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Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters

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Abstract

Decolorization of azo dye red 3BN by three fungal species Penicillium chrysogenum, Aspergillus niger and Cladosporium sp. has been analyzed using potato dextrose agar (PDA) medium containing 0.01% of Red 3 BN. Physico-chemical parameters like carbon source, nitrogen source, temperature, pH and inoculum volume are optimized for the decolorization process by changing one parameter at a time. Optimal condition for P. chrysogenum was found to be 1% maltose 1% yeast extract, pH 8, 27°C and 2% inoculums. Ideal condition for A. niger was found to be1% maltose , 1% yeast extract, pH 8, 27°C and 10% inoculum and that for Cladosporium sp. was found to be1% maltose, 1% peptone, pH 6, 37°C and 10% inoculum . Extent of decolorization recorded by P. chrysogenum under ideal conditions was 99.56%, A. niger was 98.64% and that by Cladosporium sp. was 98.18%. The study has confirmed the potential of the above fungi in the decolorization of azo dye Red 3BN and opened scope for future analysis of their performance in the treatment of textile effluent.

Keywords: Azo dye, Red 3BN, p*enicillium chrysogenum, aspergillus niger,* decolorization.

Introduction

Environmental pollution from human activities is a major challenge of civilization today[1,2,3]. Textile dyes constitute a major source of pollution. Textile industries consume a major share of dyes in India [4]. Textile dyes are classified as azo, diazo, cationic, basic, anthraquinone base and metal complex dyes based on the nature of their chemical structure. Synthetic dyes such as azo dyes, xanthenes dyes and anthraquinone dyes are very toxic to living organisms. Azo dyes constitute a major class of environmental pollutants. Some of the azo dyes or their breakdown products are known to be highly toxic and mutagenic on living organisms[5]. Characteristics of the waste water from textile industries vary depending on the process employed[6]. Accordingly wastewater generated from of the operations in wet processing such as desiring, scouring, bleaching, mercerizing, dyeing, printing and finishing differ considerably[7, 8]. The concentration of dye contained in the effluent varies between 10-200mg/ml depending on the dyeing process[9]. Many dyes and pigments are hazardous and toxic for human as well as aquatic life at the concentration at which they are being discharged to receiving water[10]. The high concentration of dyes is known to cause ulceration of skin, and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause omitting, pain, haemorrhage and sharp diarrhea[11]. Dyes used in the textile industry are difficult to remove by conventional waste water treatment methods since they are stable to light and oxidizing agents and are resistant to aerobic digestion. Presence of carcinogens has also been reported in combined waste water of dyeing and printing units[12]. The slow rate of decomposition of dyes present in waste water necessitates treatment methods to accelerate the process[13]. The methods employed for alleviating the environmental problems caused by the textile dye effluent include physical, chemical and biological treatment processes. The physico-chemical methods include adsorption, chemical precipitation, flocculation, electro floatation, oxidation via chlorine, peroxide, electrolysis and ozone treatment, reduction, electrochemical destruction and ion-pair extraction[14,15,16]. Biological methods of removal involve use of microorganisms such as bacteria and fungi to convert the pollutants into non-toxic harmless substances. Biological processes convert organic compounds to water and carbon dioxide, have low cost sustainable and are easy to use[17]. Microbial degradation and decolorization of dyes have received much attention from the viewpoint of treating industrial wastewater containing dyes[18]. Azo dyes are the largest class of dyes, which are not readily degraded by microorganisms. Microorganisms those are able to degrade azo dyes anaerobically, have been isolated[19]. However aromatic amines produced by all these anaerobic microorganisms may be toxic and carcinogenic. Current study aims to investigate the potential of fungi for decolorization of textile dyes under aerobic conditions.

Material and Methods

All chemicals used in this study were of AR grade. Textile dye, Red 3BN and effluent sample were collected from a dying industry located at Peenya, Bangalore (Karnataka). The sample was collected from the effluent disposal site of the industry. Carbon and nitrogen sources used were purchased from Himedia Laboratories (Mumbai, India).

One ml of effluent was transferred into 9 ml of distilled water in sterile test tubes. This stock solution was by serially diluted to

get concentration ranging from. 0.1 ml of sample from each dilution was spread on potato dextrose agar (PDA) plates containing chloramphenicol with the help of L-rod. The petriplates were incubated at room temperature for 5 days. A plug of mycelium of the fungal isolate was placed on a clean slide containing a drop of lacto phenol cotton blue (LCB) solution. The mycelium was spread using a sterile needle and a clean cover slip was placed above the preparation and observed under the light microscope for the identification of fungal isolate.

Pure fungal isolates were obtained on the PDA plates by sub culturing. The isolates were further sub-cultured on PDA slants and incubated at room temperature. After sufficient growth of the colonies, the slants were stored in refrigerator and served as stock cultures. Subcultures were routinely made every 30 to 60 days.

A mycelium disc of 1.2 cm diameter obtained from a 4 to 5 days old culture plates of fungus were transferred to 25 ml PDA in a 250 ml conical flask and incubated at room temperature for 4 to 5 days. At the end of the incubation period 30 ml sterile water was added to each culture and the flasks were shaken in a shaker. Then the content of each conical flasks were filtered through glass wool. The spores contained in the filtrate were used for spore count. The same spore suspension was used in the experiments described below.

All the isolates were selected for screening of decolorizing activity of the dye red 3BN. Inoculums (10 spores/ml) of each isolate were added to 100 ml of sabouraud dextrose (SD) broth supplemented with 10% dye effluent and incubated at 27°C for 6 days. After 6 days, effective decolorization was seen visually. Those isolates showing decolorization of textile dye effluent were selected for further studies on optimization of physic-chemical parameters. Three fungal strains used for extensive studies were identified on the basis of morphological characteristics.

Dye degradation activity in terms of percent decolorization was determined by following method described by Moorthi et al.[20]. 10 ml of fungal culture with dye in SD broth was centrifuged at 8000 rpm for 15 minutes. Spectrophotometer was used for absorbance measurement. The decrease in absorbance was monitored by measuring absorbance of the supernatant at 600nm for Red 3BN. Decolorization activity was calculated according to the following formula[20].

$D= [(A_0-A_1) /A_0] \times 100$

Where, D=decolorization in %; A_0=initial absorbance; A_1= final absorbance

Decolorization of red 3BN textile dye (0.02g) in SD broth by all three isolates was optimized with respect to the effect of 1%, carbon sources (maltose, fructose, sucrose), 1%, nitrogen sources (beef extract, yeast extract, peptone), pH (4, 6, 8) and temperature (27, 37°C). All experiments were carried out with 1%, (v/v) inoculum of 10 spores/ml concentration and SD broth without culture was served as control. Influence of the volume of inoculums was evaluated by inoculating 2%, 4%, 6%, 8% and 10% of respective cultures to PDA media containing red 3BN. All the flasks were incubated at respective temperature mentioned above under shaking conditions for 6 days.

The time course of decolorization was monitored under optimum conditions. Flasks were incubated up to 144h at their respective temperature and samples were removed after every 24 h and analyzed for decolorization activity as described above.

Results and Discussion

Colony morphology, microscopic observation and culture characteristics have confirmed the identity of the fungi as *P. chrysogenum, A. niger* and *Cladosporium species.* (table - 1 and figure - 1). Decolorization of the dyes is accepted as one of the indications of degradation of the dyes and hence this has been considered as the major parameter for the evaluation of dye degradation in the current study.

Figure-2 illustrates the effect of different carbon sources on decolorization of Red 3BN by *P .chrysogenum, A. niger,* and *Cladosporium sp.* Maltose has emerged as the ideal carbon source for all the 3 strains of fungi, all recording highest rate of decolorization. Fructose recoded least percentage decolorization by all the 3 strains and sucrose supported medium level of activity. Of the three fungal strains tested, *A. niger* exhibited highest activity recording 98.2% reduction in color among all the combinations tried.

Effect of Nitrogen source: Effect of different nitrogen sources on decolorization of red 3BN by *P. chrysogenum, A. niger* and *Cladosporium sp.* is illustrated in figure 3. Among the three nitrogen sources evaluated, peptone appeared to support the decolorization process by all the 3 fungal strains, recording more than 90% decolorization. However, *P .chrysogenum* exhibited highest percentage decolorization when yeast extract was used as nitrogen source, and also emerged as the strain recording highest activity.

Effect of Temperature: Evaluation of the effect of temperature on dye decolorization with reference to red 3BN by the fungi, *P.chrysogenum, A.niger* and *Cladosporium sp.* is presented in figure 4. The results have indicated 27°C as better than 37°C for *P.chrysogenum,* and *A.niger*. However, *Cladosporium sp.* showed highest activity at 37 °C. Further it has been observed that *P.chrysogenum.*is more efficient in decolorizing Red 3BN than other two species under in vitro condition.

Effect of pH: Figure – 5 illustrates the effect of different pH on decolorization of Red 3BN by *P.chrysogenum, A.niger* and *Cladosporium sp.* From the above data it can be inferred that *A .niger* is more efficient in decolorizing red 3BN than other two species and pH 8 is the ideal for its activity under in vitro condition, recording 96.67% decolorization of the dye. Maximum activity of *P.chrysogenum* was also recorded at pH 8, but extent of decolorization of the dye was only 90. 91%. Maximum decolorization activity of *Cladosporium sp*. was observed at pH 6 with 89.29% decolorization.

Effect of inoculums: Influence of the volume of inoculum on decolorization of the dye by *P.chrysogenum, A.niger* and *Cladosporium sp.* is presented in figure 6. From the data it is observed that *A.niger and P.chrysogenum* are equally effective in the decolorizing Red 3BN recording more than 95% decolorization of the dye. The ideal volume of inoculum was found to be 2% for *P.chrysogenum* and 10% for *A.niger.*

The experiments on optimization of culture conditions for the decolorization of the dye by the above three strains of fungi have identified Maltose at 1% concentration as the ideal carbon source for all the fungi tested. Peptone acted as ideal nitrogen source for *A.niger* and *Cladosporium sp.* while yeast extract promoted maximum activity by *P.chrysogenum.* Optimum temperature for *P.chrysogenum* and *A.niger* was found to be 27ºC and that for *Cladosporium sp.* as 37ºC. *P.chrysogenum* and *A.niger* recorded highest activity at pH 8 and *Cladosporium sp.* at pH 6. Optimum volume of inoculums was 10% for *A.niger* and *Cladosporium sp.* and that for *P.chrysogenum* was found to be 2%.

Evaluation of Time course of dye decolorization: The time course of decolorization of red 3BN under optimum conditions by *P.chrysogenum, A.niger* and *Cladosporium sp.* is illustrated in figure 7. The results have indicated the fact that both *P. chrysogenum* and *A.niger* are capable of executing nearly 100% decolorization of red 3 BN under their respective optimal conditions while *Cladosporium sp.* exhibited slightly lower level of decolorization activity.

Decolorization of textile dyes by fungi has been investigated extensively, reporting wide range of combinations of dyes and fungi[21,22]. Current study has confirmed the decolorization efficiency of 3 fungal strains, *P.chrysogenum, A.niger* and *Cladosporium sp.* in decolorizing the textile azo dye red 3 BN. The study has further revealed the influence of physicochemical parameters on the process. This result is in agreement with earlier reports on optimization of conditions for dye degradation[23]. Comparative analysis of the time course of decolorization by the 3 fungi under their respective optimal conditions has revealed high order of activity by *P.chrysogenum,* and *A.niger* recording almost 100% decolorization. *Cladosporium sp.* also recorded considerably good level of activity. Earlier reports have indicated the capability of decolorization of the dye reactive blue MR by *Aspergillus* spp., including *A.niger*[18]. Similarly, *Penicillium* spp. also known to decolorize different azo dyes and textile effluent[24]. Therefore, it can be concluded that species of *Aspergillus* and *Penicillium* are good source of natural microflora for exploitation in the bioremediation of textile effluent.

Table 1
Identification of dye decolorizing fungi from effluent

Isolate No.	Microscopic observations	Cultural characterstics	Organisms identified
1	Hyline and septate hyphae. conidiophores are long. They are branch and give the brush like appearance, sterigmata are long and produce chain of conidia. Conidia are spherical or oval	Initially white and fluffy, later produced pigmented spores turn into green or bluish green.	*Penicillium chrysogenum*
2	Aseptate, short conidiophores and terminally with globose vesicle. Sterigmata are doubled and covered with entire vesicle.	Cottony growth with green or yellow colour covered with black spores	*Aspergillus Niger*
3	Branching chains of conidia, showing conidiogenous loci with disjuncture conidiogenous loci at apex of a secondary ramoconidium, two conidiogenous loci at apex of a conidiophore, the one facing the viewer is clearly coronate	light green to grayish surface; gray to black back surface; blastoconidia.	*Cladosporium sp.*

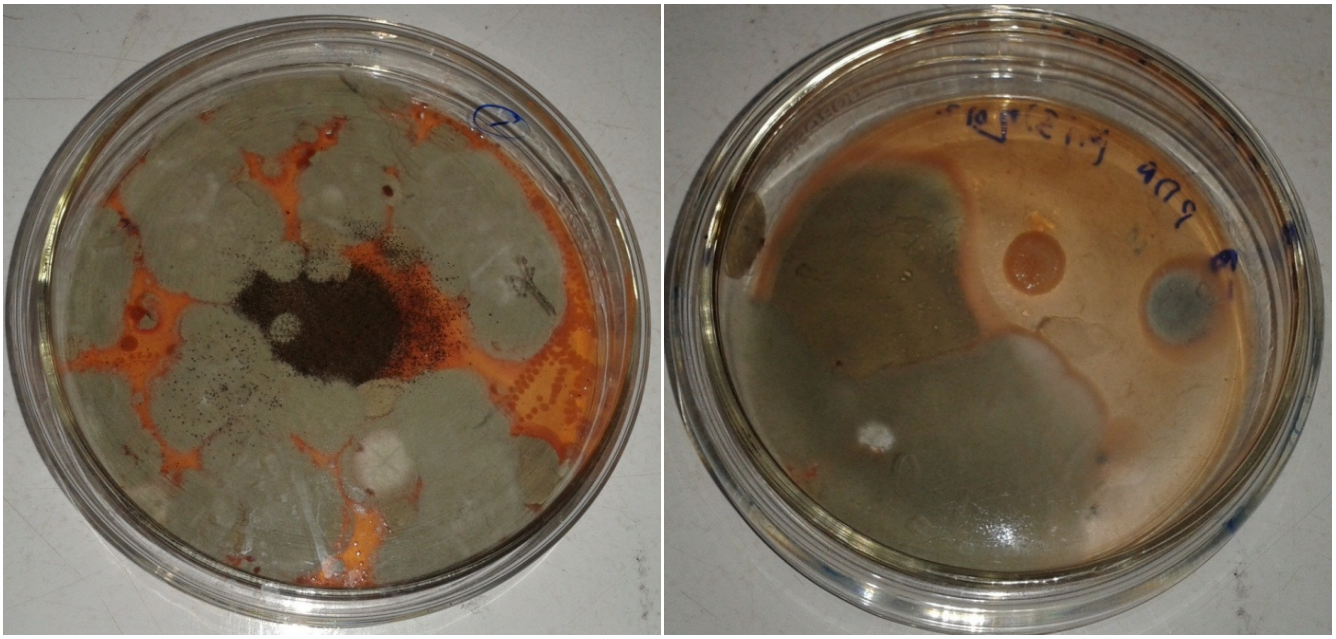

Figure-1
Petri plates containing colonies of fungi decolorizing red 3BN *P. chrysogenum, A.niger and Cladosporium sp*

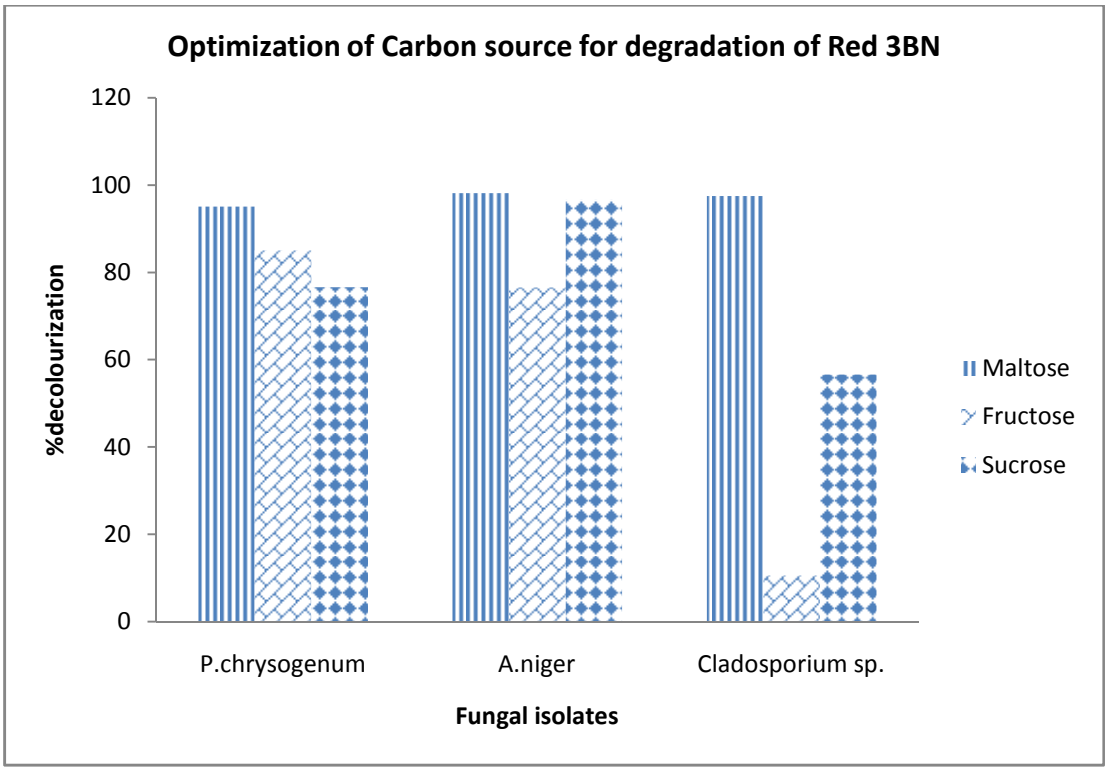

Figure-2
Effect of different carbon sources on decolorization of red 3BN dye by fungal isolates (pH 5.6, 30^{o}C , 120rpm, 144h)

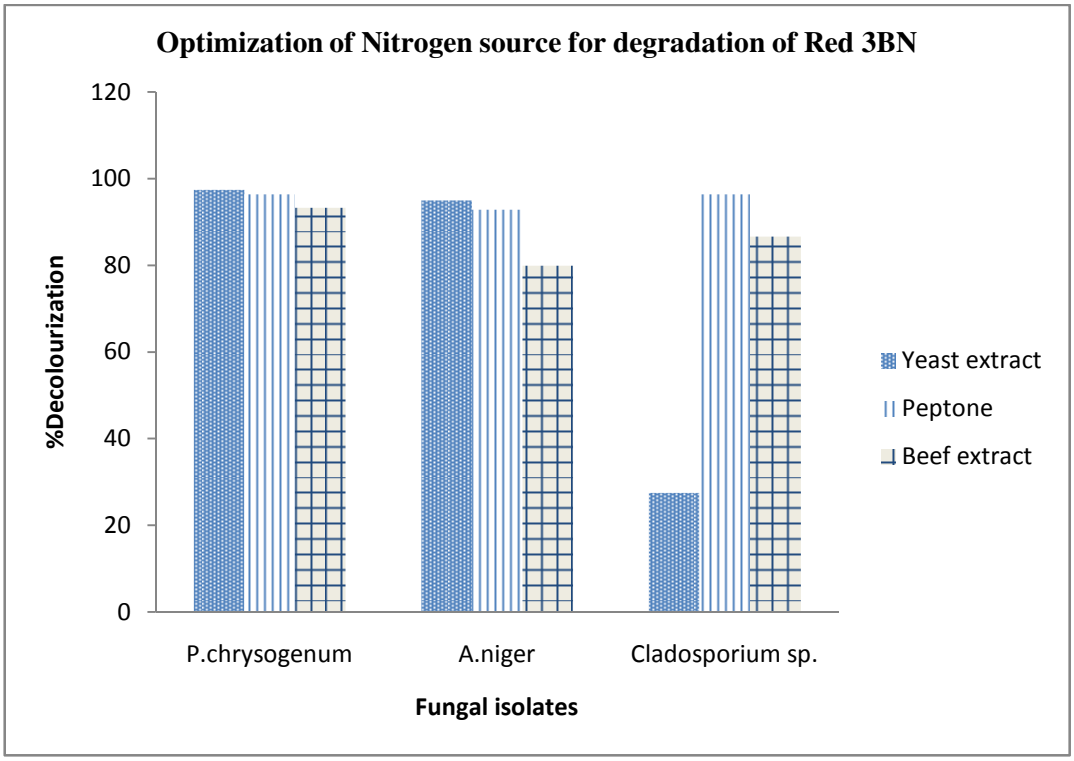

Figure-3
Effect of different nitrogen sources on decolorization of red 3BN dye by fungal isolates (pH 5.6, 30^{o}C, 120rpm, 144h)

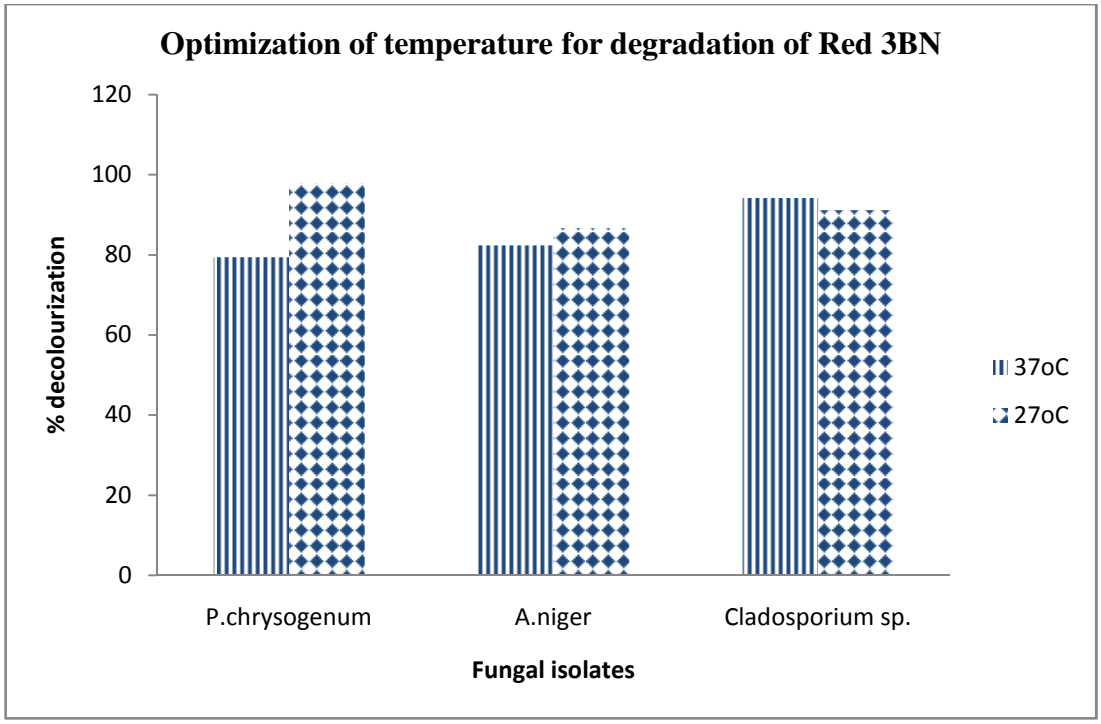

Figure-4
Effect of different temperature on decolorization of red 3BN dye by fungal isolates (pH 5.6, 120rpm, 144h)

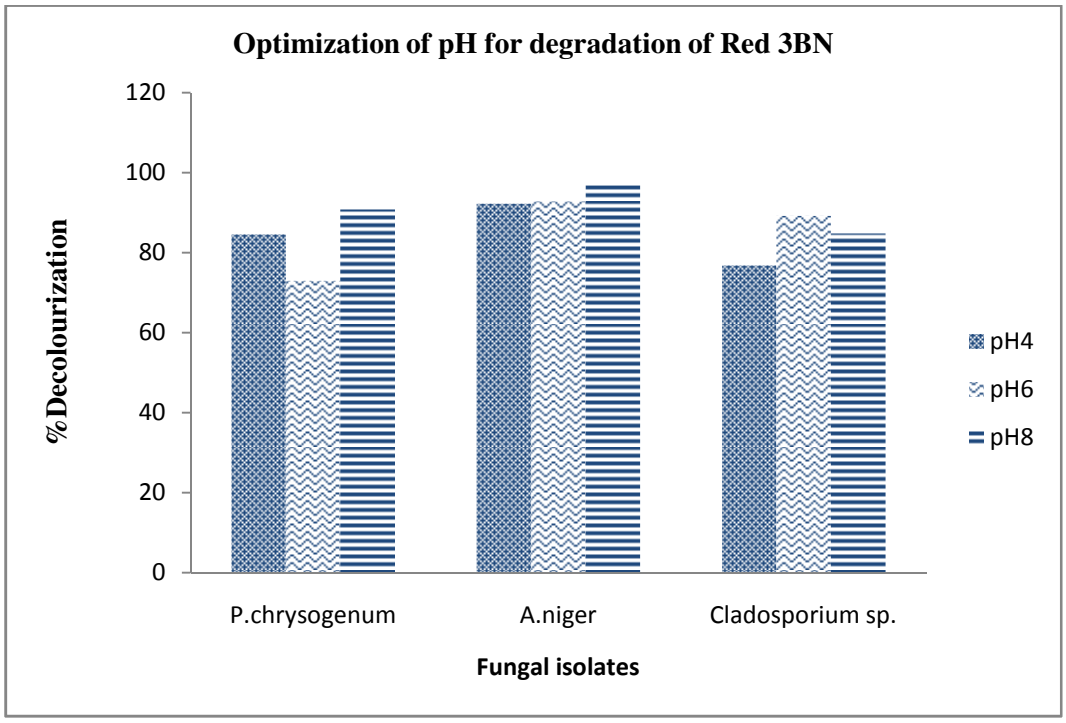

Figure-5
Effect of different pH on decolorization of red 3BN dye by fungal isolates (30°C, 120rpm, 144h)

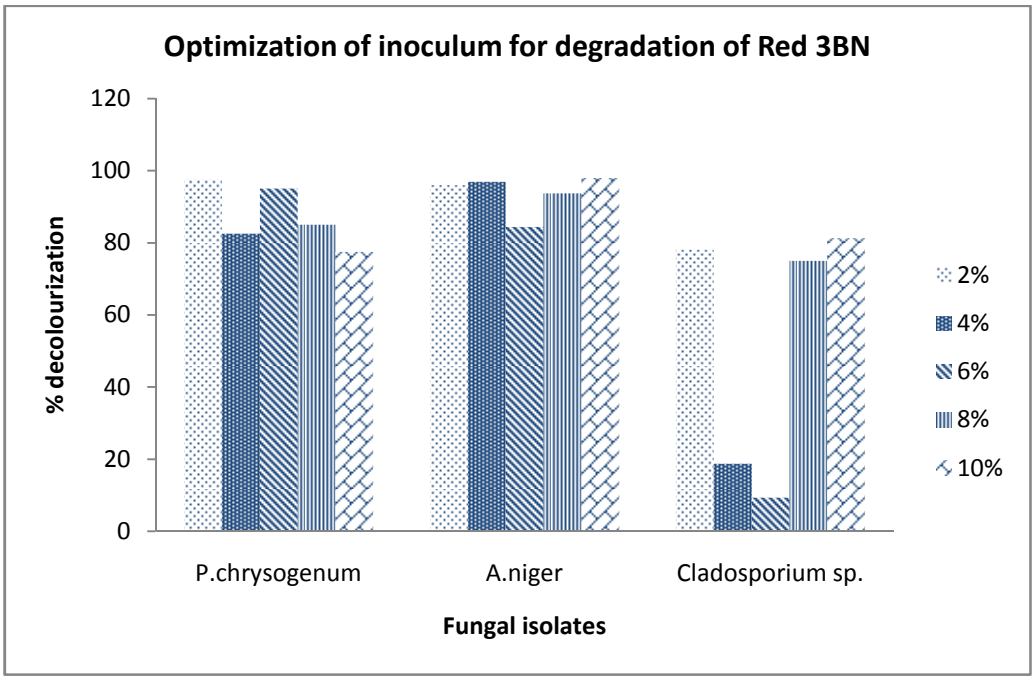

Figure-6
Effect of different inoculum on decolorization of red 3BN dye by fungal isolates (pH 5.6, 30°C, 120rpm, 144h)

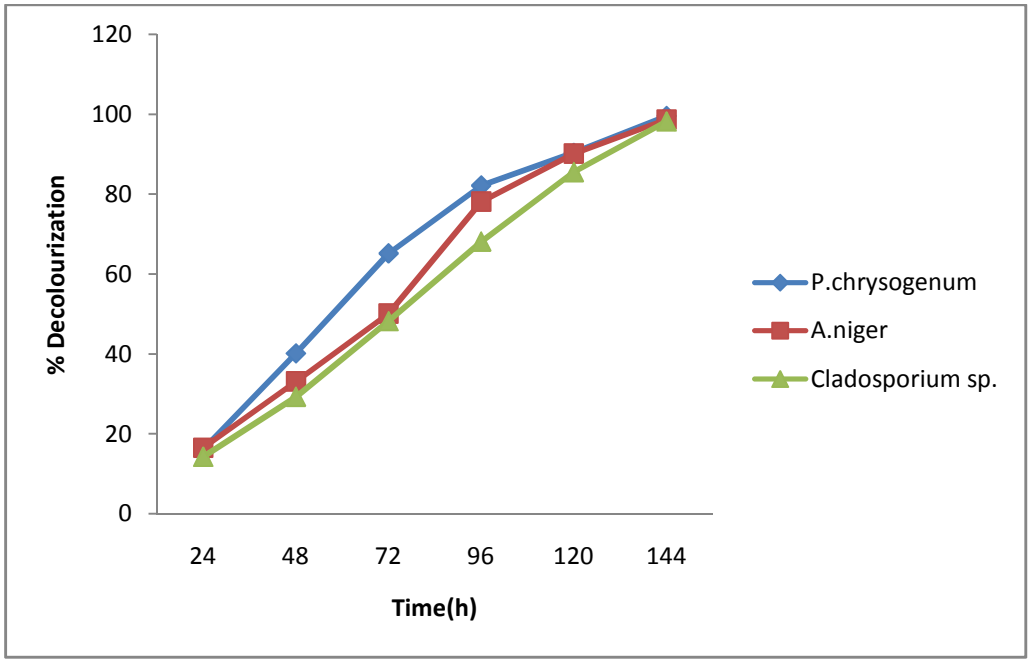

Figure-7
Time course of decolorization of red 3BN dye by fungal isolates under optimum condition

Conclusion

Current study has isolated, identified and proved the decolorization activity of textile azo dye red 3 BN by *P.chrysogenum, A.niger* and *Cladosporium sp*. and optimized the physicochemical parameters for the same. Further investigation on enzymes and mechanisms involved in decolorization is in progress.

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