromatic amines from the aquatic ecosphere is obligatory because high accumulation of textile dyes disrupt the biological activity of aquatic life **[2]**. ME_4BL is a group under reactive dyes which are cationic dyes, widely used for dyeing cellulose, protein and polyamide fibers and its dyeing process produces a large amount of colored sewage. Bioremediation of dye effluents by microorganisms is cost effective and eco-friendly **[3][4]**.Treating the textile effluent by bacteria, fungi, yeast or any consortia can be used as an alternative efficacious tool against the burgeoning water pollution **[5]**. Microbial cells are effective in the synthesis of AgNPs **[6]**. Currently, it was found that due to the high surface area AgNPs are more reactive towards aromatic chemicals compounds and are a potent tool in treatment of effluent within a short stretch of time **[7]**. In the recent study stated herein, fungi were isolated and identified from unprocessed textile effluents. Different physicochemical parameters were optimized for decolorization of ME_4BL dye, one of the commonly used reactive dye in textile industries, and fungal biodegradation was shown as the mechanism of decolorization of ME_4BL. The various factors affecting dye degradation such as agitation speed, pH, initial concentration and temperature were optimized. The intermediate degraded products were determined by UV-visible Spectrophotometer and HPLC. The combination of AgNPs with microorganisms exhibited good results in the degradation of ME_4BL dye.

MATERIALS AND METHODS

2.1 Sample collection

The effluent sample was collected from the textile industrial regions

(a) Sample 1 was collected from Pandesara G.I.D.C, Surat (G1)

(b) Sample 2 was collected from Navjivan synthetic, Surat (N1)

2.2 Dyes and Chemicals

The textile dye, reactive dye (ME_4BL) was procured from phase 2 G.I.D.C, Vatva Ahmedabad Gujarat, India. The molecular formula of ME_4BL is $\mathbf{C_{31}H_{19}ClN_7Na_3O_{21}S_6}$ and the molecular structure is shown below:

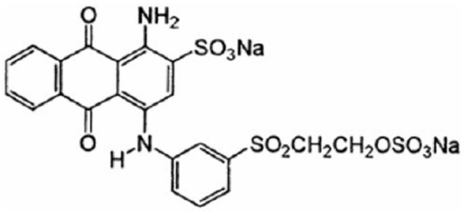

Figure 1: Molecular Structure of ME_4BL dye

2.3 Preparation of dye solution

100 mg of solid dye powder was dissolved in 100 ml of distilled water followed by filter through 0.01 Whatman filter. The solutions of the desired concentration for various experiments were obtained by successive dilution.

2.4 Determination of the dye maximum absorbance

The maximum absorbance of each dye was determined spectrophotometrically using a spectrophotometer.

2.5 Isolation of efficient dye decolorizing bacteria/fungi

The nutrient was supplemented with 100 mg ME_4BL dye and combined with distilled water and was inoculated in Nutrient broth. 10 gm of soil sample from various sites was added into the flask and incubated at 28±2 and 37±2 in a rotatory shaker upto 72 hours condition to enrich the dye-degrading bacterial/fungal population. One milliliter of the culture was withdrawn after 2 days of incubation and it was serially diluted and plated on nutrient agar medium. After incubation, the bacterial colonies were isolated and purified from the plates..

2.5.1 Screening of soil-derived fungi for dye decolorization activities

Screening was done by preparing the fungal culture and cup borer method was carried out on Bushnell and Hass Medium (BHM) +Dye (100 ppm). After incubation the observations for the zone of decolorization on the respective plates were recorded.

2.6 Effect of various Physico-chemical Analysis of waste water

The sample of effluent was analyzed for various Physico-Chemical parameters. pH, Conductivity, TDS, TSS, Total Alkalinity, Total hardness, Chloride, BOD, COD were analyzed as per the standard procedures prescribed by APHA **[8]**.

2.7 Bacterial /Fungal isolation and cultivation

Initially the samples were enriched by using nutrient broth and numerous colonies were obtained through serial dilution and streaking methods. Each strain was then inoculated into nutrient broth and incubated for 24 hrs. at room temperature under shaking

condition.1ml nutrient broth inoculums was transferred into 150 ml flask containing 100 ml BHM broth with dyes and incubated for 24 hours at room temperature under shaking condition.

2.8 Dye decolorization assays

The flask containing BHM medium with dye (150 ml) was inoculated using 1 ml of fungal culture. These flasks were incubated at 24 hrs at room temperature under static condition. Controls without inoculation were also kept for reference. The decolorization was determined by measuring the difference between initial and final optical density at specified nm **[9]**.

The percent of Decolourization was calculated as:

% Decolourization = (Initial absorbance – observed absorbance) × 100) /Initial absorbance

2.9 Effect and characterization of decolorization and degradation

2.9.1 Decolorization / degradation under stationary / agitation condition

Decolorization of dyes was studied at different physico-chemical conditions like under agitation and stationary conditions to find out the pattern of decolorization / degradation. Influence of static & shaking conditions was studied. Shaking condition was maintained at 150 rpm at room temperature and the static condition was maintained in the laboratory at room temperature **[10]**.

2.9.2 Effect of different pH

The effect of various pH on the decolorization process was studied, BHM dye media having different pH 4, 7, 9 were studied. The pH of medium was adjusted with 1 N hydrochloric acid or sodium hydroxide. The flask was inoculated and incubated at room temperature under static condition and the pattern of decolorization was observed **[11]**.

2.9.3 Effect of different Temperature

To study the effect of different temperatures on the decolorization process, BHM dye mediums were inoculated and kept at different temperatures 30℃, 50℃, 80℃ and desired pH under shaking condition to observe the pattern of decolorization **[10]**.

2.9.4 Effect of different Carbon source

BHM dye medium inoculated with the different co-substrates individually about 0.5% (Glucose, lactose, sucrose) and were further studied for the degradation / decolorization. At different time intervals, the samples were withdrawn from the flasks and centrifuged at 7700 rpm for 15 min and their absorbance were measured **[12]**.

2.9.5 Effect of different Nitrogen sources

BHM dye medium was inoculated with the different co-substrate individually about

0.5% (Beef extract, peptone, and urea) and were further studied for the degradation /decolorization **[13]**.

2.9.6 Effect of different dye concentration

BHM dye medium was inoculated with different dye concentrations of (100ppm, 200ppm, 300ppm) and were further studied for the dye degradation / decolorization **[14]**.

2.10. Biodegradation analysis

Untreated and treated effluents were analyzed by the HPLC. Experiments were carried out on Shimadzu Instrument with a UV detector at 250 nm. Each 5 ml of untreated and treated effluent was taken, centrifuged and filtered through a 0.45 µm membrane filter (Millipore make). The filtrate was then extracted by Methanol. The obtained solid mass was dissolved in methanol and was analyzed by HPLC. A 20 µl sample was injected into Octa Decyl Sinane (ODS -C18) column (4.6mm ID x 250 mm length). Methanol of purity 100% was used as a mobile phase with the flow rate of 1 ml/min for 10 minutes and UV detector was kept at 250 nm **[15]**.

2.11 Synthesis of silver Nanoparticles ($AgNO_3$)

The organism was allowed to grow in the biomass production broth containing 5g/l malt extract powder and 10g/l glucose and after 7 days the biomass was separated and allowed to grow in deionized water for 3 days. 1mM final concentration $AgNO_3$ was added to the filtered biomass and incubated in dark conditions at 30°C under shaking conditions and the formation of nanoparticles was examined under UV-visible spectrophotometer at 24hr time interval. The scanning range of the samples was 200-800 nm in the spectrophotometer **[16]**.

2.12 Characterization of the nanoparticle

Nanoparticles were characterized by UV-Visible studies; the particles were subjected to TEM studies for their size and shape determination **[17]**.

3. RESULT AND DISCUSSION

3.1 Sample collection

For the isolation of the dye decolorizing microorganism effluent were collected from different contamination sites, pH of all collected effluent samples were alkaline in nature. Colors of collected samples were reddish and bluish.

3. RESULTS

3.1 Sample collection

For the isolation of the dye decolorizing microorganism effluent were collected from different contamination sites, pH of all collected effluent sample were alkaline in nature. Colors of collected samples were reddish and bluish.

3.2 Isolation and screening of bacterial/fungal isolates

The selective enrichment of soil samples and textile effluent led to the isolation of 5 morphologically distinct fungi (A1 to A5). It was observed that out of five fungi three (A1, A3 and A5) were not able to degrade dye. A4 Fungi was subjected for further studies as it was showing highest dye degradation efficiency.

3.3 Effect of different parameter for A4

3.3.1 Effect of Static and Shaking Conditions

The effect of static and shaking conditions on dye decolourization is shown below in **(Figure 2)** which states that shaking condition was much effective in case of A4 fungi**.**. The biodegradation of the Red ME_4BL dye was monitored by UV-Vis analysis. The untreated Red ME_4BL dye presented absorbance peaks at 540, 289 and 290 nm. For treated dye, after biodegradation of the reactive dye in the static and shaking treated solution, the absorbance peaks in the visible region disappeared, indicating complete decolorization. It was noted that shaking condition favored the decolorization of Reactive violet - I dye by *Ganoderma cupreum* AG-1 **[18]**. It was observed that static condition was much favorable than shaking condition. Under static condition, a rapid inflation in the percent of decolourization by 12.63 % by *Streptomyces DJP15* for azo blue dye was observed **[19]**. It was also noted that *Micromonospora spp* was more effective and potential in decolourising reactive azo red dye under static than shaking conditions **[20]**. Some scientists has also explored static condition for bacterial dye decolorization **[21]**.

3.3.2 Effect of pH on Dye decolourization:

The maximum decolorization of dyes was recorded at pH range of 4-7-9 and any increase or decrease pH from the optimal pH range reduces the decolorization efficiency **(Figure 4)**. Literature review showed that the *P. otitidis* have an excellent degradation capacity at pH 7.0 which shows degradation of RB 250 textile dye within 8 hours **[11]**. It was also noted that *P.chrysogenum* showed maximum decolorization of azo dye Red 3BN at pH - 8 **[22]**.

3.3.3 Effect of Temperature

The A4 isolate showed maximum decolorization at 37°C **(Figure 4)**. It was observed that rate of decolorization increased from 37°C, 50°C, 80°C but it has been significantly affected when temperature increased above the 50°C. In the present study, all the isolates showed decolourization

of Reactive red ME4BL at 37°C, an increase in temperature above 37°C decreases the decolourization activity of the fungi. Similarly, decolorization of Acid Red 151 dye by *Aspergillus terreus SA*3 was optimum at 30°C and reduced above 50°C **[23]**. The studies on decolorization of Red RBN dye by *A. hydrophilla* showed decolorization in the range of 20 - 35^{o} C **[9]**.

3.3.4 Effect of Carbon source

While trying to enhance decolourization performance of Reactive red ME4BL, extra carbon source were supplied in medium. Present of decolourization was observed maximum with Lactose (95.35%), Glucose and (70.35%) while less decolourization with other supplements of carbon sources sucrose (55%) within 72 hrs. **(Figure 5)**. The consortium-GR showed moderate decolorization in the presence of different carbon sources like sucrose (77%), glucose (57%), urea (67%), lactose (70%) and yeast extract (48%), whereas addition of malt extract (10%) showed negligible decolorization after 24 hrs. incubation **[24]**. Glucose was proved as the ideal carbon for *Bacillus licheniformis* and *Pseudomonas putida* **[25]**.

3.3.5 Effect of Nitrogen source

In present study decolourization was observed maximum with Beef extract (86.76%) and Urea (65.35%), while there least decolourization was noted in the presence of Peptone within 72 hrs. **(Figure 6)**. It was noted that many bacteria such as *Bacillus, Klebseilla, Salmonella* gave the best result using beef extract but the results and effect varies according to the species **[13]**. For the *E.cloacae* urea was found to be optimum nitrogen source in degradation point of view **[26]**. The existence of supplementary organic or inorganic nitrogen sources, repressed the ligninolytic system of the white-rot fungi **[27]**.

3.3.6 Effect of Dye concentration

After incubation of 72 hr of A4 fungi in three dye concentration of 100ppm, 200ppm, 300ppm, showed good ability to decolourize the dyes reactive red in 300 ppm **(Figure 7)** .The obtained results showed that the time required for decolourization of the studied dyes was directly proportional to the concentration of dye in the system. The concentration of dye plays an important in the decolorization process by biological method. Around 93.5% decolorization of Acid Red 151 dye was obtained by *Aspergillus flavus* followed by *A. terreus* (88%) and *A. niger* (82 %) at 50 ppm dye concentration **[23]**. It was stated that around 34% decolorization was achieved upon 200

mg /L of the dye after 4 days using *Pseudomonas Spp.* ETL-1982 **[28]**.

3.4 HPLC Analysis

HPLC analysis of control displayed a peak at a retention time of 2.43, 2.94, 3.56 and 4.24 min **(Figure 8)** whereas that of the extracted metabolites after degradation there is the appearance of new peaks at retention times of 2.21, 3.16 and 4.17 min **(Figure 9)**. HPLC analysis showed prominent peak at retention time at 2.21 min when products were separated from the sample obtained after decolourization compared to control peaks. Similarly, HPLC analysis of the reactive red dye also reported that the retention times showed the disappearance of the dye after microbial treatment. Overall HPLC study concluded degradation product (Reactive Red ME_4BL) showed major peaks at 4.91 min which were not observed in case of treated samples at 3.09 min which confirms the degradation of dye **[15]**. Some HPLC analysis reported two peaks of standard/untreated Navy N5RL1 carpet dye at the retention time 5.99 and 6.00 min and peaks at lower retention time at 3.99 and 4.10 min after microbial treatment due to the degradation of dye into small intermediate products **[30]**.

3.6 Biosynthesis of nanoparticles:

However, with 1 mM $AgNO_3$ solution no colour change was observed. Therefore, the experiment was carried out by taking 2 mM solution and the extract was added dropwise to the solution. The AgNPs formation was indicated by the gradual colour change of the solution from light to dark brown, and the characteristic surface plasmon resonance (SPR) peak around 430 nm further confirmed the presence of AgNO3 in the suspension **[16]**.

3.7 Characterization of Silver Nanoparticles

3.7.1 Effect of Nanoparticles with A4 Isolate:

Different concentrations of $AgNO_3$ solution were also optimized for synthesis of $AgNO_3$ nanoparticles. Synthesis of AgNO3 started at a concentration of 1.5 mM and showed maximum absorbance at highest (2 mM) concentration. The reaction was performed for 100 min and a characteristic SPR band was observed around 430 nm, indicating efficient formation of AgNO3. Similarly, different concentrations of extract were optimized with 2 mM silver nitrate solution. From the graph **(Figure 10)**, it is clear that the yield of AgNO3 increased decolorization when the concentration of the A4 was increased. Some literature has reported that the presence of silver nanoparticles increased

the efficiency of decolourization of Acid Orange dye **[30]**.

3.7 TEM

Further, the AgNO3 characterized on TEM showed average particle size of ~100nm **(Figure 11)**. It is noted that AgNPs of 10 nm size or below display activity by itself owing to its smaller particle size and proper cell interaction. G. Sathiyanarayanan reported the presence of spherical- shaped polymeric nano particles produced from *Bacillus subtilis MSBN17* of 30-60 nm size by TEM images **[31]**. In previous studies, TEM images exhibited a size of distributed spherical shaped particles having 35nm average size with various sizes ranging from 30 to 200nm **[32]**.

3.8 Identification of the isolate based on 18 ITS

A4 isolate was further characterized using molecular basis of identification 18 ITS and it was identified as *Aspergillus terreus*.

Table 1: Physiochemical parameters of collected sample

Sample	pH	Colour	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	Hardness (mg/l)
(1) Pandesara G.I.D.C	8.4	Bluish	176.33	529	4870	2190
(2) Sachin G.I.D.C	7.6	Reddish	165.66	497	5570	2980

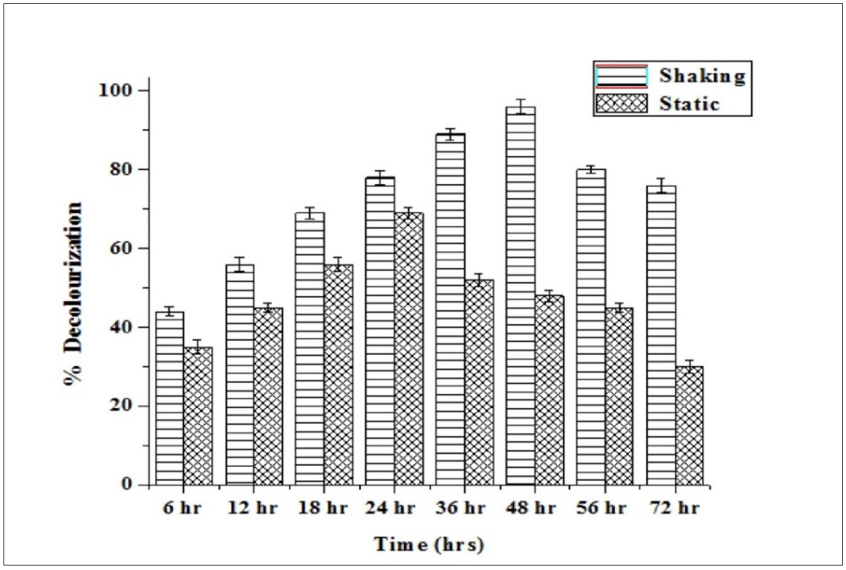

Figure 2: Effect of static and shaking on dye decolourization (A4)

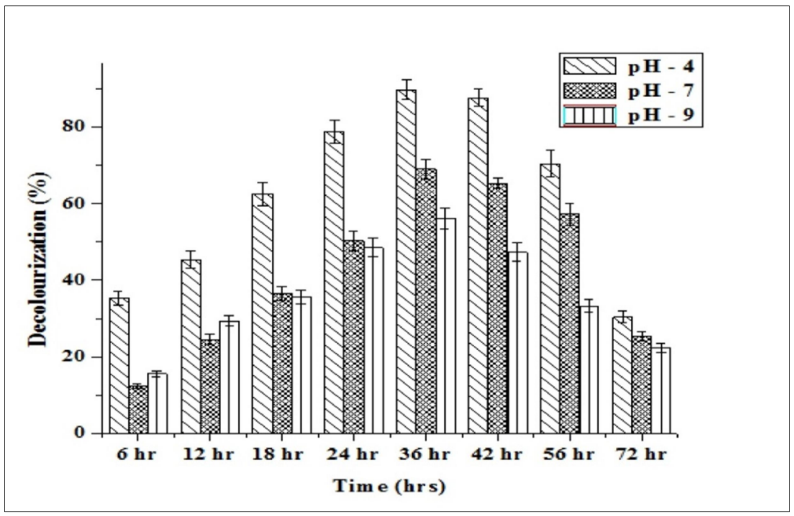

Figure 3: Effect of pH on dye decolourization (A4)

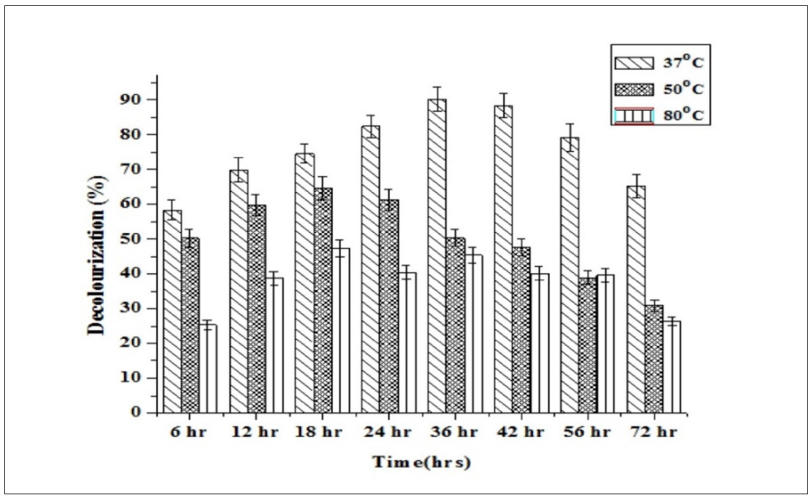

Figure 4: Effect of Temperature on dye decolourization (A4)

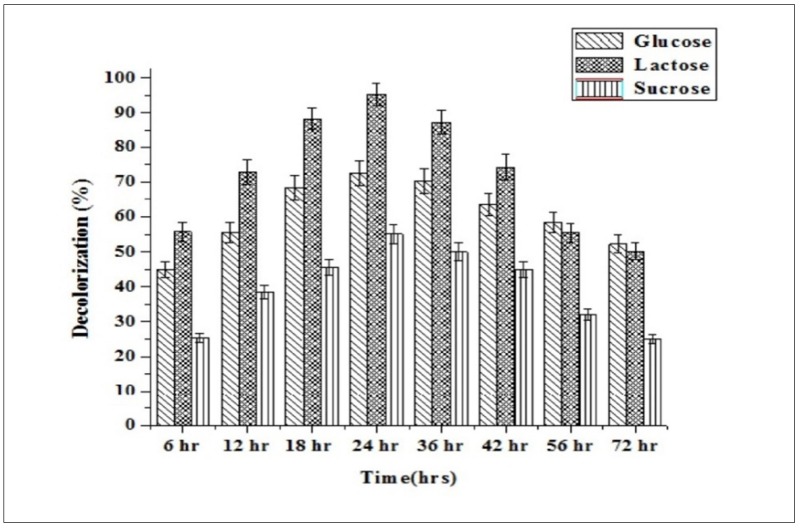

Figure 5: Effect of Carbon Sources on dye decolourization

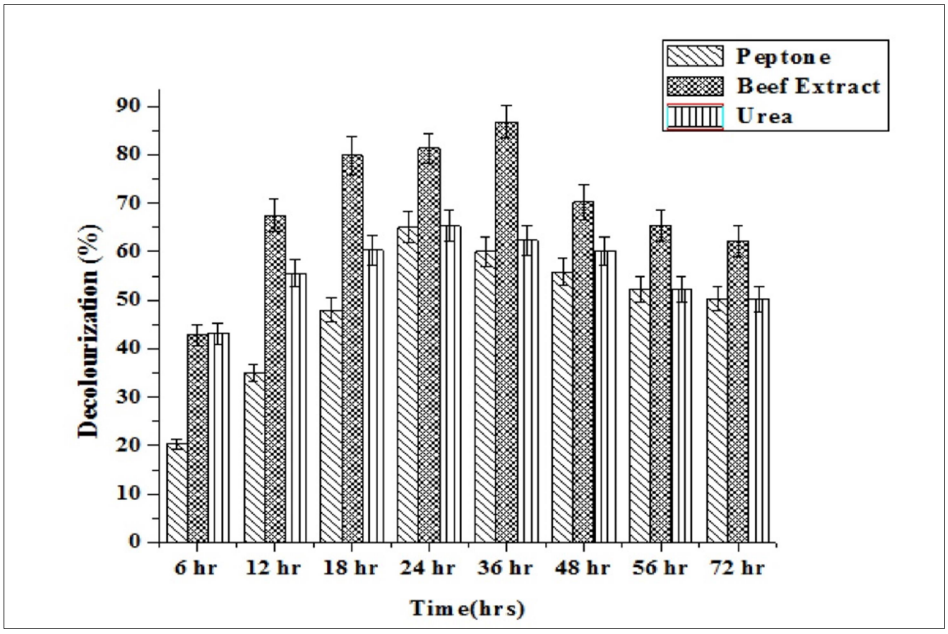

Figure 6: Effect of Nitrogen Source on dye decolourization

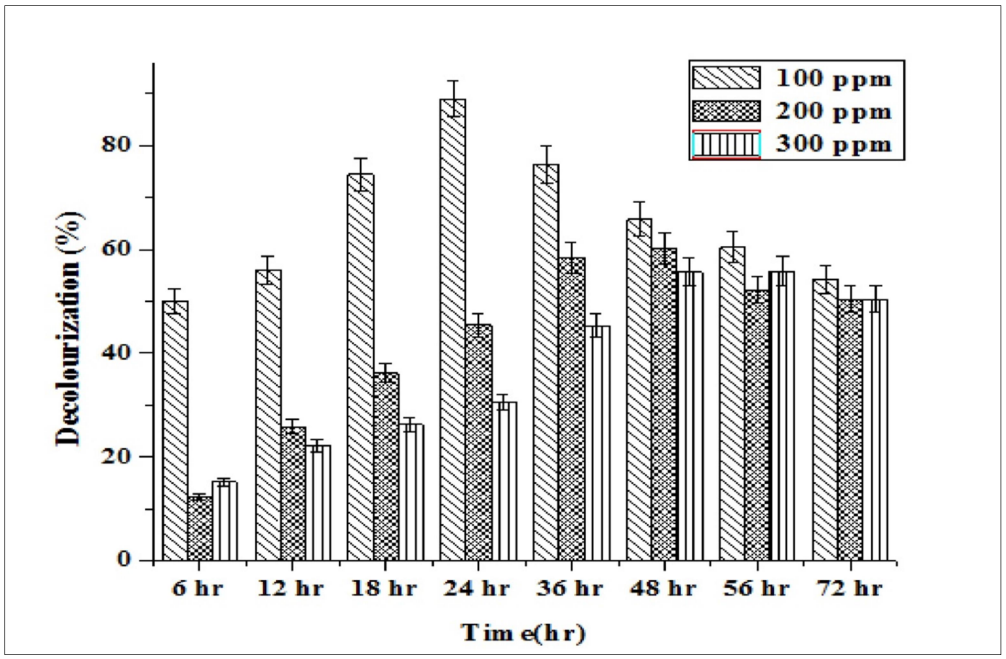

Figure 7: Effect of Dye Concentration on dye decolourization

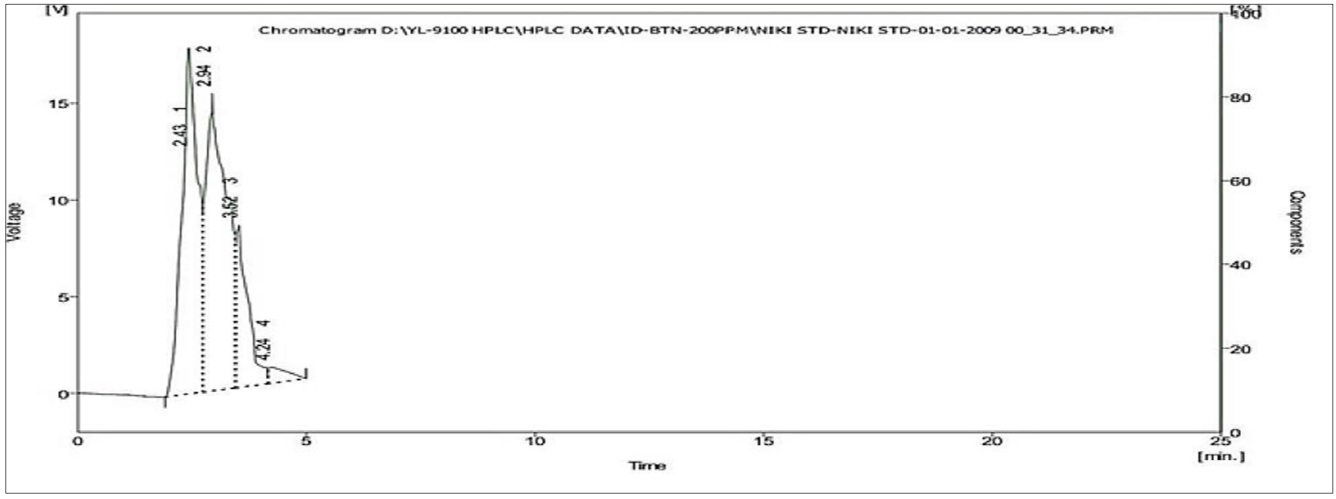

Figure 8: HPLC Chromatogram of Standard Dye

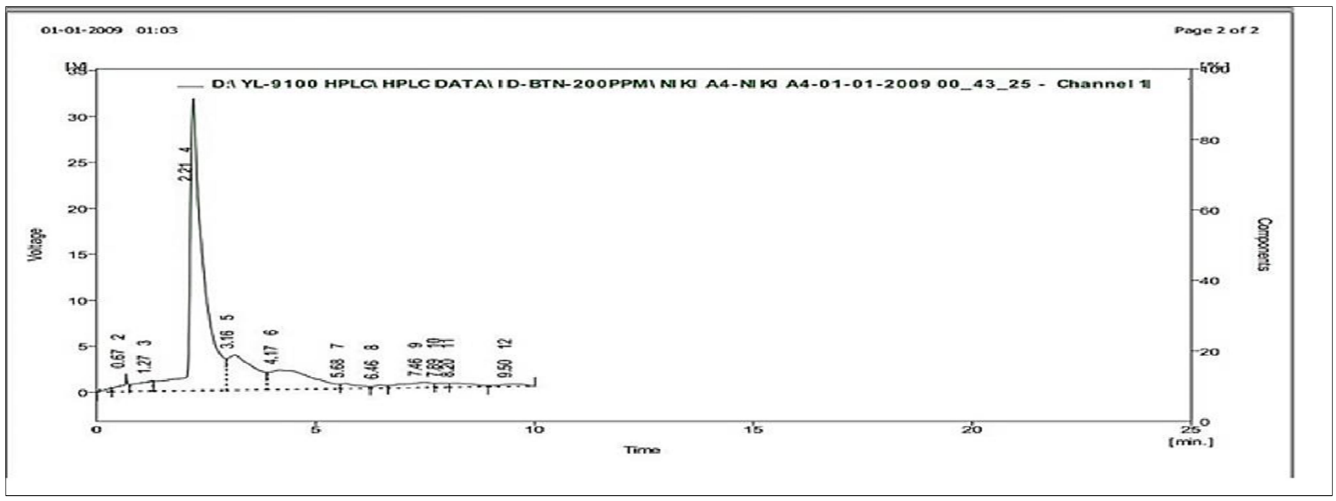

Figure 9: HPLC chromatogram after degradation using A4

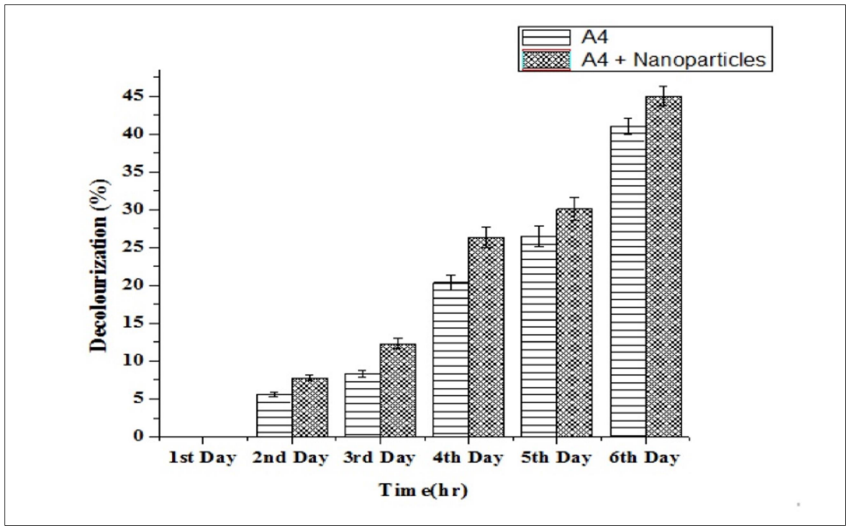

Figure 10: Effect of Nano Particles on dye decolourization with A4 fungi

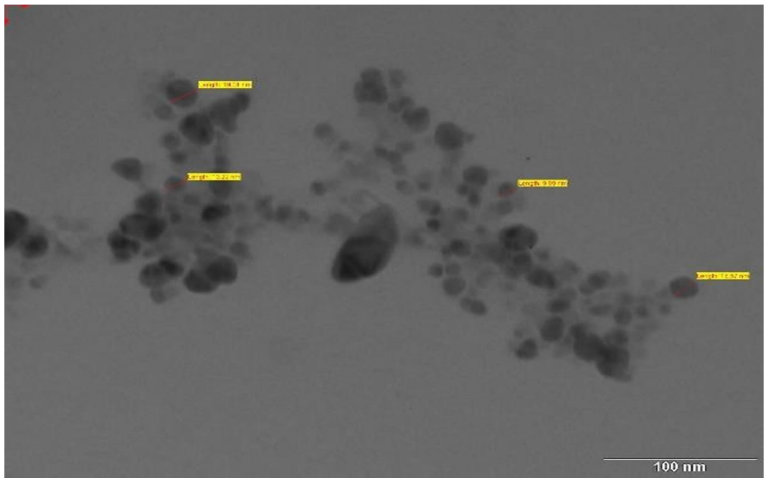

Figure 11: TEM Analysis of A4 fungi

4.0 CONCLUSION

The present study confirms the ability of isolated fungi *Aspergillus terreus* to decolorize the textile dye ME_4BL with decolorization efficiency of 74.36 %, thus suggesting its application for decolorization and degradation of textile effluents. Static condition was proved to be much efficient than shaking condition. The optimum temperature and pH was reported as 37˚C and 4 respectively. The UV spectrum and HPLC confirms the decolourization. The rate of decolourization was effective using silver nanoparticles along with the fungal strain. Thus, AgNPs can be used as effective tool and a low cost strategy for degradation of textile effluents.

5. Acknowledgement

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