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**Research Article**

# PRODUCTION OF LACCASE BY *COCHLIOBOLUS HAWANIES* AND THEIR ABILITY TO DEGRADE TEXTILE DYE

**Sumathi Tirupati and Sai gopal D.V.R***

Department of Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh

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**ABSTRACT**

The assessment of effluents generated from textile industries. The textile dyes in these present study was C1 Vat Brown, reactive black5, Violet, Indigo, acid blue1. The effluent with concentration of 50 and 100 ppm was analysed for decolourization with laccase producing fungal culture of *Cochliobolus hawanies*. The product of biological treatments was analysed after different days of intervals (3rd, 6th, 9th, 12th and 15th) and the effluents with 50 and 100ppm initially colour absorption was measured. The protein, laccase and biomass also calculated, graphs were plotted by using origin. The decolourization used in this work has significantly reduced in 50ppm compared with 100ppm concentration that is 68.15%, .88.30%, 79.72%, 78.99, and 70.97%.



## INTRODUCTION

A dye is defined as a substrate widely used for colouring diverse material [1]. Dyed flax fibers have been found in the republic of Georgia dated back in prehistoric cave to 36,000 BC [2]. Archaeological evidence shows, in India and Phoenicia, dyeing has been widely carried out forever 5,000 years [3]. There are more than 10,000 commercially available dyes and pigments which are used in dyeing and printing industries around $7\times10^5$tonnes of dyes produced annually of which 10-15% lost in the effluents during process [4]. The first human made organic dye, mauveine was discovered by William Henry Perkin in 1856, the result of failed attempt at the total synthesis of Quinine [5], and many thousands of synthetic dyes have been since prepared [6]. Synthetic dyes quickly replaced the traditional natural dyes and the cost less, they offered a vast range of new colours and they imported better properties to the dyes materials [7]. Dyes are divided into acidic, basic, azoic, chromic, diazoic dispersive and reactive sulphur and vat dyes [8]. Majority of textile industries are major contribution to the disposal of toxic dyes into natural water [9]. A special problem is encountered in the application of synthetic dyes having a complex aromatic molecular structure and designed to be resistant to physical ,chemical and microbial fading [10]. The common waste water treatment is not effective and different physical, chemical, biological treatment [11]. Now a day's microbial decourisation involving suitable bacteria, fungi has attracted increasing interest [12, 13]. The decolourisation by fungi using an oxidative mechanism has the advantage of giving products they are less toxic compare with initial dyes [14]. There are two mechanisms for treatment of dye by white rot fungi which are by biosorption of dye to the fungal biomass and biodegradation of dye into another compound by extracellular enzymes [15]. The removal of dyes in wastewater treatment is important because dyes are very toxic and are synthesised by high chemical oxygen demand, biological oxygen demand and highly aromatic conjugated and carcinogenic that can endanger human life [16].

White rot fungi have specific ability to produce nonspecific extracellular enzymes such as Laccases (EC 1.10.3.2) Lignin peroxidase (EC 1.11.1.4) Manganese peroxidase (EC 1.11.1.3) enhances the decolourization of dyes [17, 18, and 19]. Thus the present research work is carried for biodegradation of textile dyes by using different carbon sources by *Cochliobolus hawanies.* This is the first report about the decolourization different textile dyes by *Cochliobolus hawanies.*

## MATERIAL AND METHODS

### *Microorganisms and culture conditions*

The various white rot fungi were isolated from soil sample collected from renigunta in Tirupati. The fungal spores [*Cochliobolus hawanies*] harvested from seven day old slants was used as inoculum for dye decolourization.

### *Enzyme Assay*

The flasks containing homogenised suspension of fungal spores was incubated in orbital shaker at $29 \pm 2\ ^0$C and 120 rpm. The flasks with growing culture of *Cochliobolus hawanies* were withdrawn at regular intervals [3rd, 6th, 9th, 12th and 15th day] during the course of the experiment for processing. The fungal

*Corresponding author: ***Sai gopal D.V.R***
*Department of Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh*

culture was aseptically filtered through preweighed Whatman No.1 filter paper to separate mycelium mat and the culture filtrate. The filter paper along with mycelium mat was dried at $70^0$C in an oven until constant weight. Difference between the weight of the filter paper having mycelial mat and weight of only filter paper represented biomass of fungal mat. The fungal mat can be expressed in terms of gm. /flask. $P^H$ of the culture filtrate was measured. Content of extracellular protein in culture filtrates of fungi was estimated according to Lowry *et al* [20].

***Laccase activity:*** Laccase activity was assayed using 10mM guaiacol in 100mM acetate buffer (pH 5.0) containing 10% (V/V) acetone. The change in absorbance of the reaction mixture containing guaiacol was monitored at 470nm ($\varepsilon$ = 6740M−1 cm−1) for five minutes of incubation [21]. Laccase activity was expressed in International Units (IU), where one unit corresponded to the amount of enzyme that oxidized one micromole of guaiacol per minute.

***Dye Decolourization:*** The synthetic dyes Reactive Black 5, C1 Vat Brown, Violet, Indigo, Acid blue1 were obtained from Dharmavaram in Anantapur District. The stock solutions (100mg/L) were prepared by dissolving them in Milli-Q water.

***Statistical Analysis***

Each result was an average of three analyses, by using the origin pro -7 2010 application was reported along with the average value.

## RESULTS AND DISCUSSION

Biomass of culture, of *Cochliobolus hawanies* (Accession. No:KF805051) depends upon growth in liquid medium under shaking conditions was determined and is presented in (Figure. 2, 3 and 50 ppm, 100ppm concentration of dye). Growth of culture was initially slow in all textile dyes for 3rd day and then picked up and remained steady from 9th day of incubation and declined to 15th day. *Cochliobolus hawanies* produced maximum biomass in C1 vat Brown and Indigo textile dye of 0.72 g/flask and 0.85 g/flask on the 9th day.

The secretion of extracellular protein into liquid medium under shaking conditions for 10 days was measured (Figure 4, 5 and 50ppm and 100ppm concentration of textile dyes). The secretion of extracellular protein by both fungal cultures increased with increase in incubation time and reached maximum on 6th day of incubation and there onwards dropped

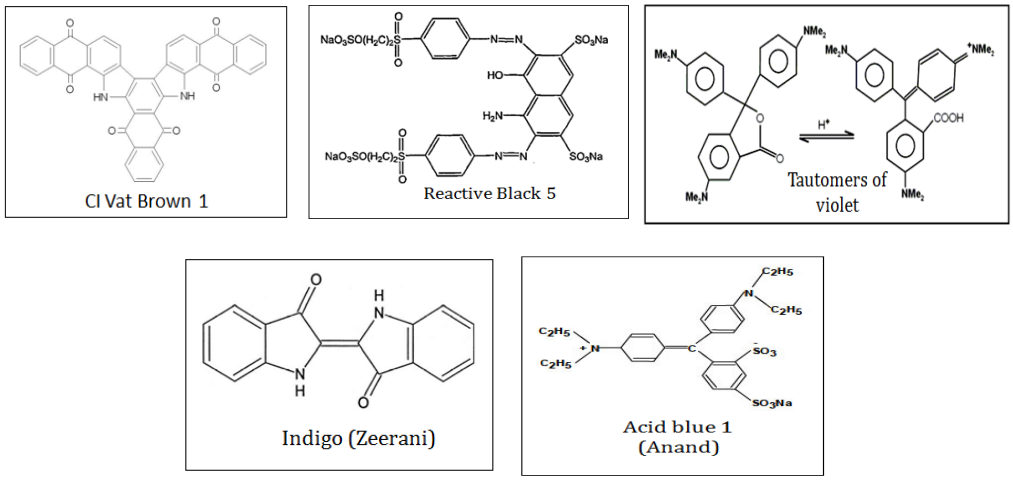


**Figure 1** Synthetic dyes structures.

The flasks were incubated for 7 days to screen the best performance of *Cochliobolus hawanies*. The experiment was conducted by growing fungal cultures since the Czapex-dox liquid medium in the presence of different dyes (at concentration with 50 to 100 ppm) in 250 ml of Erlenmeyer flasks. Dye –amended medium without inoculum was maintained as control. The flasks were withdrawn at regular intervals (3rd, 6th, 9th, 12th and 15th day) for determination of dyes decolourization, the additional parameters like biomass, protein content, and laccase activity was also determined. Absorbance of colour of dye in the culture filtrate derived from the growth of fungi was measured against uninoculated medium without dye at 483, 396, 547, 545, and 636 at the respective time intervals and was considered as observed absorbance.

Decolourization was expressed as activity (%).

$$\% \text{ Decolourization} = \frac{\text{Control absorbance} - \text{Observed absorbance}}{\text{Control absorbance}} \times 100.$$ [22].

*Cochliobolus hawanies* secreted maximum protein content in C1 Vat Brown of 0.680 $\mu$g/mL into medium as against 0.780 $\mu$g/mL and Indigo textile dye of6th day of incubation.

Both 50 ppm and 100 ppm concentration was exhibited laccase activity when grown on medium under non inducing conditions (Figure 6 and 7). Like extracellular protein secretion, laccase production by both concentration of textile dyes culture on 6th day of incubation and there onwards declined.

*Cochliobolus hawanies* gave titres of laccase 5 times higher than 100 ppm concentration of textile dye (C1 Vat Brown) Maximum yields of laccase to the tune of 0.85 Units/mL at 50 ppm concentration were recorded as against only 0.62 units/mL by 100ppm concentration (Indigo). Thus, results clearly show that 50ppm textile dye was better than the 100ppm concentration on the score of laccase production.

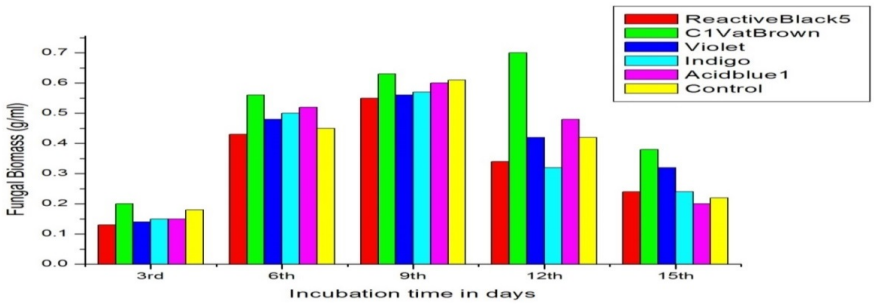


**Fig.2** Biomass of fungal cultures in 50ppm concentration of textile dyes.

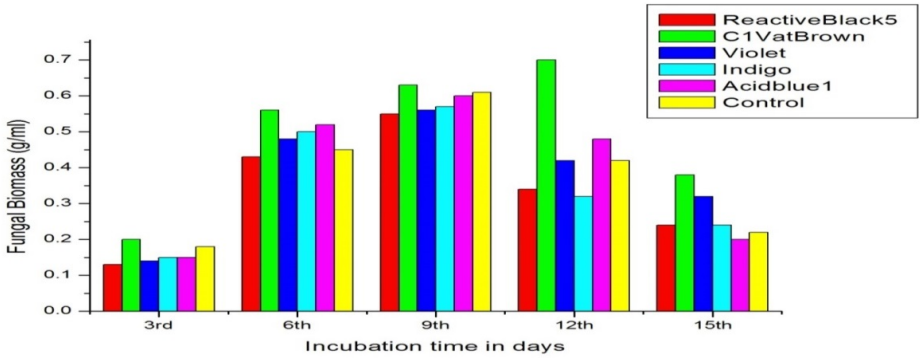


**Fig.3** Biomass of fungal culture in 100ppm concentration of textile dyes.

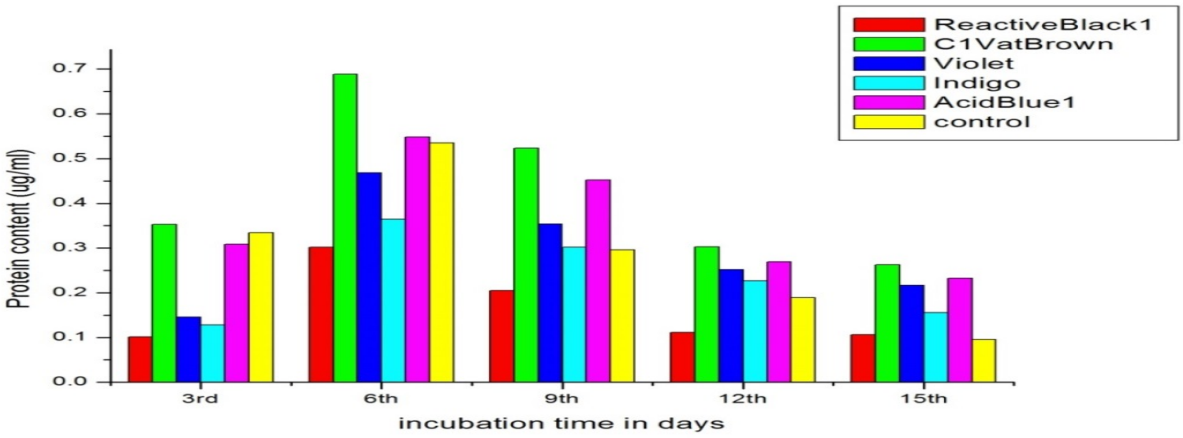


**Fig. 4** Extra cellular protein of fungal culture in 50ppm concentration of textile dye.

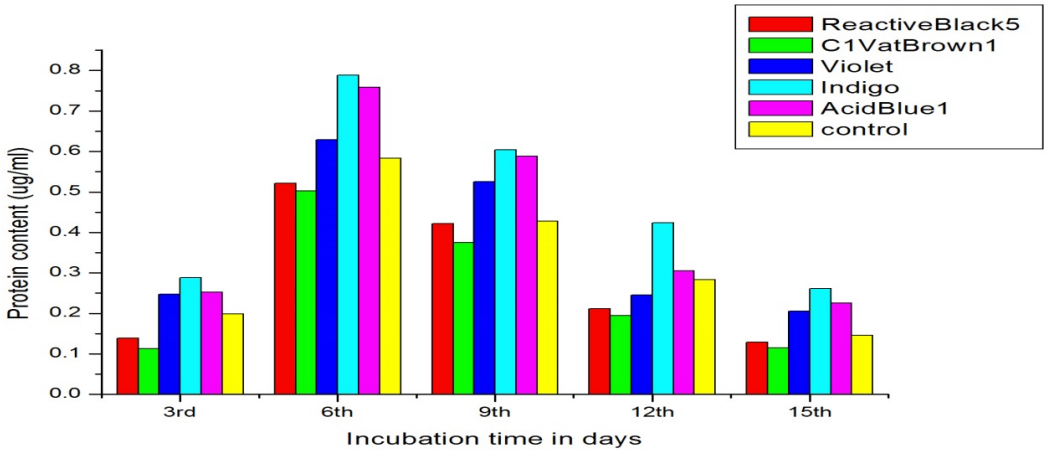


**Fig.5** Extracellular protein of fungal culture in 100ppmconcentration of textile dyes.

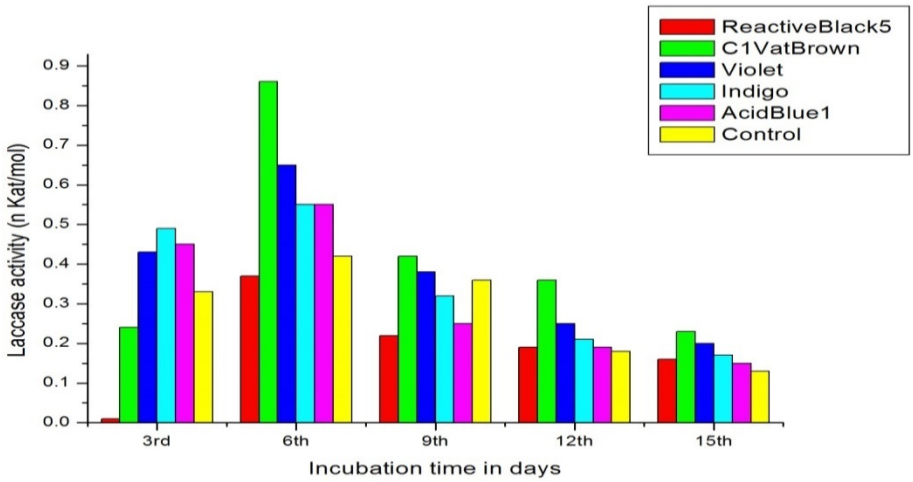


**Fig .6** Laccase activity of the fungal culture in 50 ppm concentration of textile dyes.

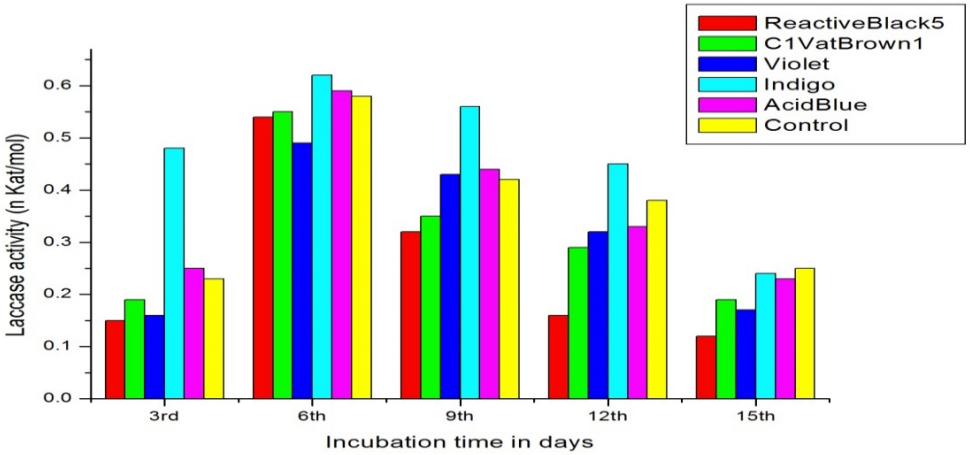


**Fig.7** Laccase activity of the fungal culture in 100 ppm concentration of textile dyes.

Textile dyes Reactive Black 5, C1 Vat Brown, Violet, Indigo, Acid Blue 1 has undergone decolourisation even at the 50, 100 ppm concentration the fungal culture was incubated (Table 1and 2). Decolourisation of dye by both concentration were followed the pattern of growth. Decolourisation was initially slow later picked up and reached maximum on 6$^{th}$ day of incubation in both concentration, but values were lower side in case 100 ppm concentration of textile dyes. The maximum decolourization of textile dyes by *Cochliobolus hawanies* at two different concentrations - 50 ppm and 100ppm concentration was found to be 68.15%, .88.30%, 79.72%, 78.99, 70.97% and 60.19%, 73.53, 74.03, 83.39%, 65.03%.

**Table.1** Decolourisation of textile dyes at 50ppm concentration by using the fungal culture.

| Incubation time (in days) | Synthetic textile dyes | | | | |
|---|---|---|---|---|---|
| | Reactive Black 5 | C1 Vat Brown | Violet | Indigo | Acid blue |
| 3$^{rd}$ | 35.12±0.30 | 50.25±0.40 | 48.13±0.36 | 39.30±0.40 | 36.31±0.20 |
| 6$^{th}$ | 68.15±0.36 | 88.30±0.36 | 79.72±0.40 | 78.99±0.25 | 70.97±0.35 |
| 9$^{th}$ | 67.29±0.30 | 87.32±0.30 | 78.31±0.15 | 78.92±0.40 | 70.93±0.26 |
| 12$^{th}$ | 66.91±0.25 | 87.19±0.37 | 78.64±0.17 | 77.51±0.26 | 69.95±0.25 |
| 15$^{th}$ | 66.82±0.37 | 86.89±0.25 | 77.69±0.10 | 77.32±0.37 | 69.93±0.40 |

**Table.2** Decolourisation of textile dyes at 100ppm concentration by using the fungal culture.

| Incubation time (in days) | Synthetic textile dyes | | | | |
|---|---|---|---|---|---|
| | Reactive Black 5 | C1 Vat Brown | Violet | Indigo | Acid blue |
| 3$^{rd}$ | 29.60±0.51 | 20.93±0.25 | 25.90±0.25 | 30.97±0.40 | 35.10±0.20 |
| 6$^{th}$ | 60.19±0.46 | 73.53±0.30 | 74.30±0.40 | 83.39±0.25 | 65.03±0.40 |
| 9$^{th}$ | 60.33±0.30 | 72.91±0.26 | 72.70±0.30 | 83.19±0.40 | 64.73±0.35 |
| 12$^{th}$ | 60.30±0.34 | 72.62±0.45 | 72.30±0.40 | 83.16±0.26 | 64.53±0.30 |
| 15$^{th}$ | 60.32±0.15 | 72.59±0.15 | 72.27±0.40 | 83.12±0.37 | 64.52±0.20 |

## CONCLUSION

The following conclusions can be drawn from the results of the present study. The white-rot fungus *Cochliobolus hawanies* produces a complete Laccase under conditions of vegetative growth. Laccase appears to be a dominant component in the production of lignolytic enzymes, *Cochliobolus hawanies* culture is more promising and potential culture in the 50 ppm concentration than the 100 ppm concentration of textile dyes.

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